DEPARTAMENTO DE FISIOLOGÍA Y
FARMACOLOGÍA.
ÁREA DE TOXICOLOGÍA

VNiVERSiDAD
D SALAMANCA

TESIS DOCTORAL

MARCADORES URINARIOS DE PREDISPOSICIÓN AL FRACASO RENAL AGUDO INDUCIDA POR NEFROTÓXICOS

R. Laura Vicente Vicente
2011

VNiVERSiDAD DE SALAMANCA

DEPARTAMENTO DE FISIOLOGÍA Y FARMACOLOGÍA.
ÁREA DE TOXICOLOGÍA

MARCADORES URINARIOS DE PREDISPOSICIÓN AL FRACASO RENAL AGUDO INDUCIDA POR NEFROTÓXICOS

MEMORIA PRESENTADA POR R. LAURA VICENTE VICENTE

PARA OPTAR AL GRADO DE DOCTOR POR LA UNIVERSIDAD DE SALAMANCA

Salamanca, a 20 de Octubre de 2011

VNiVERSiDAD
D SALAMANCA

DEPARTAMENTO DE FISIOLOGÍA Y FARMACOLOGÍA

LA DOCTORA DÑA. MARIA JESÚS MONTES RIOS, DIRECTORA DEL DEPARTAMENTO DE FISIOLOGÍA Y FARMACOLOGÍA DE LA UNIVERSIDAD DE SALAMANCA,

CERTIFICA:

Que el presente trabajo, elaborado por la Lda. en Farmacia R. Laura Vicente Vicente para optar al Grado de Doctor, con el título "MARCADORES URINARIOS DE PREDISPOSICIÓN AL FRACASO RENAL AGUDO INDUCIDA POR NEFROTÓXICOS", ha sido realizado bajo la dirección de los Doctores Dña. Ana Isabel Martín Morales y D. Francisco J. López Hernández, en el Departamento de Fisiología y Farmacología de la Universidad de Salamanca.

Y para que así conste, expido y firmo el presente certificado en Salamanca, a 20 de Octubre de 2011.

Fdo.: Dr. Dña. Maria Jesús Montes Rios.

VNiVERSiDAD
D SALAMANCA

Departamento de Fisiología y Farmacología

LA DOCTORA DÑA. ANA ISABEL MORALES MARTÍN, TITULAR DE TOXICOLOGÍA DEL DEPARTAMENTO DE FISIOLOGÍA Y FARMACOLOGÍA DE LA UNIVERSIDAD DE SALAMANCA, Y EL DOCTOR D. FRANCISCO J. LÓPEZ HERNÁNDEZ, INVESTIGADOR DEL HOSPITAL UNIVERSITARIO DE SALAMANCA Y PROFESOR ASOCIADO DE FISIOLOGÍA DEL DEPARTAMENTO DE FISIOLOGÍA Y FARMACOLOGÍA DE LA UNIVERSIDAD DE SALAMANCA

CERTIFICAN:

Que el presente trabajo, titulado "MARCADORES URINARIOS DE PREDISPOSICIÓN AL FRACASO RENAL AGUDO INDUCIDA POR NEFROTÓXICOS", presentado por la Licenciada en Farmacia R. Laura Vicente Vicente para optar al Grado de Doctor, ha sido realizado bajo nuestra dirección en el Área de Toxicología del Departamento de Fisiología y Farmacología de la Universidad de Salamanca, consideramos que cumple las condiciones necesarias y autorizamos su presentación con el fin de que pueda ser defendido ante el tribunal correspondiente.

Y para que así conste, expiden y firman el presente certificado en Salamanca, a 20 de Octubre de 2011

Fdo.: Dr. Dña Ana Isabel Morales Martín

Fdo.: Dr. D. Francisco J. López Hernández

ÍNDICE

INDICE

iNTRODVCCiÓN

1. EL SISTEMA RENAL.

El aparato urinario humano está constituido por dos riñones, dos uréteres, una vejiga y la uretra (Figura 1). A partir de un filtrado inicial del plasma sanguíneo, por medio de procesos de reabsorción y secreción, los riñones producen la orina que llega a la vejiga a través de los uréteres y se elimina al exterior por la uretra.

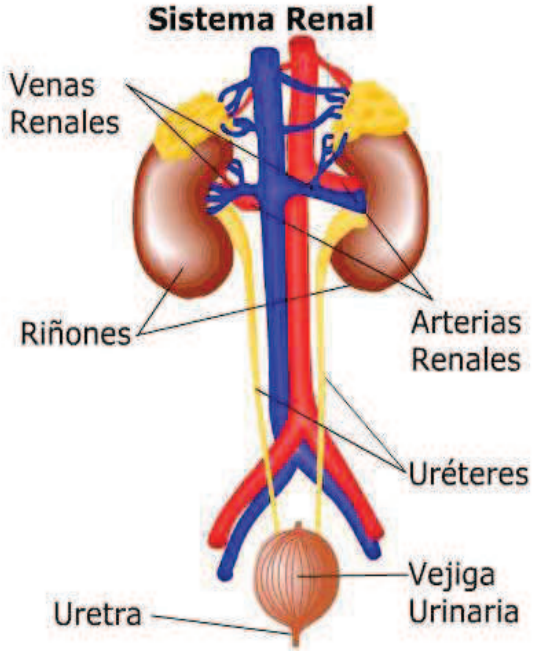


Figura 1. Representación de las partes del aparato urinario.

1.1. Anatomía del riñón.

Los riñones son dos órganos de color pardo-rojizo situados retroperitonealmente en la parte dorsal del abdomen, a cada lado de la columna vertebral. Los riñones están recubiertos por una *cápsula* de tejido fibroso.

En una sección longitudinal (Figura 2) se distinguen dos regiones: la corteza en la parte externa y la médula en la parte interna. La médula está formada por varias pirámides renales. Los ápices de éstas se proyectan dentro de los cálices menores. Cada pirámide medular, coronada por una región de corteza renal constituye un *lóbulo sencillo*. El extremo de cada pirámide (llamada papila) se vacía en un caliz y los cálices se vacían en la pelvis renal. La pelvis transmite la orina a la vejiga urinaria vía el uréter.

Un examen macroscópico permite distinguir que la corteza tiene un aspecto ligeramente granular que no se observa en la médula. Cada pirámide medular puede dividirse en una zona exterior, adyacente a la corteza, y una zona interior que incluye la papila (Bulger y Doyban, 1982, Tisher, 1981). Las estrías que aparecen en las pirámides corresponden con la porción tubular de las nefronas.

1.2. Anatomía de la nefrona.

La nefrona es la unidad anatómica y funcional del riñón. El riñón humano está formado por cerca de un millón y medio de nefronas. Cada una de ellas consta de un elemento filtrante denominado *corpúsculo renal*, y un *sistema tubular* que se extiende por fuera del corpúsculo renal, en el cual se realizan los procesos de secreción y reabsorción (Madsen y Tisher, 1986), que determinarán la composición final de la orina.

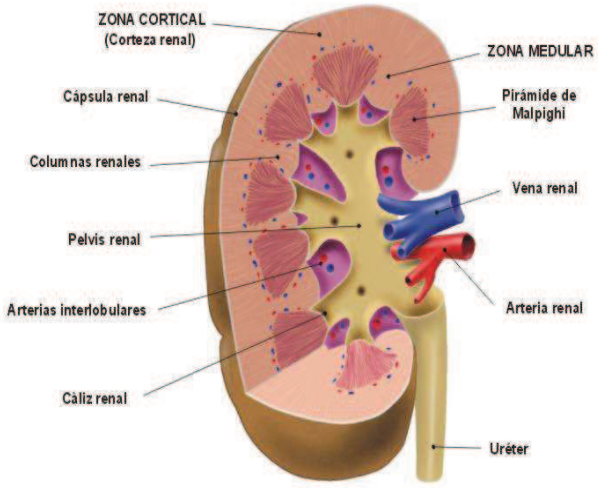


Figura 2. Estructura general de un riñón humano (adaptada de Brady y cols., 1996).

1.2.1. El corpúsculo renal.

El corpúsculo renal o de Malpighi está compuesto por el *glomérulo capilar* y la *cápsula de Bowman* que lo recubre (Figura 3). Existe un espacio dentro de la cápsula, *espacio de Bowman*, hacia donde pasa el líquido filtrado procedente del glomérulo. La barrera de filtración del corpúsculo renal o membrana glomerular, consta de tres capas: el endotelio de los capilares glomerulares, la membrana basal y una capa de células epiteliales especializadas con fenestraciones. Las células epiteliales que descansan sobre la membrana basal son muy diferentes de las células simples y aplanadas que revisten el resto de la cápsula de Bowman y se denominan *podocitos*, tienen gran número de extensiones podálicas o *pedicelos* integradas en la membrana basal. Las hendiduras entre pedicelos adyacentes constituyen la vía de paso del filtrado, que una vez atraviesa las células endoteliales y la membrana basal, penetra en el espacio de Bowman y desde allí pasa a la primera porción del túbulo proximal (Berne y Levi, 2001).

1.2.2. El sistema tubular.

La pared tubular está constituida por una sola capa de células epiteliales que descansan sobre una membrana basal. La estructura y función de esas células epiteliales varía mucho de un segmento a otro del túbulo, pero tienen una característica común: la presencia de uniones estrechas entre células adyacentes. El segmento del túbulo donde drena la cápsula de Bowman se denomina *túbulo contorneado proximal*, que inicialmente forma varias espiras, y es el segmento más largo y grueso de la nefrona. Esta tapizado por un epitelio cúbico simple, con células que presentan un borde en cepillo muy desarrollado en su superficie luminar, numerosas invaginaciones en la membrana basolateral, un gran aparato endo-lisosómico, y numerosos peroxisomas y mito-condrias, que va seguido por un segmento recto que

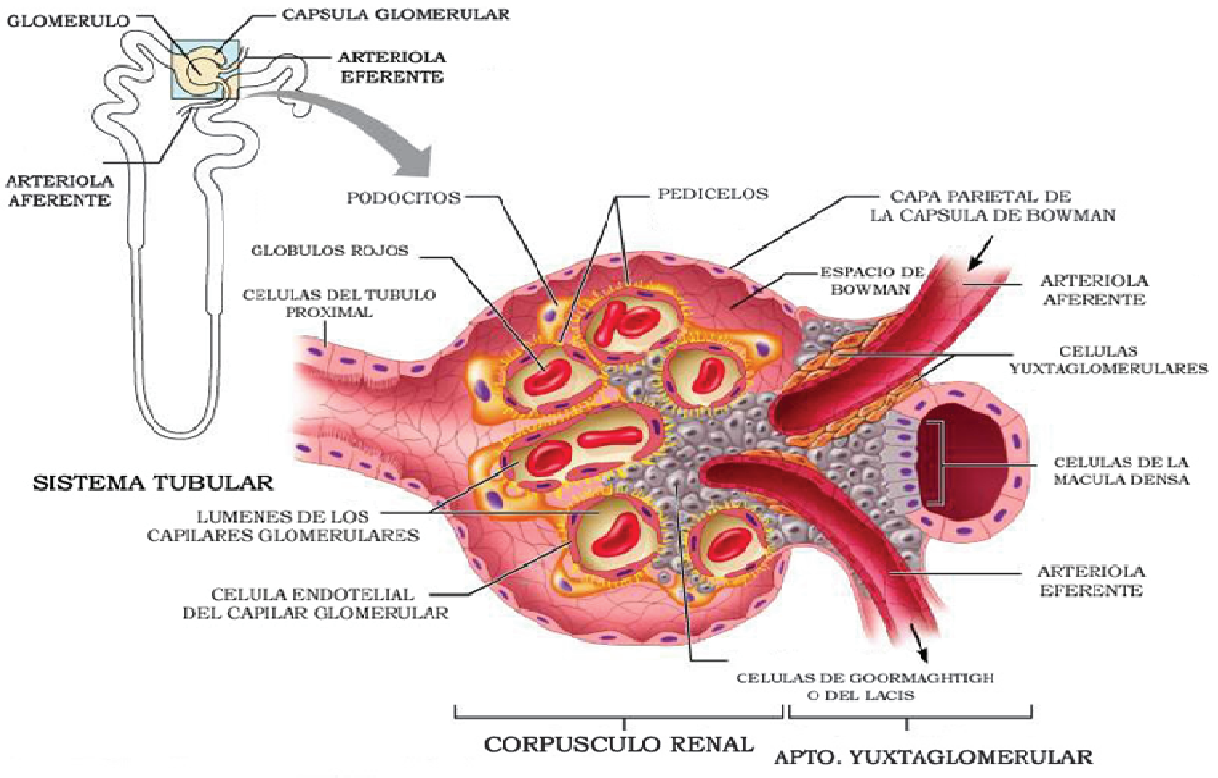


Figura 3. Representación esquemática de una nefrona y de un corpúsculo (adaptada de Netter, 2003).

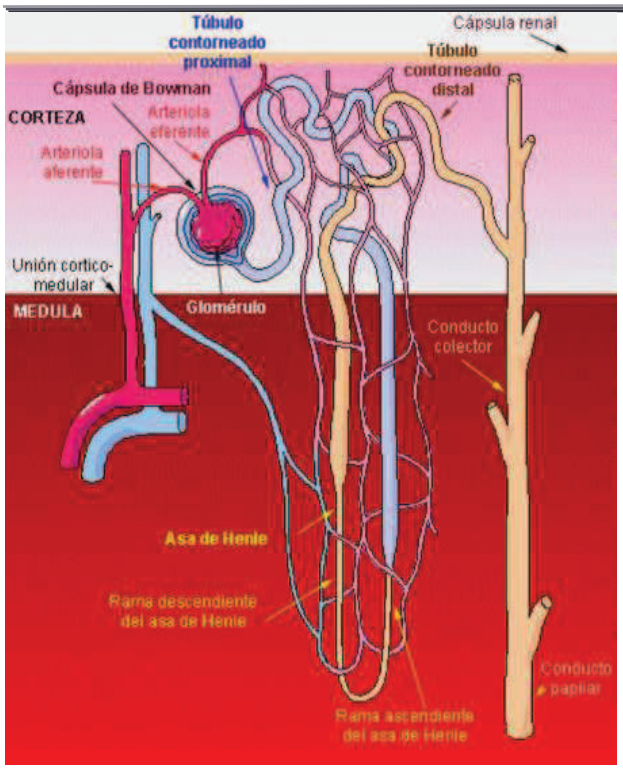


Figura 4. Esquema del sistema tubular y de la red vascular asociada.

desciende hacia la médula, es la *rama estrecha descendente del asa de Henle*. Esta rama termina en horquilla y entonces comienza un ascenso paralelo a la rama descendente. A continuación de este segmento se encuentra la *rama gruesa ascendente del asa de Henle*, que se continúa con el *túbulo contorneado distal*. Seguidamente se encuentra el *túbulo colector*, el cual vacía su contenido en cada uno de los cálices de la pelvis renal. Los distintos segmentos del túbulo están representados en la figura 4. La pelvis se continúa con el uréter, y éste con la vejiga urinaria, donde la orina se almacena de manera transitoria y de la cual se elimina en forma intermitente. Después de penetrar a un cáliz, la orina ya no sufre alteraciones. A partir de ese punto, el resto del sistema urinario sirve sólo como tubo conductor (Bulger y Doblan, 1982; Tisher, 1981).

1.2.3. El aparato yuxtaglomerular.

El aparato yuxtaglomerular (Figura 3.) está situado entre la primera porción del túbulo contorneado distal y la arteriola aferente y eferente pertenecientes al corpúsculo renal de su propia nefrona (Barajas, 1979). Está formado por tres tipos de células:

- Las *células yuxtaglomerulares*: son células mioepiteliales que rodean el final de la arteriola aferente, ricas en gránulos de secreción que producen el 90% de la renina.

- Las *células de la mácula densa*: son células epiteliales diferenciadas de la pared del túbulo recto distal ascendente, que controlan la secreción de renina y la velocidad de filtración glomerular.

- Las *células de Goormaghtigh o del lacis:* son células mesangiales extraglomerulares, que responden a múltiples mediadores y controlan la superficie de filtrado.

1.3 Procesos renales básicos

El riñón lleva a cabo sus funciones generales mediante dos procesos fundamentales que tienen lugar en cada una de las nefronas: el **filtrado glomerular**, que origina un gran volumen diario de filtrado de líquido extracelular (180 litros), y el **transporte tubular (reabsorción** y **secreción tubular)**. A continuación se describen estos procesos.

1.3.1. Filtración glomerular.

La formación de la orina comienza por la filtración en el glomerulo hacia la capsula de Bowman de unos 125 mL de plasma por minuto, lo que corresponde aproximadamente a un 20% del plasma que pasa por el riñón Más del 95% del filtrado se reabsorbe normalmente en los túbulos y el resto se elimina en forma de orina (Vander, 2006).

El proceso de formación de ultrafiltrado a través de las membranas capilares glomerulares recibe el nombre de ultrafiltrado glomerular. No obstante, a pesar de su gran permeabilidad, la membrana glomerular tiene una cierta selectividad en función del diámetro, configuración molecular y carga eléctrica de las moléculas.

La permeabilidad de la membrana glomerular está condicionada en parte por los pesos moleculares de las sustancias. Siendo ésta prácticamente impermeable a las proteínas plasmáticas, pero muy permeable al resto de las sustancias disueltas en el plasma normal. Por otra parte, los poros de la membrana son suficientemente grandes como para permitir el paso de moléculas con diámetro de hasta 8 nm. La membrana basal glomerular está recubierta por una red de proteoglicanos cargados negativamente. Así pues, las proteínas que presentan carga negativa producen una repulsión electrostática con las paredes de los poros. Esto evita prácticamente que todas las proteínas pasen a su través. Otro factor que influye es la presión de perfusión, sobre todo cuando, atendiendo a los factores anteriores, la molécula se encuentra en el límite de paso (Guyton, 2006).

El filtrado glomerular tiene casi la misma composición que el plasma, desde el punto de vista electrolítico, excepto por la ausencia de hematíes y proteínas de alto y medio peso molecular (Guyton, 2006). Su concentración de proteínas es del orden de 0,03 %, es decir, unas 240 veces menos que el contenido proteico del plasma. La relación entre el filtrado glomerular y el flujo plasmático renal se denomina *fracción de filtración.*

El proceso de filtración glomerular está condicionado por la suma neta de las diferentes fuerzas que se originan en los capilares glomerulares y en la cápsula de Bowman (Vander, 2006). Favorecen la filtración la presión hidrostática de los capilares glomerulares (Pg), que es la presión media en los capilares glomerulares y tiene un valor de 60 torr, y la presión oncótica de la Cápsula de Bowman (πi), sin embargo como la concentración de proteínas en la cápsula de Bowman es muy pequeña, este factor es despreciable y se considera cero. Se oponen a la filtración la presión oncótica de los capilares glomerulares (πg), que es la presión que ejercen las proteínas del plasma no filtradas y tiene un valor de 32 torr, y la presión hidrostática de la cápsula de Bowman (Pi), que es la presión que ejerce el líquido filtrado en la cápsula de Bowman y tiene un valor de 18 torr. La *presión efectiva de ultrafiltración* (PEF), se define como la diferencia entre la presión que favorece la filtración, es decir Pg, y las que se oponen, que son Pi y πg. Su valor aproximado es de 10 torr.

$$PEF = Pg - (Pi + \pi g)$$

El *coeficiente de filtración* (Kf), es el producto de la permeabilidad hidráulica de la membrana por el área disponible para la filtración. En función de Kf y PEF, se define la *tasa filtración glomerular* (TFG):

$$TFG = Kf \acute{} PEF$$

La *tasa filtración glomerular* (TFG) o cantidad de filtrado glomerular que se forma por minuto en todas las nefronas de ambos riñones, es un índice de la función renal y es esencial para evaluar la severidad y evolución de los trastornos renales.

1.3.2. Reabsorción y secreción tubular.

El filtrado glomerular pasa a través de todo el segmento tubular. El epitelio tubular reabsorbe y secreta de forma selectiva distintos compuestos, de tal modo que el líquido que resulta de este proceso entra en la pelvis renal como orina.

El papel de la reabsorción es mucho mayor que el de la secreción en la formación de la orina, pero la secreción es muy importante para determinar las cantidades finales urinarias de potasio, hidrogeniones y algunas otras sustancias.

Habitualmente, más del 90% del agua contenida en el filtrado glomerular se reabsorbe en los túbulos. De esta forma, si algunos de los solutos contenidos en el filtrado glomerular no se reabsorben en el trayecto tubular, la reabsorción de agua hace que se concentren de forma muy importante aumentando su concentración unas 99 veces. Por otra parte,

algunos componentes del filtrado, como la glucosa, los aminoácidos y el sodio se reabsorben casi por completo, de forma que la concentración disminuye hasta ser prácticamente igual a cero en la orina. Así, los túbulos separan las sustancias que deben ser eliminadas por la orina de las que deben ser conservadas por el organismo sin perder una cantidad excesiva de agua (Guyton, 2006).

1.4. Funciones de los riñones.

1.4.1. Regulación del equilibrio hidroeléctrico.

La función primaria de los riñones es lograr un equilibrio entre el agua corporal y los iones inorgánicos para mantener estable la concentración de esas sustancias en el líquido extracelular. Dicho equilibrio se logra mediante cambios en la composición de la orina excretada. Algunas de las sustancias del medio interno reguladas en gran parte por los riñones son el agua, sodio, potasio, cloro, calcio, magnesio, sulfato, fosfato y el ión hidrógenión. No obstante, los riñones no son los únicos reguladores de las sustancias inorgánicas esenciales. De igual manera, los riñones también participan en la regulación de algunos nutrientes orgánicos, como son la glucosa, aminoácidos, proteínas, urea, ácido úrico y otros (Vander, 2006).

1.4.2. Excreción y concentración de catabolitos y sustancias tóxicas.

Esta función consiste en retirar los productos metabólicos de la sangre y excretarlos por la orina. Entre estos productos de desecho que son eliminados se encuentran la urea (resultante del catabolismo de las proteínas), el ácido úrico (resultante del catabolismo de los ácidos nucleicos), la creatinina (resultante del metabolismo del músculo), la bilirrubina (procedente del metabolismo de la hemoglobina), metabolitos de hormonas y otros. Algunos de ellos son relativamente inocuos, pero la acumulación de otros cuando la función renal está dañada causa algunos de los trastornos de las funciones corporales observadas en las enfermedades renales graves (Vander, 1993). Los riñones tienen otra función excretora general: la eliminación de productos químicos extraños, fármacos, pesticidas, aditivos de alimentos, etc. (Vander, 1993).

1.4.3. Regulación de la presión arterial.

Los riñones participan estrechamente en la regulación de la presión arterial mediante varios mecanismos que actúan a corto, medio o largo plazo. A continuación se describen brevemente los principales.

Regulación de la excreción de sodio: se trata del principal mecanismo de regulación arterial a largo plazo del organismo (López-Hernández y López-Novoa, 2006). El equilibrio del sodio es un factor determinante crítico del gasto cardiaco. El balance positivo de sodio aumenta el volumen extracelular. Cuando se acumula mucho líquido extracelular en el organismo la presión arterial aumenta debido al mayor volumen sanguíneo, que eleva la presión media de llenado circulatorio y el retorno venoso de sangre hacia el corazón. En consecuencia aumenta el gasto cardiaco y a su vez la presión arterial. (Knox y Granger, 1987; Guyton, 2006; Vander, 2006).

Sistema renina-angiotensina (SRA): el riñón funciona como una glándula endocrina del SRA, un complejo hormonal de enzimas, proteínas y péptidos importantes en la regulación de la presión arterial. La renina es una enzima proteolítica segregada a la sangre por los riñones, específicamente por las células granulares del aparato yuxtaglomerular. Una vez en la corriente sanguínea la renina actúa el angiotensinógeno producido por el hígado, dando lugar a la angiotensina I (Ag I). La Ag I es una sustancia inactiva que a su paso por el lecho vascular, la enzima convertidora de angiotensina (ECA) separa sus dos aminoácidos terminales para producir la angiotensina II (Ag II), muy activa. En el plasma se encuentra una cierta cantidad de ECA, pero la mayor parte está en el endotelio de los vasos sanguíneos, en particular, en los capilares pulmonares. No obstante, como los riñones producen renina y el tejido renal

también contiene angiotensinógeno y ECA, las reacciones generadoras de Ag I y II ocurren en cierta medida dentro de los riñones. La Ag II ejerce muchos efectos sobre diversos tejidos, pero el objetivo final de gran parte de ellos es incrementar la presión arterial. (Dzau y cols., 1988; Vander, 2006).

Otras sustancias vasoactivas: es muy probable que los riñones segreguen a la sangre o retiren de ella otras sustancias vasoactivas diferentes de la renina. Así, los riñones pueden sintetizar varios eicosanoides de acción vasodilatadora o vasoconstrictora. De la misma manera se segregan lípidos vasodilatadores (Vander, 2006).

1.4.4. Síntesis y secreción de otras sustancias.

Secreción de eritropoyetina: Los riñones segregan eritropoyetina (EPO), esta hormona relacionada con el control de la producción de eritrocitos en la médula ósea, y por tanto con el mantenimiento de niveles de oxígeno adecuados para el funcionamiento del organismo. Las enfermedades renales pueden reducir la secreción de EPO, con la consecuente producción de anemia grave (Vander, 2006).

Secreción de 1,25-dihidroxivitamina D3: los riñones producen 1,25-dihidroxivitamina D3, la forma activa de la vitamina D, la cual interviene en el metabolismo del calcio aumentando la absorción del calcio intestinal y la movilización del calcio óseo (Vander, 2006).

***Gluconeogénesis*:** durante el ayuno prolongado los riñones sintetizan glucosa a partir de aminoácidos y otros precursores y la liberan a la sangre. Por tanto, el riñón es un órgano gluconeogénico (Vander, 2006).

Eicosanoides: se trata de un grupo de compuestos derivados del ácido araquidónico, entre los que se incluyen las prostaglandinas E2 y F2, prostaciclina y tromboxano (Levenson, 1983). Realizan diversas funciones entre las que se encuentra el control del flujo sanguíneo y del filtrado glomerular.

Metalotioneínas: se trata de una familia de proteínas con afinidad a ciertos metales como el cadmio, el cobre y el zinc, participando en su homeostasis. Ciertas situaciones fisiopatológicas como el estrés, la inflamación o la intoxicación por algunos metales aumentan su síntesis.(Okabe y cols. 1996).

2. LA NEFROTOXICIDAD COMO CAUSA DEL FRACASO RENAL AGUDO.

El fracaso renal agudo (FRA) es un tipo de lesión de gravedad clínica en la que la función excretora renal se reduce súbitamente tanto que los riñones son incapaces de depurar la sangre de los fármacos, tóxicos y productos nocivos de deshecho procedentes del metabolismo, y de conseguir el equilibrio electrolítico. Como consecuencia de ello, la función de muchos otros órganos y tejidos y, con ella, la vida del paciente se ven seriamente comprometidas. El FRA se caracteriza por una disfunción renal aguda (que surge horas o pocos días después del inicio del daño) derivada de un estímulo patológico rápido e intenso (Esteller y Cordero, 1998; Rivero-Sánchez y cols., 2000).

EL FRA se puede producir como consecuencia de cualquier proceso que disminuya la función renal (medida por la tasa de filtración glomerular). Supone un deterioro brusco de la función excretora renal con aparición de uremia, oliguria, anuria o diuresis renal. La función renal puede normalizarse si se descubre y se trata satisfactoriamente la causa subyacente del problema. El pronóstico depende fundamentalmente de la intensidad y el tipo de lesión. En general, una lesión tisular leve o una disfunción moderada desaparecen con la retirada del agente nefrotóxico, mientras que la destrucción extensa de uno o varios compartimentos renales (p.e. la necrosis tubular

aguda) puede originar una deficiente reparación e incluso un deterioro progresivo y crónico.

2.1 Incidencia y morbimortalidad.

El FRA presenta todavía una alta morbi-mortalidad con consecuencias humanas y sociales muy importantes. Se calcula que aproximadamente un 1-7% de los pacientes que ingresan en los hospitales presentan FRA (Chertow y cols., 2001; Liangos y cols., 2006). Más concretamente, un 1-25% de los pacientes ingresados en la unidad de cuidados intensivos (UCI) desarrollan FRA en algún momento (Mendonça y cols., 2000). También se estima que aproximadamente un 15% de los pacientes sometidos a *bypass* y de las mujeres embarazadas sufren algún grado de FRA. La tasa de mortalidad debido al FRA se mantiene alarmantemente constante alrededor del 50% de los casos, que asciende al 80% entre los pacientes que desarrollan fallo multiorgánico (Rivero-Sánchez y cols., 2000). En la mayoría de los casos es necesario aplicar diálisis, lo que supone un gran carga humana y socioeconómica.

Como se indica en las secciones siguientes, una causa importante de FRA, relacionada directamente con el objeto de esta tesis doctoral, es la nefrotoxicidad aguda, o daño renal agudo producido por fármacos y toxinas. La nefrotoxicidad constituye un problema de salud y socioeconómico muy serio en todo el mundo. Aproximadamente el 25% de los 100 fármacos más utilizados en las UCIs son potencialmente nefrotóxicos (Taber y Mueller, 2006). Además, se estima que la nefrotoxicidad es la causa del 10-20% de los casos de FRA (Brivet y cols., 1996).

2.2 Fisiopatología general del FRA.

Esta enfermedad se clasifica en pre-renal, renal o post-renal, de acuerdo con el mecanismo que la desencadena (Figura 5). Las causas pre-renales y renales, representan el mayor porcentaje de casos, aunque no es infrecuente observar que la etiología sea multifactorial y que un mismo agente nocivo produzca simultáneamente efectos pre-renales y renales (Esteller y Cordero, 1998; Singri, 2003). También es posible que en un mismo paciente se encuentren a la vez diferentes formas de FRA.

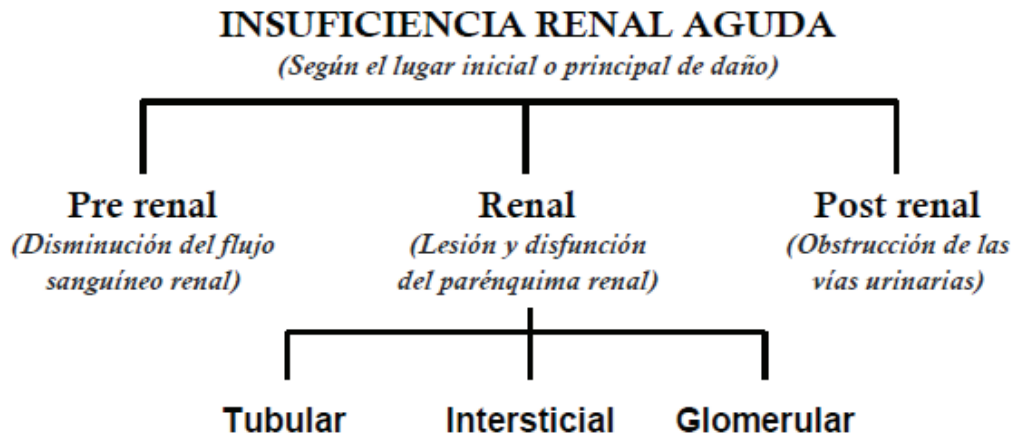


Figura 5. Principales categorías de insuficiencia renal aguda.

2.3. FRA pre-renal.

Constituye la forma de FRA más prevalente (55-60% de los casos). Se debe a una perfusión sanguínea renal deficiente que resulta de una disminución súbita y aguda de la presión arterial (shock) o de una interrupción del flujo de sangre a los riñones debido a un traumatismo o enfermedad grave o a un procedimiento quirúrgico. Esta situación lleva a una caída en la filtración glomerular. Cuando la presión arterial cae, ocurre una dilatación gradual de la arteriola aferente (autorregulación del flujo sanguíneo renal) mediada por la generación de óxido nítrico y una constricción eferente concomitante mediada por la Ag II, que tienden a mantener una presión hidrostática capilar constante. Sin embargo, hay un punto en que la hipoperfusión es tal, que estos cambios compensatorios se hacen insuficientes y comienza a caer rápidamente la presión hidrostática capilar, con la consiguiente caída de la filtración glomerular y el aumento de reabsorción tubular de agua, electrolitos y otros integrantes de la orina primaria, que producen oliguria.

Además, como consecuencia de la hipoperfusión se produce una isquemia renal que da lugar a una necrosis tubular aguda cortical que, dependiendo del grado de isquemia, puede afectar también a otras estructuras. La patogenia tubular isquémica da cuenta de una lesión de la célula epitelial tubular, que incluye turgencia celular, pérdida del borde en cepillo, pérdida de la polaridad por una redistribución de las proteínas de membrana (por ejemplo la bomba Na+-K+, que aumenta la liberación distal de sodio, lo que activa la retroalmentación tubuloglomerular contribuyendo a la vasoconstricción), además de necrosis y apoptosis. Como consecuencia de esto, se agotan todas las reservas de ATP, se acumula calcio, y se activan enzimas que alteran y dañan la estructura de la célula y que inducen apoptosis. Las células dañadas se desprenden y obstruyen la luz del túbulo, aumentando la presión intratubular con la consiguiente disminución del filtrado glomerular. Además se producen lesiones endoteliales que aumentan la liberación de endotelina (vasocontrictor); a esto se le suma la disminución de la producción de óxido nítrico (NO) y prostaglandina I2 (Rivero-Sánchez y cols., 2000; Esteller y Cordero, 1998).

Otro aspecto importante a tener en cuenta es que la hipoperfusión prolongada puede ser una causa de necrosis tubular aguda o de un agravamiento de la misma. Este hecho dificulta en la práctica clínica la diferenciación del fracaso renal agudo pre-renal del renal.

Entre las principales causas de hipoperfusión renal destacan las siguientes:

-La hipovolemia (por hemorragias, deshidratación, uso de diuréticos, etc.).

-La insuficiencia cardiaca.

-El uso de ciertos medicamentos como los antiinflamatorios no esteroideos (AINEs) que disminuyen la producción de las prostaglandinas que normalmente dilatan la arteriola aferente y aumentan la presión hidrostática capilar, o los inhibidores de la enzima convertidora de angiotensina (IECAs), que disminuyen la producción de angiotensina II, que contrae la arteriola eferente y aumenta la presión hidrostática glomerular (Singri, 2003).

-Otras causas como cirugía mayor (transplante renal), trauma y sepsis (Brady y cols., 1996).

2.4. FRA renal o intrínseco.

Se trata de una enfermedad del parénquima renal debida a inflamación, toxinas, medicamentos, infecciones o disminución del riego sanguíneo. El FRA renal o intrínseco puede deberse a alteraciones de los glomérulos, de los túbulos y del intersticio renales. Esta causa supone aproximadamente el 25% de los casos de FRA.

2.4.1. Afecciones glomerulares.

El glomérulo es el primer sitio de la nefrona que se pone en contacto con los agentes químicos. La acción directa de determinados fármacos y tóxicos sobre las células que forman la barrera de filtración glomerular produce alteración de las propiedades físico-químicas (p.e. eléctricas) de la barrera, o contracción o relajación de sus estructuras, que determinan alteraciones en la selectividad del filtrado y en la tasa de filtración glomerular, respectivamente. Concretamente una hipofiltración puede deberse a lesión glomerular que se manifiesta como una disminución del K_f que puede estar provocado por alteraciones en la permeabilidad hidráulica de la barrera de filtración, o bien por una vasoconstricción y proliferación de las células mesangiales intraglomerulares. Son ejemplos de este tipo de daño, la ciclosporina, la anfotericina B y la gentamicina.

La lesión glomerular inducida por sustancias químicas también puede estar mediada por factores endógenos extrarrenales, como ocurre en las reacciones de hipersensibilidad tipo III. Los complejos inmunes circulantes pueden depositarse en los glomérulos. En la glomerulonefritis membranosa suelen observarse neutrófilos y macrófagos dentro de los glomérulos, y la liberación de citocinas y de radicales libres de oxígeno (ROS) puede contribuir a causar la lesión glomerular. Los metales pesados, los hidrocarburos, la penicilina y el captopril pueden producir también esta clase de lesión. Por último, ciertas infecciones y enfermedades inmunológicas (p.e. autoinmunes) producen una inflamación del glomérulo (glomerulonefritis) que altera la filtración (Rivero-Sánchez y cols., 2000; Esteller y Cordero, 1998).

2.4.2. Afecciones tubulares.

Ésta es la causa renal más frecuente de FRA intrínseco en los adultos representando el 75% de los casos. Son tres los desencadenantes más importantes de enfermedad tubular aguda:

1. La obstrucción tubular. Esto puede ocurrir por la precipitación de ácido úrico (como efecto secundario de la quimioterapia), de proteínas (mieloma) o de pigmentos (en casos de hemólisis masiva). por precipitación del xenobiótico o bien por un depósito del propio epitelio lesionado (Sierra y cols., 2000).

2. La isquemia. Normalmente los túbulos renales están irrigados por los vasos rectos (ramas de las arteriolas eferentes), recibiendo el O_2 necesario para el transporte activo de sustancias en el proceso de reabsorción, especialmente el de sodio. En la necrosis tubular por isquemia hay falta de oxigenación de las células tubulares, lo que lleva a necrosis tubular y a que las células muertas se desprendan hacia el túbulo; esto lleva a la caída de la filtración glomerular tanto por la obstrucción del túbulo (restos celulares y tisulares) como por la vasoconstricción de la arteriola aferente desencadenada por el retrocontrol túbulo-glomerular, mediado por la mácula densa que detecta la gran concentración de sodio que no puede reabsorberse por el daño de las células tubulares y la falta de oxígeno (Lamiere y Vanholder, 2004; Rivero-Sánchez y cols., 2000; Valdivielso y cols., 2001).

3. La alteración de la función tubular causada por acción directa de fármacos o sustancias tóxicas sobre dianas moleculares tubulares (Sierra y cols., 2009).

Lesión del túbulo proximal: Los túbulos son las estructuras más susceptibles al daño renal por efectuar principalmente la reabsorción isoosmótica y la secreción. Es el lugar donde con más frecuencia actúan los tóxicos, y esto se debe en parte a la acumulación de éstos en esta parte de la nefrona. El transporte tubular de aniones o cationes orgánicos, sustancias de bajo peso molecular, péptidos y metales pesados se hace fundamentalmente en este tramo. El poder nefrotóxico de los xenobióticos depende de la capacidad intrínseca de cada sustancia para reaccionar con las dianas subcelulares o moleculares. Además, las células del túbulo proximal parecen ser más

sensibles a las lesiones isquémicas que las del túbulo distal (Rivero-Sáncez y cols., 2000).

***Lesiones del Asa de Henle y de los túbulos distal y colector*:** Las alteraciones funcionales de estos tramos de la nefrona se manifiestan principalmente por disminución de la capacidad de concentración, por defectos de acidificación o por ambas cosas. La anfotericina B, el cisplatino y el metoxiflurano producen poliuria resistente a la hormona antidiurética (ADH), lo que sugiere que el defecto de concentración tiene lugar en la porción gruesa de la rama ascendente del asa de Henle o bien en el conducto colector (Rivero-Sánchez y cols., 2000).

Lesión papilar: La toxicidad papilar suele ser consecuencia de tratamientos de larga duración con fármacos tales como los analgésicos y los AINEs. Las concentraciones elevadas de estos tóxicos y la inhibición de las prostaglandinas vasodilatadoras comprometen el flujo sanguíneo de la médula y las papilas renales y provocan isquemia tisular (Rivero-Sánchez y cols., 2000).

2.4.3. Afecciones del intersticio.

Normalmente se caracterizan por una inflamación del intersticio (nefritis intersticial aguda). Esto ocurre en casos de alergia a medicamentos, entre otras causas. Tras la administración de algunos xenobióticos se han registrado trastornos renales con oliguria, proteinuria, hematuria y elevación de la tensión arterial que puede llegar al cuadro de insuficiencia renal aguda. La biopsia de estos casos ha mostrado infiltración del intersticio por linfocitos y plasmocitos, mientras el hemograma suele presentar una eosinofilia indicativa de afectación alérgica

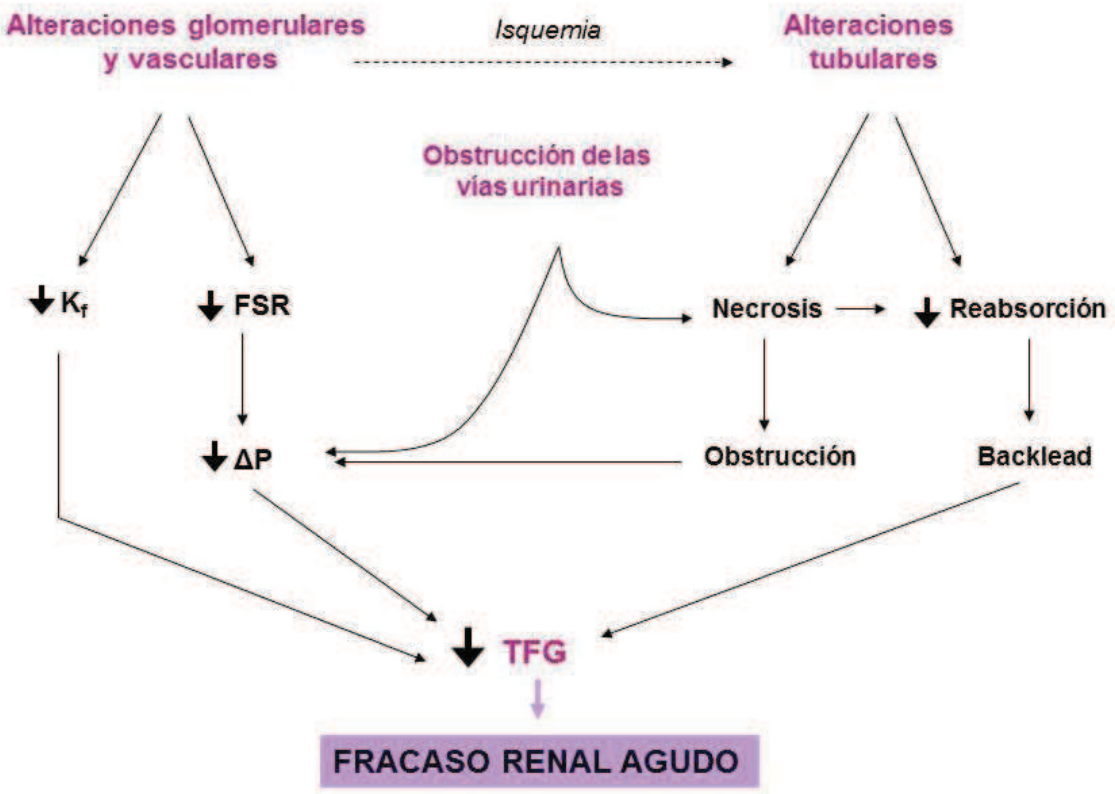


Figura 6. Mecanismos frecuentes que conducen a la disminución del filtrado glomerular durante el daño renal agudo**.** Abreviaturas: K_f: Coeficiente de ultrafiltración; FSR: Flujo sanguíneo renal; ΔP: Presión neta de ultrafiltración; TFG: Tasa de filtración glomerular (Adaptado de Rivas y cols., 1995).

(Klaasen y watkins, 2005).

2.5. FRA post-renal.

Esta forma de FRA es consecuencia de una obstrucción súbita del flujo de orina debido a aumento de tamaño de la próstata, cálculos, tumores o traumatismos de la vejiga y las vías urinaria. El FRA post-renal es la responsable al menos del 5% de los casos. Dado que un solo riñón posee la capacidad de depuración suficiente para excretar los productos de desecho, para que se produzca un FRA de causa obstructiva es necesario que exista una obstrucción en la uretra, en ambos uréteres o una obstrucción unilateral en un paciente con un solo riñón.

El mecanismo principal que conduce al FRA post-renal es la hipertensión retrógrada; esto significa que por la obstrucción aumenta la presión en las vías urinarias y ésta es transmitida hacia las zonas más proximales, hacia los túbulos renales y glomérulo; se produce un aumento de la presión hidrostática en el espacio de Bowman, que disminuyen el gradiente de presión de filtración, y con ello la filtración glomerular.

2.6. Agentes nefrotóxicos y susceptibilidad renal.

Multitud de fármacos y contaminantes medioambientales (metales, insecticidas, etc.) pueden alterar rápidamente la función renal, modificando directamente los procesos renales básicos, dañando las estructuras renales, o mediante ambas acciones. La tabla 1 recopila las características fundamentales del efecto nefrotóxico de muchos de estos agentes, la mayoría de los cuales han sido presentados en las secciones anteriores.

EFECTO PATOLÓGICO	MECANISMO PRIMARIO	NEFROTÓXICOS
Hipoperfusión/ Hipofiltración	Vasoconstricción renal Daño glomerular	Anfotericina B Aminoglucósidos Ciclosporina AINEs Agentes de radiocontraste
Necrosis tubular directa	Daño tubular directo	Aminoglucósidos Anfotericina B Acetaminofeno Cisplatino Metales pesados β-lactámicos
Obstrucción Nefritis Tubulo intersticial	Obstrucción intratubular Inmunológica Inflamatoria	Agentes de radiocontraste β-lactámicos AINEs Sulfamidas

Tabla 1. Efectos renales de los principales nefrotóxicos.

A pesar de que los riñones constituyen sólo el 0.5% de la masa corporal total, sus características anatómicas y fisiológicas les proporcionan una especial susceptibilidad a los efectos tóxicos de muchos de estos xenobióticos, ya que son los órganos que reciben mayor irrigación por gramo de tejido (alrededor del 20 al 25% del gasto cardíaco en reposo) y son la principal vía de eliminación de fármacos y de sus metabolitos.

Los procesos que intervienen en la concentración de la orina sirven también para concentrar los tóxicos en el interior del túbulo. Por lo tanto, puede ocurrir que un agente químico cuya concentración no llega a ser tóxica en el plasma alcance concentraciones tóxicas en el riñón. (Klaassen y Watkins, 2005).

Los lugares concretos en los que se produce el daño van a depender de las características del agente tóxico, especialmente de sus propiedades fisicoquímicas, que en definitiva determinan su interacción con sistemas de transporte, receptores, enzimas y estructuras celulares y titulares específicas, y por lo tanto su distribución en los distintos compartimentos del organismo y sus efectos tóxicos.

Para llevar a cabo esta Tesis Doctoral se ha precisado del uso de diferentes agentes nefrotóxicos, más concretamente dos fármacos, la gentamicina y el cisplatino, y un contaminante medioambiental, el uranio. Para comprender mejor los resultados obtenidos y a modo de consulta, se han adjuntado cuatro anexos en los que se desarrolla información precisa a cerca de la nefrotoxicidad que producen estos agentes:

2.6.1. Gentamicina

La gentamicina es un antibiótico aminoglucósido ampliamente utilizado en la práctica clínica para el tratamiento de infecciones por microorganismos gram negativos y de la endocarditis bacteriana. La nefrotoxicidad es su principal limitación terapéutica, que afecta a de un 10-25% de los pacientes tratados con este fármaco, y puede dar lugar a un FRA. A pesar de ello, los aminoglucósidos continúan siendo la única alternativa terapéutica efectiva contra los gérmenes sensibles a otros antibióticos, e incluso los fármacos de elección en muchas circunstancias, por su eficacia y bajo coste. La nefrotoxicidad de la gentamicina se describe en dos de nuestros artículos de revisión, que se incluyen en dos anexos.

Anexo I: proporciona información sobre la integración de los mecanismos fisiopatológicos de la nefrotoxicidad de la gentamicina mediante el artículo de revisión titulado **"New insights into the mechanism of aminoglycoside nephrotoxicity. an integrative point of view."**

Anexo II: se trata de una revision sobre los mecanismos de citotoxicidad de la gentamicina, el artículo se ha titulado **"An integrative overview on the mechanisms underlying the renal tubular cytotoxicity of gentamicin."**

2.6.2. Cisplatino

El cisplatino es uno de los agentes antineoplásicos más efectivos en el tratamiento de tumores sólidos, con un amplio espectro de acción, sin embargo, la nefrotoxicidad acumulativa dosis-dependiente, es la principal limitación terapéutica de este caso. Es por ello que, en algunos casos, se precisa de una reducción en la dosis administrada o incluso la suspension del tratamiento. A pesar de las intensas medidas profilácticas encaminadas a proteger las complicaciones renales, aproximadamente un tercio de los pacientes tratados con cisplatino padece fallo renal irreversible. La relevancia terapeútca y la serveridad de la nefrotoxicidad producida por cisplatino se describen en el anexo III.

Anexo III: caracterización de los mecanismos fisiopatológicos implicados en la nefrotoxicidad del cisplatino, mediante la información adjunta que se ha

titulado **"Aspectos generales de la nefrotoxicidad del cisplatino."**

2.6.3. Uranio

El uranio es un elemento natural que se encuentra ampliamente distribuido en la corteza terrestre. Cierta cantidad de este metal está presente en los alimentos, en el aire, en el suelo y en el agua, por lo que el ser humano se encuentra expuesto al mismo de forma natural. Pero también puede ser objeto de una sobreexposición patológica como consecuencia de la deposición de uranio natural desde la atmósfera o debido a actividades industriales humanas que vierten productos de desecho directamente sobre el terreno. La nefrotoxicidad es el principal efecto observado tras exposición aguda a uranio. Este efecto se ha descrito en múltiples estudios realizados en animales de experimentación y en algunos casos de humanos expuestos a dosis elevadas de uranio de forma accidental. Sin embargo, la producción de daño renal por exposición crónica está poco documentada, lo que se debate en el artículo de revisión adjuntado en el anexo IV.

Anexo VI: análisis detallado sobre la controversia existente en cuanto a la nefrotoxicidad que produce la exposición crónica a uranio, el artículo de revisión se ha titulado **"Nephrotoxicity of uranium: Pathophysiological, Diagnostical and Terapeutic perspectives."**

3. EVALUACIÓN DEL DAÑO RENAL.

Existen diversos procedimientos para evaluar el daño renal, que van desde sencillos análisis cualitativos y ensayos bioquímicos hasta estudios anatomo-patológicos más complejos. A continuación se describen los más comúnmente utilizados.

3.1. Medida de la filtración glomerular.

La filtración glomerular se puede medir directamente calculando el aclaramiento de la inulina (sustancia exógena) o la creatinina (producto endógeno derivado de la musculatura esquelética), pues ambas sustancias se filtran fácilmente y no se reabsorben ni secretan en gran medida. Las concentraciones del nitrógeno ureico en sangre (BUN), de la creatinina sérica y de la urea son marcadores indirectos de la filtración glomerular. Así, un aumento de su concentración en sangre sugiere una disminución de la filtración glomerular.

Actualmente se recomienda, con limitaciones, el uso de determinadas ecuaciones para valorar la filtración glomerular y, en general, la función excretora renal. Estas ecuaciones toman como base los valores de la concentración plasmática de creatinina, pero los corrigen con ciertos datos antropométricos de los pacientes, como la edad, el sexo y el peso. Las ecuaciones más utilizadas son las denominadas Ecuación de la Modificación de la Dieta en la Enfermedad Renal (MDRD, por sus siglas en inglés) y la Ecuación de Cockcroft-Gault en los adultos, y la Schwartz en los niños (Herget-Rosenthal y cols., 2007).

3.2. Análisis del sedimento urinario.

El análisis del sedimento urinario también proporciona una cierta información pues la presencia de células epiteliales indica una lesión tóxica, mientras que la presencia de células sanguíneas es indicativa de una posible infección.

3.3. Estudio histopatológico del riñón.

El análisis histopatológico del riñón es muy útil para identificar la localización, la naturaleza y la intensidad de la lesión nefrotóxica. Sin embargo su uso está muy restringido por la dificultad que entraña la obtención de muestras tisulares (biopsias). La mera observación de una preparación de tejido renal, debidamente procesada y teñida (normalmente con hematoxilina y eosina) proporciona una idea de las estructuras más afectadas. Mediante estudios histoquímicos e inmunocitoquímicos puede detectarse

la presencia o ausencia de antígenos marcadores de daño menos evidente, de forma muy localizada en cada estructura y tipo celular.

3.4. Estudio de la composición de la orina

La lesión renal puede ser detectada también mediante la evaluación de una serie de compuestos presentes en la orina, como proteínas, glucosa y electrolitos. Una elevada concentración de glucosa en la orina (glucosuria), en tanto que su concentración plasmática es normal, puede estar relacionada con defectos de reabsorción de los azúcares en el túbulo proximal causados por un tóxico o puede ser secundaria a hiperglucemia.

La excreción urinaria de proteínas de elevado peso molecular, como la álbumina, sugiere la existencia de lesiones glomerulares, mientras que la excreción de proteínas de bajo peso molecular, como la b-2-microglobulina debe hacer sospechar una lesión del túbulo proximal. Además son marcadores de proteinuria tubular: alfa-1-microglobulina, proteína unida a retinol (RBP), cistatina C, amilasa, etc., así como las proteínas *villin* (del citoesqueleto tubular e intestinal) y Tamm-Horsfall (THP) (del asa de Henle). La eliminación por la orina de enzimas que ocupan el borde en cepillo de las células tubulares, como la fosfatasa alcalina (FAL) y la gamma-glutamil-transferasa (GGT), se debe a lesiones del borde en cepillo, mientras que la excreción de otras enzimas, como la lactatodeshidrogenasa (LDH), glutatión transferasa (GST) y la alanina-aminopeptidasa (AAP), puede reflejar una lesión celular más generalizada. La lesión de la papila renal por agentes papilotóxicos libera N-acetil-beta-D-glucosaminidasa (NAG), una hidrolasa lisosómica que posee varias isoenzimas (presente también tras la lesión del túbulo proximal), acompañadas por un aumento del volumen de orina con osmolalidad baja.

Sin embargo, hay que tener en cuenta que a pesar de que los marcadores mencionados anteriormente parecen proporcionarnos información más precisa del lugar donde se produce el daño, éstos poseen limitaciones, su especificidad es muy relativa, aparecen elevadas indistintamente de cual sea la etiología del daño renal. Un ejemplo es el caso de la enzima NAG, se han observado aumentos de esta enzima en orina de pacientes con nefropatía membranosa, glomelulonefritis membranoproliferativa, glomeruloesclerosis segmentaria focal, pielonefritis obstructiva, nefroesclerosis, nefropatía diabética y nefropatía de cambios mínimos (Sherman y cols., 1983).

4. PERSPECTIVAS EN EL DIAGNÓSTICO DEL FRA.

En la práctica clínica, el fallo renal agudo se diagnostica cuando la disfunción renal produce síntomas que se pueden medir. Estos típicamente se basan en la determinación de los niveles plasmáticos de creatinina y urea. Lo más habitual es que sus concentraciones en la sangre aumenten a medida que la TFG disminuye. Sin embargo, en ese estado en el que ya se observa aumento de los niveles séricos de urea y creatinina, el fallo renal agudo resulta difícil de tratar y se ha perdido mas del 50 % de la función renal. Así, las tendencias actuales en el diagnóstico buscan detectar eventos fisiopatológicos incipientes producidos en etapas tempranas, cuando el daño está menos extendido (Vaidya y cols., 2008), a través de marcadores muy tempranos (figura 7).

Otro aspecto importante en la búsqueda de marcadores de daño renal, es la capacidad de diferenciar la nefrotoxicidad producida por unos fármacos de la producida por otros, p.e. en pacientes polimedicados, mediante marcadores diferenciales o etiológicos de daño renal (Ferreira y cols., 2011).

Además de los marcadores arriba indicados, recientemente ha surgido un nuevo concepto, el de predisposición al daño renal (Quirós y cols., 2010). Esto sucede cuando la admnistación de fármacos a dosis que no deberían de ser tóxicas, o la exposición a sustancias ambientales que en un principio no producen ninguna alteración renal predisponen a

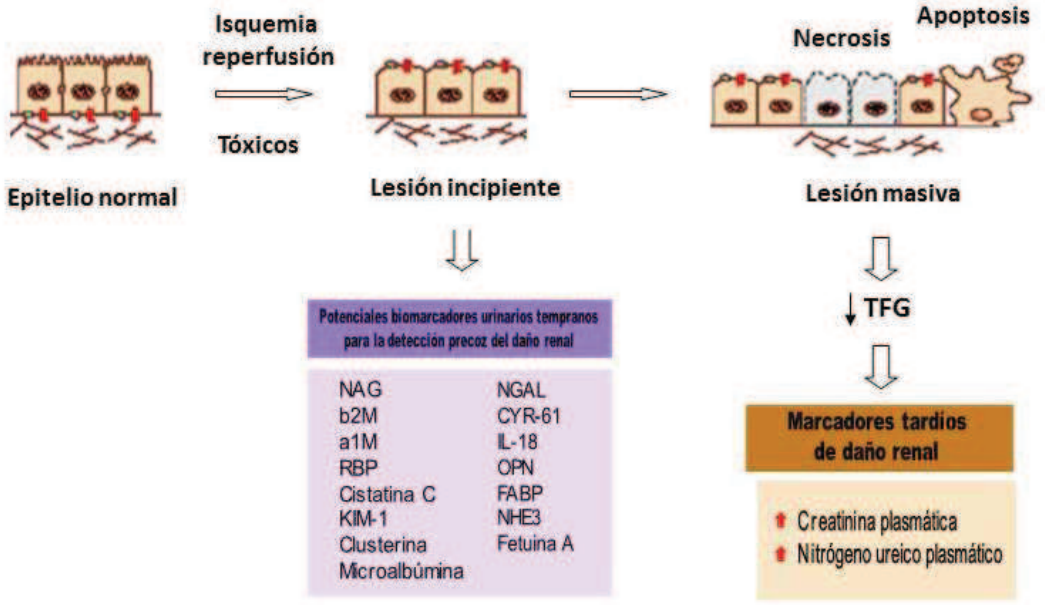


Figura 7. Biomarcadores para el diagnóstico de la lesión renal. Abreviaturas: NAG, N-acetyl-beta-D-glucosaminidasa; b2M, beta-2-microglobulina, a1M, alfa-1-microglobulina; RBP, proteína asociada al retinol; KIM-1, molécula de daño renal 1; NGAL, lipocalina asociada a la gelatinasa de neutrófilos; CYR61, proteína rica en cisteína 61 ; IL-18, interleuquina 18; OPN, osteopontina; FABP, proteína de unión a ácidos grasos; NHE3, intercambiador 3 de Na/H (adaptada de Vaidya y cols., 2008)

sufrir insuficiencia renal aguda cuando el indivíduo se expone a un segundo nefrotóxico. La búsqueda de biomarcadores capaces de detectar dicha condición también supone un reto en la mejora del manejo de esta enfermedad.

4.1. Una nueva generación de marcadores sensibles y tempranos de daño renal agudo.

Los procesos fisiopatológicos característicos de la insuficiencia renal aguda comprenden tanto mecanismos relacionados con el daño como con la respuesta reparadora del organismo. Durante el daño a las estructuras renales, sus componentes o derivados metabólicos, compuestos de degradación o restos de ellos podrían verterse a la orina donde podrían ser detectados y utilizados como marcadores de lesión. El contacto directo de los epitelios renales con la orina facilita la aparición en ésta de moléculas y fracciones celulares procedentes de los procesos fisiopatológicos de estos tejidos. Los posibles marcadores pueden tener su origen, entre otros, en la síntesis, activación o inhibición de mediadores de los procesos bioquímicos y de constituyentes estructurales celulares relacionados con procesos como la apoptosis y la regeneración tisular. Así mismo, podrían encontrarse en la orina indicios de la destrucción de los tejidos (células, matriz extracelular, membranas basales, etc.), bien como moléculas enteras, fracciones de éstas, organelas o restos de ellas y de fracciones celulares o tisulares.

El biomarcador ideal de FRA sería aquel de alta sensibilidad y especificidad, fácil de cuantificar, reproducible, barato, específico para el riñón, que aparezca precozmente en el curso del fracaso renal. A su vez también es importante que el biomarcador

tenga la capacidad de valoración del pronóstico de la enfermedad (duración y gravedad del FRA), que defina el curso del FRA y que pueda monitorizar las respuestas al tratamiento (si existe mejora o empeoramiento de la funcionalidad renal). Sin embargo, resulta difícil pensar que un único marcador pueda llegar a acometer todas estas funciones. Es por esto que actualmente se está instaurando la idea de que, para hacer un seguimiento eficaz del daño renal desde que aparece el riesgo hasta que se produce la reparación del daño renal, es necesario el uso de una colección de biomarcadores.

Identificar marcadores en muestras de orina resulta ventajoso frente a otras muestras biológicas ya que se obtiene fácilmente y es una técnica no invasiva. Además contiene marcadores que se liberan directamente del riñón, útiles para el diagnóstico precoz y monitorización de enfermedades en la práctica clínica diaria.

La detección en la orina de ciertas enzimas celulares procedentes de la lesión de células renales, es actualmente el procedimiento más refinado para la detección temprana del FRA que cursa con daño tubular, como se comentó anteriormente en el apartado 3.4. Estas enzimas incluyen la NAG, pero también otras como LDH, FAL o GGT. La mayor parte de estas enzimas tienen un valor moderado como marcadores urinarios tempranos y sensibles del fallo renal agudo, debido principalmente a problemas de especificidad, estabilidad e inhibición por otros componentes de la orina (Vaidya y cols., 2008). La determinación de la actividad de la NAG en la orina es una de las técnicas más finas para la detección de daño leve, aunque su uso todavía no está consolidado como técnica diagnóstica habitual (Price, 1982, 1992, 2002, Vaidya y cols., 2008)

Atendiendo a las necesidades de encontrar nuevos biomarcadores que se aproximen al concepto de biomarcador ideal, en la última década se ha identificado, validado y desarrollado una nueva generación de marcadores sensibles y tempranos. Estos novedosos marcadores son capaces de detectar el daño renal agudo en sus fases iniciales, mucho antes de que se agote la reserva funcional renal y, por tanto, se manifieste la disfunción. Éstos incluyen, entre otros, la medida en la orina de la molécula de lesión renal 1 (KIM-1, por sus siglas en inglés *kidney injury molecule 1*), la lipocalina asociada a la gelatinasa neutrófila (NGAL, por sus siglas en inglés *neutrophil gelatinase associated lipocalin*), inhibidor 1 del activador del plasminógeno (PAI-1, por sus siglas en inglés *plasminogen activator inhibitor 1*), cistatina C, interleuquina 18 (IL-18), proteína unida al retinol (RBP, por sus siglas en ingles, *retinol binding protein*) y otros. Es probable que estos marcadores sean útiles para medir el tiempo inicial del daño y la evaluación de la FRA, así como para distinguir entre los diversos tipos y etiologías de FRA (Bonventre, 2007; Nguyen y Devarajan, 2007; Vaidya y cols., 2008 y Waikar y Bonventre., 2008).

KIM-1, es una glicoproteína de membrana de tipo I, que se ha desarrollado como un marcador precoz. El ectodominio de KIM-1 aparece antes que la NAG en la progresión del daño renal agudo, como resultado de una gran variedad de daños (Ichimura, 2004, Waikar y Bonventre, 2008), incluyendo el tratamiento con la gentamicina (Zhou y cols., 2008), tanto en animales de experimentación como en humanos (Van Timmeren y cols., 2007). Un análisis de 31 estudios publicados sobre la capacidad de diagnóstico de varios marcadores de última generación en la orina humana, ha puesto de manifiesto que KIM-1 es un buen marcador para diferenciar el daño renal agudo (especialmente asociado a la necrosis tubular aguda) y otros tipos de daño renal, como la enfermedad renal crónica (Coca y cols., 2008).

NGAL, es una lipocalina que se sintetiza rápidamente en el epitelio dañado, se detecta en la orina y en el plasma tras el inicio del daño renal (Devarajan., 2008) independientemente del mecanismo de daño (Nickolas y cols., 2008), y de forma sustancial es detectado antes que otros

marcadores (Devarajan, 2008). Hasta el momento, diferentes estudios de cohortes de tamaño medio en pacientes, no sólo han corroborado la utilidad de NGAL como una herramienta de diagnóstico y pronóstico, sino también como uno de los mejores marcadores predictivos de daño renal agudo (Nickolas y cols., 2008). Desafortunadamente, la utilidad diagnóstica de esta proteína se ve limitada puesto que presenta baja selectividad. Este marcador aumenta con los síndromes inflamatorios.

La ***cistatina C*** es una proteína producida por las células nucleadas y se cree que es uno de los inhibidores más importantes de la proteasa cisterina. Su bajo peso molecular y alto punto isoeléctrico le permiten ser filtrada libremente y se reabsorbe casi totalmente en el epitelio tubular donde se cataboliza. Además, su producción es estable por lo que es un buen indicador para evaluar la tasa de filtración glomerular. Algunos estudios demuestran que es un marcador más sensible que la creatinina sérica y el aclaramiento de creatinina en diversas enfermedades renales como por ejemplo la nefropatía por depósitos de inmunoglobulina A (IgA), que puede ser utilizada para predecir el pronóstico de una manera más temprana en estos pacientes. (Shimizu-Tokiwa y cols., 2002, Arias y cols., 2005). Otros autores han postulado que en presencia de alteraciones tubulares se produce una disminución de la degradación de la cistatina C, por lo que sus niveles urinarios aumentan. Este incremento se ha propuesto como posible método diagnostico de disfunción tubular (Conti y cols., 2006, Uchida y Gotoh, 2002).

La ***vimentina*** es una proteína del citoesqueleto de las células poco diferenciadas, y por ello es un marcador de células regenerativas y desdiferenciadas, y un marcador de regeneración tisular (Gröne y cols., 1987). Aumenta en los riñones tras el daño agudo y crónico, como consecuencia de diferentes etiologías (tóxica, isquémica, etc.) (Villanueva y cols., 2006; Yang y cols., 2007). Incluso parece que esta molécula es importante para la reparación renal tras el daño, ya que los ratones deficientes en ella recuperan peor la función renal tras la isquemia (Runembert y cols., 2004).

IL-18 es un mediador de inflamación y lesiones del tejido isquémico en muchos órganos. Recientemente se ha demostrado que la IL-18 es un mediador del FRA en ratones. En el trabajo Melnikov y cols (2001) se observó presencia de IL-18 en células epiteliales del túbulo proximal de ratones sham. Las concentraciones de IL-18 en orina humana predicen la lesión proximal tubular tras isquemia o trasplante renal (Melnikov y cols., 2001, Parikh y cols., 2004), tras observar mayores niveles de IL-18 urinaria en pacientes que presentaban enfermedades renales. La rápida disminución en la orina tras el trasplante predice una mejora en la función renal y se asocia a la normalización en plasma de los niveles de creatinina (Parikh y cols., 2004). Por lo tanto, la detección de incrementos en la IL-18 urinaria puede ser una herramienta valiosa en el diagnostico diferencial de la disfunción renal aguda, sobre todo después del trasplante.

Algunos de estos marcadores disponen ya de kits comerciales de inmunohistoquímica (como NGAL) o de química seca (para KIM-1) que permiten utilizarlos en la escala del análisis poblacional, aunque todavía deben consolidarse en la práctica clínica habitual para la detección precoz del daño renal agudo.

4.2 Diagnóstico etiológico diferencial del FRA.

Otra posibilidad para mejorar el diagnostico del daño renal agudo es el diagnóstico etiológico diferencial, es decir, la identificación de la causa. Por ejemplo, sería de gran utilidad poder diferenciar el daño renal causado por un fármaco o agente determinado del ejercido por otros (Cataldi y cols., 2002). Actualmente esto es casi imposible ya que casi todas las formas de daño renal agudo dan lugar a los mismos marcadores, especialmente dentro del grupo de causas de un mismo tipo de FRA, como por ejemplo, la necrosis tubular aguda.

Esto sería de gran utilidad en aquellas situaciones clínicas en las que en un mismo paciente convergen a la vez diferentes fármacos y procedimientos potencialmente nefrotóxicos. En ese contexto, en aquellos pacientes en los que aparecen síntomas precoces de daño renal, sería muy útil poder conocer cuál de todas las causas potenciales de daño renal es la responsable principal del daño. De esta manera se podría actuar específicamente sobre esa y respetar las demás. Un ejemplo específico de estas situaciones es el caso de los pacientes polimedicados. En ellos, cuando aparecen síntomas de daño renal, es imposible determinar con la tecnología existente cuál de esos fármacos es el desencadenante del efecto tóxico. La identificación de marcadores específicos de cada fármaco y de cada causa de FRA permitirá realizar un tratamiento más racional, individualizado y específico de éstas situaciones clínicas cotidianas.

Recientemente, en nuestro grupo de investigación, se han identificado dos nuevos biomarcadores urinarios candidatos para el diagnóstico diferencial, que son las proteínas Reg IIIb (del inglés regenerating islet-derived protein III) y gelsolina (Ferreira y cols., 2011). Estas proteínas aparecían en la orina de ratas con daño renal producido por la administración de gentamicina y sin embargo no estaban presentes en la orina de ratas con daño renal inducido por la administración de cisplatino. Los resultados obtenidos en este estudio proporcionan una prueba de concepto sobre la posibilidad de diferenciar la nefrotoxicidad de unos fármacos de la de otros y abre nuevos caminos en el estudio de los biomarcadores.

4.3. Diagnóstico de la predisposición adquirida al FRA, aguda y crónica.

Los tratamientos con fármacos potencialmente nefrotóxicos constituyen factores de riesgo de FRA para la administración conjunta de otros fármacos potencialmente nefrotóxicos. Así mismo, la exposición crónica a sustancias medioambientales, como puede ser el caso de los metales pesados, también podría actuar como un factor de riesgo de sufrir FRA cuando existe administración conjunta de otros fármacos nefrotóxicos. En general, tanto en un caso como en otro, esta circunstancia se ha considerado tradicionalmente como la suma de dos elementos que desencadenan un FRA. Sin embargo, los estudios de esta Tesis Doctoral demuestran que el tratamiento con un fármaco (el cisplatino) o la exposición crónica a una sustancia ambiental (el uranio), ambos potencialmente nefrotóxicos, pero a dosis que no produce ningún síntoma de daño renal, predisponen al desarrollo de FRA.

Dicha predisposición se pone de manifiesto cuando los animales previamente tratados bien con el cisplatino o bien con el uranio, se someten a un segundo agente potencialmente nefrotóxico, en un régimen que en un animal no predispuesto no produce alteraciones importantes de la función renal. Así, más que de sumación de efectos, debemos hablar de una sinergia entre los dos agentes, que tiene consecuencias farmacológicas, clínicas y socioeconómicas de gran importancia. Por ejemplo, un 0.6-2.3% de los pacientes sometidos a una radiografía de contraste, sin historia previa de enfermedad renal, desarrollan algún grado de FRA (Mehran y cols., 2006). Algunos de estos pacientes podrían cursar silenciosamente con un incremento del riesgo al FRA debido a un tratamiento previo con un nefrotoxico como la gentamicina, el cisplatino o por la exposición a un agente ambiental (como el cadmio o el uranio) sin ninguna evidencia clínica, ni síntomas de lesiones renales, donde la TFG y los niveles urinarios de marcadores sensibles al daño renal (por ejemplo, KIM-1, NGAL) se mantienen en los valores normales. Así, estos pacientes tendrían un riesgo teórico incrementado, pero difícil de evaluar individualmente.

Por este motivo, la identificación de marcadores o sistemas de diagnóstico que sean capaces de detectar la predisposición al FRA adquirida mediante tratamientos farmacológicos o agentes potencialmente nefrotóxicos podrían ser de gran utilidad para identificar los pacientes de riesgo y estratificar su condición de una manera personalizada,

antes de someterlos a nuevas intervenciones, procesos o tratamientos que puedan desencadenar el daño. Los datos presentados en esta Tesis Doctoral reafirman el concepto de predisposición al daño renal. Para ello hemos utilizado dos modelos de predisposición diferentes, uno de predisposición aguda y otro de predisposición crónica.

En el primer modelo experimental hemos utilizado el fármaco cisplatino, administrado en una sola dosis. Esta situación tiene un paralelismo de gran relevancia clínica para el ser humano. Millones de pacientes reciben cada año tratamientos con cisplatino en los que, en principio, y a dosis subtóxicas, no se detecta ninguna alteración renal. Sin embargo, nuestros resultados indican que pueden estar en mayor riesgo de sufrir un grave FRA por exposición paralela o subsiguiente a otros agentes potencialmente nefrotóxicos que en condiciones normales no afectarían a la función renal, pero que, por acción del cisplatino ven reducido su umbral de nefrotoxicidad .

Para el segundo modelo experimental se ha utilizado un nefrotóxico medioambiental administrado de forma crónica, el uranio. La importancia de este estudio radica en que podría poseer un paralelismo con situaciones concretas en los humanos, como por ejemplo los trabajadores de las fábricas de uranio o los residentes cercanos a ellas, que generalmente están expuestos a elevados niveles de este metal. Esta población podría no tener ninguna afectación clínica evidente en los controles médicos habituales, y sin embargo, estar desarrollando una predisposición al daño renal que se manifestaría en determinadas circunstancias en las que se viesen sujetos a la acción de un segundo nefrotóxico, como por ejemplo un fármaco. El hallazgo de marcadores de predisposición crónica al daño renal sería interesante para detectar esta condición y poder prevenir la aparición de un fracaso renal agudo en este tipo de pacientes.

Pero estos dos modelos no son los únicos en los que se pone de manifiesto el concepto de predisposición al daño renal. Nuestro grupo de investigación tiene sólidas evidencias de que este no es un caso aislado (Quirós y cols., 2010). Otro tóxico, la gentamicina, también causa este tipo de predisposición. En este modelo se han encontrado marcadores con posibilidades de uso como método de diagnóstico. La investigación para la identificación y desarrollo de marcadores de predisposición debe, según nuestra opinión, extenderse a otras causas de daño renal de diferente naturaleza, e incluso a la predisposición de otros efectos tóxicos de los fármacos, como la hepato, cardio o neurotoxicidad. Esto dará lugar a un nuevo concepto “teranóstico” en el que nuestra capacidad diagnóstica se anticipe a los primeros signos o síntomas de las enfermedades y prevenga su aparición mediante la identificación de individuos con alto riesgo adquirido.

5. BIBLIOGRAFÍA

Arias, I. M., Pobes, A., Baños, M. (2005). Cystatin C. New marker of renal function *Nephrology*. **25**, 217 -20.

Barajas, L. (1979). Anatomy of the juxtaglomerular apparatus. *Am J Physiol*. **237**, 333-43.

Berne, R. M., Levy, M. N. (2001). Fisiología. Ediciones Harcourt, S.A. Tercera edición.

Bonventre, J. V. (2007). Diagnosis of acute kidney injury: from classic parameters to new biomarkers. *Contrib Nephrol.* **156**, 213-9.

Brady, H. R., Brenner, B. M., Lieberthal, W. L. (1996). Acute renal failure. In The kidney of Brenner and Rector. 5th. Edition. Philadelphia. Saunders company, pp1200-1255.

Brivet, F., Loirat, P., Kleinknecht, D., Landais, P. (1996). Biocompatible dialysis membrane in acute renal failure: the best choice. French Study Group on Acute Renal Failure. *Intensive Care Med*. **22**, 833-4, 1996.

Bulger, R. E., Dobyan, D. C. (1982). Recent advances in renal morphology. *Annu Rev Physiol*. **44**, 147-79.

Cataldi, L., Mussap, M., Verlato, G., Plebani, M.,

Fanos, V. (2002). Netilmicin effect on urinary retinol binding protein (RBP) and N-acetyl-beta-D-glucosaminidase (NAG) in preterm newborns with and without anoxia. *J Chemother*. **14**, 76-83.

Chertow, G. M., Lee, J., Kuperman, G. J., Burdick, E., Horsky, J., Seger, D. L., Lee, R., Mekala, A., Song, J., Komaroff, A. L., Bates, D. W. (2001). Guided medication dosing for inpatients with renal insufficiency. *Jama*. **286**, 2839-44.

Coca, S. G., Yalavarthy, R., Concato, J., Parikh, C. R. (2008). Biomarkers for the diagnosis and risk stratification of acute kidney injury: a systematic review. *Kidney Int*. **73**: 1008-16.

Conti, M., Moutereau, S., Zater, M., Lallali, K., Durrbach, A., et al. (2006). Urinary cystatin C as a specific marker of tubular dysfunction. *Clin. Chem. Lab. Med*. **44**, 288–91.

De Mendonça, A., Vincent, J. L., Suter, P. M., Moreno, R., Dearden, N. M., Antonelli, M., Takala, J., Sprung, C., Cantraine, F. (2000). Acute renal failure in the ICU: risk factors and outcome evaluated by the SOFA. Score. *Intensive Care Med.* **26**, 915–21, 2000.

Devarajan, P. (2007). Neutrophil gelatinase-associated lipocalin: new paths for an old shuttle. *Cancer Ther*. **5**, 463-470.

Devarajan, P. (2008). Neutrophil gelatinase-associated lipocalin (NGAL): a new marker of kidney disease. *Scand J Clin Lab Invest Suppl*. **241**, 89-94, 2008.

Dzau, V. J., Burt, D. W., Pratt, R. E. (1988). Molecular biology of the renin angiotensin system. *Am. J. Physiol.* **255**, 563-73.

Esteller, A., Cordero, M. (1998). Fundamentos de Fisiopatología. McGraw-Hill. Interamericana . 1ra Edición.

Ferreira, L., Quiros, Y., Sancho-Martinez, S. M., García-Sánchez, O., Raposo, C., López-Novoa, J. M., González-Buitrago, J. M., López-Hernández, F. J. (2011). Urinary levels of regenerating islet-derived protein IIIβ and gelsolin differentiate gentamicin from cisplatin -induced acute kidney injury in rats. *Kidney Int.* **79**(5): 528-28.

Gröne, H. J., Weber, K., Gröne, E., Helmchen, U., Osborn, M. (1987). Coexpression of keratin and vimentin in damaged and regenerating tubular epithelia of the kidney. *Am J Pathol.* **129**: 1-8.

Guyton, A. C. (2006). Tratado de Fisiología Médica. McGraw-Hill. Interamericana. 11na edición.

Herget-Rosenthal, S., Bökenkamp, A., Hofmann, W. (2007). How to estimate GFR-serum creatinine, serum cystatin C or equations?. *Clin Biochem*. **40**, 153-61.

Ichimura, T., Hung, C. C., Yang, S. A., Stevens, J. L., Bonventre, J. V. (2004). Kidney injury molecule-1: a tissue and urinary biomarker for nephrotoxicant-induced renal injury. *Am J Physiol Renal Physiol*. **286** F552-63.

Klaassen, C. D., Watkins, J. B. (2005). Casarett y Doull. Fundamentos de Toxicología. McGraw-Hill. Interamericana. 1ª Edición, Madrid.

Knox, F. G., Granger, J. P. (1987). Control of sodium excretion. The kidney produces under pressure. *News Physiol Sci*. **2**, 26.

Lameire, N. H., Vanholder, R. (2004). Pathophysiology of ischaemic acute renal failure. *Best Pract Res Clin Anaesthesiol*. **18**, 21-36, 2004.

Levenson, D. J., Simon, C. E. Jr, Benner, B. M. (1982). Arachidonic acid metabolism, prostaglandins and the kidney. *Am J Med*. **72**, 354.

Liangos, O., Wald, R., O'Bell, J. W., Price, L., Pereira, B. J., Jaber, B. L. (2006). Epidemiology and outcomes of acute renal failure in hospitalized patients: a national survey. *Clin J Am Soc Nephrol*. **1**, 43-51.

Lopez-Hernandez, F. J., Lopez-Novoa, J. M. (2006). The lord of the ring: Mandatory role of the kidney in drug therapy of hypertension. *Pharmacol Ther*. **111**, 53-80.

Madsen, K. M., Tisher, C. C. (1986). Structural-functional relationships along the distal nephron. *Am J Physiol*. **250**: 1-15.

Mehran, R., Nikolsky, E.(2006). Contrast-induced nephropathy: definition, epidemiology, and patients at risk. *Kidney Int* **100**, S11-5.

Melnikov, V. Y., Ecder, T., Fantuzzi, G., Siegmund, B., Lucia, M. S., Dinarello, C. A., Schrier, R. W.,

Edelstein, C. L. (2001). Impaired IL-18 processing protects caspase-1-deficient mice from ischemic acute renal failure. *J Clin Invest.* **107**, 1145-52.

Molitoris, B. A. (1991). New insights into the cell biology of ischemic acute renal failure. *J Am Soc Nephrol.* **1**, 1263-70.

Netter, J. T. (2003). Atlas of human anatomy. Ciba-Geigy limited.

Nguyen, M. T., Devarajan, P. (2008). Biomarkers for the early detection of acute kidney injury *Pediatr Nephrol.* **23**, 2151-7.

Nickolas, T. L., O'Rourke, M. J., Yang, J., Sise, M. E,, Canetta, P. A., Barasch, N., Buchen, C., Khan, F., Mori, K., Giglio, J., Devarajan, P., Barasch, J. (2008). Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. *Ann Intern Med.* **148**, 810-9.

Okabe, M., Nakayama, K., Kurasaki, M., Yamasaki, F., Aoyagi, K., Yamanoshita, O., Sato, S., Okui, T., Ohyama, T., Kasai, N. (1996). Direct visualization of copper-metallothionein in LEC rat kidneys: application of autofluorescence signal of copper-thiolate cluster. *J Histochem Cytochem*. **44**(8), 865-73.

Parikh, C. R., Jani, A., Melnikov, V. Y., Faubel, S., Edelstein, C. L. (2004). Urinary interleukin-18 is a marker of human acute tubular necrosis. *Am J Kidney Dis*. **43**: 405-14.

Price, R. G. (2002). Early Markers of Nephrotoxicity. *Comp Clin Path*. **11**, 2-7.

Price, R. G. (1992). The measurement of urinary N-acetyl-bd- glucosaminidase NAG) amd its applications. *Eur J Clin ChemClin Biochem.* **30**, 693-705.

Price, R. G. (1982). Urinary enzymes, nephrotoxicity and renal disease. *Toxicology*. **23**, 99-134.

Quiros, Y., Ferreira, L., Sáncho-Martínez, S. M., González-Buitrago, J. M., López-Novoa, J. M., López-Hernandez, F. J. (2010). Sub-nephrotoxic doses of gentamicin predisposes animals to developing acute kidney injury and to excrete ganglioside M2 activator protein. *Kindey Int*. **78** (10), 1006-15.

Rivas-Cabañero, L., Rodríguez-Barbero, A., Arévalo, M., López-Novoa, J. M. (1995). Effect of NG-nitro-arginine methyl ester on nephrotoxicity induced by gentamicin in rats. *Nephron*. **71**, 203-7.

Rivero-Sánchez, M., Rubio-Quiñones, J., Cozar-Carrasco, J., García-Gil, D. Insuficiencia renal aguda (sitio en internet). Principios de urgencias, emergencias y cuidados críticos. Disponible en: http//www.uninet.edu/tratado. Acceso: 28 de septiembre del 2008.

Runembert, I., Couette, S., Federici, P., Colucci-Guyon, E., Babinet, C., Briand, P., Friedlander, G., Terzi, F. (2004). Recovery of Na-glucose cotransport activity after renal ischemia is impaired in mice lacking vimentin. *Am J Physiol Renal Physiol*. **287**, 960-8.

Sherman, R. L., Drayer, D. E., Leyland-Jones, B. R., Reidenberg, M. M. (1983). N-acetyl-beta-glucosaminidase and beta-2-microglobulin. Their urinary excretion in patients with renal parenchymal disease. *Arch intern med.* **143**(6), 1183-5.

Shimizu-Tokiwa, A., Kobata, M., Io, H., Kobayashi, N., Shou, I., Funabiki, K., Fukui, M., Horikoshi, S., Shirato, I., Saito, K., Tomino, Y. (2002). Serum cystatin C is a more sensitive marker of glomerular function than serum creatinine. *Nephron*. **92**, 224-6.

Sierra Camerino R., Pedraza López S., Pérez Ruilópez M.A., Cózar Carrasco J.J. Principios de Urgencias, Emergencias y Cuidados Críticos: Insuficiencia Renal Aguda. Capitulo. 7.2, Disponible en: http:// tratado.uninet.edu/c0702i.html. Acceso: 28 de septiembre del 2009.

Singri, N., Ahya. S. N., Levin, M.L. (2003). Acute renal failure. *JAMA*. **289**, 747-51.

Taber, S. S., Mueller, B. A. (2006). Drug-associated renal dysfunction. *Crit Care Clin. Apr*. **22**, 357-74, viii.

Tisher, C. C. (1981). Anatomy of the Kidney, Brenner BM. Rector FC (ediciones). The Kidney Philadelphia Saunders.

Uchida, K., Gotoh, A. (2002). Measurement of cystatin-C and creatinine in urine. *Clin. Chim. Acta*. **323**, 121–28, 2002.

Vaidya, V. S., Ferguson, M. A., Bonventre, J. V. (2008). Biomarkers of Acute Kidney Injury. *Annu Rev Pharmacol Toxicol*. **48**, 17.1–17.31.

Valdivielso, J.M., Crespo, C., Alonso, J. R., Martínez-Salgado, C., Eleno, N., Arévalo, M., Pérez-Barriocanal, F., López-Novoa, J. M. (2001). Renal ischemia in the rat stimulates glomerular nitric oxide síntesis. *Am J Physiol Regul Integr Comp Physio*. **280**, R771-9.

Van Timmeren, M. M., Vaidya, V. S., van Ree, R. M., Oterdoom, L. H., de Vries, A. P., Gans, R. O., van Goor, H., Stegeman, C. A., Bonventre, J. V., Bakker, S. J. (2007). High Urinary Excretion of Kidney Injury Molecule-1 Is an Independent Predictor of Graft Loss in Renal Transplant Recipients. *Transplantation*. **84**, 1625-30.

Vander, A. J. (2006). Fisiología Renal. McGraw-Hill. Interamericana. Sexta edición.

Villanueva, S., Cespedes, C., Vio, C. P. (2006). Ischemic acute renal failure induces the expression of a wide range of nephrogenic proteins. *Am J Physiol Regul Integr Comp Physiol*. **290**, R861–70.

Waikar, S. S., Bonventre, J. V. (2008). Biomarkers for the diagnosis of acute kidney injury. *Nephron Clin Pract*. **109**, c192-7.

Yang, A., Trajkovic, D., Illanes, O., Ramiro-Ibáñez, F. (2007). Clinicopathological and tissue indicators of para-aminophenol nephrotoxicity in sprague-dawley rats. *Toxicol Pathol*. **35**, 521-32.

Zhou, Y., Vaidya, V. S., Brown, R. P., Zhang, J., Rosenzweig, B. A., Thompson, K. L., Miller, T. J., Bonventre, J. V., Goering, P. L. (2008). Comparison of kidney injury molecule-1 and other nephrotoxicity biomarkers in urine and kidney following acute exposure to gentamicin, mercury, and chromium. *Toxicol Sci*. **101**, 159-70.

ANEXO i

NEW INSIGHTS INTO THE MECHANISM OF AMINOGLYCOSIDE NEPHROTOXICITY: AN INTEGRATIVE POINT OF VIEW

José Miguel López-Novoa, Yaremi Quiros, Laura Vicente-Vicente, Ana Isabel Morales and Francisco J. López-Hernández.

Revista: Kidney International; 2010

doi: 10.1038/ki.2010.337

New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view

Jose M. Lopez-Novoa[1,2], Yaremi Quiros[1,2], Laura Vicente[1,2], Ana I. Morales[1,2] and Francisco J. Lopez-Hernandez[1,2,3]

[1]*Unidad de Fisiopatología Renal y Cardiovascular, Departamento de Fisiología y Farmacología, Universidad de Salamanca, Salamanca, Spain;* [2]*Instituto Reina Sofía de Investigación Nefrológica, Fundación íñigo Álvarez de Toledo, Madrid, Spain and* [3]*Unidad de Investigación, Hospital Universitario de Salamanca, Salamanca, Spain*

Nephrotoxicity is one of the most important side effects and therapeutical limitations of aminoglycoside antibiotics, especially gentamicin. Despite rigorous patient monitoring, nephrotoxicity appears in 10–25% of therapeutic courses. Traditionally, aminoglycoside nephrotoxicity has been considered to result mainly from tubular damage. Both lethal and sub-lethal alterations in tubular cells handicap reabsorption and, in severe cases, may lead to a significant tubular obstruction. However, a reduced glomerular filtration is necessary to explain the symptoms of the disease. Reduced filtration is not solely the result of tubular obstruction and tubular malfunction, resulting in tubuloglomerular feedback activation; renal vasoconstriction and mesangial contraction are also crucial to fully explain aminoglycoside nephrotoxicity. This review critically presents an integrative view on the interactions of tubular, glomerular, and vascular effects of gentamicin, in the context of the most recent information available. Moreover, it discusses therapeutic perspectives for prevention of aminoglycoside nephrotoxicity derived from the pathophysiological knowledge.

Kidney International advance online publication, 22 September 2010; doi:10.1038/ki.2010.337

KEYWORDS: aminoglycoside antibiotics; gentamicin; nephrotoxicity; pathophysiology; prevention

Correspondence: *Jose M. Lopez-Novoa, Unidad de Fisiopatología Renal y Cardiovascular, Departamento de Fisiología y Farmacología, Universidad de Salamanca, Campus Miguel de Unamuno, E-37007 Salamanca, Spain. E-mail: jmlnovoa@usal.es*

Received 29 April 2010; revised 8 June 2010; accepted 23 June 2010

INTRODUCTION: AMINOGLYCOSIDE ANTIBIOTICS AND NEPHROTOXICITY

Aminoglycoside antibiotics (AG) are widely used in the treatment of a variety of infections (for example, ocular, pulmonary, and intestinal infections) produced by Gram-negative bacteria and bacterial endocarditis.[1] Their cationic structure, which depends on the number of amino groups and on their distribution within the molecule, seems to have an important role in their toxicity, mostly affecting renal (nephrotoxicity[2]) and hearing (ototoxicity) tissues in which they accumulate. In spite of their undesirable toxic effects, AGs still constitute the only effective therapeutic alternative against germs insensitive to other antibiotics. This is primarily because of their chemical stability, fast bactericidal effect, synergy with betalactamic antibiotics, little resistance, and low cost.[3] In spite of being one of the most nephrotoxic AG, gentamicin is still frequently used as a first- and second-choice drug in a vast variety of clinical situations. Moreover, this aminoglycoside has been widely used as a model to study the nephrotoxicity of this family of drugs, both in experimental animals and human beings.[4–6] Most of the available data on the mechanisms responsible for AG nephrotoxicity has been obtained from gentamicin, especially at the preclinical level, in animal models or cell culture studies.

Although there are some reviews about the mechanisms explaining the toxic effects of gentamicin in the tubular epithelium, renal vasculature, and glomeruli, they lack an integrative view that brings together glomerular and tubular effects and their possible interplays. Thus, the purpose of this article is to review the effects of gentamicin in several kidney compartments with an integrative approach in order to further explain its nephrotoxicity.

NEPHROTOXICITY OF GENTAMICIN

Incidence and risk factors

The incidence of aminoglycoside nephrotoxicity has progressively increased since its introduction, until reaching 10–25% of the treatments, despite the accurate control and follow-up exercised on patients.[5–9] Clinical studies lead to the conclusion that the incidence of renal damage varies depending on the target population,[10–13] which indicates that some

individuals seem to be more sensitive than others. Table 1 shows the most important risk factors for the nephrotoxicity of gentamicin and, in general, of AGs.[14–17]

Clinical manifestations

The typical clinical manifestation of aminoglycoside toxicity is nonoliguric or even polyuric renal excretion dysfunction,[10,18–20] accompanied by an increase in plasma creatinine, urea and other metabolic products of the organism, proteinuria, enzymuria, aminoaciduria, glycosuria, and electrolyte alterations (hypercalciuria, hypermagnesuria, hypocalcemia, and hypomagnesemia).[21,22]

Table 1 | Risk factors of aminoglycoside antibiotics related to patient and treatment characteristics, and to the concomitant administration of other drugs

Patient	Treatment	Other drugs
Older age	Longer treatment	NSAIDs
Reduced renal function	Higher dosage	Diuretics
Pregnancy	Split dosage	Amphotericin
Dehydration	—	Cisplatin
Renal mass reduction	—	Cyclosporin
Hypothyroidism	—	Iodide contrast media
Hepatic dysfunction	—	Vancomycin
Metabolic acidosis	—	Cephalosporin
Sodium depletion	—	—

Abbreviation: NSAIDs, nonsteroidal anti-inflammatory drugs.

TUBULAR EFFECTS

The tubular toxicity of gentamicin presents two aspects: (i) the death of tubular epithelial cells, mainly within the proximal segment, with a very important inflammatory component associated and (ii) the nonlethal, functional alteration of key cellular components involved in water and solute transport.

Mechanisms of tubular cell death

A central aspect of aminoglycoside nephrotoxicity is their tubular cytotoxicity. Treatment of experimental animals with gentamicin results in apoptosis[23–25] as well as necrosis[26] of tubular epithelial cells. In culture, gentamicin also causes both apoptosis[27] and necrosis of these cells.[28] The phenotype of death might depend on the concentration of the drug, as with other cytotoxic compounds such as cisplatin and H_2O_2.[29,30] It might also depend on the concurrence of other triggering or predisposing factors, such as the degree of ischemia, on specific points of the renal parenchyma. Apoptosis is an ATP-requiring process. When the cell's ATP reserve drops, the death mode loses the typical characteristics of apoptosis and acquires those of necrosis.[31] Hypoxia inhibits respiration, ATP production, and sensitizes cells to Fas ligand[32] and induces cell death.[33,34] However, the most commonly observed phenotype *in vitro* is apoptosis, probably because it is necessary to expose cultured cells to high concentrations of the drug (>1 to 2 mg/ml) to observe a modest cytotoxic effect.[28,35,36] Figure 1 graphically depicts the

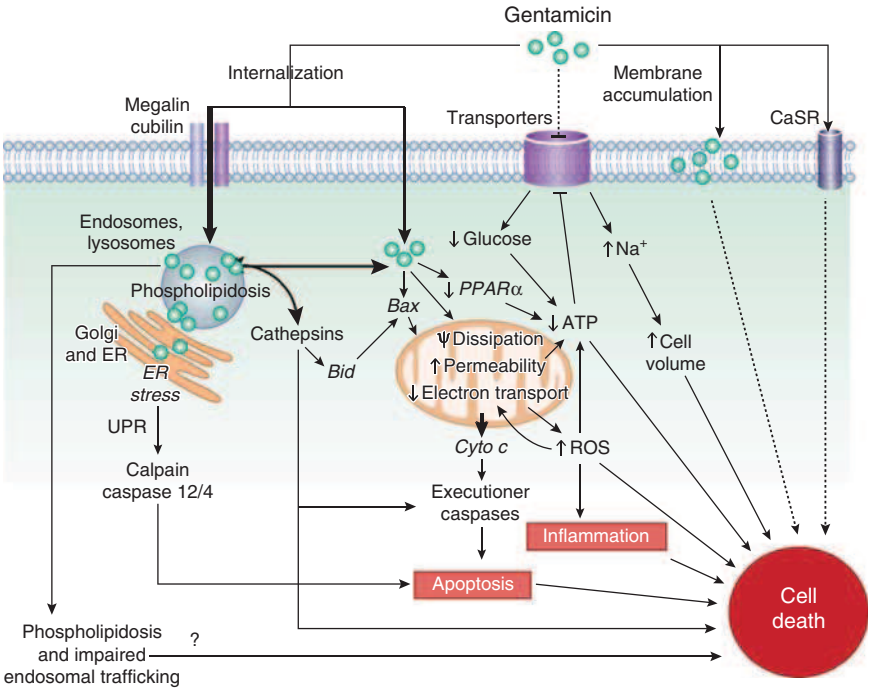


Figure 1 | Mechanisms and cell signaling pathways underlying the cytotoxic effect of gentamicin. ATP, adenosine triphosphate; CaSR, extracellular calcium-sensing receptor; *Cyto c*, cytochrome *c*; ER, endoplasmic reticulum; *PPARα*, peroxisome proliferator-activated receptor-α; ROS, reactive oxygen species; UPR, unfolded protein response; ?, The contribution of these mechanisms to cell death is not completely known.

mechanisms of cytotoxicity detailed in the following paragraphs.

Gentamicin cytotoxicity occurs in those cell types in which the drug accumulates. In the kidneys, these cells constitute the epithelial cells in the cortex, mainly in the proximal tubule of experimental animals[37] and humans,[16] and also in the distal and collecting ducts.[38] A higher accumulation of gentamicin in these cells is consistent with the expression of a transporter of proteins and cations, namely, the giant endocytic complex formed by megalin and cubilin, which is restricted to the proximal tubule. This complex is known to transport gentamicin and, in general, AGs, by endocytosis.[39] These drugs then traffic through the endosomal compartment and accumulate mostly in lysosomes, the Golgi, and endoplasmic reticulum.[40–41] Gentamicin binds to membrane phospholipids, alters their turnover and metabolism, and, as a consequence, causes a condition known as phospholipidosis that has been observed in humans[7] and experimental animals treated with the drug.[42,43] Lysosomal phospholipidosis results from (i) the reduction in the available negative charge necessary for the correct function of phospholipases[44] and (ii) inhibition of A1, A2, and C1 phospholipases.[4,45,46] Phospholipidosis correlates tightly with the level of toxicity of aminoglycosides.[43,47,48] Moreover, agents protecting from phospholipidosis, such as polyaspartic acid, also prevent aminoglycoside nephrotoxicity.[49–51] However, the effect of polyaspartic acid has been ascribed to its capacity to bind gentamicin and thus to prevent its union to phospholipids.[52] Binding to phospholipids is also a requirement for gentamicin endocytosis,[53,54] indicating that further investigation is necessary to ascertain the exact role of phospholipidosis in tubular cell death.

When the concentration of aminoglycoside in endosomal structures exceeds an undetermined threshold, their membrane is disrupted and their content, along with the drug, is poured into the cytosol.[55,56] Cytosolic gentamicin then acts on mitochondria directly and indirectly,[57,58] and thus activates the intrinsic pathway of apoptosis, interrupts the respiratory chain, impairs ATP production,[58,59] and produces oxidative stress by increasing superoxide anions and hydroxyl radicals,[60,61] which further contributes to cell death. The indirect mitochondrial effect is mediated by increasing Bax levels[62] through the inhibition of its proteosomal degradation.[35] In addition, the lysosomal content bears highly active proteases named cathepsins, which are capable of producing cell death.[63] Cathepsin-mediated cell death occurs through apoptosis by directly cleaving active executioner caspases and indirectly unleashing the intrinsic pathway through the proteolytic activation of Bid.[64,65] In high amounts, cathepsins also cause a massive proteolysis that, especially under low ATP conditions, leads to a rapid, necrotic-like mode of cell death.[66]

In the endoplasmic reticulum, gentamicin inhibits protein synthesis,[67,68] impairs translational accuracy,[69] and might interfere with the correct posttranslational protein folding.[62] This generates endoplasmic reticulum stress and activates the unfolded protein response that, on continuous stimulation, activates apoptosis through calpains and caspase 12.[70–72] Finally, activation of the extracellular calcium-sensing receptor (CaSR) with gentamicin and other aminoglycosides has also been shown to induce a mild degree of apoptosis in CaSR-expressing tubule cells and not in those lacking it. However, CaSR is also expressed in gentamicin-resistant cells including bone, brain, colon, parathyroid gland, smooth muscle, endothelial cells, and so on. Clearly, more information is necessary to clarify the exact role and the relative weight of CaSR stimulation in tubule cell death induced by aminoglycosides.

Sub-lethal alterations in tubular reabsorption

In experiments carried out with cultured cells or membrane vesicles from tubular cells, it has been shown that gentamicin, independently of cell injury, inhibits a variety of cell membrane transporters of both the brush-border and the basolateral membrane (reviewed in Mingeot-Leclercq and Tulkens[20]) including (i) Na-Pi cotransporter[73] and Na-H exchange;[74] (ii) carrier-mediated dipeptide transport;[75] (iii) electrogenic Na transport;[76] and (iv) Na-K adenosine triphosphatase.[77,78] Transport inhibition affects tubular reabsorption, but it may also compromise cell viability (Figure 1). For example, Na-K adenosine triphosphatase is a key component of cell volume homeostasis, and deregulated swelling may lead to necrosis or apoptosis.[79,80] As early as 30 min after gentamicin renal perfusion[21] or 3 h after gentamicin administration to rats,[81] deficient reuptake of calcium and magnesium is observed, leading to hypercalciuria, hypermagnesiuresis, and hypomagnesemia, before alterations in renal handling of Na^+ and K^+, and before detectable signs of renal damage and toxicity are evident. Gentamicin is transported by and also competes with proteins, organic cations, and other molecules for the megalin–cubilin endocytic complex in the proximal tubule, and thus impairs their reabsorption.[82–85]

Tubular effects cannot solely explain the reduced glomerular filtration rate

The spilling of tissue and cellular residues to the tubular lumen partially or totally obstructs the tubules.[86,87] Tubular obstruction reduces, or even voids, the excretory function of the affected nephrons. In addition, it increases the hydrostatic pressure inside the tubule and in the Bowmans' capsule, which reduces filtration pressure gradient and, therefore, the glomerular filtration rate (GFR). Moreover, the increase of intratubular pressure increases the leak of the ultrafiltrate toward the interstitial space (backleak) and peritubular capillaries, and, thus, decreases excretion of the filtrate products.[86] Accordingly, tubular obstruction may account for a part of the reduced filtration caused by gentamicin. However, in mild cases and early stages of severe cases, that is, in the absence of significant tubular obstruction, a relevant accumulation of creatinine and uremic products can be detected in the blood, which is usually the evidence that alerts

on the underlying renal damage, and indicates that, by that time, GFR is already reduced. In the absence of significant nephron obstruction, an increase in plasma creatinine (and other products) can only be explained by a reduced GFR.

Tubular damage leads to a dysfunctional reabsorption process that produces an excessive delivery of water and electrolytes to the distal part of the nephron, which in turn triggers the tubuloglomerular feedback (TGF) mechanism. TGF is brought about by an angiotensin-II and adenosine-mediated afferent and efferent arteriole effects, and the subsequent decrease in GFR.[88,89] TGF is activated as a protective mechanism to avoid massive loss of water and electrolytes.[90] The TGF mechanism is known to adapt in a period of time ranging from 1 to 24 h.[91,92] Therefore, its role in the reduction of glomerular filtration should, theoretically, disappear after this interval. However, GFR continues to decrease as long as gentamicin treatment is maintained. As described in the following sections, oxidative stress, inflammation, and the release of vasoconstrictors induce mesangial and vascular contraction (see below). These may explain why GFR remains low even in the absence of an active TGF and of significant tubular obstruction. In addition, it can also be hypothesized that gentamicin might inhibit or modulate TGF-adaptive mechanisms.

GLOMERULAR EFFECTS

The glomerulus is the first part of the nephron to come into contact with chemical agents. Gentamicin has glomerular effects that alter filtration (Figure 2). (i) Gentamicin produces mesangial contraction (reviewed in Martínez-Salgado *et al.*[93]) and results in K_f (ultrafiltration coefficient) and GFR reduction;[94,95] (ii) gentamicin also stimulates mesangial proliferation paralleled by an increase in apoptosis of these cells, which basically compensate each other;[93,96] (iii) despite the fact that gentamicin does not generate significant morphological changes in the glomerulus, in high-dose treatments, a slight increase in size, alteration of their round shape and density, and a diffuse swelling of the filtration barrier associated with neutrophil infiltration have been detected,[97] although their pathophysiological significance is uncertain; and (iv) loss of glomerular filtration barrier selectivity, due to the neutralization of its negative charges,[98] contributes to proteinuria, especially under circumstances in which tubular reabsorption is impaired such as in tubular necrosis.

Early studies demonstrated that gentamicin reduces the number and pore size of glomerular endothelial fenestrae,[99–101] correlating with a decrease in the sieving coefficient of low-molecular-weight proteins such as lysozyme,[100] and supporting a reduction in GFR. These effects seem to be the consequence of mesangial contraction. Gentamicin activates contraction of cultured mesangial cells and isolated glomeruli,[102,103] and thus reduces K_f. Several factors induced by gentamicin increase intracellular calcium concentration and cause mesangial cell contraction (reviewed in Martínez-Salgado *et al.*[93]; Figure 2). They include (i) platelet-activating factor (PAF) secretion and autocrine action;[102] (ii) activation of the renal renin–angiotensin system; (iii) production and action of vasoconstrictors such as endothelin-1 and

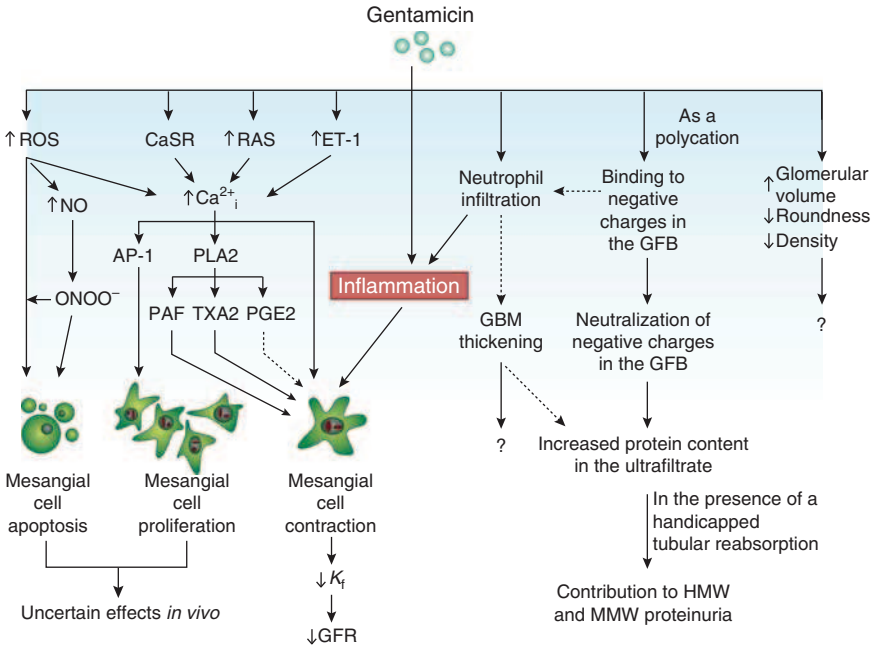


Figure 2 | Glomerular effects of gentamicin. AP-1, activator protein 1; CaSR, extracellular calcium-sensing receptor; ET-1, endothelin-1; GBM, glomerular basement membrane; GFB, glomerular filtration barrier; GFR, glomerular filtration rate; HMW, high molecular weight; K_f, ultrafiltration coefficient; MMW, medium molecular weight; NO, nitric oxide; PAF, platelet activating factor; PGE2, prostaglandin E2; PLA2, phospholipase A2; RAS, renin–angiotensin system; ROS, reactive oxygen species; TXA2, thromboxane A2; ?, Unknown pathophysiological consequences.

thromboxane A2 arising from endothelial dysfunction or imbalance;[104] (iv) CaSR stimulation; and (v) increase in reactive oxygen species (ROS) production and oxidative stress.[105]

Activation of phospholipase A2 has also been associated with the synthesis of some of the above mediators and with the effect of gentamicin on mesangial cells.[103] Phospholipase A2 catalyzes the formation of arachidonic acid, a soluble phospholipid. Arachidonic acid generates, through cyclooxygenase, the synthesis of thromboxane A2 which leads to mesangial contraction. PAF is also synthesized from the soluble phospholipids that result from phospholipase A2 activity. PAF is recognized as an important mediator of mesangial contraction, which decreases K_f and GFR.[106–108] In fact, PAF antagonists partially inhibit gentamicin-induced reduction in GFR,[95,109,110] and mesangial contraction in isolated glomeruli and cultured mesangial cells.[92,102,110]

In rats treated with gentamicin, both proliferation and apoptosis take place at the same time in the mesangial compartment. Both effects apparently compensate one another, because no net variation in the number of mesangial cells has been reported.[93,111] Mesangial proliferation is mediated by calcium-dependent AP-1 activation.[96] Mesangial cell apoptosis is mediated by increased ROS[96,111] and probably by nitric oxide (NO) overproduction.[93] Gentamicin stimulates inducible nitric oxide synthase (iNOS) expression and NO production in isolated glomeruli and mesangial cells.[112–114] Excessive NO production due to expression of iNOS, especially under oxidative stress circumstances, interacts with superoxide anion to forms peroxynitrite, which causes nitrosative stress and cytotoxic effects.[115] The role of mutually counterbalancing mesangial apoptosis and proliferation is not clear. Probably, one is the homeostatic consequence of the other, in order to maintain tissue integrity. Gentamicin might cause a mild degree of apoptosis in mesangial cells followed by a repairing proliferation. Alternatively, gentamicin might promote the proliferation of mesangial cells (through the increment in Ca_i^{2+}) that, in the absence of tissue damage, would lead to apoptosis.[93] However, both increased proliferation and apoptosis have been detected in cultured mesangial cells treated with gentamicin,[111] which obscures both of these interpretations. As argued in Martínez-Salgado *et al.*,[93] *in vivo* the primary effect would be apoptosis, with subsequent homeostatic proliferation.

VASCULAR EFFECTS

Gentamicin induces a reduction in renal blood flow (RBF),[116,117] which is the consequence of an increased resistance of the renal vascular bed rather than that of a lower perfusion pressure.[118] A lower RBF causes GFR to fall[119] (see Figure 3), and sensitizes tubule cells to cell death by reduction of oxygen and ATP availability (as explained above). RBF reduction arises initially (i) from the activation of TGF by the handicapped tubular reabsorption, in order to prevent massive fluid and electrolyte loss and (ii) progressively, superseding TGF adaptation, by production of vasoconstrictors within the renal vascular tree and mesangial compartment; and by direct effects of gentamicin on vascular cells (Figure 3).

The production of several vasocontrictors is increased on gentamicin treatment, including endothelin-1,[104] PAF, and arachidonic acid metabolites, mainly prostaglandins and thromboxane A2,[103,120,121] arising from endothelial and mesangial cells,[93] as explained in the previous section. They act in a paracrine manner on vascular myocytes and cause vasoconstriction. In addition to stimulating the production

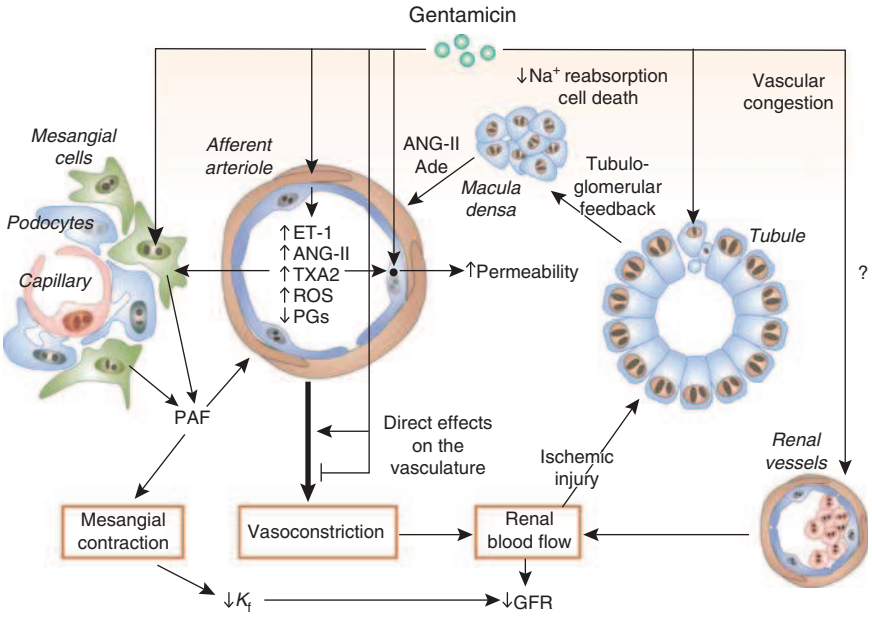


Figure 3 | Vascular effects of gentamicin. Ade, adenosine; ANG-II, angiotensin-II; ET-1, endothelin-1; GFR, glomerular filtration rate; K_f, ultrafiltration coefficient; PAF, platelet-activating factor; PGs, prostaglandins; ROS, reactive oxygen species; TXA2, thromboxane A2.

of vasoconstrictors, gentamicin also blocks the synthesis of vasodilator prostaglandins.[120] Endothelial NO synthase-derived NO, at low levels, mediates physiological vasodilatation, whereas excessive NO production due to the over-expression of iNOS (see above, section 'Vascular effects') can cause cytotoxic effects in surrounding cells. NO interacts with superoxide anion to form peroxynitrite, which induces protein and cell damage and uncouples endothelial NO synthase to become a dysfunctional superoxide-generating enzyme that contributes to vascular oxidative stress.[122]

Gentamicin also impairs vascular smooth muscle-relaxing capacity through an unraveled mechanism, theoretically contributing to vasoconstriction and RBF reduction, to an undetermined extent.[123] However, gentamicin has also been shown to relax isolated, precontracted arteries,[124,125] through the inhibition of phospholipase C, protein kinase C, and calcium movements.[124,125] This relaxing effect is exerted directly on smooth muscle cells and occurs despite gentamicin inhibiting the release of endothelium-derived relaxing factor, secondary to inhibition of PLC.[126]

Finally, leukocyte margination, leading to vascular plugging, congestion, and infarction, is induced by gentamicin in retinal vessels after 48–72 h of treatment.[127] It can be speculated that vascular plugging contributing to ischemia might also occur in the kidneys, especially under a strong proinflammatory environment, although this has to be specifically corroborated.

INTEGRATIVE PATHOPHYSIOLOGY OF GENTAMICIN NEPHROTOXICITY

Classically, the nephrotoxicity of gentamicin has been considered as a tubulopathy in which tubular damage and tubular dysfunction are the main cause of renal insufficiency. This may explain some clinical observations, such as proteinuria, enzymuria, and electrolytic alterations. However, as explained in the section 'Tubular effects cannot solely explain the reduced glomerular filtration rate', in the absence of tubular obstruction, tubular damage itself cannot account for a reduced GFR without the concourse of extratubular determinants. GFR reduction needs to be justified in order to fully explain the alterations in renal excretory function, leading to the accumulation of metabolic products in the blood, azotemia, uremia, and the whole renal syndrome produced by gentamicin.

Tubular and glomerular mechanisms differentially contribute to the reduced GFR

Tubular dysfunction leads to the loss of fluid and electrolytes that swiftly fire the TGF response, which reduces RBF and GFR to the appropriate level. Because, under physiological circumstances ~99% of water and electrolytes in the ultrafiltrate are reabsorbed along the tubule, a drastic reduction in GFR must be accomplished to compensate for a small reduction in tubular reabsorption, thus preventing the life-threatening loss of water and electrolytes. That is why even a mild injury to the tubular epithelium may bring about a pathological reduction in GFR and renal failure. However, TGF adapts within hours and its control over GFR is lost even in the presence of an increasing tubular incompetence. Yet, clinical and experimental observations demonstrate that, despite TGF adaptation, GFR grows lower as gentamicin-induced damage progresses, as described in previous sections.

Figure 4 shows the mechanisms leading to a reduced GFR. It can be observed that tubular malfunction leading to a defective reabsorption is the only mechanism that causes no GFR reduction directly, although it decreases GFR indirectly by activating the TGF mechanism, at least transitorily. Tubular obstruction increases progressively with tubular damage, as does its contribution to the reduced GFR. As such, it only partially explains the whole reduction in GFR, especially in the initial phase of acute kidney injury, which is the most relevant clinical situation. In these circumstances (Figure 5), a number of factors may hold GFR low in the absence of TGF-mediated control. Contracting factors produced by mesangial, vascular, and tubular cells, including ROS, PAF, angiotensin-II, and endothelin-1 act in an autocrine and paracrine manner to induce contraction of glomerular vessels and mesangial cells, which reduce RBF and K_f, respectively, and lower GFR. A question for the future is if a part of the reduction in GFR caused by gentamicin would still occur, should tubular alterations be completely and specifically prevented, or, whether most glomerular and vascular effects are, at least partially, independent of tubular damage. As explained above, gentamicin-induced mesangial activation and contraction have been documented in cultured, isolated mesangial cells,[93] indicating that no tubular-derived stimulation is necessary for these effects. In addition, reduced GFR and RBF may contribute to aggravating gentamicin-induced tubular damage,[128] probably because they limit oxygen and nutrient availability to tubular cells and facilitate oxidative stress, as it has been demonstrated in the ischemic renal failure.

Central role of oxidative stress and inflammation: a loop of damage amplification and a connection between tubular and glomerular mechanisms

Oxidative stress has been suggested to have a key role in gentamicin nephrotoxicity.[129–131] This is mainly based on a myriad of studies conducted in experimental models demonstrating that cotreatment with a variety of antioxidants protects from gentamicin-induced renal damage,[61,117,132,133] although clinical data is not so conclusive.[134] Gentamicin directly increases the production of mitochondrial ROS,[58] which (i) are able of damaging many cellular molecules including proteins, lipids, and nucleic acids, thus impairing cell function and leading to cell death; (ii) contribute to mesangial and vascular contraction (as described in sections 'Glomerular effects' and 'Vascular effects'); and (iii) participate in inflammation.

The nephrotoxicity of gentamicin has been shown to involve an inflammatory response in experimental animals[135,136] and humans,[137] with cell infiltration, activation

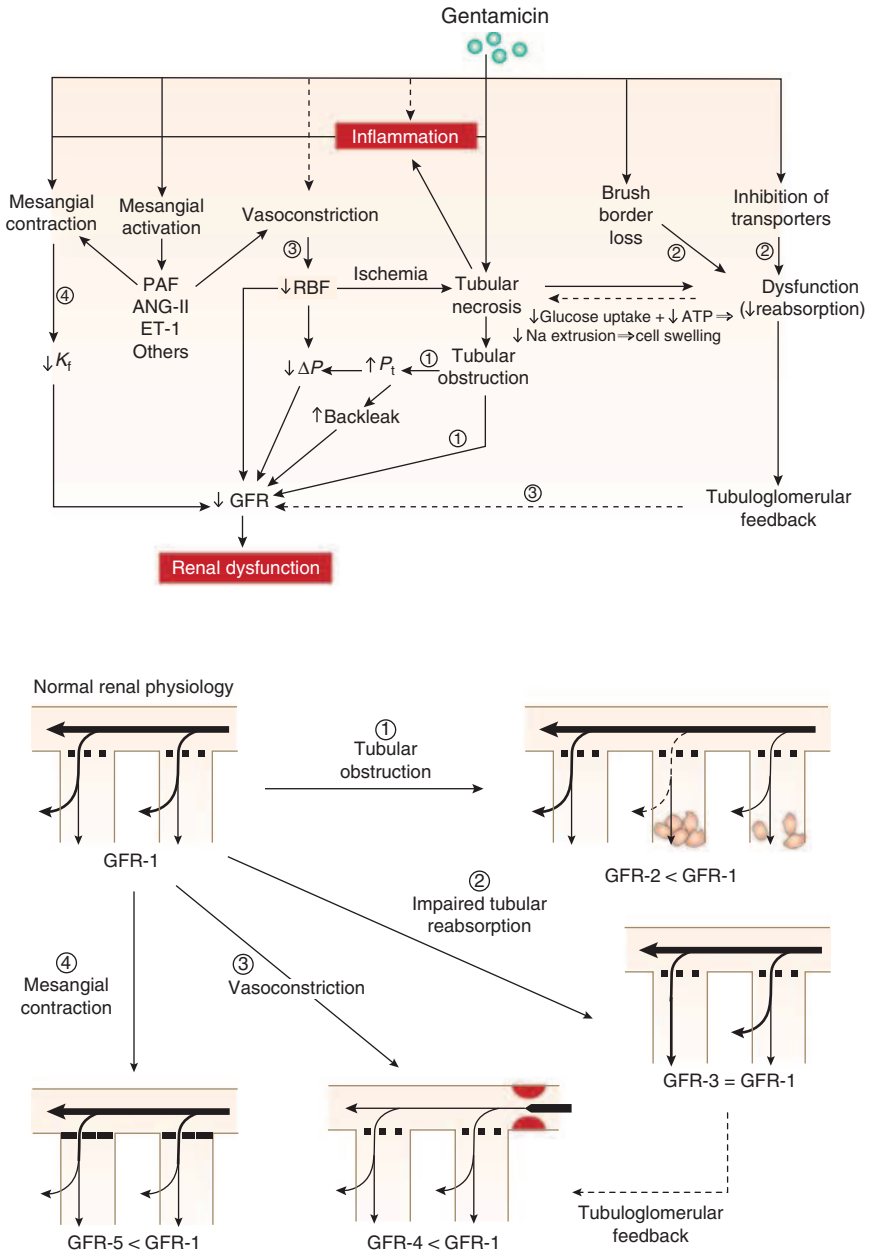


Figure 4 | Integrative view of the mechanisms leading to gentamicin nephrotoxicity. It can be appreciated that, in the absence of a significant tubular obstruction, vascular and mesangial mechanisms are necessary to explain the reduction in glomerular filtration (GFR) and renal excretion, once the tubuloglomerular feedback adapts. ANG-II, angiotensin-II; ATP, adenosine triphosphate; ET-1, endothelin-1; GFR, glomerular filtration rate; K_f, ultrafiltration coefficient; ΔP, net ultrafiltration pressure; PAF, platelet-activating factor; P_t, intratubular pressure; RBF, renal blood flow.

of resident cells, increased cytokine production,[138,139] and capillary hyperpermeability.[140] The inflammatory response, initially unleashed as a defense and repair mechanism, when globally considered seems to contribute to renal damage progression. In fact, strategies that protect from gentamicin-induced renal damage usually inhibit the inflammatory response.[135,141] In this sense, ROS are known to participate in the inception and signaling of inflammation,[142] which might explain why antioxidants are very effective at softening the renal damage inflicted by gentamicin[117,143,144] (Figure 6) and, in general, by other tubular necrosis-inducing nephrotoxins.[134,145] ROS such as superoxide anion[146] and hydrogen peroxide[147] activate nuclear factor κB, which has a key role in the inception of the inflammatory process. Indeed, nuclear factor κB inhibitors protect the kidney against gentamicin-induced damage.[148] Nuclear factor κB induces the expression of proinflammatory cytokines[149] and iNOS.[150] As described above, iNOS-derived NO can react with

superoxide anion and produce peroxinitrite, a highly reactive radical that contributes to cell damage and reduced vascular relaxation.

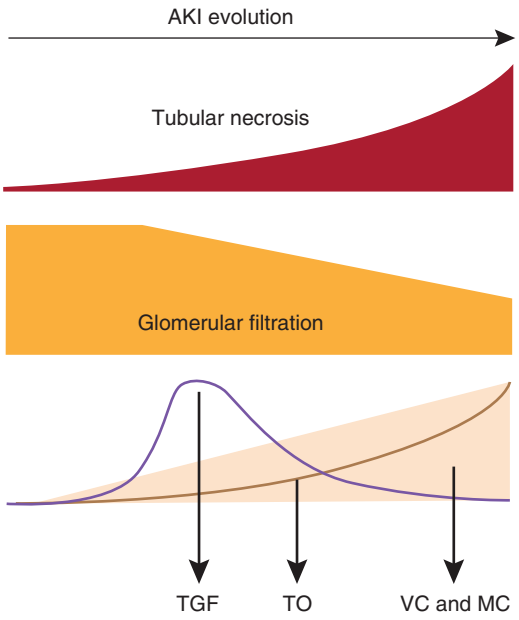


Figure 5 | Comparative temporal evolution of the acute kidney injury (AKI), tubular necrosis, glomerular filtration, tubuloglomerular feedback, and vascular and mesangial contraction on treatment with gentamicin. Initially, tubuloglomerular feedback (TGF) controls glomerular filtration rate. As TGF adapts, increasing tubular obstruction (TO), and vascular and mesangial contraction (VC and MC) take over and make GFR progressively lower.

It can be speculated that the effect of antioxidants might be related to a combined action at different levels, including the following: (i) softening of gentamicin's direct cytotoxicity (as explained above); (ii) inhibiting vasoconstriction and mesangial contraction; and (iii) an antiinflammatory action. However, there is little information on the ability of antioxidants to modulate the direct cytotoxic effect of gentamicin on cultured tubule cells. To our knowledge, only Juan *et al.*[151] have reported a protective effect in this sense. In their article, tetramethylpyrazine reduces ROS accumulation and apoptotic events in rat renal NRK-52E cells. However, the effect of tetramethylpyrazine on cell viability is not reported. Because there are many apoptotic and necrotic pathways leading to cell death as a consequence of gentamicin action, and because their redundancy and hierarchical organization are not well understood, the magnitude of the direct cytoprotection afforded by ROS inhibition is unknown.

In any case, it is reasonable to think that the inflammatory response acts as an amplifying mechanism of damage (Figure 6). Initially, cell destruction through necrosis would lead to the onset of an inflammatory response. Tissue debris and cell content shed into the extracellular space trigger inflammation,[152] whereas an exaggerated inflammation would contribute to further damage that, in turn, would exacerbate the inflammatory response.[153] Inflammation also activates glomerular cells, such as mesangial cells, podocytes and epithelial cells, endothelial cells, and resident and infiltrated leukocytes. These, in turn, produce cytokines and growth factors that contribute to the pathophysiological process with different effects (Figure 6), including amplification of tubular damage.[154] As such, inflammation and

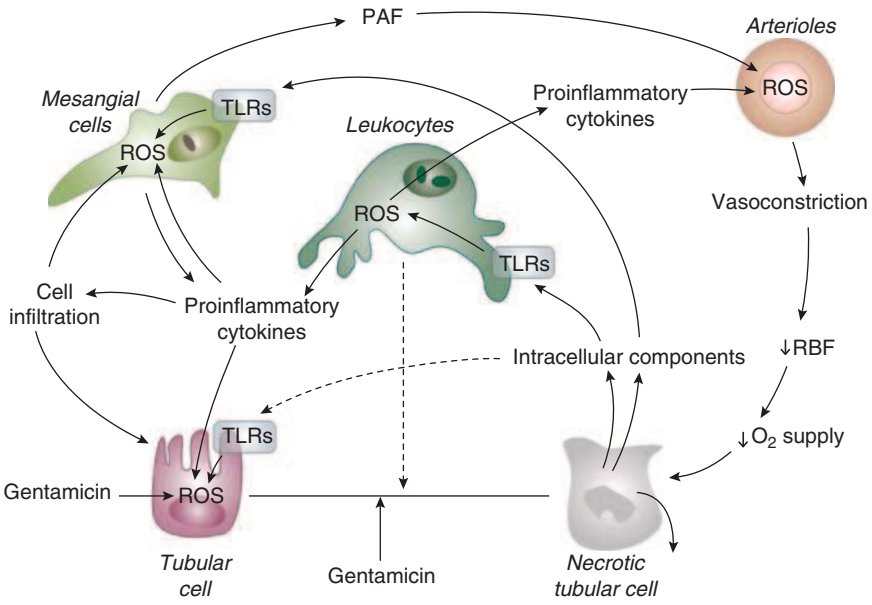


Figure 6 | Role of inflammation in the amplification of tubular, glomerular, and vascular effects of gentamicin. PAF, platelet-activating factor; RBF, renal blood flow; ROS, reactive oxygen species; TLRs, toll-like receptors.

oxidative stress provide a connection between tubular necrosis and glomerular and vascular activation and contraction, which ultimately further contribute to tubular damage, mainly through a reduction in RBF.

CLINICAL IMPLICATIONS FOR THE PREVENTION OF NEPHROTOXICITY

Prevention of nephrotoxicity is an unmet therapeutic objective that will improve the pharmacotoxicological profile and the clinical utility of many drugs significantly, including AGs. In many cases, nephrotoxicity is the most important limitation to the dosage or intensity of the therapeutic regimen, and may lead to serious health complications and even death in determined cases. Nephrotoxicity is a concern in all clinical settings, but takes special relevance among critically ill patients. Indeed, it is estimated that ~25% of the 100 most used drugs in intensive care units are potentially nephrotoxic,[155] and that nephrotoxicity is responsible for 10–20% of acute renal failure cases.[156] Besides a correct monitoring, maintenance of patient's hydration, and application of dialysis when necessary, there are no therapeutic tools available to prevent or palliate drug nephrotoxicity. There are no or very few tailored preventive strategies for individual nephrotoxic drugs, based on specific mechanisms of action. Nonetheless, this is another challenge for the future. In addition to the identification of less toxic compounds, several new strategies for the prevention of aminoglycoside nephrotoxicity are currently under different degrees of development, mostly at the preclinical level.

Inhibition of tubular accumulation

A proposed strategy focuses on finding drugs that prevent the accumulation of aminoglycosides by interfering with transport mechanisms. An obvious target is the megalin-related endocytic machinery responsible for AG transport and accumulation in tubular and auditory cells. Inhibition of aminoglycoside transport can be approached by administering (i) competitors for the receptor that displace aminoglycosides from binding to it or (ii) specific inhibitors of this endocytic pathway. Certain protein, fragments thereof and basic peptide ligands of megalin reduce the accumulation of gentamicin in cultured tubular cells and renal tubuli *in vivo* by inhibiting drug binding to the brush border.[85,157,158] Statins have been shown to reduce gentamicin accumulation in tubule cells and renal damage through a mechanism involving geranyl isoprenoids.[159] Megalin-mediated endocytosis involves other proteins with binding, adaptor, and unknown functions, such as cubilin, disabled-2, nonmuscle myosin heavy chain IIA and β-actin, which seem to participate in endocytic trafficking.[160] These proteins, and others resulting from a deeper knowledge of the endocytic mechanisms, are potential targets for pharmacological prevention of aminoglycoside accumulation. Indeed, genetic disruption of myosin VI[161] or treatment with the myosin inhibitor blebbistatin[160] reduces the uptake of proteins transported by the megalin complex. Myosin VI knockout mice show albuminuria with no alterations in urine output or electrolyte excretion. These initial results show a potential avenue for further exploration. Yet, the clinical consequences (for example, proteinuria) of interfering with megalin-mediated endocytosis as a mechanism of nephroprotection need to be determined in the short- and long term. In this line, myosin VI knockout mice show tubular dilation and fibrosis, consistent with persistent proteinuria.[161]

Cotreatment with renoprotective drugs

Another strategy relies on nephroprotective drugs for cotreatment along with aminoglycosides. At the preclinical level, many molecules have been shown to exert protective effects on drug nephrotoxicity and, specifically on aminoglycoside nephrotoxicity. By far, most of the studies have tested the ability of antioxidants to alleviate aminoglycoside nephrotoxicity. With one exception studied in patients,[162] all of them have been conducted in experimental animals. Preclinical studies offer unambiguous information on the beneficial effects of antioxidants. However, these results need to be further explored in the clinical setting, as promising, although inconsistent, results have been obtained on the protection exerted by antioxidants on the nephrotoxicity of other drugs.[134] In most studies in which inflammation has been evaluated, it is concluded that they might exert their effects through a cytoprotective and antiinflammatory action.

Improvement of RBF may also attenuate aminoglycoside nephrotoxicity, even independently from tubular damage.[162] An increased RBF by preglomerular or general vasodilatation can enhance GFR and attenuate the tubular damage caused or amplified by the reduced flow. In this sense, promising results have been obtained in animals with PAF inhibitors, although they have not progressed further into human investigation. This could be an attractive strategy to pursue. In general, vasodilators also relax mesangial cells and augment K_f. As such, the increase in GFR is not only the result of hemodynamic improvement but also of K_f modulation. Thromboxane A2 inhibitors have been used in one study with protective results.[120] Calcium antagonists have also been used with contradictory results at the preclinical level. We have found only two studies conducted in humans. They document protection afforded by calcium channel blockers verapamil and nifedipine on gentamicin nephrotoxicity.[162,163] The effect of calcium antagonists may depend on the relative level of contraction of preglomerular and postglomerular vessels and mesangial cells, and on the weight of vasoconstriction and mesangial contraction in the overall effect of a determined experimental or clinical therapeutic regimen with aminoglycosides. This, in turn, may also depend on the dose and length of treatment, drug accumulation, and so on. A note of caution should also be introduced here, because the clinical consequences of augmenting GFR without a parallel amelioration of tubular damage may result in massive proteinuria, and water and electrolytic loss, which need to be addressed.

Other strategies

Another potentially nephroprotective effect that should be pursued is the blockade of the immune response. In fact, genetic knockdown of toll-like receptor-4 has been shown to alleviate the renal lesion induced by cisplatin[164] and ischemia reperfusion[165] in mice, in which inflammation has a central pathological role. Indeed, ROS are involved in toll-like receptor-mediated inflammation.[166] Perhaps, cocktails containing several drugs aimed at providing protection against tubular damage and inflammation, and improvement of renal hemodynamics should be evaluated at the preclinical and clinical levels.

CONCLUSIONS AND PERSPECTIVES

An integration of tubular, glomerular, and vascular effects of aminoglycosides based on the evidence discussed in this paper is consistent with an important component of tubular injury. In severe degrees of acute kidney injury induced by gentamicin, tubular obstruction may account, at least partly, for the reduced GFR. However, in mild cases and early stages of severe cases, that is, in the absence of significant tubular obstruction, GFR reduction can only be explained by extratubular mechanisms, namely, mesangial and vascular contraction. These later result from (i) the TGF mechanism, with the temporal restriction explained in section 'Tubular effects cannot solely explain the reduced glomerular filtration rate'; (ii) direct mesangial and vascular contraction; and (iii) indirect mesangial and vascular contraction produced by inflammation and paracrine mediators. Inflammation is known to result from tissue damage, specially arising from cell necrosis. Still, it remains to be elucidated (i) whether all the inflammatory responses are the consequences of tubular damage or whether they are also partly activated or amplified by tubular necrosis-independent mechanisms; and (ii) what is the contribution of direct extratubular effects of gentamicin to the overall syndrome, which are completely independent of tubular damage, and of mechanisms derived from tubular damage that alter glomerular and vascular function.

Finally, it should be stressed that known and new nephroprotective strategies should also be tested for their potential effects on the bactericidal effect of aminoglycosides. This issue has not been addressed in renal studies. For example, oxidative stress has been proposed to contribute to aminoglycoside bactericidal effect.[167] Then, treatment with antioxidants with the objective of reducing their nephrotoxicity may also impair their antibiotic activity. Thus, combined models of nephrotoxicity/nephroprotection and sepsis should be developed.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

Studies from the authors' laboratory were supported by grants from the Instituto de Salud Carlos III (Retic 016/2006, RedinRen to JML-N, and FIS grant PI081900 to FJL-H), Junta de Castilla y Leon (Excellence Group GR-100), and Ministerio de Ciencia y Tecnología (BFU2004-00285/BFI and SAF2007-63893).

REFERENCES

1. Chen LF, Kaye D. Current use for old antibacterial agents: polymyxins, rifamycins, and aminoglycosides. *Infect Dis Clin North Am* 2009; **23**: 1053–1075.
2. Moore RD, Lietman PS, Smith CR. Clinical response to aminoglycoside therapy: importance of the ratio of peak concentration to minimal inhibitory concentration. *J Infect Dis* 1987; **155**: 93–99.
3. Edson RS, Terrell CL. The aminoglycosides. *Mayo Clin Proc* 1999; **74**: 519–528.
4. Laurent G, Carlier MB, Rollman B *et al.* Mechanism aminoglycoside-induced lysosomal phospholipidosis: *in vitro* and *in vivo* studies with gentamicin and amikacin. *Biochem Pharmacol* 1982; **31**: 3861–3870.
5. Laurent G, Kishore BK, Tulkens PM. Aminoglycoside-induced renal phospholipidosis and nephrotoxicity. *Biochem Pharmacol* 1990; **40**: 2383–2392.
6. Kacew S, Bergeron MG. Pathogenic factors in aminoglycoside induced nephrotoxicity. *Toxicol Lett* 1990; **51**: 241–259.
7. De Broe ME, Paulus GJ, Verpooten GA *et al.* Early effects of gentamicin, tobramycin, and amikacin on the human kidney. *Kidney Int* 1984; **25**: 643–652.
8. Leehey DJ, Braun BI, Tholl DA *et al.* Can pharmacokinetic dosing decrease nephrotoxicity associated with aminoglycoside therapy. *J Am Soc Nephrol* 1993; **4**: 81–90.
9. Bertino Jr JS, Booker LA, Franck PA *et al.* Incidence of and significant risk factors for aminoglycoside-associated nephrotoxicity in patients dosed by using individualized pharmacokinetic monitoring. *J Infect Dis* 1993; **167**: 173–179.
10. Kays SE, Crowell WA, Johnson MA. Iron supplementation increases gentamicin nephrotoxicity in rats. *J Nutr* 1992; **121**: 1869–1872.
11. Madsen KM, Park CH. Lysosome distribution and cathepsin B and L activity along the rabbit proximal tubule. *Am J Physiol* 1987; **253**: 290–301.
12. Schentag JJ, Plaut ME, Cerra FB. Comparative nephrotoxicity of gentamicin and tobramycin: pharmacokinetic and clinical studies in 201 patients. *Antimicrob Agents Chemother* 1981; **19**: 859–866.
13. Plaut ME, Schentag JJ, Jusko WJ. Nephrotoxicity with gentamicin or tobramycin. *Lancet* 1979; **2**: 526–527.
14. Moore RD, Smith CR, Lipsky JJ *et al.* Risk factors for nephrotoxicity in patients treated with aminoglycosides. *Ann Intern Med* 1984; **100**: 352–357.
15. Prins JM, Weverling GJ, de Blok K *et al.* Validation and nephrotoxicity of a simplified once-daily aminoglycoside dosing schedule and guidelines for monitoring therapy. *Antimicrob Agents Chemother* 1996; **40**: 2494–2499.
16. Verpooten GA, Giuliano RA, Verbist L *et al.* Once-daily dosing decreases renal accumulation of gentamicin and netilmicin. *Clin Pharmacol Ther* 1989; **45**: 22–27.
17. De Broe ME, Verbist L, Verpooten GA. Influence of dosage schedule on renal cortical accumulation of amikacin and tobramycin in man. *J Antimicrob Chemother* 1991; **27**(Suppl C): 41–47.
18. Trollfors B, Alestig K, Krantz I *et al.* Quantitative nephrotoxicity of gentamicin in nontoxic doses. *J Infect Dis* 1980; **141**: 306–309.
19. Klastersky J, Hensgens C, Henri A *et al.* Comparative clinical study of tobramycin and gentamicin. *Antimicrob Agents Chemother* 1974; **5**: 133–138.
20. Mingeot-Leclercq MP, Tulkens PM. Aminoglycosides: nephrotoxicity. *Antimicrob Agents Chemother* 1999; **43**: 1003–1012.
21. Parsons PP, Garland HO, Harpur ES *et al.* Acute gentamicin-induced hypercalciuria and hypermagnesiuria in the rat: dose-response relationship and role of renal tubular injury. *Br J Pharmacol* 1997; **122**: 570–576.
22. Banday AA, Farooq N, Priyamvada S *et al.* Time dependent effects of gentamicin on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in rat kidney tissues. *Life Sci* 2008; **82**: 450–459.
23. Li J, Li QX, Xie XF *et al.* Differential roles of dihydropyridine calcium antagonist nifedipine, nitrendipine and amlodipine on gentamicin-induced renal tubular toxicity in rats. *Eur J Pharmacol* 2009; **620**: 97–104.
24. Laurent G, Maldague P, Carlier MB *et al.* Increased renal DNA synthesis *in vivo* after administration of low doses of gentamicin to rats. *Antimicrob Agents Chemother* 1983; **24**: 586–593.
25. El Mouedden M, Laurent G, Mingeot-Leclercq MP *et al.* Apoptosis in renal proximal tubules of rats treated with low doses of aminoglycosides. *Antimicrob Agents Chemother* 2000; **44**: 665–675.

26. Edwards JR, Diamantakos EA, Peuler JD *et al.* A novel method for the evaluation of proximal tubule epithelial cellular necrosis in the intact rat kidney using ethidium homodimer. *BMC Physiol* 2007; **7**: 1.
27. El Mouedden M, Laurent G, Mingeot-Leclercq MP *et al.* Gentamicin-induced apoptosis in renal cell lines and embryonic rat fibroblasts. *Toxicol Sci* 2000; **56**: 229–239.
28. Pessoa EA, Convento MB, Silva RG *et al.* Gentamicin-induced preconditioning of proximal tubular LLC-PK1 cells stimulates nitric oxide production but not the synthesis of heat shock protein. *Braz J Med Biol Res* 2009; **42**: 614–620.
29. Shibuya H, Kato Y, Saito M *et al.* Induction of apoptosis and/or necrosis following exposure to antitumour agents in a melanoma cell line, probably through modulation of Bcl-2 family proteins. *Melanoma Res* 2003; **13**: 457–464.
30. Saito Y, Nishio K, Ogawa Y *et al.* Turning point in apoptosis/necrosis induced by hydrogen peroxide. *Free Radic Res* 2006; **40**: 619–630.
31. Chiarugi A. 'Simple but not simpler': toward a unified picture of energy requirements in cell death. *FASEB J* 2005; **19**: 1783–1788.
32. Steinbach JP, Wolburg H, Klumpp A *et al.* Hypoxia sensitizes human malignant glioma cells towards CD95L-induced cell death. *J Neurochem* 2005; **92**: 1340–1349.
33. Khan S, Cleveland RP, Koch CJ *et al.* Hypoxia induces renal tubular epithelial cell apoptosis in chronic renal disease. *Lab Invest* 1999; **79**: 1089–1099.
34. Módis K, Gero D, Nagy N *et al.* Cytoprotective effects of adenosine and inosine in an *in vitro* model of acute tubular necrosis. *Br J Pharmacol* 2009; **158**: 1565–1578.
35. Servais H, Jossin Y, Van Bambeke F *et al.* Gentamicin causes apoptosis at low concentrations in renal LLC-PK1 cells subjected to electroporation. *Antimicrob Agents Chemother* 2006; **50**: 1213–1221.
36. Wu Y, Connors D, Barber L *et al.* Multiplexed assay panel of cytotoxicity in HK-2 cells for detection of renal proximal tubule injury potential of compounds. *Toxicol In Vitro* 2009; **23**: 1170–1178.
37. Pattyn VM, Verpooten GA, Giuliano RA *et al.* Effect of hyperfiltration, proteinuria and diabetes mellitus on the uptake kinetics of gentamicin in the kidney cortex of rats. *J Pharmacol Exp Ther* 1988; **244**: 694–698.
38. Fujiwara K, Shin M, Matsunaga H *et al.* Light-microscopic immunocytochemistry for gentamicin and its use for studying uptake of the drug in kidney. *Antimicrob Agents Chemother* 2009; **53**: 3302–3307.
39. Schmitz C, Hilpert J, Jacobsen C *et al.* Megalin deficiency offers protection from renal aminoglycoside accumulation. *J Biol Chem* 2002; **277**: 618–622.
40. Silverblatt FJ, Kuehn C. Autoradiography of gentamicin uptake by the rat proximal tubule cell. *Kidney Int* 1979; **15**: 335–345.
41. Silverblatt F. Pathogenesis of nephrotoxicity of cephalosporins and aminoglycosides: a review of current concepts. *Rev Infect Dis* 1982; **4**(Suppl): S360–S365.
42. Giuliano RA, Paulus GJ, Verpooten GA *et al.* Recovery of cortical phospholipidosis and necrosis after acute gentamicin loading in rats. *Kidney Int* 1984; **26**: 838–847.
43. Nonclercq D, Wrona S, Toubeau G *et al.* Tubular injury and regeneration in the rat kidney following acute exposure to gentamicin: a time-course study. *Ren Fail* 1992; **14**: 507–521.
44. Mingeot-Leclercq MP, Brasseur R, Schanck A. Molecular parameters involved in aminoglycoside nephrotoxicity. *J Toxicol Environ Health* 1995; **44**: 263–300.
45. Ramsammy LS, Josepovitz C, Lane B *et al.* Effect of gentamicin on phospholipid metabolism in cultured rabbit proximal tubular cells. *Am J Physiol* 1989; **256**: C204–C213.
46. Abdel-Gayoum AA, Ali BH, Ghawarsha K *et al.* Plasma lipid profile in rats with gentamicin-induced nephrotoxicity. *Hum Exp Toxicol* 1993; **12**: 371–375.
47. Tulkens PM. Nephrotoxicity of aminoglycoside antibiotics. *Toxicol Lett* 1989; **46**: 107–123.
48. Kaloyanides GJ. Drug-phospholipid interactions: role in aminoglycoside nephrotoxicity. *Ren Fail* 1992; **14**: 351–357.
49. Ramsammy LS, Josepovitz C, Lane BP *et al.* Polyaspartic acid protects against gentamicin nephrotoxicity in the rat. *J Pharmacol Exp Ther* 1989; **250**: 149–153.
50. Beauchamp D, Laurent G, Maldague P *et al.* Protection against gentamicin-induced early renal alterations (phospholipidosis and increased DNA synthesis) by coadministration of poly-L-aspartic acid. *J Pharmacol Exp Ther* 1990; **255**: 858–866.
51. Swan SK, Kohlhepp SJ, Kohnen PW *et al.* Long-term protection of polyaspartic acid in experimental gentamicin nephrotoxicity. *Antimicrob Agents Chemother* 1991; **35**: 2591–2595.
52. Ramsammy L, Josepovitz C, Lane B *et al.* Polyaspartic acid inhibits gentamicin-induced perturbations of phospholipid metabolism. *Am J Physiol* 1990; **258**: C1141–C1149.
53. Lipsky JJ, Cheng L, Sacktor B *et al.* Gentamicin uptake by renal tubule brush border membrane vesicles. *J Pharmacol Exp Ther* 1980; **215**: 390–393.
54. Frommer JP, Senekjian HO, Babino H *et al.* Intratubular microinjection study of gentamicin transport in the rat. *Miner Electrolyte Metab* 1983; **9**: 108–112.
55. Ngaha EO, Ogunleye IO. Studies on gentamicin-induced labilization of rat kidney lysosomes *in vitro*. Possible protection by selenium. *Biochem Pharmacol* 1983; **32**: 2659–2664.
56. Regec AL, Trump BF, Trifillis AL. Effect of gentamicin on the lysosomal system of cultured human proximal tubular cells. Endocytotic activity, lysosomal pH and membrane fragility. *Biochem Pharmacol* 1989; **38**: 2527–2534.
57. Mather M, Rottenberg H. Polycations induce the release of soluble intermembrane mitochondrial proteins. *Biochim Biophys Acta* 2001; **1503**: 357–368.
58. Morales AI, Detaille D, Prieto M *et al.* Metformin prevents experimental gentamicin-induced nephropathy by a mitochondria-dependent pathway. *Kidney Int* 2010; **77**: 861–869.
59. Simmons Jr CF, Bogusky RT, Humes HD. Inhibitory effects of gentamicin on renal mitochondrial oxidative phosphorylation. *J Pharmacol Exp Ther* 1980; **214**: 709–715.
60. Walker PD, Shah SV. Evidence suggesting a role for hydroxyl radical in gentamicin-induced acute renal failure in rats. *J Clin Invest* 1988; **81**: 334–341.
61. Cuzzocrea S, Mazzon E, Dugo L *et al.* A role for superoxide in gentamicin-mediated nephropathy in rats. *Eur J Pharmacol* 2002; **450**: 67–76.
62. Horibe T, Matsui H, Tanaka M *et al.* Gentamicin binds to the lectin site of calreticulin and inhibits its chaperone activity. *Biochem Biophys Res Commun* 2004; **323**: 281–287.
63. Schnellmann RG, Williams SW. Proteases in renal cell death: calpains mediate cell death produced by diverse toxicants. *Ren Fail* 1998; **20**: 679–686.
64. Chwieralski CE, Welte T, Bühling F. Cathepsin-regulated apoptosis. *Apoptosis* 2006; **11**: 143–149.
65. Yin XM. Bid, a BH3-only multi-functional molecule, is at the cross road of life and death. *Gene* 2006; **369**: 7–19.
66. Golstein P, Kroemer G. Cell death by necrosis: towards a molecular definition. *Trends Biochem Sci* 2007; **32**: 37–43.
67. Bennett WM, Mela-Riker LM, Houghton DC *et al.* Microsomal protein synthesis inhibition: an early manifestation of gentamicin nephrotoxicity. *Am J Physiol* 1988; **255**: F265–F269.
68. Monteil C, Leclere C, Fillastre JP *et al.* Characterization of gentamicin-induced dysfunctions *in vitro*: the use of optimized primary cultures of rabbit proximal tubule cells. *Ren Fail* 1993; **15**: 475–483.
69. Buchanan JH, Stevens A, Sidhu J. Aminoglycoside antibiotic treatment of human fibroblasts: intracellular accumulation, molecular changes and the loss of ribosomal accuracy. *Eur J Cell Biol* 1987; **43**: 141–147.
70. Shimizu A, Takumida M, Anniko M *et al.* Calpain and caspase inhibitors protect vestibular sensory cells from gentamicin ototoxicity. *Acta Otolaryngol* 2003; **123**: 459–465.
71. Peyrou M, Hanna PE, Cribb AE. Cisplatin, gentamicin, and p-aminophenol induce markers of endoplasmic reticulum stress in the rat kidneys. *Toxicol Sci* 2007; **99**: 346–353.
72. Peyrou M, Cribb AE. Effect of endoplasmic reticulum stress preconditioning on cytotoxicity of clinically relevant nephrotoxins in renal cell lines. *Toxicol In Vitro* 2007; **21**: 878–886.
73. Sorribas V, Halaihel N, Puttaparthi K *et al.* Gentamicin causes endocytosis of Na/Pi cotransporter protein (NaPi-2). *Kidney Int* 2001; **59**: 1024–1036.
74. Levi M, Cronin RE. Early selective effects of gentamicin on renal brush-border membrane Na-Pi cotransport and Na-H exchange. *Am J Physiol* 1990; **258**: F1379–F1387.
75. Skopicki HA, Zikos D, Sukowski EJ *et al.* Gentamicin inhibits carrier-mediated dipeptide transport in kidney. *Am J Physiol* 1996; **270**: F531–F538.
76. Todd JH, Sens DA, Hazen-Martin DJ *et al.* Aminoglycoside antibiotics alter the electrogenic transport properties of cultured human proximal tubule cells. *Toxicol Pathol* 1992; **20**: 608–616.
77. Fukuda Y, Malmborg AS, Aperia A. Gentamicin inhibition of Na+,K(+)-ATPase in rat kidney cells. *Acta Physiol Scand* 1991; **141**: 27–34.

78. Sassen MC, Kim SW, Kwon TH *et al.* Dysregulation of renal sodium transporters in gentamicin-treated rats. *Kidney Int* 2006; **70**: 1026–1037.
79. DiBona DR, Powell Jr WJ. Quantitative correlation between cell swelling and necrosis in myocardial ischemia in dogs. *Circ Res* 1980; **47**: 653–665.
80. Lieberthal W, Levine JS. Mechanisms of apoptosis and its potential role in renal tubular epithelial cell injury. *Am J Physiol* 1996; **271**: F477–F488.
81. Foster JE, Harpur ES, Garland HO. An investigation of the acute effect of gentamicin on the renal handling of electrolytes in the rat. *J Pharmacol Exp Ther* 1992; **261**: 38–43.
82. Moestrup SK, Cui S, Vorum H *et al.* Evidence that epithelial glycoprotein 330/megalin mediates uptake of polybasic drugs. *J Clin Invest* 1995; **96**: 1404–1413.
83. Cui S, Verroust PJ, Moestrup SK *et al.* Megalin/gp330 mediates uptake of albumin in renal proximal tubule. *Am J Physiol* 1996; **271**: F900–F907.
84. Nagai J, Katsube T, Murakami T *et al.* Effect of gentamicin on pharmacokinetics of lysozyme in rats: interaction between megalin substrates in the kidney. *J Pharm Pharmacol* 2002; **54**: 1491–1496.
85. Nagai J, Saito M, Adachi Y *et al.* Inhibition of gentamicin binding to rat renal brush-border membrane by megalin ligands and basic peptides. *J Control Release* 2006; **112**: 43–50.
86. Neugarten J, Aynedjian HS, Bank N. Role of tubular obstruction in acute renal failure due to gentamicin. *Kidney Int* 1983; **24**: 330–335.
87. Rivas-Cabañero L, García-Bastos JL, Arevalo M *et al.* Effect of gentamicin treatment on glutamine and lactate metabolism by the renal cortex of the rat. *Arch Int Physiol Biochim Biophys* 1993; **101**: 193–196.
88. Vallon V. Tubuloglomerular feedback and the control of glomerular filtration rate. *News Physiol Sci* 2003; **18**: 169–174.
89. Blantz RC, Deng A, Miracle CM *et al.* Regulation of kidney function and metabolism: a question of supply and demand. *Trans Am Clin Climatol Assoc* 2007; **118**: 23–43.
90. Komlosi P, Bell PD, Zhang ZR. Tubuloglomerular feedback mechanisms in nephron segments beyond the macula densa. *Curr Opin Nephrol Hypertens* 2009; **18**: 57–62.
91. Thomson SC, Vallon V, Blantz RC. Resetting protects efficiency of tubuloglomerular feedback. *Kidney Int Suppl* 1998; **67**: S65–S70.
92. Deng A, Wead LM, Blantz RC. Temporal adaptation of tubuloglomerular feedback: effects of COX-2. *Kidney Int* 2004; **66**: 2348–2353.
93. Martínez-Salgado C, López-Hernández FJ, López-Novoa JM. Glomerular nephrotoxicity of aminoglycosides. *Toxicol Appl Pharmacol* 2007; **223**: 86–98.
94. Schor N, Ichikawa I, Rennke HG *et al.* Pathophysiology of altered glomerular function in aminoglycoside-treated rats. *Kidney Int* 1981; **19**: 288–296.
95. Dos Santos OF, Boim MA, Barros EJ *et al.* Role of platelet activating factor in gentamicin and cisplatin nephrotoxicity. *Kidney Int* 1991; **40**: 742–747.
96. Martínez-Salgado C, Rodríguez-Barbero A, Eleno N *et al.* Gentamicin induces Jun-AP1 expression and JNK activation in renal glomeruli and cultured mesangial cells. *Life Sci* 2005; **77**: 2285–2298.
97. Stojiljkovic N, Mihailovic D, Veljkovic S *et al.* Glomerular basement membrane alterations induced by gentamicin administration in rats. *Exp Toxicol Pathol* 2008; **60**: 69–75.
98. De-Barros-e-Silva ML, Varanda WA, Lachat JJ *et al.* Glomerular permeability to macromolecules in gentamicin-treated rats. *Braz J Med Biol Res* 1992; **25**: 409–417.
99. Luft FC, Aronoff GR, Evan AP *et al.* The effect of aminoglycosides on glomerular endothelium: a comparative study. *Res Commun Chem Pathol Pharmacol* 1981; **34**: 89–95.
100. Cojocel C, Docius N, Maita K *et al.* Renal ultrastructural and biochemical injuries induced by aminoglycosides. *Environ Health Perspect* 1984; **57**: 293–299.
101. Maita K, Cojocel C, Dociu N *et al.* Effects of aminoglycosides on glomerular ultrastructure. *Pharmacology* 1984; **29**: 292–300.
102. Rodriguez-Barbero A, Rodriguez-Lopez AM, Gonzalez-Sarmiento R *et al.* Gentamicin activates rat mesangial cells. A role for platelet activating factor. *Kidney Int* 1995; **47**: 1346–1353.
103. Martínez-Salgado C, Rodríguez-Barbero A, Rodríguez-Puyol D *et al.* Involvement of phospholipase A2 in gentamicin-induced rat mesangial cell activation. *Am J Physiol* 1997; **273**: F60–F66.
104. Valdivielso JM, Rivas-Cabañero L, Morales AI *et al.* Increased renal glomerular endothelin-1 release in gentamicin-induced nephrotoxicity. *Int J Exp Pathol* 1999; **80**: 265–270.
105. Duque I, García-Escribano C, Rodríguez-Puyol M *et al.* Effects of reactive oxygen species on cultured rat mesangial cells and isolated rat glomeruli. *Am J Physiol* 1992; **263**: F466–F473.
106. Friedlander G, Pirotzky E, Amiel C *et al.* Renal effects of platelet-activating factor in the rat. *Agents Actions* 1987; **22**: 165–170.
107. Santos CX, Tanaka LY, Wosniak J *et al.* Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. *Antioxid Redox Signal* 2009; **11**: 2409–2427.
108. López-Novoa JM. Potential role of platelet activating factor in acute renal failure. *Kidney Int* 1999; **55**: 1672–1682.
109. Rodriguez-Barbero A, Bosque E, Rivas-Cabañero L *et al.* Effect of platelet activating factor antagonist treatment on gentamicin nephrotoxicity. *Mediators Inflamm* 1992; **1**: 23–26.
110. Rodriguez-Barbero A, López-Novoa JM, Arévalo M. Involvement of platelet-activating factor in gentamicin nephrotoxicity in rats. *Exp Nephrol* 1997; **5**: 47–54.
111. Martínez-Salgado C, Eleno N, Morales AI *et al.* Gentamicin treatment induces simultaneous mesangial proliferation and apoptosis in rats. *Kidney Int* 2004; **65**: 2161–2171.
112. Rivas-Cabañero L, Montero A, López-Novoa JM. Increased glomerular nitric oxide synthesis in gentamicin-induced renal failure. *Eur J Pharmacol* 1994; **270**: 119–121.
113. Rivas-Cabañero L, Rodríguez-López AM, Martínez-Salgado C *et al.* Gentamicin treatment increases mesangial cell nitric oxide production. *Exp Nephrol* 1997; **5**: 23–30.
114. Leung JC, Marphis T, Craver RD *et al.* Altered NMDA receptor expression in renal toxicity: protection with a receptor antagonist. *Kidney Int* 2004; **66**: 167–176.
115. Pedraza-Chaverrí J, Barrera D, Maldonado PD *et al.* S-allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen *in vitro* and attenuates gentamicin-induced oxidative and nitrosative stress and renal damage *in vivo*. *BMC Clin Pharmacol* 2004; **30**: 4–5.
116. Hishida A, Nakajima T, Yamada M *et al.* Roles of hemodynamic and tubular factors in gentamicin-mediated nephropathy. *Ren Fail* 1994; **16**: 109–116.
117. Morales AI, Buitrago JM, Santiago JM *et al.* Protective effect of trans-resveratrol on gentamicin-induced nephrotoxicity. *Antioxid Redox Signal* 2002; **4**: 893–898.
118. Klotman PE, Yarger WE. Reduction of renal blood flow and proximal bicarbonate reabsorption in rats by gentamicin. *Kidney Int* 1983; **24**: 638–643.
119. Persson PB. Physiological regulation of renal blood flow and glomerular filtration rate by the endothelium and smooth muscle. *Blood Purif* 1997; **15**: 219–227.
120. Papanikolaou N, Peros G, Morphake P *et al.* Does gentamicin induce acute renal failure by increasing renal TXA2 synthesis in rats? *Prostaglandins Leukot Essent Fatty Acids* 1992; **45**: 131–136.
121. Assael BM, Chiabrando C, Gagliardi L *et al.* Prostaglandins and aminoglycoside nephrotoxicity. *Toxicol Appl Pharmacol* 1985; **78**: 386–394.
122. Förstermann U. Nitric oxide and oxidative stress in vascular disease. *Pflugers Arch* 2010; **459**: 923–939.
123. Yorulmaz O *et al.* Protective effect of L-arginine intake on the impaired renal vascular responses in the gentamicin-treated rats. *Nephron Physiol* 2005; **100**: 13–20.
124. Gergawy M, Vollrath B, Cook D. The mechanism by which aminoglycoside antibiotics cause vasodilation of canine cerebral arteries. *Br J Pharmacol* 1998; **125**: 1150–1157.
125. Wickman G, Nessim MA, Cook DA *et al.* The polycationic aminoglycosides modulate the vasoconstrictive effects of endothelin: relevance to cerebral vasospasm. *Br J Pharmacol* 2001; **133**: 5–12.
126. De Nucci G, Gryglewski RJ, Warner TD *et al.* Receptor-mediated release of endothelium-derived relaxing factor and prostacyclin from bovine aortic endothelial cells is coupled. *Proc Natl Acad Sci USA* 1988; **85**: 2334–2338.
127. Hines J, Vinores SA, Campochiaro PA. Evolution of morphologic changes after intravitreous injection of gentamicin. *Curr Eye Res* 1993; **12**: 521–529.
128. Moran K, Mulhall J, Kelly D *et al.* Morphological changes and alterations in regional intrarenal blood flow induced by graded renal ischemia. *J Urol* 1992; **148**: 463–466.
129. Ali BH. Gentamicin nephrotoxicity in humans and animals: some recent research. *Gen Pharmacol* 1995; **26**: 1477–1487.
130. Marumo F *et al.* Increased renal susceptibility to gentamicin in rat with obstructive jaundice. Role of lipid peroxidation. *Dig Dis Sci* 1995; **40**: 1060–1064.
131. Abdel-Naim AB, Abdel-Wahab MH, Attia FF. Protective effects of vitamin E and probucol against gentamicin-induced nephrotoxicity in rats. *Pharmacol Res* 1999; **40**: 183–187.

132. Martínez-Salgado C, Eleno N, Tavares P *et al.* Involvement of reactive oxygen species on gentamicin-induced mesangial cell activation. *Kidney Int* 2002; **62**: 1682–1692.
133. Ali BH. Agents ameliorating or augmenting experimental gentamicin nephrotoxicity: some recent research. *Food Chem Toxicol* 2003; **41**: 1447–1452.
134. Koyner JL, Sher Ali R, Murray PT. Antioxidants. Do they have a place in the prevention or therapy of acute kidney injury? *Nephron Exp Nephrol* 2008; **109**: e109–e117.
135. Bledsoe G, Crickman S, Mao J *et al.* Kallikrein/kinin protects against gentamicin-induced nephrotoxicity by inhibition of inflammation and apoptosis. *Nephrol Dial Transplant* 2006; **21**: 624–633.
136. Kalayarasan S, Prabhu PN, Sriram N *et al.* Diallyl sulfide enhances antioxidants and inhibits inflammation through the activation of Nrf2 against gentamicin-induced nephrotoxicity in Wistar rats. *Eur J Pharmacol* 2009; **606**: 162–171.
137. Kourilsky O, Solez K, Morel-Maroger L *et al.* The pathology of acute renal failure due to interstitial nephritis in man with comments on the role of interstitial inflammation and sex in gentamicin nephrotoxicity. *Medicine (Baltimore)* 1982; **61**: 258–268.
138. Geleilete TJ, Melo GC, Costa RS *et al.* Role of myofibroblasts, macrophages, transforming growth factor-beta endothelin, angiotensin-II, and fibronectin in the progression of tubulointerstitial nephritis induced by gentamicin. *J Nephrol* 2002; **15**: 633–642.
139. Park JW, Bae EH, Kim IJ *et al.* Renoprotective effects of paricalcitol on gentamicin-induced kidney injury in rats. *Am J Physiol Renal Physiol* 2009; **298**: F301–F313.
140. Goto T, Fujigaki Y, Sun DF *et al.* Plasma protein extravasation and vascular endothelial growth factor expression with endothelial nitric oxide synthase induction in gentamicin-induced acute renal failure in rats. *Virchows Arch* 2004; **444**: 362–374.
141. Sue YM, Cheng CF, Chang CC *et al.* Antioxidation and anti-inflammation by haem oxygenase-1 contribute to protection by tetramethylpyrazine against gentamicin-induced apoptosis in murine renal tubular cells. *Nephrol Dial Transplant* 2009; **24**: 769–777.
142. Cachofeiro V, Goicochea M, de Vinuesa SG *et al.* Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. *Kidney Int Suppl* 2008: S4–S9.
143. Maldonado PD, Barrera D, Rivero I *et al.* Antioxidant S-allylcysteine prevents gentamicin-induced oxidative stress and renal damage. *Free Radic Biol Med* 2003; **35**: 317–324.
144. Kadkhodaee M, Khastar H, Faghihi M *et al.* Effects of co-supplementation of vitamins E and C on gentamicin-induced nephrotoxicity in rat. *Exp Physiol* 2005; **90**: 571–576.
145. Servais H, Ortiz A, Devuyst O *et al.* Renal cell apoptosis induced by nephrotoxic drugs: cellular and molecular mechanisms and potential approaches to modulation. *Apoptosis* 2008; **13**: 11–32.
146. Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-κB transcription factor and HIV-1. *EMBO J* 1991; **10**: 2247–2258.
147. Meyer M, Schreck R, Baeuerle PA. H_2O_2 and antioxidants have opposite effects on activation of NF-κB and AP-1 in intact cells: AP-1 as secondary antioxidant responsive factor. *EMBO J* 1993; **12**: 2005–2015.
148. Tugcu V, Ozbek E, Tasci AI *et al.* Selective nuclear factor kappa-B inhibitors, pyrolidium dithiocarbamate and sulfasalazine, prevent the nephrotoxicity induced by gentamicin. *BJU Int* 2006; **98**: 680–686.
149. Markewitz BA, Michael JR, Kohan DE. Cytokine-induced expression of a nitric oxide synthase in rat renal tubule cells. *J Clin Invest* 1993; **91**: 2138–2143.
150. Xie QW, Kashiwabara Y, Nathan C. Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase. *J Biol Chem* 1994; **269**: 4705–4708.
151. Juan SH, Chen CH, Hsu YH *et al.* Tetramethylpyrazine protects rat renal tubular cell apoptosis induced by gentamicin. *Nephrol Dial Transplant* 2007; **22**: 732–739.
152. Colten HR. Tissue-specific regulation of inflammation. *J Appl Physiol* 1992; **72**: 1–7.
153. Karkar A. Modulation of renal inflammation: therapeutic strategies. *Saudi J Kidney Dis Transpl* 2008; **19**: 1–19.
154. García-Sánchez O, López-Hernández FJ, López-Novoa JM. An integrative view on the role of TGF-β in the progressive tubular deletion associated to chronic kidney disease. *Kidney Int* 2010; **77**: 950–955.
155. Taber SS, Mueller BA. Drug-associated renal dysfunction. *Crit Care Clin* 2006; **22**: 357–374.
156. Brivet FG, Kleinknecht DJ, Loirat P *et al.* Acute renal failure in intensive care units–causes, outcome, and prognostic factors of hospital mortality; a prospective, multicenter study. French Study Group on Acute Renal Failure. *Crit Care Med* 1996; **24**: 192–198.
157. Watanabe A, Nagai J, Adachi Y *et al.* Targeted prevention of renal accumulation and toxicity of gentamicin by aminoglycoside binding receptor antagonists. *J Control Release* 2004; **95**: 423–433.
158. Fujii K, Nagai J, Sawada T *et al.* Effect of PEGylation of N-WASP181-200 on the inhibitory potency for renal aminoglycoside accumulation. *Bioconjugate Chem* 2009; **20**: 1553–1558.
159. Antoine DJ, Srivastava A, Pirmohamed M *et al.* Statins inhibit aminoglycoside accumulation and cytotoxicity to renal proximal tubule cells. *Biochem Pharmacol* 2010; **79**: 647–654.
160. Hosaka K, Takeda T, Iino N *et al.* Megalin and nonmuscle myosin heavy chain IIA interact with the adaptor protein Disabled-2 in proximal tubule cells. *Kidney Int* 2009; **75**: 1308–1315.
161. Gotoh N, Yan Q, Du Z *et al.* Altered renal proximal tubular endocytosis and histology in mice lacking myosin-VI. *Cytoskeleton* 2010; **67**: 178–192.
162. Vlasic-Matas J, Rumboldt Z, Karelovic D. Renoprotective role of nifedipine during gentamicin therapy: randomized controlled trial. *Croat Med J* 2000; **41**: 417–422.
163. Kazierad DJ, Wojcik GJ, Nix DE *et al.* The effect of verapamil on the nephrotoxic potential of gentamicin as measured by urinary enzyme excretion in healthy volunteers. *J Clin Pharmacol* 1995; **35**: 196–201.
164. Zhang B, Ramesh G, Uematsu S *et al.* TLR4 signaling mediates inflammation and tissue injury in nephrotoxicity. *J Am Soc Nephrol* 2008; **19**: 923–932.
165. Pulskens WP, Teske GJ, Butter LM *et al.* Toll-like receptor-4 coordinates the innate immune response of the kidney to renal ischemia/reperfusion injury. *PLoS One* 2008; **3**: e3596.
166. Asehnoune K, Strassheim D, Mitra S *et al.* Involvement of reactive oxygen species in Toll-like receptor 4-dependent activation of NF-kappa B. *J Immunol* 2004; **172**: 2522–2529.
167. Kohanski MA, Dwyer DJ, Wierzbowski J *et al.* Mistranslation of membrane proteins and two-component system activation trigger aminoglycoside-mediated oxidative stress and cell death. *Cell* 2008; **135**: 679–690.

ANEXO ii

AN INTEGRATIVE OVERVIEW ON THE MECHANISM UNDERLYING THE RENAL TUBULAR CYTOTOXICITY OF GENTAMICIN.

Yaremi Quiros, Laura Vicente-Vicente, Ana Isabel Morales, José Miguel López-Novoa and Francisco J. López-Hernández.

Revista: Toxicological Sciences; 2010

doi: 10.1093/toxsci/kfq267

TOXICOLOGICAL SCIENCES **119(2)**, 245–256 (2011)
doi:10.1093/toxsci/kfq267
Advance Access publication September 9, 2010

An Integrative Overview on the Mechanisms Underlying the Renal Tubular Cytotoxicity of Gentamicin

Yaremi Quiros,*,†,‡ Laura Vicente-Vicente,*,† Ana I. Morales,*,† José M. López-Novoa,*,† and Francisco J. López-Hernández*,†,§,[1]

*Unidad de Fisiopatología Renal y Cardiovascular, Departamento de Fisiología y Farmacología, Universidad de Salamanca, 37007 Salamanca, Spain; †Instituto Reina Sofía de Investigación Nefrológica, Fundación Íñigo Álvarez de Toledo, 28003 Madrid, Spain; ‡Bio-inRen, S.L., 37007 Salamanca, Spain; and §Unidad de Investigación, Hospital Universitario de Salamanca, 37007 Salamanca, Spain

[1]To whom correspondence should be addressed at Unidad de Investigación, Hospital Universitario de Salamanca, Paseo de San Vicente, 58-182, 37007 Salamanca, Spain. Fax: +34-923-294-669. E-mail: flopezher@usal.es.

Received June 9, 2010; accepted August 25, 2010

Gentamicin is an aminoglycoside antibiotic widely used against infections by Gram-negative microorganisms. Nephrotoxicity is the main limitation to its therapeutic efficacy. Gentamicin nephrotoxicity occurs in 10–20% of therapeutic regimes. A central aspect of gentamicin nephrotoxicity is its tubular effect, which may range from a mere loss of the brush border in epithelial cells to an overt tubular necrosis. Tubular cytotoxicity is the consequence of many interconnected actions, triggered by drug accumulation in epithelial tubular cells. Accumulation results from the presence of the endocytic receptor complex formed by megalin and cubulin, which transports proteins and organic cations inside the cells. Gentamicin then accesses and accumulates in the endosomal compartment, the Golgi and endoplasmic reticulum (ER), causes ER stress, and unleashes the unfolded protein response. An excessive concentration of the drug over an undetermined threshold destabilizes intracellular membranes and the drug redistributes through the cytosol. It then acts on mitochondria to unleash the intrinsic pathway of apoptosis. In addition, lysosomal cathepsins lose confinement and, depending on their new cytosolic concentration, they contribute to the activation of apoptosis or produce a massive proteolysis. However, other effects of gentamicin have also been linked to cell death, such as phospholipidosis, oxidative stress, extracellular calcium–sensing receptor stimulation, and energetic catastrophe. Besides, indirect effects of gentamicin, such as reduced renal blood flow and inflammation, may also contribute or amplify its cytotoxicity. The purpose of this review was to critically integrate all these effects and discuss their relative contribution to tubular cell death.

Key Words: **gentamicin; aminoglycoside antibiotics; cytotoxicity; apoptosis; necrosis.**

Nephrotoxicity is one of the main side effects of the aminoglycoside antibiotics, especially of gentamicin, and also one of its main therapeutic limitations. Gentamicin accumulates in the renal cortex (see next section) and induces renal morphological changes and an overall syndrome very similar in humans and experimental animals (Luft *et al*., 1977). However, the precise characterization of the pathophysiological and molecular mechanisms underlying gentamicin's nephrotoxicity at the organism, tissue, cell, and molecular levels has been mostly obtained in animal and cellular experimental models. Gentamicin nephrotoxicity is typically characterized by tubular damage arising from tubular epithelial cell cytotoxicity. Treatment of experimental animals with gentamicin produces apoptosis (Li *et al*., 2009a) as well as necrosis (Edwards *et al*., 2007) of tubular epithelial cells *in vivo* and also in cultured cells (Pessoa *et al*., 2009). For other toxins, such as chemotherapeutic agents (Edinger and Thompson, 2004) and H_2O_2 (Saito *et al*., 2006), a relationship also exists between toxin concentration and death phenotype. Low concentrations cause apoptosis, whereas high ones cause necrosis. The death phenotype strongly depends on the cell energy status and adenosine triphosphate (ATP) reserve. Apoptosis requires ATP, at least for the initial steps. At such, other circumstances different from drug concentration may modulate the death mode. For example, a severely diminished renal blood flow (RBF) may lower oxygen availability in some areas of the kidneys and limit respiration and ATP pool. In these circumstances, cell death may lose the typical characteristics of apoptosis and acquires those of necrosis (Chiarugi, 2005). Still, the most commonly observed phenotype *in vitro* is apoptosis, an observation that is in agreement with the fact that high concentrations of the drug (>1–2 mg/ml) are necessary to induce a modest cytotoxic effect in cultured cells (Pessoa *et al*., 2009; Servais *et al*., 2006).

ACCUMULATION OF GENTAMICIN IN TUBULAR CELLS

In the kidneys, aminoglycosides distinctively accumulate in epithelial cells of the proximal tubule (PTECs). This has been verified both in humans and in experimental animals (Luft *et al.*, 1977). However, the mechanism of accumulation has been mostly studied in animals. This specific accumulation is because of the existence in these cells of a membrane endocytic complex involving the proteins megalin and cubilin (Cui *et al.*, 1996; Moestrup *et al.*, 1995; Nagai *et al.*, 2002, 2006), which has also been described as an endocytic receptor in human proximal tubules (Lee *et al.*, 2009). This complex transports cations present in the ultrafiltrate, such as a vast variety of proteins and certain xenobiotics, as for example aminoglycoside antibiotics (Schmitz *et al.*, 2002; Fig. 1). Accumulation of aminoglycosides inside the PTECs alters the function of several organelles and processes that are crucial for cell viability. Moreover, gentamicin activates the extracellular calcium–sensing receptor (CaSR), a membrane receptor sensitive to the amount of extracellular calcium, which has also been associated with tubular cell death.

It has been demonstrated in animal models and cultured cells that, quantitatively, most gentamicin enters tubular cells via endocytosis mediated by the megalin/cubilin complex. This process requires the electrostatic binding of gentamicin to the negative charges of membrane phospholipids (Frommer *et al.*, 1983; Lipsky *et al.*, 1980). Gentamicin then passes via pinocytosis to the endosomal compartment. The drug mostly accumulates in the lysosomes, travels retrograde through the secretory pathway to the Golgi and endoplasmic reticulum (ER; Sandoval and Molitoris, 2004; Silverblatt, 1982; Silverblatt and Kuehn, 1979) and alters vesicular traffic (Giurgea-Marion *et al.*, 1986; Jones and Wessling-Resnick, 1998). In the lysosomes, gentamicin produces membrane destabilization, lysosomal aggregation (De Broe *et al.*, 1984), alteration of lipid metabolism, and phospholipidosis, which have been associated with cell death (see below). It also generates multilamelar structures known as myeloid bodies (Edwards *et al.*, 1976; Houghton *et al.*, 1978; Silverblatt, 1982), whose pathophysiological role is uncertain.

ER STRESS AND UNFOLDED PROTEIN RESPONSE

Accumulation of gentamicin in the ER may originate ER stress (Fig. 2). ER stress activates the unfolded protein response (UPR) and cell cycle arrest (Zhang *et al.*, 2006). Under circumstances of UPR overload, the cell undergoes apoptosis (Fribley *et al.*, 2009), which is mediated by the classical route of calpains and caspase 12 (maybe caspase 4 in humans) activated by the release of Ca from the ER; UPR-activated apoptosis also involves Jun kinase and C/EBP homologous protein transcription factor (Kim *et al.*, 2008; Lai *et al.*, 2007; Peyrou and Cribb, 2007; Peyrou *et al.*, 2007). In this line, a calpain inhibitor reduces the cytotoxicity of gentamicin in cultured auditory hair cells (Shimizu *et al.*, 2003). Once activated, these enzymes promote the proteolytic activation of executor caspases and unleash the mitochondrial pathway of apoptosis (Kerbiriou *et al.*, 2009; Peyrou *et al.*, 2007). In fact, gentamicin joins calreticulin and inhibits its necessary chaperon activity for a correct posttranslational protein folding (Horibe *et al.*, 2004). It is well known that the bactericidal effect of gentamicin is related to its capacity to bind the small subunit of the ribosome and skew protein translation (Recht *et al.*, 1999). However, it is not yet well characterized whether gentamicin exerts similar effects in mammalian cells, which could be the cause or participate in cell death. Recht *et al.* (1999) reported that the minimum inhibitory concentration of gentamicin for the eukaryotic 16S

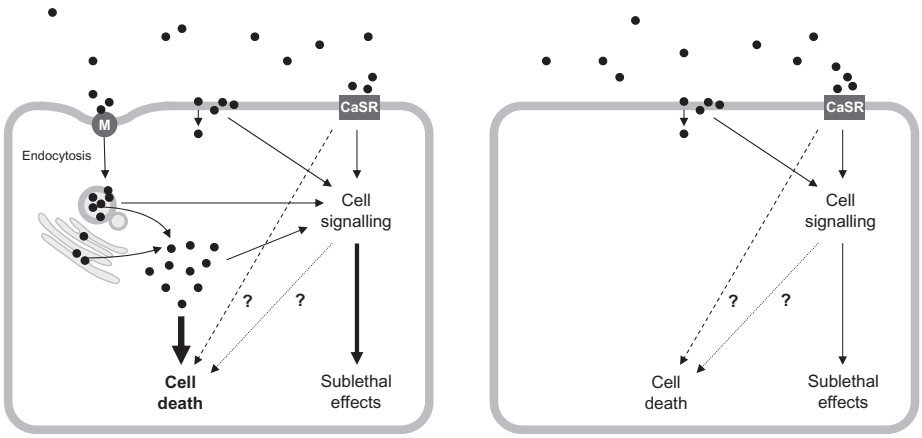


FIG. 1. Mechanisms of uptake and subcellular redistribution of gentamicin in tubular and other renal cells. M, Megalin.

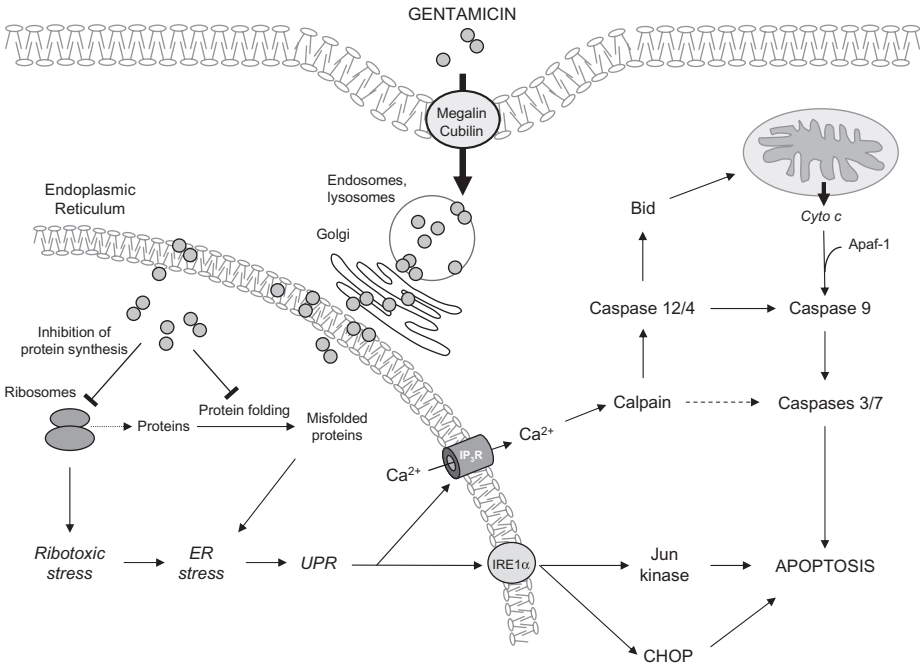


FIG. 2. Schematic representation of the ER stress and UPR caused by gentamicin. Cyto c, Cytochrome c.

ribosomal ribonucleic acid (rRNA) was 0.23mM, 128 times higher than that for the prokaryotic rRNA. Despite this, different reports have suggested that aminoglycosides alter ribosomal accuracy (Buchanan *et al*., 1987) and inhibit protein synthesis (Bennett *et al*., 1988; Monteil *et al*., 1993; Sundin *et al*., 2001). Protein synthesis is reduced by 50% before gross cellular morphological alterations appear (Sundin *et al*., 2001). The implications of these effects need to be further clarified.

CYTOSOLIC REDISTRIBUTION AND MITOCHONDRIAL TARGETING

Recent studies with cultured cells have shown that a critical aspect of gentamicin's tubular cytotoxicity is its cytosolic concentration and not, as previously thought, its accumulation in lysosomes (Servais *et al*., 2006, 2008; Fig. 3). In comparison, a small amount of gentamicin directly enters the cytosol and nucleus independently from the endocytosis mediated by the megalin/cubilin complex (Myrdal *et al*., 2005). Very recently, it has been demonstrated that gentamicin also enters cultured tubule cells through an unspecific cation channel, namely the transient receptor potential vanilloid type 4 (Karasawa *et al*., 2008) channel. However, this channel is expressed in epithelial cells of the distal tubule but not in the proximal tubule (Karasawa *et al*., 2008). Besides, the relative contribution of this entry mechanism is probably small.

The most important effect occurs when the concentration of gentamicin inside the lysosomes, the Golgi, and ER exceeds a threshold and destabilizes their membrane (Ngaha and Ogunleye, 1983; Regec *et al*., 1989; Fig. 3). The accumulated gentamicin is released into the cytosol from where it acts on mitochondria and activates the mitochondrial pathway of apoptosis, produces oxidative stress, and reduces the ATP reserve (Morales *et al*., 2010; Simmons *et al*., 1980). On the other hand, the rupture of lysosomes causes the release of proteases into the cytosol, such as L, B, D, and other cathepsins, which intervene in the induction of cell death (Schnellmann and Williams, 1998). Cathepsins catalyze the proteolytic activation of executor caspases 3 and 7 and activate the mitochondrial pathway of apoptosis through the activation of Bid (Chwieralski *et al*., 2006; Yin, 2006). In the absence of ATP, cathepsins in the cytosol produce a massive proteolysis that leads to necrotic cell death (Golstein and Kroemer, 2007).

In cell cultures, cytosolic gentamicin acts on mitochondria and triggers the translocation of cytochrome c and other proapoptotic proteins, such as apoptosis-inducing factor (AIF). In the cytosol, cytochrome c activates caspase 9 and, finally, the executor caspases 3 and 7, which result in cellular death by apoptosis (Servais *et al*., 2008). The effect of gentamicin on mitochondria is produced in a direct and also in an indirect fashion. The mechanism of the direct action is unknown. However, it has been demonstrated that incubation of isolated mitochondria with gentamicin induces the release of proapoptotic proteins from the intermembrane space (Mather and Rottenberg, 2001), a requisite for the activation of the intrinsic

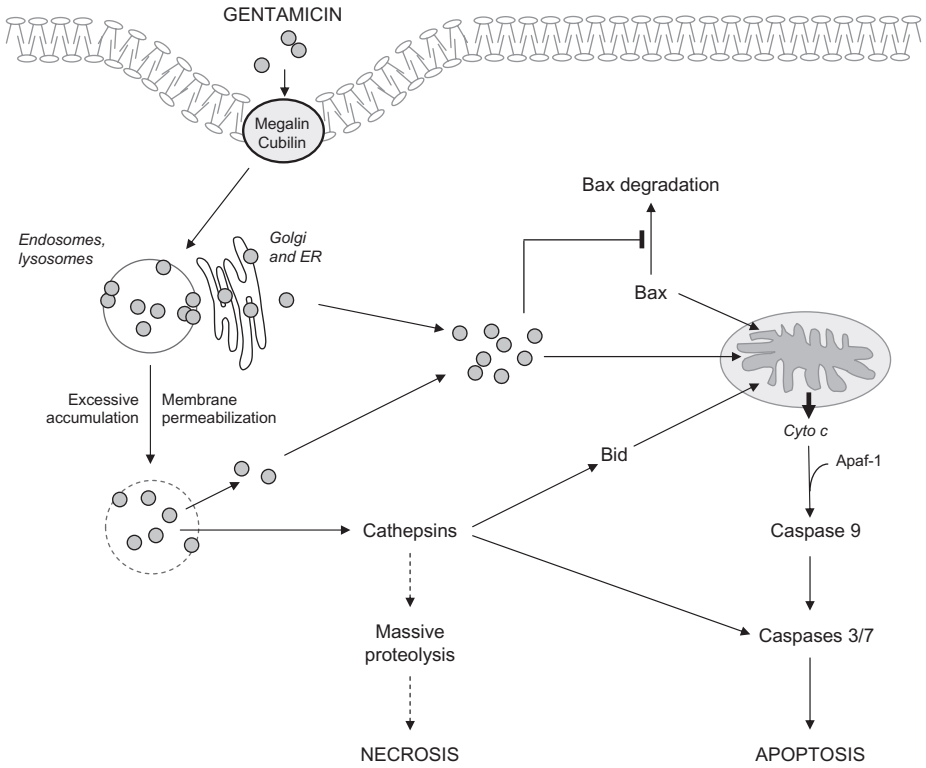


FIG. 3. Cytosolic redistribution of gentamicin and mechanisms leading to cell death through necrosis and the apoptotic intrinsic pathway.

pathway of apoptosis. The indirect action is mediated by Bax, and it is inhibited by overexpression of Bcl-2. In this sense, gentamicin binds the proteasome (Horibe *et al*., 2004), which might affect the degradation of Bax and increase its cellular levels (Servais *et al*., 2006).

CELL ENERGY STATUS IMPAIRMENT

Studies carried out in rats and mice demonstrate that peroxisome proliferator–activated receptor alpha (PPAR-α) activation (1) maintains ATP production by sustaining fatty acid oxidation; (2) prevents the increase in reactive oxygen species (ROS) and oxidative stress; and (3) reduces apoptosis of tubule cells, both *in vitro* and *in vivo*, during the acute kidney injury induced by ischemia and a variety of drugs, including cisplatin (Li *et al*., 2004, 2009b), doxorubicin (Lin *et al*., 2007), and gentamicin (Hsu *et al*., 2008). Indeed, these drugs reduce the level of PPAR-α in tubular cells through ubiquitination-dependent degradation, which has been shown to be crucial for their tubular toxicity (Lopez-Hernandez and Lopez-Novoa, 2009). The inhibition of cell membrane transporters might also contribute to an undetermined extent to the cytotoxicity of gentamicin. Indeed, both glucose intake inhibition and reduced Na+ efflux can theoretically lead to decreased cellular ATP levels and cell swelling. Glucose transport in proximal tubule cells depends on the sodium gradient generated by adenosin triphosphatases (ATPases). Deficient sodium extrusion caused by gentamicin may (1) indirectly reduce intracellular glucose availability and contribute to ATP pool reduction and (2) lead to sodium and, consequently, water accumulation, cell swelling, and necrotic death. Na–K ATPase is a key component of cell volume homeostasis, and deregulated swelling may lead to necrosis (DiBona and Powell, 1980; Lieberthal and Levine, 1996). In experiments carried out with cultured cells or membrane vesicles from tubular cells, it has been shown that gentamicin inhibits a variety of cell membrane transporters (reviewed in Mingeot-Leclercq and Tulkens, 1999) of both the brush border and the basolateral membrane, such as the Na–Pi cotransporter and Na–H exchanger (Levi and Cronin, 1990), brush-border dipeptide transporters (Skopicki *et al*., 1996), electrogenic Na transport (Todd *et al*., 1992), and the Na–K ATPase (Fukuda *et al*., 1991; Lipsky *et al*., 1980). Figure 4 schematically represents the cellular events activated by gentamicin that lead to ATP exhaustion.

MEMBRANE DESTABILIZATION AND PHOSPHOLIPIDOSIS

Another mechanism potentially involved in its cytotoxicity is the accumulation of gentamicin in cell membranes. Because of its polycationic properties, gentamicin binds to phospholipids.

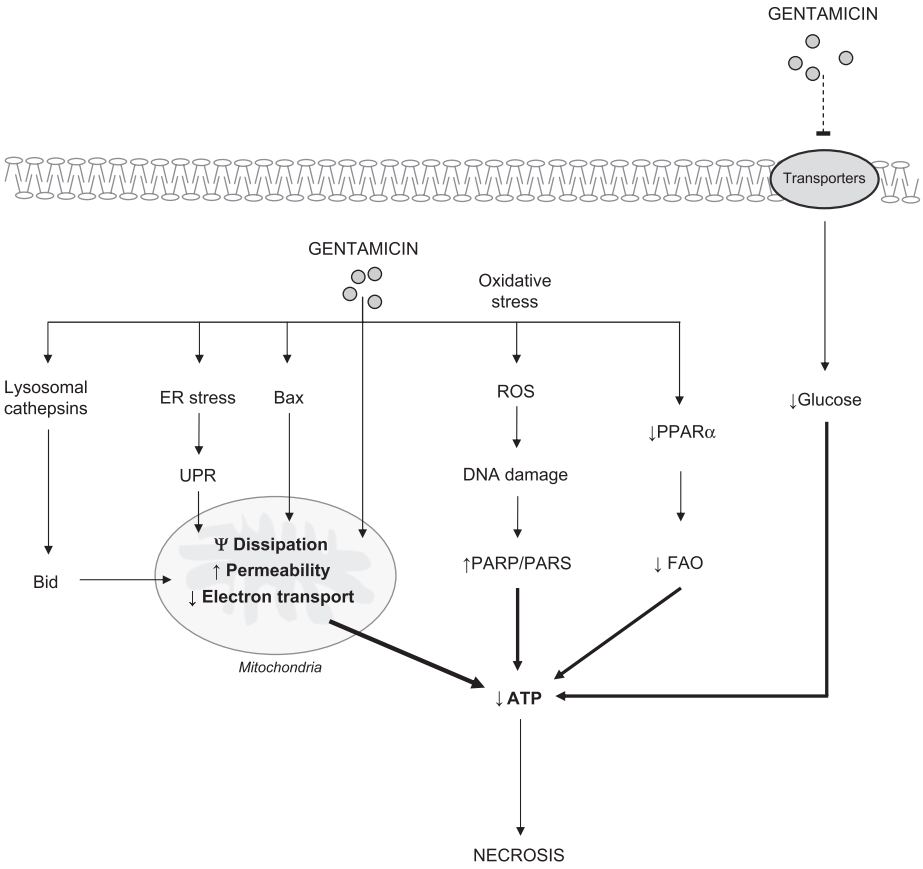


FIG. 4. Mechanisms contributing to the energetic catastrophe caused by gentamicin. FAO, fatty acid oxidation; PARP, poly (ADP-ribose) polymerase; PARS, poly (ADP-ribose) synthase. Ψ, mitochondrial transmembrane potential.

This has been shown to cause cell membrane structure alterations (Forge *et al*., 1989) and a condition known as phospholipidosis, which has been observed in humans (De Broe *et al*., 1984) and experimental animals treated with the drug (Giuliano *et al*., 1984; Nonclercq *et al*., 1992). Phospholipidosis is derived from (1) the disruption of phosphatidylinositol signalling pathways (Ramsammy *et al*., 1988), (2) the reduction of phospholipid turnover (Ramsammy *et al*., 1989a) and phospholipid accumulation in cell membranes (Kacew, 1987; Laurent *et al*., 1982), (3) the reduction in the available negative charge necessary for the correct function of phospholipases (Mingeot-Leclercq *et al*., 1995), and (4) the inhibition of calcium-dependent phosphodiesterases by competing with and displacing calcium from the enzyme (van Rooijen and Agranoff, 1985). Binding to plasmalemmal phospholipids and plasma membrane accumulation occurs in other cell types exposed to the drug, in which intracellular accumulation and cell death are comparatively much less significant or absent. This indicates that these effects initiated in the cell membrane might not contribute largely to tubule cell death.

However, because aminoglycosides accumulate in lysosomes, lysosomal phospholipidosis has been more closely linked to cell death. In fact, lysosomal phospholipidosis correlates tightly with the level of toxicity of aminoglycosides (Kaloyanides, 1992; Nonclercq *et al*., 1992; Tulkens, 1989). Precisely, lysosomal phospholipidosis has been proposed to be the result of (1) the reduction in the available negative charge, which is necessary for the proper function of lysosomal phospholipases (Mingeot-Leclercq *et al*., 1995) and (2) the direct inhibition of A1, A2, and C1 phospholipases (Abdel-Gayoum *et al*., 1993; Laurent *et al*., 1982; Ramsammy *et al*., 1989a). Support for a role of phospholipidosis in cell death comes from experiments in which rats were treated with polyaspartic acid (PAA), which has been shown to mitigate (Ramsammy *et al*., 1989b) or to completely prevent the nephrotoxicity of gentamicin (Swan *et al*., 1991). The effect of PAA has been ascribed to its capacity to bind gentamicin and thus to prevent its union to phospholipids (Ramsammy *et al*., 1990). However, binding to phospholipids is also a requirement for gentamicin endocytosis (as described in section "Accumulation of Gentamicin in Tubular Cells"), which blurs conclusions. As such, it is not known to what extent (if to any) lysosomal or endosomal phospholipidosis contribute to cell death or to other sublethal alterations.

A glimpse of light on this issue was provided by the study of Kishore *et al.* (1990). These authors used three different polyanionic peptides, namely poly-L-Asp with poly-L-Glu and

poly-D-Glu to inhibit the nephrotoxicity and lysosomal phospholipidosis caused by gentamicin in rats. These peptides showed similar capacity to bind gentamicin, and thus to displace it from phospholipids in wide range of pH, including acidic pH. However, they showed a significantly different degree of hydrolysis in the presence of lysosomal extracts. Interestingly, their capacity to prevent gentamicin-induced phospholipidosis and gentamicin's nephrotoxicity was inversely proportional to their hydrolysis rate, supporting the hypothesis that their site of action was inside the lysosomes and not at the level of other renal membranes. Clearly, further research is necessary to shed light on this matter.

CaSR STIMULATION

CaSR, a member of the family C of cell membrane G-protein–coupled receptors (Trivedi *et al*., 2008), has also been implicated in gentamicin-induced tubule cell death (Fig. 1). *In vitro* experiments using HEK-293 cells have shown that gentamicin induces the death of cells expressing CaSR but not of those lacking it (Ward *et al*., 2005). Moreover, pharmacological antagonism of CaSR prevents the cell death induced by gentamicin in CaSR-expressing cells (Gibbons *et al*., 2008). However, a number of issues invites to caution when interpreting these results. First, there has been some controversy about the origin and phenotype of HEK-293 cells. Second, the extent of cell death induced by gentamicin in CaSR-expressing cells is low. Finally, *in vivo* evidence is missing because there are no useful tools to manipulate the CaSR. Moreover, an important pathophysiological role of gentamicin-induced CaSR–mediated tubule cell death odds with the evidence showing that the critical event is its cytosolic concentration, as explained above. In addition, the CaSR has been found in many other cell types outside the kidneys, where gentamicin has no evident cytotoxicity, including bone, brain, colon, parathyroid gland, smooth muscle, endothelial cells, etc. Clearly, more information is necessary to clarify the exact role of CaSR in tubule cell death.

OXIDATIVE STRESS

Treatment with gentamicin produces oxidative stress in tubular cells, both *in vivo* (in rats; Karataş *et al*., 2004) and in cultured tubular cells (Juan *et al*., 2007). This oxidative stress is mediated by hydroxyl radicals from hydrogen peroxide and by superoxide anions (Basnakian *et al*., 2002; Nakajima *et al*., 1994) from mitochondrial origin (Yang *et al*., 1995). Gentamicin directly increases the production of mitochondrial ROS from the respiratory chain (Morales *et al*., 2010). Reduced glutathione (GSH; Ali *et al*., 1992; Sandhya and Varalakshmi, 1997) and superoxide dismutase (SOD; Nakajima *et al*., 1994; Kadkhodaee *et al*., 2007) levels have been found to be low in the kidneys upon treatment with gentamicin.

Oxidative stress plays an important role in the nephrotoxicity of gentamicin (Koyner *et al*., 2008). Cotreatment of rats with a variety of antioxidants significantly reduces renal dysfunction and tissue damage (Ali, 2003; Cuzzocrea *et al*., 2002; Koyner *et al*., 2008; Martínez-Salgado *et al*., 2002; Morales *et al*., 2002). However, a note of caution was introduced by the study of Stratta *et al*. (1994), who demonstrated that GSH administration had no effect on the nephrotoxicity of gentamicin, despite reducing lipid peroxidation and increasing renal GSH content. Interestingly, this absence of effect was observed with a dosage of gentamicin that apparently resulted in an excess of the drug. With a lower dosage, GSH implementation softened renal damage, which curiously correlated with a lower accumulation of gentamicin in the kidneys. It is thus possible that (1) the critical level of highly cytotoxic oxidative stress induced by gentamicin depends on the dose or accumulation of gentamicin, and consequently, that the weight of oxidative stress in the nephrotoxicty of gentamicin depends on the dose of the drug and (2)the mechanism of damage is, at least partially, derived from the prevention of its renal accumulation. These aspects need further investigation.

ROS, mainly superoxide anions and hydroxyl radicals, cause cellular damage and death through diverse mechanisms, including the following (Cuzzocrea *et al*., 2004; Morgan *et al*., 2007; Ott *et al*., 2007; Ryter *et al*., 2007): (1) inhibition of the electron transport chain and suppression of cellular respiration and ATP production; (2) stimulation of the release of cytochrome c, AIF, etc. from the mitochondrial intermembrane space; (3) DNA damage, which triggers an increase in poly ADP ribose synthase activity, a decrease in the cell's ATP reserve, and cell cycle arrest; (4) lipid peroxidation, destabilization of the cellular membrane, activation of death receptors (Fas, etc.) by alteration of lipid rafts, and generation of proapoptotic lipid metabolites, such as 4-hydroxynonenal and ceramide; (5) stress on different organelles and cellular structures, such as the ER (Yokouchi *et al*., 2008; Santos *et al*., 2009) and (6) inhibition of transmembrane sodium flow, by oxidative inhibition of the Na+/K+ ATPase pump and of sodium channels, which originates cellular swelling, loss of membrane integrity, and necrosis.

However, there is little information on the ability of antioxidants to modulate the direct cytotoxic effect of gentamicin on cultured tubule cells. To our knowledge, only Juan *et al*. (2007) have reported a protective effect in this sense. In their article, tetramethylpyrazine (TTP) reduces ROS accumulation and apoptotic events in rat renal NRK-52E cells. However, the effect of TTP on cell viability is not reported. Because there are many apoptotic and necrotic pathways leading to cell death as a consequence of gentamicin action, and because their redundancy and hierarchical organization are not well understood, the magnitude of the direct cytoprotection afforded by ROS inhibition is unknown. The question that remains to be solved is, is the increment in ROS production the consequence of the mitochondrial injury directly and indirectly exerted by gentamicin?

Or are ROS increased by gentamicin previously to or independently from its mitochondrial proapoptotic effects, which in turn the trigger apoptosis? Speculatively, oxidative stress can be viewed at least as an amplification factor.

INTEGRATIVE OVERVIEW OF TUBULAR CELL DEATH

From the information presented above, it can be concluded that gentamicin needs to accumulate inside the cells to a significant level in order to induce cell death. CaSR stimulation (from the outside) has also been shown to induce some degree of cell death in tubule cells and might participate in mesangial and tubular cell death (Martínez-Salgado *et al*., 2007). However, this is proportionally small compared with the cytotoxicity caused by intracellular accumulation, and might show cell type dependency, because many other CaSR-expressing cells do not die when exposed to gentamicin.

Inside the cells, a critical factor appears to be its cytosolic concentration rather than its accumulation in endosomal structures. Cytosolic gentamicin directly and indirectly attacks mitochondria, inhibits respiration and ATP production, and produces oxidative stress (Morales *et al*., 2010), all of which activate the intrinsic pathway of apoptosis. These data indicate that cytosolic gentamicin has the ability to trigger apoptosis. However, they do not discard contributions from other damaged structures or signalling pathways. In fact, the cytosolic redistribution of gentamicin probably coincides with the leakage from the ER, permeabilization of lysosomes, and the release of lysosomal proteases (i.e., cathepsins) into the cytosol, which may add a redundant mediation toward cell death. Gentamicin also induces stress of other cellular structures, such as the ER, including protein synthesis inhibition, which, depending on the intensity, can affect cell viability. Unresolved and persistent stress also unleashes apoptosis from the damaged structures. Because the route to cytosolic accumulation goes through accumulation in intracellular membrane structures, including ER, it is difficult to imagine how gentamicin can accumulate in the cytosol without inducing some degree of ER stress. As such, we propose that besides mitochondrial damage, gentamicin also activates other pathways of cell death resulting from stress to other structures and organelles, which add an unknown level of redundancy. Probably, the predominance of some over the others, as well as the phenotype of cell death (highly dependent on energy status), might be a matter of concentration of the drug to which the cell is exposed. It can be hypothesized that low concentrations of the drug would traffic through the endocytic pathway and leak through the ER into the cytosol to a sufficient amount to active mitochondrial apoptosis, without inducing a significant injury to the ER and without causing lysosomal breakage or energetic catastrophe. High concentrations would cause further leakage through the ER, significant ER stress and protein synthesis inhibition, lysosomal rupture, and redundant apoptotic stimulation. In extreme cases of drug accumulation, massive and rapid cathepsin-driven proteolysis and ATP exhaustion may abort the execution of apoptosis and cause necrotic-like cell death. Also, as a result of accumulation in endosomal vesicles and lysosomes, phospholipidosis may also contribute to an undetermined extent to tubular cell death. A challenge for the coming future is to elucidate the relative contribution of all these mechanisms of cytotoxicity to the different cell death phenotypes, under a range of drug concentrations. This will unravel the key targets for the pharmacological prevention of the tubular cytotoxicity of aminoglycosides, which cannot be achieved with the present level of knowledge.

INDIRECT DETERMINANTS OF CYTOTOXICITY

In general, cultured tubular cells exhibit a significant resistance to cell death by exposure to gentamicin. Only very high concentrations of the drug (>1–3mM), over long periods of time (>1–4 days), cause a mild degree of cell death (<20%), only in determined cell lines (El Mouedden *et al*., 2000; Pessoa *et al*., 2009; Servais *et al*., 2006; Wu *et al*., 2009). As such, other factors independent from a direct cytotoxic action of gentamicin might exist, which would amplify deadly stimulation, and which are present *in vivo* and absent in cultured cells. One hypothesis (Fig. 5) is that inflammation and ischemia may be two of those amplification factors. Alternatively, it might be speculated that tubule cells in culture lose their capacity to efficiently accumulate gentamicin (Servais *et al*., 2006). This topic is of special interest because therapeutic targets to prevent gentamicin-induced tubular cell death should be sought out of target cells if additional factors are essential for an extensive tubular necrosis.

Gentamicin causes a reduction in RBF in experimental animals (Hishida *et al*., 1994; Morales *et al*., 2002), which has been associated to tubular damage (Moran *et al*., 1992). Although the mechanisms linking reduced RBF to tubular cell death are not well understood, it is hypothesized that limitation in O_2 and glucose supply lead to a diminished ATP production, all of which causes or sensitizes to cell death (Jeong *et al*., 2003; Sato *et al*., 2010; Seppet *et al*., 2009). In fact, hypoxia activates inducible nitric oxide synthase (iNOS) expression, which leads to cell death by inducing oxidative stress, inhibiting ATP synthesis, and activating the mitochondrial pathway of apoptosis (Kiang and Tsen, 2006). Platelet-activating factor (PAF) and thromboxane A2 (TXA2) are two mediators of gentamicin-induced renal vasoconstriction (López-Novoa, 1999; Martínez-Salgado *et al*., 2007). PAF and TXA2 inhibitors improve RBF and lessen tubular damage (Dos Santos *et al*., 1991; Papanikolaou *et al*., 1992; Rodriguez-Barbero *et al*., 1992). Gentamicin is also known to impair vascular smooth muscle relaxing capacity, contributing to the reduced RBF (Seçilmiş *et al*., 2005). Coadministration of

L-arginine normalizes vascular relaxation and softens tubular injury (Seçilmiş *et al*., 2005). However, results from Hishida *et al*. (1994) contradict this notion. These authors found that cotreatment with desoxycorticosterone acetate or SOD normalized gentamicin-induced RBF decline but did not reduce the severity of tubular necrosis. Moreover, cotreatment with dimethylthiourea, a hydroxyl radical scavenger, attenuated tubular necrosis but did not ameliorate the reduction in RBF. These data break the link between tubular necrosis and reduced RBF but, interestingly, indicate that intervention on different ROS species may have preferential vascular or tubular effects in gentamicin's nephrotoxicity. Clearly, more investigation is necessary.

The nephrotoxicity of gentamicin has been shown to involve an inflammatory response in experimental animals (Bledsoe *et al*., 2006; Kalayarasan *et al*., 2009; Kourilsky *et al*., 1982). An exaggerated or pathologically skewed inflammatory response seems to be involved in tubular injury and contribute to renal damage progression (Karkar, 2008). In fact, strategies that protect from gentamicin-induced renal damage usually inhibit the inflammatory response (Bledsoe *et al*., 2006; Sue *et al*., 2009) An increased or unbalanced ROS production and oxidative stress mediate the inflammatory response unleashed by gentamicin (Kadkhodaee *et al*., 2005; Maldonado *et al*., 2003; Morales *et al*., 2002; Fig. 5). Superoxide anion (Schreck *et al*., 1991) and hydrogen peroxide (Meyer *et al*., 1993; Lu *et al*., 2010) activate nuclear factor κB (NFκB), a key mediator of several inflammatory pathways. Indeed, NFκB inhibitors protect the kidney against gentamicin-induced damage (Tugcu *et al*., 2006). NFκB induces the expression of proinflammatory cytokines (Sánchez-López *et al*., 2009) and iNOS (Xie *et al*., 1994). Endothelial NOS-derived NO, at low levels, mediates physiological vasodilatation, whereas excessive NO production because of the overexpression of iNOS can cause cytotoxic effects in surrounding cells. Excessive iNOS-derived NO can react with superoxide anion and produce peroxinitrite, a highly reactive radical that contributes to cell damage (Pedraza-Chaverrí *et al*., 2004) and reduced vascular relaxation (Förstermann, 2010; Fig. 5). Inflammatory cytokines, such as tumor necrosis factor alpha can activate tubular apoptosis, especially in the pathological environment (Justo *et al*., 2006).

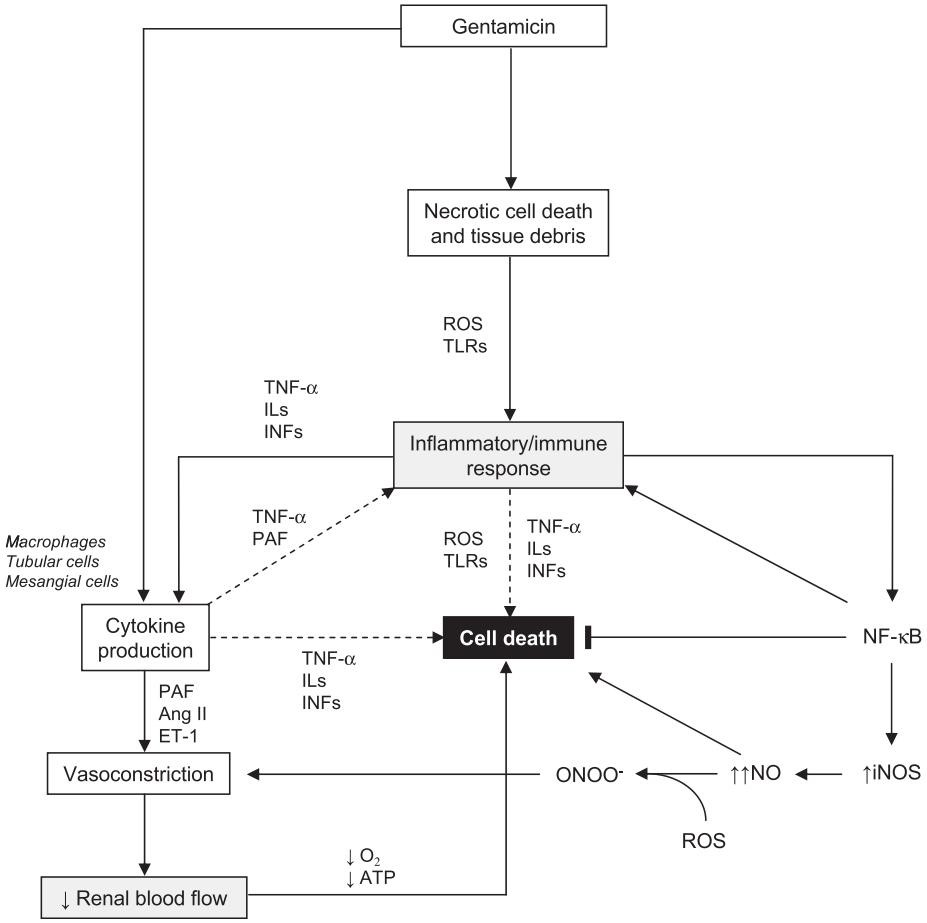


FIG. 5. Indirect mediators of gentamicin's cytotoxicity: inflammation and reduced RBF. Ang II, angiotensin II; ET-1, endothelin-1; Ils, interleukins; INFs, interferons; TLRs, toll-like receptors; TNF-α, tumor necrosis factor alpha.

PERSPECTIVES

Many cellular effects of gentamicin have the capacity to cause cell death or contribute significantly to it, including activation of the mitochondrial pathway of apoptosis, ER stress, and onset of an UPR and phospholipidosis. Others have an uncertain capacity to lead directly to cell death, such as oxidative stress and ATP-depleting mechanisms. However, besides the relative contribution of these pathways considered individually, the hierarchic relation among them is still unknown. For example, can gentamicin pass through the endosomal vesicles (endosomes, lysosomes, Golgi, etc.) toward the cytosol without producing ER stress leading to cell death? If mitochondrial effects were inhibited, would other mechanisms lead the way to cell death? To what extent are some of these mechanisms redundant? Does the participation of each individual mechanism vary depending on the level of stimulation (i.e., gentamicin dosage)? After reviewing the existing information on gentamicin tubular cytotoxicity, it must be concluded that these questions remain incompletely answered. These are important aspects of future research, which will yield critical information on the key mechanisms that should be targeted for the pharmacological prevention of gentamicin's undesired renal side effects. Selective inhibition of specific mediators of individual mechanisms will led further light on these issues.

A potential limitation to progression in this line is the uncertainty on the reliability of the available tubular cell lines and primary cultures at reproducing the effects of gentamicin in tubular cells *in vivo*. The relative resistance of cultured cells to gentamicin cytotoxicity might be the result of an experimental artifact or it might reflect the real nature of tubular cells in their tissue environment. If this is the case, indirect mechanisms of cytotoxicity, as those addressed in the previous section (i.e., reduced RBF, inflammation, and the immune response), will need to be invoked to fully explain tubular necrosis and may gain a central role in therapeutics.

FUNDING

Instituto de Salud Carlos III (Retic 016/2006); RedinRen to JML-N; FIS (PI081900 to F.J.L.-H.); Junta de Castilla y Leon (Excellence Group GR-100); Ministerio de Ciencia y Tecnologia (BFU2004-00285/BFI, SAF2007-63893).

REFERENCES

Abdel-Gayoum, A. A., Ali, B. H., Ghawarsha, K., and Bashir, A. A. (1993). Plasma lipid profile in rats with gentamicin-induced nephrotoxicity. *Hum. Exp. Toxicol.* **12,** 371–375.

Ali, B. H. (2003). Agents ameliorating or augmenting experimental gentamicin nephrotoxicity: some recent research. *Food. Chem. Toxicol.* **41,** 1447–1452.

Ali, B. H., Abdel Gayoum, A. A., and Bashir, A. A. (1992). Gentamicin nephrotoxicity in rat: some biochemical correlates. *Pharmacol. Toxicol.* **70,** 419–423.

Basnakian, A. G., Kaushal, G. P., and Shah, S. V. (2002). Apoptotic pathways of oxidative damage to renal tubular epithelial cells. *Antioxid. Redox Signal.* **4,** 915–924.

Bennett, W. M., Mela-Riker, L. M., Houghton, D. C., Gilbert, D. N., and Buss, W. C. (1988). Microsomal protein synthesis inhibition: an early manifestation of gentamicin nephrotoxicity. *Am. J. Physiol.* **255,** F265–F269.

Bledsoe, G., Crickman, S., Mao, J., Xia, C. F., Murakami, H., Chao, L., and Chao, J. (2006). Kallikrein/kinin protects against gentamicin-induced nephrotoxicity by inhibition of inflammation and apoptosis. *Nephrol. Dial. Transplant.* **21,** 624–633.

Buchanan, J. H., Stevens, A., and Sidhu, J. (1987). Aminoglycoside antibiotic treatment of human fibroblasts: intracellular accumulation, molecular changes and the loss of ribosomal accuracy. *Eur. J. Cell Biol.* **43,** 141–147.

Chiarugi, A. (2005). "Simple but not simpler": toward a unified picture of energy requirements in cell death. *FASEB J.* **19,** 1783–1788.

Chwieralski, C. E., Welte, T., and Bühling, F. (2006). Cathepsin-regulated apoptosis. *Apoptosis* **11,** 143–149.

Cui, S., Verroust, P. J., Moestrup, S. K., and Christensen, E. I. (1996). Megalin/gp330 mediates uptake of albumin in renal proximal tubule. *Am. J. Physiol.* **271,** F900–F907.

Cuzzocrea, S., Mazzon, E., Dugo, L., Serraino, I., Di Paola, R., Britti, D., De Sarro, A., Pierpaoli, S., Caputi, A., Masini, E., *et al.* (2002). A role for superoxide in gentamicin-mediated nephropathy in rats. *Eur. J. Pharmacol.* **450,** 67–76.

Cuzzocrea, S., Thiemermann, C., and Salvemini, D. (2004). Potential therapeutic effect of antioxidant therapy in shock and inflammation. *Curr. Med. Chem.* **11,** 1147–1462.

De Broe, M. E., Paulus, G. J., Verpooten, G. A., Roels, F., Buyssens, N., Wedeen, R., Van Hoof, F., and Tulkens, P. M. (1984). Early effects of gentamicin, tobramycin, and amikacin on the human kidney. *Kidney Int.* **25,** 643–652.

DiBona, D. R., and Powell, W. J., Jr. (1980). Quantitative correlation between cell swelling and necrosis in myocardial ischemia in dogs. *Circ. Res.* **47,** 653–665.

Dos Santos, O. F., Boim, M. A., Barros, E. J., and Schor, N. (1991). Role of platelet activating factor in gentamicin and cisplatin nephrotoxicity. *Kidney Int.* **40,** 742–747.

Edinger, A. L., and Thompson, C. B. (2004). Death by design: apoptosis, necrosis and autophagy. *Curr. Opin. Cell Biol.* **16,** 663–669.

Edwards, C. Q., Smith, C. R., Baughman, K. L., Rogers, J. F., and Lietman, P. S. (1976). Concentrations of gentamicin and amikacin in human kidneys. *Antimicrob. Agents Chemother.* **9,** 925–927.

Edwards, J. R., Diamantakos, E. A., Peuler, J. D., Lamar, P. C., and Prozialeck, W. C. (2007). A novel method for the evaluation of proximal tubule epithelial cellular necrosis in the intact rat kidney using ethidium homodimer. *BMC Physiol.* **7,** 1.

El Mouedden, M., Laurent, G., Mingeot-Leclercq, M. P., and Tulkens, P. M. (2000). Gentamicin-induced apoptosis in renal cell lines and embryonic rat fibroblasts. *Toxicol. Sci.* **56,** 229–239.

Forge, A., Zajic, G., Davies, S., Weiner, N., and Schacht, J. (1989). Gentamicin alters membrane structure as shown by freeze-fracture of liposomes. *Hear. Res.* **37,** 129–139.

Förstermann, U. (2010). Nitric oxide and oxidative stress in vascular disease. *Pflugers Arch.* **459,** 923–939.

Fribley, A., Zhang, K., and Kaufman, R. J. (2009). Regulation of apoptosis by the unfolded protein response. *Methods Mol. Biol.* **559,** 191–204.

Frommer, J. P., Senekjian, H. O., Babino, H., and Weinman, E. J. (1983). Intratubular microinjection study of gentamicin transport in the rat. *Miner. Electrolyte Metab.* **9,** 108–112.

Fukuda, Y., Malmborg, A. S., and Aperia, A. (1991). Gentamicin inhibition of Na+, K(+)-ATPase in rat kidney cells. *Acta Physiol. Scand.* **141,** 27–34.

Gibbons, C. E., Maldonado-Pérez, D., Shah, A. N., Riccardi, D., and Ward, D. T. (2008). Calcium-sensing receptor antagonism or lithium treatment ameliorates aminoglycoside-induced cell death in renal epithelial cells. *Biochim. Biophys. Acta* **1782,** 188–195.

Giuliano, R. A., Paulus, G. J., Verpooten, G. A., Pattyn, V. M., Pollet, D. E., Nouwen, E. J., Laurent, G., Carlier, M. B., Maldague, P., Tulkens, P. M., *et al*. (1984). Recovery of cortical phospholipidosis and necrosis after acute gentamicin loading in rats. *Kidney Int.* **26,** 838–847.

Giurgea-Marion, L., Toubeau, G., Laurent, G., Heuson-Stiennon, J. A., and Tulkens, P. M. (1986). Impairment of lysosome-pinocytic vesicle fusion in rat kidney proximal tubules after treatment with gentamicin at low doses. *Toxicol. Appl. Pharmacol.* **86,** 271–285.

Golstein, P., and Kroemer, G. (2007). Cell death by necrosis: towards a molecular definition. *Trends Biochem. Sci.* **32,** 37–43.

Hishida, A., Nakajima, T., Yamada, M., Kato, A., and Honda, N. (1994). Roles of hemodynamic and tubular factors in gentamicin-mediated nephropathy. *Ren. Fail.* **16,** 109–116.

Horibe, T., Matsui, H., Tanaka, M., Nagai, H., Yamaguchi, Y., Kato, K., and Kikuchi, M. (2004). Gentamicin binds to the lectin site of calreticulin and inhibits its chaperone activity. *Biochem. Biophys. Res. Commun.* **323,** 281–287.

Houghton, D. C., Campbell-Boswell, M. V., Bennett, W. M., Porter, G. A., and Brooks, R. E. (1978). Myeloid bodies in the renal tubules of humans: relationship to gentamicin therapy. *Clin. Nephrol.* **10,** 140–145.

Hsu, Y. H., Chen, C. H., Hou, C. C., Sue, Y. M., Cheng, C. Y., Cheng, T. H., Lin, H., Tsai, W. L., Chan, P., and Chen, T. H. (2008). Prostacyclin protects renal tubular cells from gentamicin-induced apoptosis via a PPARalpha-dependent pathway. *Kidney Int.* **73,** 578–587.

Jeong, J. I., Lee, Y. W., and Kim, Y. K. (2003). Chemical hypoxia-induced cell death in human glioma cells: role of reactive oxygen species, ATP depletion, mitochondrial damage and Ca2+. *Neurochem. Res.* **28,** 1201–1211.

Jones, A. T., and Wessling-Resnick, M. (1998). Inhibition of in vitro endosomal vesicle fusion activity by aminoglycoside antibiotics. *J. Biol. Chem.* **273,** 25301–25309.

Juan, S. H., Chen, C. H., Hsu, Y. H., Hou, C. C., Chen, T. H., Lin, H., Chu, Y. L., and Sue, Y. M. (2007). Tetramethylpyrazine protects rat renal tubular cell apoptosis induced by gentamicin. *Nephrol. Dial. Transplant.* **22,** 732–739.

Justo, P., Sanz, A. B., Sanchez-Niño, M. D., Winkles, J. A., Lorz, C., Egido, J., and Ortiz, A. (2006). Cytokine cooperation in renal tubular cell injury: the role of TWEAK. *Kidney Int.* **70,** 1750–1758.

Kacew, S. (1987). Cationic amphiphilic drug-induced renal cortical lysosomal phospholipidosis: an in vivo comparative study with gentamicin and chlorphentermine. *Toxicol. Appl. Pharmacol.* **91,** 469–476.

Kadkhodaee, M., Khastar, H., Arab, H. A., Ghaznavi, R., Zahmatkesh, M., and Mahdavi-Mazdeh, M. (2007). Antioxidant vitamins preserve superoxide dismutase activities in gentamicin-induced nephrotoxicity. *Transplant. Proc.* **39,** 864–865.

Kadkhodaee, M., Khastar, H., Faghihi, M., Ghaznavi, R., and Zahmatkesh, M. (2005). Effects of co-supplementation of vitamins E and C on gentamicin-induced nephrotoxicity in rat. *Exp. Physiol.* **90,** 571–576.

Kalayarasan, S., Prabhu, P. N., Sriram, N., Manikandan, R., Arumugam, M., and Sudhandiran, G. (2009). Diallyl sulfide enhances antioxidants and inhibits inflammation through the activation of Nrf2 against gentamicin-induced nephrotoxicity in Wistar rats. *Eur. J. Pharmacol.* **606,** 162–171.

Kaloyanides, G. J. (1992). Drug-phospholipid interactions: role in aminoglycoside nephrotoxicity. *Ren. Fail.* **14,** 351–357.

Karasawa, T., Wang, Q., Fu, Y., Cohen, D. M., and Steyger, P. S. (2008). TRPV4 enhances the cellular uptake of aminoglycoside antibiotics. *J. Cell Sci.* **121,** 2871–2879.

Karataş, Y., Seçilmiş, M. A., Karayaylali, I., Doran, F., Büyükafşar, K., Singirik, E., Sagliker, Y., and Dikmen, A. (2004). Effect of tempol (4-hydroxy tempo) on gentamicin-induced nephrotoxicity in rats. *Fundam. Clin. Pharmacol.* **18,** 79–83.

Karkar, A. (2008). Modulation of renal inflammation: therapeutic strategies. *Saudi J. Kidney Dis. Transpl.* **19,** 1–19.

Kerbiriou, M., Teng, L., Benz, N., Trouvé, P., and Férec, C. (2009). The calpain, caspase 12, caspase 3 cascade leading to apoptosis is altered in F508del-CFTR expressing cells. *PloS One* **4,** e8436.

Kiang, J. G., and Tsen, K. T. (2006). Biology of hypoxia. *Chin. J. Physiol.* **49,** 223–233.

Kim, I., Xu, W., and Reed, J. C. (2008). Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. *Nat. Rev. Drug Discov.* **7,** 1013–1030.

Kishore, B. K., Lambricht, P., Ibrahim, S., Laurent, G., Tulkens, P. M., and Maldague, P. (1990). Inhibition of aminoglycoside-induced nephrotoxicity in rats by polyanionic peptides. *Contrib. Nephrol.* **83,** 191–201.

Kourilsky, O., Solez, K., Morel-Maroger, L., Whelton, A., Duhoux, P., and Sraer, J. D. (1982). The pathology of acute renal failure due to interstitial nephritis in man with comments on the role of interstitial inflammation and sex in gentamicin nephrotoxicity. *Medicine (Baltimore)* **61,** 258–268.

Koyner, J. L., Sher Ali, R., and Murray, P. T. (2008). Antioxidants. Do they have a place in the prevention or therapy of acute kidney injury? *Nephron. Exp. Nephrol.* **109,** e109–e117.

Lai, E., Teodoro, T., and Volchuk, A. (2007). Endoplasmic reticulum stress: signalling the unfolded protein response. *Physiology (Bethesda)* **22,** 193–201.

Laurent, G., Carlier, M. B., Rollman, B., Van Hoof, F., and Tulkens, P. (1982). Mechanism aminoglycoside-induced lysosomal phospholipidosis: in vitro and in vivo studies with gentamicin and amikacin. *Biochem. Pharmacol.* **31,** 3861–3870.

Lee, B. H., Lee, S. H., Choi, H. J., Kang, H. G., Oh, S. W., Lee, D. S., Ha, I. S., Choi, Y., and Cheong, H. I. (2009). Decreased renal uptake of (99m)Tc-DMSA in patients with tubular proteinuria. *Pediatr. Nephrol.* **24,** 2211–2216.

Levi, M., and Cronin, R. E. (1990). Early selective effects of gentamicin on renal brush-border membrane Na-Pi cotransport and Na-H exchange. *Am. J. Physiol.* **258,** F1379–F1387.

Li, J., Li, Q. X., Xie, X. F., Ao, Y., Tie, C. R., and Song, R. J. (2009a). Differential roles of dihydropyridine calcium antagonist nifedipine, nitrendipine and amlodipine on gentamicin-induced renal tubular toxicity in rats. *Eur. J. Pharmacol.* **620,** 97–104.

Li, S., Nagothu, K. K., Desai, V., Lee, T., Branham, W., Moland, C., Megyesi, J. K., Crew, M. D., and Portilla, D. (2009b). Transgenic expression of proximal tubule peroxisome proliferator-activated receptor-alpha in mice confers protection during acute kidney injury. *Kidney Int.* **76,** 1049–1062.

Li, S., Wu, P., Yarlagadda, P., Vadjunec, N. M., Proia, A. D., Harris, R. A., and Portilla, D. (2004). PPAR alpha ligand protects during cisplatin-induced acute renal failure by preventing inhibition of renal FAO and PDC activity. *Am. J. Physiol. Renal Physiol.* **286,** F572–F580.

Lieberthal, W., and Levine, J. S. (1996). Mechanisms of apoptosis and its potential role in renal tubular epithelial cell injury. *Am. J. Physiol.* **271,** F477–F488.

Lin, H., Hou, C. C., Cheng, C. F., Chiu, T. H., Hsu, Y. H., Sue, Y. M., Chen, T. H., Hou, H. H., Chao, Y. C., Cheng, T. H., *et al*. (2007). Peroxisomal proliferator-activated receptor-alpha protects renal tubular cells from doxorubicin-induced apoptosis. *Mol. Pharmacol.* **72,** 1238–1245.

Lipsky, J. J., Cheng, L., Sacktor, B., and Lietman, P. S. (1980). Gentamicin uptake by renal tubule brush border membrane vesicles. *J. Pharmacol. Exp. Ther.* **215,** 390–393.

Lopez-Hernandez, F. J., and Lopez-Novoa, J. M. (2009). Potential utility of PPARalpha activation in the prevention of ischemic and drug-induced acute renal damage. *Kidney Int.* **76,** 1022–1024.

López-Novoa, J. M. (1999). Potential role of platelet activating factor in acute renal failure. *Kidney Int.* **55,** 1672–1682.

Lu, H., Zhen, J., Wu, T., Peng, A., Ye, T., Wang, T., Yu, X., Vaziri, N. D., Mohan, C., and Zhou, X. J. (2010). Superoxide dismutase mimetic drug tempol aggravates anti-GBM antibody induced glomerulonephritis in mice. *Am. J. Physiol. Renal Physiol.* **299,** F445–F452.

Luft, F. C., Yum, M. N., Walker, P. D., and Kleit, S. A. (1977). Gentamicin gradient patterns and morphological changes in human kidneys. *Nephron* **18,** 167–174.

Maldonado, P. D., Barrera, D., Rivero, I., Mata, R., Medina-Campos, O. N., Hernández-Pando, R., and Pedraza-Chaverrí, J. (2003). Antioxidant S-allylcysteine prevents gentamicin-induced oxidative stress and renal damage. *Free Radic. Biol. Med.* **35,** 317–324.

Martínez-Salgado, C., Eleno, N., Tavares, P., Rodríguez-Barbero, A., García-Criado, J., Bolaños, J. P., and López-Novoa, J. M. (2002). Involvement of reactive oxygen species on gentamicin-induced mesangial cell activation. *Kidney Int.* **62,** 1682–1692.

Martínez-Salgado, C., López-Hernández, F. J., and López-Novoa, J. M. (2007). Glomerular nephrotoxicity of aminoglycosides. *Toxicol. Appl. Pharmacol.* **223,** 86–98.

Mather, M., and Rottenberg, H. (2001). Polycations induce the release of soluble intermembrane mitochondrial proteins. *Biochim. Biophys. Acta* **1503,** 357–368.

Meyer, M., Schreck, R., and Baeuerle, P. A. (1993). H2O2 and antioxidants have opposite effects on activation of NF-kappa B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J.* **12,** 2005–2015.

Mingeot-Leclercq, M. P., Brasseur, R., and Schanck, A. (1995). Molecular parameters involved in aminoglycoside nephrotoxicity. *J. Toxicol. Environ. Health* **44,** 263–300.

Mingeot-Leclercq, M. P., and Tulkens, P. M. (1999). Aminoglycosides: nephrotoxicity. *Antimicrob. Agents Chemother.* **43,** 1003–1012.

Moestrup, S. K., Cui, S., Vorum, H., Bregengard, C., Bjorn, S. E., Norris, K., Gliemann, J., and Christensen, E. I. (1995). Evidence that epithelial glycoprotein 330/megalin mediates uptake of polybasic drugs. *J. Clin. Invest.* **96,** 1404–1413.

Monteil, C., Leclere, C., Fillastre, J. P., and Morin, J. P. (1993). Characterization of gentamicin-induced dysfunctions in vitro: the use of optimized primary cultures of rabbit proximal tubule cells. *Ren. Fail.* **15,** 475–483.

Morales, A. I., Buitrago, J. M., Santiago, J. M., Fernández-Tagarro, M., López-Novoa, J. M., and Pérez-Barriocanal, F. (2002). Protective effect of trans-resveratrol on gentamicin-induced nephrotoxicity. *Antioxid. Redox Signal.* **4,** 893–898.

Morales, A. I., Detaille, D., Prieto, M., Puente, A., Briones, E., Arévalo, M., Leverve, X., López-Novoa, J. M., and El-Mir, M. Y. (2010). Metformin prevents experimental gentamicin-induced nephropathy by a mitochondria-dependent pathway. *Kidney Int.* **77,** 861–869.

Moran, K., Mulhall, J., Kelly, D., Sheehan, S., Dowsett, J., Dervan, P., and Fitzpatrick, J. M. (1992). Morphological changes and alterations in regional intrarenal blood flow induced by graded renal ischemia. *J. Urol.* **148,** 463–466.

Morgan, M. J., Kim, Y. S., and Liu, Z. (2007). Lipid rafts and oxidative stress-induced cell death. *Antioxid. Redox Signal.* **9,** 1471–1483.

Myrdal, S. E., Johnson, K. C., and Steyger, P. S. (2005). Cytoplasmic and intranuclear binding of gentamicin does not require endocytosis. *Hear. Res.* **204,** 156–169.

Nagai, J., Katsube, T., Murakami, T., and Takano, M. (2002). Effect of gentamicin on pharmacokinetics of lysozyme in rats: interaction between megalin substrates in the kidney. *J. Pharm. Pharmacol.* **54,** 1491–1496.

Nagai, J., Saito, M., Adachi, Y., Yumoto, R., and Takano, M. (2006). Inhibition of gentamicin binding to rat renal brush-border membrane by megalin ligands and basic peptides. *J. Control. Release* **112,** 43–50.

Nakajima, T., Hishida, A., and Kato, A. (1994). Mechanisms for protective effects of free radical scavengers on gentamicin-mediated nephropathy in rats. *Am. J. Physiol.* **266,** F425–F431.

Ngaha, E. O., and Ogunleye, I. O. (1983). Studies on gentamicin-induced labilization of rat kidney lysosomes in vitro. Possible protection by selenium. *Biochem. Pharmacol.* **32,** 2659–2664.

Nonclercq, D., Wrona, S., Toubeau, G., Zanen, J., Heuson-Stiennon, J. A., Schaudies, R. P., and Laurent, G. (1992). Tubular injury and regeneration in the rat kidney following acute exposure to gentamicin: a time-course study. *Ren. Fail.* **14,** 507–521.

Ott, M., Gogvadze, V., Orrenius, S., and Zhivotovsky, B. (2007). Mitochondria, oxidative stress and cell death. *Apoptosis* **12,** 913–922.

Papanikolaou, N., Peros, G., Morphake, P., Gkikas, G., Maraghianne, D., Tsipas, G., Kostopoulos, K., Arambatze, C., Gkika, E. L., and Bariety, J. (1992). Does gentamicin induce acute renal failure by increasing renal TXA2 synthesis in rats? *Prostaglandins Leukot. Essent. Fatty Acids* **45,** 131–136.

Pedraza-Chaverrí, J., Barrera, D., Maldonado, P. D., Chirino, Y. I., Macías-Ruvalcaba, N. A., Medina-Campos, O. N., Castro, L., Salcedo, M. I., and Hernández-Pando, R. (2004). S-allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen in vitro and attenuates gentamicin-induced oxidative and nitrosative stress and renal damage in vivo. *BMC Clin. Pharmacol.* **4,** 5.

Pessoa, E. A., Convento, M. B., Silva, R. G., Oliveira, A. S., Borges, F. T., and Schor, N. (2009). Gentamicin-induced preconditioning of proximal tubular LLC-PK1 cells stimulates nitric oxide production but not the synthesis of heat shock protein. *Braz. J. Med. Biol. Res.* **42,** 614–620.

Peyrou, M., and Cribb, A. E. (2007). Effect of endoplasmic reticulum stress preconditioning on cytotoxicity of clinically relevant nephrotoxins in renal cell lines. *Toxicol. In Vitro* **21,** 878–886.

Peyrou, M., Hanna, P. E., and Cribb, A. E. (2007). Cisplatin, gentamicin, and p-aminophenol induce markers of endoplasmic reticulum stress in the rat kidneys. *Toxicol. Sci.* **99,** 346–353.

Ramsammy, L. S., Josepovitz, C., and Kaloyanides, G. J. (1988). Gentamicin inhibits agonist stimulation of the phosphatidylinositol cascade in primary cultures of rabbit proximal tubular cells and in rat renal cortex. *J. Pharmacol. Exp. Ther.* **247,** 989–996.

Ramsammy, L. S., Josepovitz, C., Lane, B., and Kaloyanides, G. J. (1989a). Effect of gentamicin on phospholipid metabolism in cultured rabbit proximal tubular cells. *Am. J. Physiol.* **256,** C204–C213.

Ramsammy, L. S., Josepovitz, C., Lane, B. P., and Kaloyanides, G. J. (1989b). Polyaspartic acid protects against gentamicin nephrotoxicity in the rat. *J. Pharmacol. Exp. Ther.* **250,** 149–153.

Ramsammy, L., Josepovitz, C., Lane, B., and Kaloyanides, G. J. (1990). Polyaspartic acid inhibits gentamicin-induced perturbations of phospholipid metabolism. *Am. J. Physiol.* **258,** C1141–C1149.

Recht, M. I., Douthwaite, S., and Puglisi, J. D. (1999). Basis for prokaryotic specificity of action of aminoglycoside antibiotics. *EMBO J.* **18,** 3133–3138.

Regec, A. L., Trump, B. F., and Trifillis, A. L. (1989). Effect of gentamicin on the lysosomal system of cultured human proximal tubular cells. Endocytotic activity, lysosomal pH and membrane fragility. *Biochem. Pharmacol.* **38,** 2527–2534.

Rodriguez-Barbero, A., Bosque, E., Rivas-Cabañero, L., Arévalo, M., and López-Novoa, J. M. (1992). Effect of platelet activating factor antagonist treatment on gentamicin nephrotoxicity. *Mediators Inflamm.* **1,** 23–26.

Ryter, S. W., Kim, H. P., Hoetzel, A., Park, J. W., Nakahira, K., Wang, X., and Choi, A. M. (2007). Mechanisms of cell death in oxidative stress. *Antioxid. Redox Signal.* **9,** 49–89.

Saito, Y., Nishio, K., Ogawa, Y., Kimata, J., Kinumi, T., Yoshida, Y., Noguchi, N., and Niki, E. (2006). Turning point in apoptosis/necrosis induced by hydrogen peroxide. *Free Radic. Res.* **40,** 619–630.

Sánchez-López, E., Rayego, S., Rodrigues-Díez, R., Rodriguez, J. S., Rodrigues-Díez, R., Rodríguez-Vita, J., Carvajal, G., Aroeira, L. S., Selgas, R., Mezzano, S. A., *et al*. (2009). CTGF promotes inflammatory cell infiltration of the renal interstitium by activating NF-kappaB. *J. Am. Soc. Nephrol.* **20,** 1513–1526.

Sandhya, P., and Varalakshmi, P. (1997). Effect of lipoic acid administration on gentamicin-induced lipid peroxidation in rats. *J. Appl. Toxicol.* **17,** 405–408.

Sandoval, R. M., and Molitoris, B. A. (2004). Gentamicin traffics retrograde through the secretory pathway and is released in the cytosol via the endoplasmic reticulum. *Am. J. Physiol. Renal Physiol.* **286,** F617–F624.

Santos, C. X., Tanaka, L. Y., Wosniak, J., and Laurindo, F. R. (2009). Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. *Antioxid. Redox Signal.* **11,** 2409–2427.

Sato, T., Oku, H., Tsuruma, K., Katsumura, K., Shimazawa, M., Hara, H., Sugiyama, T., and Ikeda, T. (2010). Effect of hypoxia on susceptibility of RGC-5 cells to nitric oxide. *Invest. Ophthalmol. Vis. Sci.* **51,** 2575–12486.

Schmitz, C., Hilpert, J., Jacobsen, C., Boensch, C., Christensen, E. I., Luft, F. C., and Willnow, T. E. (2002). Megalin deficiency offers protection from renal aminoglycoside accumulation. *J. Biol. Chem.* **277,** 618–622.

Schnellmann, R. G., and Williams, S. W. (1998). Proteases in renal cell death: calpains mediate cell death produced by diverse toxicants. *Ren. Fail.* **20,** 679–686.

Schreck, R., Rieber, P., and Baeuerle, P. A. (1991). Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO J.* **10,** 2247–2258.

Seçilmiş, M. A., Karataş, Y., Yorulmaz, O., Buyukafşar, K., Singirik, E., Doran, F., Inal, T. C., and Dikmen, A. (2005). Protective effect of L-arginine intake on the impaired renal vascular responses in the gentamicin-treated rats. *Nephron Physiol.* **100,** 13–20.

Seppet, E., Gruno, M., Peetsalu, A., Gizatullina, Z., Nguyen, H. P., Vielhaber, S., Wussling, M. H., Trumbeckaite, S., Arandarcikaite, O., Jerzembeck, D., *et al*. (2009). Mitochondria and energetic depression in cell pathophysiology. *Int. J. Mol. Sci.* **10,** 2252–2303.

Servais, H., Jossin, Y., Van Bambeke, F., Tulkens, P. M., and Mingeot-Leclercq, M. P. (2006). Gentamicin causes apoptosis at low concentrations in renal LLC-PK1 cells subjected to electroporation. *Antimicrob. Agents Chemother.* **50,** 1213–1221.

Servais, H., Ortiz, A., Devuyst, O., Denamur, S., Tulkens, P. M., and Mingeot-Leclercq, M. P. (2008). Renal cell apoptosis induced by nephrotoxic drugs: cellular and molecular mechanisms and potential approaches to modulation. *Apoptosis* **13,** 11–32.

Shimizu, A., Takumida, M., Anniko, M., and Suzuki, M. (2003). Calpain and caspase inhibitors protect vestibular sensory cells from gentamicin ototoxicity. *Acta Otolaryngol.* **123,** 459–465.

Silverblatt, F. (1982). Pathogenesis of nephrotoxicity of cephalosporins and aminoglycosides: a review of current concepts. *Rev. Infect. Dis.* **4**(Suppl), S360–S365.

Silverblatt, F. J., and Kuehn, C. (1979). Autoradiography of gentamicin uptake by the rat proximal tubule cell. *Kidney Int.* **15,** 335–345.

Simmons, C. F., Jr., Bogusky, R. T., and Humes, H. D. (1980). Inhibitory effects of gentamicin on renal mitochondrial oxidative phosphorylation. *J. Pharmacol. Exp. Ther.* **214,** 709–715.

Skopicki, H. A., Zikos, D., Sukowski, E. J., Fisher, K. A., and Peterson, D. R. (1996). Gentamicin inhibits carrier-mediated dipeptide transport in kidney. *Am. J. Physiol.* **270,** F531–F538.

Stratta, P., Segoloni, G. P., Canavese, C., Muzio, G., Dogliani, M., Serra, A., Allemandi, P., Salomone, M., Caramellino, C., and Canuto, R. (1994). Oxygen free radicals are not the main factor in experimental gentamicin nephrotoxicity. *Ren. Fail.* **16,** 445–455.

Sue, Y. M., Cheng, C. F., Chang, C. C., Chou, Y., Chen, C. H., and Juan, S. H. (2009). Antioxidation and anti-inflammation by haem oxygenase-1 contribute to protection by tetramethylpyrazine against gentamicin-induced apoptosis in murine renal tubular cells. *Nephrol. Dial. Transplant.* **24,** 769–777.

Sundin, D. P., Sandoval, R., and Molitoris, B. A. (2001). Gentamicin inhibits renal protein and phospholipid metabolism in rats: implications involving intracellular trafficking. *J. Am. Soc. Nephrol.* **12,** 114–123.

Swan, S. K., Kohlhepp, S. J., Kohnen, P. W., Gilbert, D. N., and Bennett, W. M. (1991). Long-term protection of polyaspartic acid in experimental gentamicin nephrotoxicity. *Antimicrob. Agents Chemother.* **35,** 2591–2595.

Todd, J. H., Sens, D. A., Hazen-Martin, D. J., Bylander, J. E., Smyth, B. J., and Sens, M. A. (1992). Aminoglycoside antibiotics alter the electrogenic transport properties of cultured human proximal tubule cells. *Toxicol. Pathol.* **20,** 608–616.

Trivedi, R., Mithal, A., and Chattopadhyay, N. (2008). Recent updates on the calcium-sensing receptor as a drug target. *Curr. Med. Chem.* **15,** 178–186.

Tugcu, V., Ozbek, E., Tasci, A. I., Kemahli, E., Somay, A., Bas, M., Karaca, C., Altug, T., Cekmen, M. B., and Ozdogan, H. K. (2006). Selective nuclear factor kappa-B inhibitors, pyrolidium dithiocarbamate and sulfasalazine, prevent the nephrotoxicity induced by gentamicin. *BJU Int.* **98,** 680–686.

Tulkens, P. M. (1989). Nephrotoxicity of aminoglycoside antibiotics. *Toxicol. Lett.* **46,** 107–123.

Van Rooijen, L. A., and Agranoff, B. W. (1985). Inhibition of polyphosphoinositide phosphodiesterase by aminoglycoside antibiotics. *Neurochem. Res.* **10,** 1019–1024.

Ward, D. T., Maldonado-Pérez, D., Hollins, L., and Riccardi, D. (2005). Aminoglycosides induce acute cell signalling and chronic cell death in renal cells that express the calcium-sensing receptor. *J. Am. Soc. Nephrol.* **16,** 1236–1244.

Wu, Y., Connors, D., Barber, L., Jayachandra, S., Hanumegowda, U. M., and Adams, S. P. (2009). Multiplexed assay panel of cytotoxicity in HK-2 cells for detection of renal proximal tubule injury potential of compounds. *Toxicol. In Vitro* **23,** 1170–1178.

Xie, Q. W., Kashiwabara, Y., and Nathan, C. (1994). Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase. *J. Biol. Chem.* **269,** 4705–4708.

Yang, C. L., Du, X. H., and Han, Y. X. (1995). Renal cortical mitochondria are the source of oxygen free radicals enhanced by gentamicin. *Ren. Fail.* **17,** 21–26.

Yin, X. M. (2006). Bid, a BH3-only multi-functional molecule, is at the cross road of life and death. *Gene* **369,** 7–19.

Yokouchi, M., Hiramatsu, N., Hayakawa, K., Okamura, M., Du, S., Kasai, A., Takano, Y., Shitamura, A., Shimada, T., Yao, J., *et al*. (2008). Involvement of selective reactive oxygen species upstream of proapoptotic branches of unfolded protein response. *J. Biol. Chem.* **283,** 4252–4260.

Zhang, F., Hamanaka, R. B., Bobrovnikova-Marjon, E., Gordan, J. D., Dai, M. S., Lu, H., Simon, M. C., and Diehl, J. A. (2006). Ribosomal stress couples the unfolded protein response to p53-dependent cell cycle arrest. *J. Biol. Chem.* **281,** 30036–30045.

ANEXO iii

ASPECTOS GENERALES DE LA NEFROTOXICIDAD DEL CISPLATINO

ASPECTOS GENERALES DE LA NEFROTOXICIDAD DEL CISPLATINO

INDICE

1. INTRODUCCIÓN

El cisplatino (*cis*-clorodiaminoplatinum-II) es un fármaco antineoplásico usado principalmente para el tratamiento de tumores sólidos. En humanos ha mostrado eficacia en el linfoma de Hodgkin, en cáncer de cabeza y cuello, tiroideo, de endometrio, de vejiga, de ovario (Hill y cols., 1974), de pulmón, de esófago y de cérvix (Rozencweig y cols., 1977).

El cisplatino es un complejo de un metal pesado que contiene un átomo central de platino rodeado por dos átomos de cloruro y dos moléculas de amonio en la posición *cis*. Debido a su estructura química, los átomos de cloro del cisplatino son más factibles de sufrir reacciones de sustitución por nucleófilos, por lo que generalmente son reemplazados por agua, así se forma un compuesto más reactivo. Estas moléculas reaccionan con ADN, formando uniones covalentes ("aductos") inter- o intra-catenarias. Los "aductos" formados distorsionan la estructura espacial del ADN, causando lesiones citotóxicas en células tumorales y células normales, las cuales son reconocidas por proteínas especializadas encargadas de su reparación. Si no se produce la reparación o es incompleta, la célula muere por apoptosis (Liedert y cols., 2006).

Los dos principales problemas asociados a la quimioterapia con cisplatino son la resistencia intrínseca o adquirida así como los efectos adversos que produce en tejidos normales. Los mecanismos moleculares responsables de la resistencia al cisplatino pueden ser previos a la formación de los "aductos" como la falta de captación del cisplatino por la célula, el excesivo eflujo del mismo o su inactivación por moléculas como el glutation. Una vez se han producido los "aductos" la eficacia en los mecanismos de reparación del ADN o los defectos en el proceso de apoptosis pueden ser causa de resistencia al cisplatino (Kartalou y Essigmann, 2001).

Los efectos adversos del cisplatino en tejidos normales constituyen el principal factor limitante de su uso en la práctica clínica. Entre ellos, cabe destacar la neurotoxicidad, ototoxicidad, nauseas y vómitos, mielosupresión y toxicidad renal, siendo éste último la principal limitación terapéutica de su uso y eficacia en la terapia contra el cáncer, en términos de una reducción de la dosis o una suspensión del tratamiento (Tsang y cols., 2009; Meyer y Madias, 1994).

A pesar de su toxicidad renal, en la actualidad el cisplatino sigue siendo uno de los fármacos antineoplásicos más ampliamente utilizados por su potente eficacia antitumoral (Faubel y cols., 2007).

2. NEFROTOXICIDAD. FACTOR LIMITANTE DE LA EFICACIA DEL CISPLATINO.

La utilización clínica del cisplatino no sólo depende de su eficacia terapéutica, sino también de la reducción de su nefrotoxicidad. Una de las estrategias aceptadas como estándar en la clínica es la hidratación intensiva con suero salino antes, durante y después de la administración de cisplatino y diuresis forzada mediante tratamiento simultáneo con furosemida o manitol (Launay-Vacher y cols., 2008). Sin embargo, a pesar de las intensas medidas profilácticas encaminadas a proteger las complicaciones renales, aproximadamente un 20-30% de los pacientes hospitalizados tratados con cisplatino padece algún grado de daño renal agudo, siendo en la mayoría de los casos reversible, pero puede volverse irreversible cuando se administran dosis muy elevadas o repetidas (Kuriakose y Kurup, 2008).

Los riñones acumulan cisplatino en mayor grado que otros órganos, por ser la principal vía de excreción del fármaco y por ser órganos altamente vascularizados. Esta acumulación desproporcionada en el tejido renal contribuye decisivamente a su nefrotoxicidad (Ali y cols., 2008). El cisplatino se acumula en el riñón por captación peritubular en nefronas tubulares, predominantemente en el segmento S3 de los túbulos

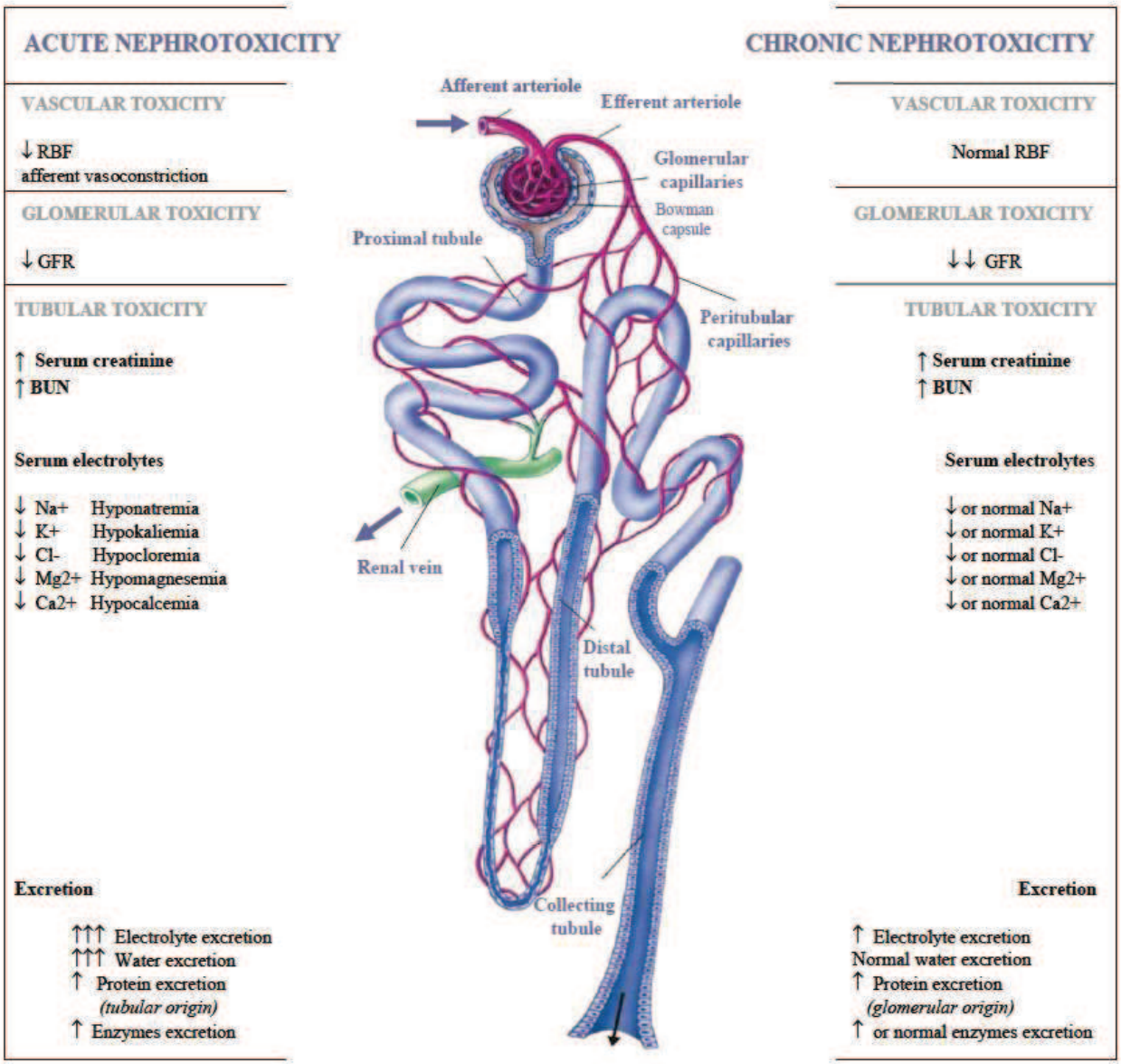


Figura 1. Cambios en la función renal por nefrotoxicidad aguda o crónica del cisplatino (adaptado de Sánchez-González y cols., 2011)

proximales, en la región corticomedular, donde ejerce sus efectos nefrotóxicos. No obstante, en algunos casos se evidencian daños distales, e incluso lesiones tóxicas en los túbulos colectores como consecuencia de un daño tubulointersticial, por tratamientos prolongados con cisplatino (Kawai y cols., 2009). Es importante destacar que la incidencia, severidad y duración de los efectos nefrotóxicos del cisplatino son variables, pudiéndose manifestar como una disfunción renal derivando en un fracaso renal agudo irreversible o incluso una insuficiencia renal crónica y progresiva, alteraciones dependientes en gran medida de las dosis quimioterápicas empleadas (Taguchi y cols., 2005).

2.1. Nefrotoxicidad aguda

En humanos, la nefrotoxicidad aguda producida por el tratamiento con cisplatino se manifiesta con una función tubular alterada y un daño renal reversible. Como se muestra en la figura 1, el daño renal agudo se presenta con una serie de alteraciones de la hemodinámica renal observándose un flujo plasmático renal reducido (FPR) que aparece a las 3 horas tras la

administración del cisplatino y precede a la disminución de la tasa de filtración glomerular (TFG). Esta hipofiltración se desarrolla en dos fases distintas. En un principio la osmolaridad de la orina disminuye durante las primeras 24-48 horas tras la administración del fármaco presentando una TFG normal. Esta poliuria temprana generalmente suele mejorar de forma espontánea. Durante la segunda fase se observa una reducción de la osmolaridad entre las 72-96 horas, en este caso se observa también una disminución de la TFG que además persiste en el tiempo (Meyer y Madias, 1994, y cols., 2000). El daño tubular que se produce puede ser detectado a través de una serie de enzimas que aparecen en orina como son la alanina aminopeptidasa (AAP), alcalino fosfatasa (AP), gamma-glutamiltranspeptidasa (γGT) y N-acetil-beta-D-glucosaminidasa (NAG) (Townsend et al., 2003, Uehara y cols., 2005, Kawai y cols., 2005).

Morfológicamente, se observa muerte celular tubular por necrosis en el segmento S3 de túbulo proximal mientras que las células del túbulo colector distal y de la rama gruesa ascendente del asa de Henle presentan muerte por apoptosis (Arany y cols., 2004, Taguchi y cols., 2005).

2.2. Nefrotoxicidad crónica

Los pacientes con nefrotoxicidad crónica producida por el cisplatino presentan alteraciones en la estructura de la nefrona, progresivo daño su funcionalidad y daño renal irreversible. Como se ilustra en la figura 1, los signos clínicos de esta patología se caracterizan por permanente e incluso a veces progresiva disminución de la TFG acompañada con un incremento de los niveles plasmáticos de creatinina y nitrógeno ureico plasmático (BUN), incremento en la excreción de electrolitos urinarios, así como proteinuria y enzimuria que pueden volver a valores normales.

Las alteraciones histopatológicas observadas tras un tratamiento prolongado con dosis repetidas de cisplatino incluyen lesiones como necrosis-apoptosis aguda tubular focal, hiperplasia intersticial, infiltración de células peritubulares, núcleo atípico o incluso fibrosis tubulointersticial. Estos cambios se han observado en riñones de humanos tratados con cisplatino 5 meses después de la quimioterapia lo que indica daño renal irreversible (Kawai y cols., 2009).

3. FISIOPATOLOGÍA

3.1. Efectos vasculares

3.1.1. Disfunción endotelial:

Una de las principales causas de la producción y mantenimiento de las distintas fases de daño renal agudo por el cisplatino, es la disfunción endotelial (Brodsky y cols., 2002). Inicialmente se manifiesta como una serie de eventos que desencadenan una vasoconstricción, que produce una disminución del flujo sanguíneo renal (FSR) poco después de la administración de cisplatino. Además, en el daño endotelial también se observa una sobreexpresión de moléculas de adhesión celular en el endotelio vascular, seguido de una adhesión de leucocitos (Luke y cols., 1992). Como consecuencia se produce una obstrucción de los pequeños vasos impidiendo el flujo sanguíneo, lo que contribuye a la producción de factores promotores de la vasoconstricción. Este aumento de la sensibilidad de la arteriola glomerular aferente a la vasoconstricción podría producir la activación de los mecanismos de retroalimentación tubuloglomerular (figura 2) con la consiguiente disminución de la TFG durante la lesión renal aguda (Schrier y cols., 2004).

El daño endotelial producido por el cisplatino puede comprender también una alteración de la respuesta de las areteriolas a las sustancias vasoactivas (Bae y cos., 2009), dando lugar a un incremento de la reactividad de agentes vasoactivos como la angiotensina II (Saad y cols., 2007), adenosina (Gill y cols., 2009), endotelina-I (Bae y cols., 2009) y factor activador de plaquetas (PAF) (dos Santos y cols., 1991b). Así mismo puede producir una disminución de la respuesta a la vasodilatación mediante un descenso de la producción de oxido nítrico endotelial por parte de la

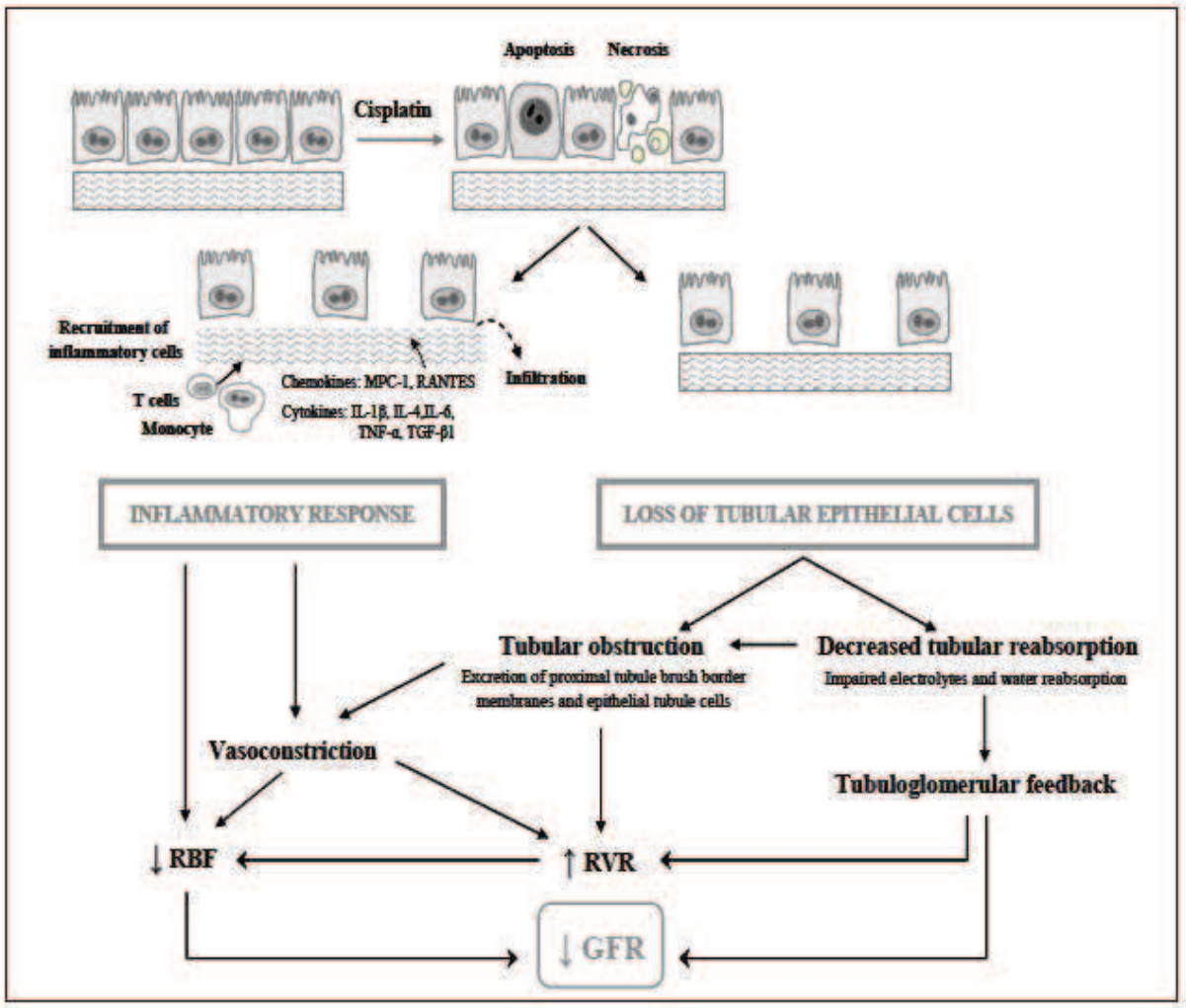


Figura 2. Fisiopatología de la nefrotoxicidad por cisplatino. Mecanismos tubulares y vasculares.

óxido nítrico sintasa endotelial (eNOS), mientras que se produce un incremento de la producción de NO por la óxido nítrico sintasa inducible (iNOS). Además existe evidencia de que la producción de prostaglandinas vasodilatatorias como es el caso de la prostaglandina E1 (PGE1) se encuentra disminuida por la administración de cisplatino.

Adicionalmente, la acumulación de citoquinas, quimioquinas y radicales libres de oxígeno (ROS), por la administración de cisplatino es posible que produzca una activación de la expresión de moléculas de adhesión en el endotelio vascular y leucocitos circulatorios, que puede favorecer a la respuesta inflamatoria y al tono vascular, contribuyendo a la reducción de la TFG (Bae y cols., 2009)

3.1.2. Autorregulación vascular anormal

Un importante componente en la fisiopatología del daño renal agudo producido por el cisplatino está relaccionada con reducciones en el FSR medular en el que se observa una disminución de la liberación de oxígeno desde las células renales (Winston y Sarfistein, 1985). El riñón isquémico muestra una vasodilatación pronunciada que puede llegar a transformarse en daño hipóxico como consecuencia de una alteración en la función vascular normal del FSR. Además se observa una reactividad vascular anormal puesto que se produce un aumento a la sensibilidad de los agentes vasoconstrictores y una mayor respuesta a la estimulación nerviosa renal en las arteriolas de los riñones isquémicos por tratamiento con cisplatino (Khan y cols., 2009). Las alteraciones hemodinámicas

renales observadas podrían ser debidas a un incremento del calcio citosólico en las arteriolas glomerulares (Kawai y cols., 2006). Por otra parte, los bloqueadores de los canales de calcio han demostrado revertir el incremento de FPR atenuando así la disfunción renal asociada con la administración de cisplatino (Khan y cols., 2009).

3.2. Efectos glomerulares

El cisplatino puede dañar directamente las células glomerulares y alterar tanto su estructura como su permeabilidad dando lugar a la aparición de proteinuria en el rango nefrótico (Skinner y cols., 2009). Las alteraciones ultraestructurales que puede causar el cisplatino muestran que existe daño en todos los componentes glomerulares. Esta lesión se manifiesta con aparición de extrusiones citoplasmáticas endoteliales dentro del lumen vascular, ensanchamiento y laminación de la membrana basal capilar, fusión focal de los pedicelos de los podocitos, vacuolización citoplasmática de las células endoteliales, podocitos epiteliales y células parietales así como la presencia de cuerpos lipídicos y mieloides en todos los tipos de células glomerulares (Skinner y cols., 2009; Kohn y cols., 2002).

La inducción de daño glomerular por el cisplatino está caracterizada por un marcado descenso de la TFG y el FPR que está asociada con un marcado descenso en el coeficiente de ultrafiltración (Kf) y un aumento de la resistencia vascular renal (RVR) (dos Santos y cols., 1991a), que sugiere que se deriva de una vasoconstricción preglomerular (Winston y cols., 1985; Safirstein y cols., 1986). Se ha comprobado que el cisplatino altera la función de filtración glomerular principalmente mediante una disminución de la permeabilidad de la membrana de filtración, como resultado de una contracción de las células mesangiales (Mene y cols., 1989).

3. 3. Efectos tubulares

El túbulo proximal es el sitio donde se produce principalmente el daño renal inducido por una gran cantidad de tóxicos. El cisplatino es captado por las células tubulares renales, alcanzando su máxima concentración en las células de la corteza interna y médula externa, donde produce mayor lesión, pero también puede dañar al túbulo distal y a los conductos colectores de forma dependiente de la dosis (Taguchi y cols., 2005, Arany y cols., 2003; Kroning y cols., 2000).

El cisplatino entra en las células tubulares renales mediante un proceso de difusión pasiva, pero también puede hacerlo mediante un proceso mediado por transportadores. Los transportadores basolaterales de cationes orgánicos (OCTs) han sido identificados como los mecanismos responsables de la captación del cisplatino (Ciarimboli y cols., 2011). Más concretamente, el transportador OCT2 es el que determina la captación de cisplatino y por lo tanto su citotoxicidad (Yokoo y cols., 2009; Burguer y cols., 2011).

Una vez dentro de las células, los iones cloruro se disocian de la molécula, debido a las bajas concentraciones de cloruro intracelular (Ekborn y cols., 2003) provocando la formación de especies acuatadas mediante reacciones hidrolíticas. Los iones de platino cargados positivamente son más tóxicos y altamente reactivos que el compuesto en sí, lo que aumenta su afinidad por el ADN, ARN y proteínas (Cohen y cols., 2001; Townsend y cols., 2003). El cisplatino forma puentes inter- e intra- catenarios con el ADN renal. Los aductos de platino-ADN activan varias respuestas celulares, incluyendo la señalización del daño del ADN, los puntos de control del ciclo celular y el arresto, reparación del ADN y muerte celular.

Los mecanismos fisiopatológicos mediante los cuales el cisplatino produce daño renal tubular se encuentran resumidos en la figura 3.

3.3.1. Alteración de los sistemas de transporte

La nefrotoxicidad del cisplatino se caracteriza por una disfunción de las células renales del túbulo proximal. En particular, interfiere con los transporta-

dores de sodio como la Na/K ATPasa, el co-transportador Na/K/2Cl y el intercambiador Na/H tipo III y también con los canales de agua de la membrana como las acuaporinas 1, 2 y 3(Pedersen y cols., 2005; Bae y cols., 2009). Como consecuencia, se produce una alteración en el transporte de agua, nutrientes y electrolitos en las células tubulares renales. En este sentido, el cisplatino inhibe la actividad de los transportadores en el borde en cepillo de la membrana renal tanto *in vivo* como *in vitro* (Yoshiki y cols., 2000). El daño producido por cisplatino puede perturbar la integridad del citoesqueleto y la polaridad celular, dando lugar a cambios en el manejo de los iones de hidrógeno, magnesio, potasio y calcio, lo que contribuye a que se produzca una disminución en la reabsorción de dichos iones en los túbulos proximal y distal y por lo tanto, un incremento de la excreción urinaria (Lajer y cols., 2005). Además puede provocar la pérdida de la barrera epitelial celular y/o las uniones estrechas entre células viables durante el proceso de inducción de daño renal por parte del cisplatino, pudiendo incluso producir una disminución de la TFG, como se ilustra en la figura 2.

3.3.2. Disfunción mitocondrial

La mitocondria es un punto clave para la nefrotoxicidad del cisplatino, por ello la aparición de disfunción mitocondrial producida por la administración de cisplatino puede suceder de forma temprana (Santos y cols., 2008). El cisplatino puede acumularse en las mitocondiras de las células renales, afectando a la bioenergética mitocondrial, lo que incrementa la generación de ROS, produce una disminución del consumo mitocondrial de calcio y una liberación de factores pro-apoptóticos, dando lugar a la muerte de las células tubulares renales (Chang y cols., 2002).

3.3.3. Estrés oxidativo y nitrosativo

Es bien conocido que el estrés oxidativo está involucrado en la producción de daño renal por administración de cisplatino (Chirino y cols., 2009). Se ha sugerido que entre los principales mecanismos mediante los cuales el cisplatino produce nefrotoxicidad se encuentran la generación de ROS, la depleción de los sistemas antioxidantes y la estimulación de la peroxidación lipídica (Aydinoz y cols., 2007). La producción de estrés oxidativo por el cisplatino se debe a la excesiva producción de ROS por la mitocondria dañada, incluyendo al anión superóxido ($O_2^{\bullet -}$), el peróxido de hidrógeno (H_2O_2) y el radical hidroxilo ($^{\bullet}OH$), y a una disminución en los sistemas de defensa antioxidante, como el glutation reducido (GSH), la superóxido dismutasa (SOD), catalasa (CAT) y glutation peroxidasa (GPx) (Badari y cols., 2005; Kawai y cols., 2009). Recientemente se ha asociado la nefrotoxicidad del cisplatino con el estrés nitrosativo. Hay evidencias de que los efectos celulares de los ROS están amplificados por una producción masiva de óxido nítrico ($NO^{\bullet}$), posiblemente por la iNOS, como consecuencia se produce una contínua formación de peroxinitritos ($ONOO^-$) (Saleh y cols., 2001), contribuyendo al daño renal producido por el cisplatino.

Esta situación en la que se ve incrementada la producción de especies reactivas del oxígeno y del nitrógeno tras el tratamiento con cisplatino es conocida como estrés oxidativo y nitrosativo y resulta en un significativo daño renal de las estructuras celulares y de sus funciones, incluyendo a la peroxidación lipídica, nitración de proteínas, inactivación enzimática y rotura del ADN. Como consecuencia, induce disfunción celular y la generación de señales intracelulares para la activación de los procesos de apoptosis y por lo tanto de muerte celular.

3.3.4. Respuesta inflamatoria

El cisplatino activa la fosforilación y la posterior translocación del factor de transcripción kappa B (NF-κB) al núcleo, a través de la degradación de la proteína inhibitoria IκBα (Lee y cols., 2006; Sung y cols., 2008). La activación de NF-κB promueve la transcripción de genes específicos que codifican mediadores inflamatorios, promoviendo las respuestas inmunológica, proliferativa, antiapoptósica e inflamatoria. (Lee y cols., 2006). Este evento da lugar a un

incremento de la expresión del factor de necrosis tubular α (TNF-α) en las células tubulares renales. El TNF-α es una citoquina muy importante involucrada en la inflamación sistémica y en la respuesta de fase aguda inducida por la administración de cisplatino. Ejerce su función directamente a través del receptor tipo 1 (TNFR1), pero también lo puede hacer de modo indirecto mediante una activación de la respuesta inmune via receptor tipo 2 (TNFR2). Adicionalmente la señal TNF- α/TNFR2 puede potenciar los efectos proapotósicos de la activación del TNFR1 lo que contribuye a la nefrotoxicidad del cisplatino (Ramesh y cols., 2003).

El TNF-α además coordina la activación de gran cantidad de citoquinas pro-inflamatorias como las interleuquinas 1, 4, 6 (IL-1β, IL-4, IL-6), factor de crecimiento transformante – β1 (TGF- β1) y las proteína quimioatractante de monolitos 1 (MCP1 del ingés *monocyte chemoattractant protein-1*) y células RANTES (del inglés *regulated upon activation normal T-cell expressed and secreted*). Además TNF-α induce la expresión de moléculas de adhesión entre las que se incluyen la molécula de adhesión intercelular 1 (ICAM-1), la molécula de adhesión vascular 1 (VCAM-1) y la E-selectina, promoviendo un influjo de células inflamatorias dentro del tejido. (Ramesh y cols., 2002; Zhang., 2007; Kang., 2009; Mukhopadhyay y cols., 2011).

La expresión de los factores de crecimiento y las citoquinas, cuando ya existe un problema inflamatorio desemboca en el desarrollo de fibrosis intersticial (Bolhe y cols., 1996), caracterizada por la expansión de la membrana basal tubular y de la matriz extracelular intersticial (Norman y cols., 1996). Estas alteraciones dan lugar a una pérdida del balance entre la tasa de producción de matriz extracelular y la inactivación de enzimas proteolíticas encargadas de degra-

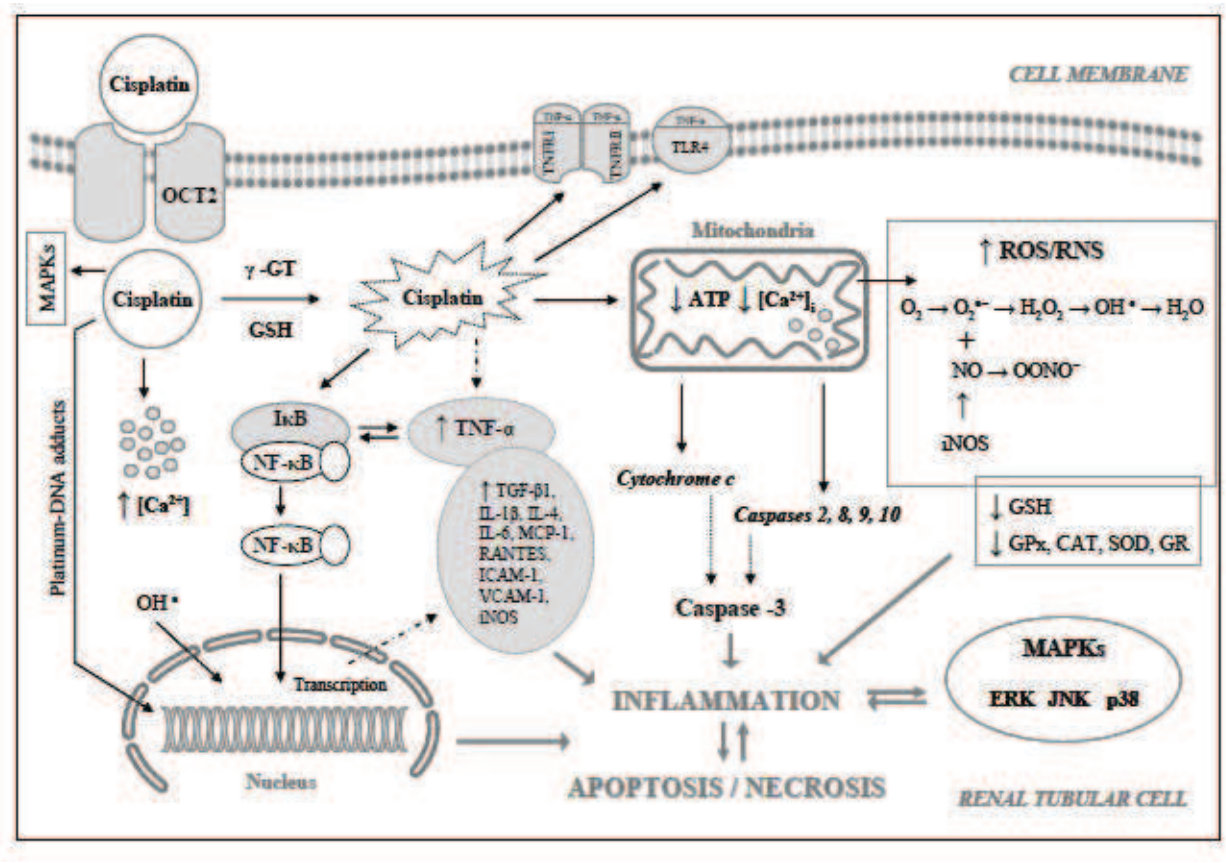


Figura 3. Principales mecanismos intracelulares involucrados en la inducción de daño renal tubular por cisplatino

dar los componentes de la matriz, con el consiguiente desarrollo de fibrosis (Norman y cols., 1995).

3.3.5. Activación de proteínas quinasas activadas por mitógeno (MAPKs)

El mecanismo de señalización de las MAPKs consiste en una serie de quinasas que se activan por diversos tipos de estrés extracelular, que regulan la homeostasis celular y procesos como los de la proliferación celular, diferenciación, apoptosis y supervivencia (Owens y cols., 2007). Investigaciones recientes con modelos experimentales de nefrotoxicidad por cisplatino *in vivo* e *in vitro* han observado una expresión diferencial de las principales quinasas: quinasa Jun N-terminal (JNK), p38 y la proteína quinasa activada por señales extracelulares (ERK). Otro estudio (Arany y cols., 2004) realizado *in vivo*, sugirió que la activación temprana de ERK, P38 y JNK por cisplatino, precedía al desarrollo de daño renal. Sin embargo, el papel que juega cada quinasa parece ser muy complicado de explicar y varía dependiendo del tipo celular, del perfil de estrés y de las señales de crecimiento a las que las células están expuestas. La activación de dichas quinasas podría ser, en parte, responsable de la activación de varios mecanismos de señalización que culminan en daño celular renal y muerte durante la nefrotoxicidad producida por cisplatino (Pabla y cols., 2009).

4. DIAGNÓSTICO DE LA NEFROTOXICIDAD PRODUCIDA POR EL CISPLATINO.

A pesar de que la administración de cisplatino se realiza de forma controlada y tras la previa realización de pruebas de funcionalidad renal, durante y después del tratamiento, la aparición de nefrotoxicidad suele aparecer en un porcentaje elevado de pacientes. Diversos estudios tratan de encontrar un marcador temprano de daño renal capaz de diagnosticarlo en sus primeras fases de desarrollo.

En humanos, antes de la administración de cisplatino, generalmente se mide BUN, creatinina plasmática y se realiza el cálculo del aclaramiento de creatinina mediante el uso de diversas fórmulas (Giannattasio y cols., 1983). Sin embargo, el cálculo de este parámetro presenta problemas puesto que no se correlaciona totalmente con la TFG. Bardi y cols., (2004) propusieron el cálculo del aclaramiento de cistatina C, en pacientes pediátricos, como mejor marcador para caracterizar la función glomerular que la creatinina, ya que la cistatina C tiene una tasa de producción mucho más estable, es independiente de la talla, del peso, de la masa muscular y del género. Además, observaron que la concentración plasmática de cistatina C aumentaba de forma significativa antes que la cratinina.

En algunos casos, también se miden iones séricos y urinarios (calcio, magnesio, fósforo y potasio) así como las enzimas hepáticas en orina: aspartato aminotransferasa (AST) y alanino aminotransferasa (ALT) (Giannattasio y cols., 1983). Estas pruebas de funcionalidad renal también han de realizarse durante y después del tratamiento con cisplatino.

La excreción urinaria de marcadores de daño tubular se ha propuesto como posible método de detección temprana como es el caso de la beta-2-microglobulina, la NAG y la alfa-1-glicoproteína ácida, de las que se ha observado un aumento tras la administración de cisplatino en humanos (Anand y cols., 2011). Pero, como se comentó anteriormente en el apartado 3.4., de la introducción, estos marcadores poseen algunos inconvenientes puesto que la información que nos proporcionan es limitada y su aparición sucede cuando ya existe algún tipo de alteración.

Un marcador más específico y temprano en la detección del daño por cisplatino es la proteína lipocalina asociada a la gelatinasa neutrófila (NGAL, por sus siglas en inglés *neutrophil gelatinase associated lipocalin*). Estudios realizados con animales de experimentación, observaron concentraciones elevadas de NGAL tras la administración de cisplatino, de forma

aguda, en ratones (20 mg/Kg o 5 mg/Kg). Compararon la excreción urinaria de NGAL con la aparición de otros marcadores como NAG en orina o creatinina en plasma. Mientras los marcadores habituales no variaban sus valores hasta 96 horas después de la administración de cisplatino, la excreción de NGAL ya se había incrementado a las 3 horas. Asimismo, en estudios clínicos se ha corroborado el incremento de ésta proteína, tras observar niveles elevados de NGAL en plasma y en orina de pacientes tratados con cisplatino, además observaron que este incremento precedía al diagnosticado por los métodos habituales de la práctica clínica (Haase y cols., 2010, Gaspary y cols., 2010).

Otra proteína estudiada como posible marcador temprano de daño renal por el cisplatino es la molécula de lesión renal 1 (KIM-1 por sus siglas en inglés *kidney injury molecule 1*). Se observó un incremento en la excreción urinaria de este polipéptido en ratones tratados con 3 o 6 mg/Kg de cisplatino vía iv a las 48 horas, mientras que la NAG no aumentó hasta las 72 horas (Espandian y cols., 2009). También se observó un aumento en la excreción urinaria de KIM-1 en ratas tratadas con 10 mg/Kg de cisplatino vía i.p., en este caso, el incremento se produjo al día siguiente de la administración del citostático, mientras que los niveles de creatinina plasmática no aumentaron hasta dos días después de dicha administración. En ratas Sprague-Dawley también se detectó el incremento de KIM-1 que se produjo tres días después de administrar 3,5mg/Kg de cisplatino vía i.p. (Vaidya y cols., 2009). Estos autores concluyen que el incremento renal de KIM-1 se correlaciona con los diferentes grados de daño tubular y que proporcionan una mejor detección del daño renal, más específica y más temprana que el BUN, la creatinina plasmática o la enzima NAG.

Otras proteínas han sido probadas también en la detección temprana de la nefrotoxicidad de cisplatino, como es el caso de la proteína netrina-1 (PM 75KDa) cuya aparición en orina sucedía antes que la de NGAL en un estudio realizado con ratones (Reeves y cols., 2008), o de la fetuina A exosomal, que apareció excretada en la orina 24 h después de la administración de cisplatino en ratas, antes de observar incrementos en la creatinina plasmática y el BUN (Zhou et al., 2008)

Tonomura y cols., 2010 realizaron una evaluación de diferentes marcadores urinarios tras tratamiento con cisplatino, encontraron que los marcadores KIM-1 y glutation-S-transferasa μ (GST-μ) presentaban una alta sensibilidad a la detección de la nefrotoxicidad del cisplatino mientras que los marcadores GST-α, lactato deshidrogenasa (LDH) y albúmina urinaria eran relativamente sensibles y que, de todos los estudiados, la proteína NGAL es el marcador más exacto y sensible.

Pero no solamente se han estudiado proteínas como posibles marcadores. En el trabajo de Portilla y cols. (2006) se observó un incremento en la excreción urinaria de glucosa, aminoácidos y metabolitos del ciclo del ácido tricarboxílico dos días después del tratamiento con cisplatino. El aumento en la excreción urinaria de aminoácidos tras la administración de cisplatino se ha observado también en otra serie de trabajos experimentales, como es el caso de la carnitina (Hascke y cols., 2010) histidina, glicil prolina (Boudonck y cols., 2010), alanina, leucina, metionina (Portilla y cols., 2006; 2007) succinato y fumarato (Xu y cols., 2008).

En perros se ha observado la excreción de citoquinas y quimioquinas urinarias [interleucinas (IL-2, IL-8, IL-7), MCP-1, chemoquina derivada de queratinocitos (KC, por sus siglas en inglés *keratinocite-derived chemokine*) y el factor estimulante de colonias de macrógos y granulocitos (GMCSF, por sus siglas en inglés *granulocyte macrophague colonyl-stimulating factor*)] en la orina tras la administración del cisplatino (vía i.v. a la dosis de 0,75 mg/Kg/día durante 5 días), y este aumento apareció antes de que se observaran incrementos de la creatinina en plasma (McDuffie y cols., 2010).

De todos los marcadores mencionados anteriormente, la agencia europea del medicamento (EMA) ha realizado una valoración y ha concluido que los biomarcadores urinarios KIM-1, Albúmina, beta-2-microglobulina, trefoil factor 3 y cistatina C urinaria son considerados como aceptables en el concepto de determinaciones no clínicas, ya que proporcionan información complementaria a la obtenida con el BUN y la creatinina, y que se correlación con las alteraciones histopatológicas observadas. Además reconocen que se deben realizar más estudios y así poder valorar si estos marcadores podrían tener utilidad en un futuro en la práctica clínica (EMA, 2011).

5. MECANISMOS NEFROPROTECTORES DEL DAÑO TÓXICO POR CISPLATINO.

En una visión histórica, los esfuerzos por controlar la toxicidad renal del cisplatino se pueden agrupar en dos grandes períodos. El primero basado en las modificaciones de los protocolos de perfusión, en cuanto a tiempos y vehículos de administración del cisplatino, y fue una mezcla de empirismo y de investigación guiada por las propiedades químicas y cinéticas del fármaco. Pertenecen a esta estrategia el fraccionamiento de la dosis, la intensificación de la diuresis, y el uso concomitante de suero salino hipertónico.

La segunda época o de modulación farmacológica, en la que aún nos encontramos, se caracterizaría por el empleo concurrente con el cisplatino de diversos fármacos no citostáticos, que pudieran inhibir los efectos tóxicos del cisplatino sin alterar su función terapéutica.

Hasta la fecha, la administración de coadyuvantes nefroprotectores no ha logrado resultados clínicos satisfactorios. Existe un amplio arsenal de compuestos en fase de desarrollo experimental, encaminados a facilitar la eliminación y evitar la acumulación del cisplatino en los túbulos renales, a formar complejos menos tóxicos, o a modular las vías de señalización implicadas en su efecto tóxico. Entre ellos se incluyen: (1) la *cimetidina*, que compite con el cisplatino por el OTC2; (2) la *procainamida*, que forma complejos menos tóxicos con el cisplatino; (3) la *L-arginina*, como modulador del óxido nítrico; (4) los *salicilatos*, la *hormona estimulante de melanocitos* y la IL-10, como supresores de mediadores proinflamatorios; (5) la *amifostina*, como citoprotector por donación de un grupo tiol; (6) los inhibidores de la ruta de señalización intracelular de las MAPKs, como son PD98059 y UO126 [inhibidores de MEK/ERK], SKF-86002 y SB203580 [inhibidores de p38] o SP600125 [inhibidor de JNK] (Pabla y Dong, 2008; Ali y Al Moundhri, 2006); o (7) la *quercetina* como antioxidante, que además ejerce mejoras en la hemodinámica renal y reduce la inflamación en el riñón (Sánchez-González y cols., 2011)

La amifostina, un tiofosfato orgánico, es el único fármaco aprobado por la FDA para su uso en pacientes sometidos a tratamiento con cisplatino, con el objetivo de reducir su toxicidad (Capizzi, 1999). No obstante, el descubrimiento y desarrollo de fármacos nefroprotectores realmente útiles en la clínica sigue siendo un objetivo pendiente.

6. BIBLIOGRAFÍA

Ali, B. H., Al-Moundhri, M., Tageldin, M., Al Husseini, I. S., Mansour, M. A., Nemmar, A., and Tanira, M. O. (2008). Ontogenic aspects of cisplatin-induced nephrotoxicity in rats. *Food Chem Toxicol* **46**, 3355-9.

Arany, I., Megyesi, J. K., Kaneto, H., Price, P. M., and Safirstein, R. L. (2004). Cisplatin-induced cell death is EGFR/src/ERK signaling dependent in mouse proximal tubule cells. *Am J Physiol Renal Physiol* **287**, F543-9.

Arany, I., and Safirstein, R. L. (2003). Cisplatin nephrotoxicity. *Seminars in nephrology* **23**, 460-4.

Aydinoz, S., Uzun, G., Cermik, H., Atasoyu, E. M., Yildiz, S., Karagoz, B., and Evrenkaya, R. (2007). Effects of different doses of hyperbaric oxygen on cisplatin-induced nephrotoxicity. *Renal failure* **29**, 257-63.

Bae, E. H., Lee, J., Ma, S. K., Kim, I. J., Frokiaer, J., Nielsen, S., Kim, S. Y., and Kim, S. W. (2009). alpha-Lipoic acid prevents cisplatin-induced acute kidney injury in rats. *Nephrol Dial Transplant* **24**, 2692-700.

Bohle, A., Muller, G. A., Wehrmann, M., y cols. (1996). Pathogenesis of chronic renal failure in the primary glomerulopathies, renal vasculopathies, and chronic interstitial nephritides. *Kidney Int* **54**, S2–S9,

Boudonck, K. J., Mitchell, M. W., Nemet, L., Keresztes, L., Nyska, A., Shinar, D., and Rosenstock, M. (2009). Discovery of metabolomics biomarkers for early detection of nephrotoxicity. *Toxicol Pathol* **37**, 280-92.

Brodsky, S. V., Yamamoto, T., Tada, T., Kim, B., Chen, J., Kajiya, F., and Goligorsky, M. S. (2002). Endothelial dysfunction in ischemic acute renal failure: rescue by transplanted endothelial cells. *Am J Physiol Renal Physiol* **282**, F1140-9.

Burger, H., Zoumaro-Djayoon, A., Boersma, A. W., Helleman, J., Berns, E. M., Mathijssen, R. H., Loos, W. J., and Wiemer, E. A. Differential transport of platinum compounds by the human organic cation transporter hOCT2 (hSLC22A2). *British journal of pharmacology* **159**, 898-908.

Ciarimboli, G., Deuster, D., Knief, A., Sperling, M., Holtkamp, M., Edemir, B., Pavenstadt, H., Lanvers-Kaminsky, C., am Zehnhoff-Dinnesen, A., Schinkel, A. H., Koepsell, H., Jurgens, H., and Schlatter, E. Organic cation transporter 2 mediates cisplatin-induced oto- and nephrotoxicity and is a target for protective interventions. *Am J Pathol* **176**, 1169-80.

Cohen, S. M., and Lippard, S. J. (2001). Cisplatin: from DNA damage to cancer chemotherapy. *Progress in nucleic acid research and molecular biology* **67**, 93-130.

Chang, B., Nishikawa, M., Sato, E., Utsumi, K., and Inoue, M. (2002). L-Carnitine inhibits cisplatin-induced injury of the kidney and small intestine. *Archives of biochemistry and biophysics* **405**, 55-64.

Chirino, Y. I. and Pedraza-Chaverri, J. (2009) Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity. *Exp Toxicol Pathol* **61**, 223-242.

Dos Santos, O. F., Boim, M. A., Barros, E. J., Pirotzky, E., Braquet, P., and Schor, N. (1991a). Effect of platelet-activating factor antagonist BN 52063 on the nephrotoxicity of cisplatin. *Lipids* **26**, 1324-8.

Dos Santos, O. F., Boim, M. A., Barros, E. J., and Schor, N. (1991b). Role of platelet activating factor in gentamicin and cisplatin nephrotoxicity. *Kidney Int* **40**, 742-7.

Ekborn, A., Lindberg, A., Laurell, G., Wallin, I., Eksborg, S., and Ehrsson, H. (2003). Ototoxicity, nephrotoxicity and pharmacokinetics of cisplatin and its monohydrated complex in the guinea pig. *Cancer Chemother Pharmacol* **51**, 36-42.

EMEA,(20011)http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/10/WC500004205.pdf

Engineer, M. S., Bodey, G. P., Sr., Newman, R. A., and Ho, D. H. (1987). Effects of cisplatin-induced nephrotoxicity on gentamicin pharmacokinetics in rats. *Drug Metab Dispos* **15**, 329-34.

Espandiari, P., Rosenzweig, B., Zhang, J., Zhou, Y., Schnackenberg, L., Vaidya, V. S., Goering, P. L., Brown, R. P., Bonventre, J. V., Mahjoob, K., Holland, R. D., Beger, R. D., Thompson, K., Hanig, J., and Sadrieh, N. (2009). Age-related differences in susceptibility to cisplatin-induced renal toxicity. *J Appl Toxicol* **30**, 172-182.

Faubel, S., Lewis, E. C., Reznikov, L., Ljubanovic, D., Hoke, T. S., Somerset, H., Oh, D. J., Lu, L., Klein, C. L., Dinarello, C. A., and Edelstein, C. L. (2007). Cisplatin-induced acute renal failure is associated with an increase in the cytokines interleukin (IL)-1beta, IL-18, IL-6, and neutrophil infiltration in the kidney. *The Journal of pharmacology and experimental therapeutics* **322**, 8-15.

Giannattasio, M., Rizzi, R., Coratelli, P., Restaino, A., Selvaggi, L., Ferreri, R., Orlando, E., Lerdardi, G. M., Grannata, A., Bettochi, S. (1983). Use of cisplatinum in the treatment of ovarian carcinoma. Clical results and evaluation of nephrotoxicity. *Eur J Gynaelcol Oncol*. **4**(1), 1-3.

Gill, A., Wortham, K., Costa, D., Davis, W., Ticho, B., and Whalley, E. (2009). Protective effect of tonapofylline (BG9928), an adenosine A1 receptor antagonist, against cisplatin-induced acute kidney injury in rats. *American journal of nephrology* **30**, 521-6.

Gonzalez, R., Romay, C., Borrego, A., Hernandez, F., Merino, N., Zamora, Z., and Rojas, E. (2005). Lipid peroxides and antioxidant enzymes in cisplatin-induced chronic nephrotoxicity in rats. *Mediators Inflamm* **2005**, 139-43.

Haschke, M., Vitins, T., Lude, S., Todesco, L., Novakova, K., Herrmann, R., and Krahenbuhl, S. Urinary excretion of carnitine as a marker of proximal tubular damage associated with platin-based antineoplastic drugs. *Nephrol Dial Transplant* **25**, 426-33.

Hellwig-Burgel, T., Stiehl, D. P., Katschinski, D. M., Marxsen, J., Kreft, B., and Jelkmann, W. (2005). VEGF production by primary human renal proximal tubular cells: requirement of HIF-1, PI3-kinase and MAPKK-1 signaling. *Cell Physiol Biochem* **15**, 99-108.

Hill, J. M., Loeb, E., MacLellan, A.S., Hill, N. O., Khan, A., and Kogler, J. (1974). Further clinical experience with cis-platinum II diamminedichloride. In Platinium Coordination Complexes in Cancer Chemoterapy, T. A. Conners and J. J. Roberts. Eds. New York, Springer-Verlag, pp 145 -152.

Kang, D. G., Lee, A. S., Mun, Y. J., Woo, W. H., Kim, Y. C., Sohn, E. J., Moon, M. K., and Lee, H. S. (2004). Butein ameliorates renal concentrating ability in cisplatin-induced acute renal failure in rats. *Biological & pharmaceutical bulletin* **27**, 366-70.

Kang, K. P., Kim, D. H., Jung, Y. J., Lee, A. S., Lee, S., Lee, S. Y., Jang, K. Y., Sung, M. J., Park, S. K., and Kim, W. (2009). Alpha-lipoic acid attenuates cisplatin-induced acute kidney injury in mice by suppressing renal inflammation. *Nephrol Dial Transplant* **24**, 3012-20.

Kartalou, M., and Essigmann, J. M. (2001). Mechanisms of resistance to cisplatin. *Mutation research* **478**, 23-43.

Kaushal, G. P., Kaushal, V., Hong, X., and Shah, S. V. (2001). Role and regulation of activation of caspases in cisplatin-induced injury to renal tubular epithelial cells. *Kidney Int* **60**, 1726-36.

Kawai, Y., Nakao, T., Kunimura, N., Kohda, Y., and Gemba, M. (2006). Relationship of intracellular calcium and oxygen radicals to Cisplatin-related renal cell injury. *Journal of pharmacological sciences* **100**, 65-72.

Kawai, Y., Satoh, T., Hibi, D., Ohno, Y., Kohda, Y., Miura, K., and Gemba, M. (2009). The effect of antioxidant on development of fibrosis by cisplatin in rats. *Journal of pharmacological sciences* **111**, 433-9.

Kawai, Y., Taniuchi, S., Okahara, S., Nakamura, M., and Gemba, M. (2005). Relationship between cisplatin or nedaplatin-induced nephrotoxicity and renal accumulation. *Biological & pharmaceutical bulletin* **28**, 1385-8.

Khan, A. H., Sattar, M. A., Abdullah, N. A., and Johns, E. J. (2009). Effect of calcium channel blockade on adrenergically induced renal vasoconstriction in rat models of renal impairment. *Clinical and experimental pharmacology & physiology* **36**, 501-8.

Kishore, B. K., Krane, C. M., Di Iulio, D., Menon, A. G., and Cacini, W. (2000). Expression of renal aquaporins 1, 2, and 3 in a rat model of cisplatin-induced polyuria. *Kidney Int* **58**, 701-11.

Kohn, S., Fradis, M., Ben-David, J., Zidan, J., and Robinson, E. (2002). Nephrotoxicity of combined treatment with cisplatin and gentamicin in the guinea pig: glomerular injury findings. *Ultrastruct Pathol* **26**, 371-82.

Kroning, R., Lichtenstein, A. K., and Nagami, G. T. (2000). Sulfur-containing amino acids decrease cisplatin cytotoxicity and uptake in renal tubule epithelial cell lines. *Cancer Chemother Pharmacol* **45**, 43-9.

Kuriakose, G. C., and Kurup, M. G. (2008). Evaluation of renoprotective effect of Aphanizomenon flos-aquae on cisplatin-induced renal dysfunction in rats. *Renal failure* **30**, 717-25.

Lajer, H., Kristensen, M., Hansen, H. H., Nielsen, S., Frokiaer, J., Ostergaard, L. F., Christensen, S., Daugaard, G., and Jonassen, T. E. (2005). Magnesium depletion enhances cisplatin-induced nephrotoxicity. *Cancer Chemother Pharmacol* **56**, 535-42.

Launay-Vacher, V., Isnard-Bagnis, C., Janus, N., Karie, S., and Deray, G. (2008). [Chemotherapy and renal toxicity]. *Bulletin du cancer* **95 FMC Onco**, F96-103.

Lee, C. K., Park, K. K., Hwang, J. K., Lee, S. K., and Chung, W. Y. (2009). Extract of Prunus persica flesh (PPFE) improves chemotherapeutic efficacy and protects against nephrotoxicity in cisplatin-treated mice. *Phytother Res* **23**, 999-1005.

Lee, S., Kim, W., Moon, S. O., Sung, M. J., Kim, D. H., Kang, K. P., Jang, Y. B., Lee, J. E., Jang, K. Y., and Park, S. K. (2006). Rosiglitazone ameliorates cisplatin-induced renal injury in mice. *Nephrol Dial Transplant* **21**, 2096-105.

Liedert, B., Pluim, D., Schellens, J., and Thomale, J. (2006). Adduct-specific monoclonal antibodies for the measurement of cisplatin-induced DNA lesions in individual cell nuclei. *Nucleic acids research* **34**, e47.

Luke, D. R., Vadiei, K., and Lopez-Berestein, G. (1992). Role of vascular congestion in cisplatin-induced acute renal failure in the rat. *Nephrol Dial Transplant* **7**, 1-7.

Markowitz, G. S., Appel, G. B., Fine, P. L., Fenves, A. Z., Loon, N. R., Jagannath, S., Kuhn, J. A., Dratch, A. D., and D'Agati, V. D. (2001). Collapsing focal segmental glomerulosclerosis following treatment with high-dose pamidronate. *J Am Soc Nephrol* **12**, 1164-72.

Mene, P., Simonson, M. S., and Dunn, M. J. (1989). Physiology of the mesangial cell. *Physiological reviews* **69**, 1347-424.

Meyer, K. B., and Madias, N. E. (1994). Cisplatin

nephrotoxicity. *Mineral and electrolyte metabolism* **20**, 201-13.

Miyamoto, Y., Shimada, K., Sakaguchi, Y., and Miyamoto, M. (2007). Cisplatin (CDDP)-induced acute toxicity in an experimental model of hepatic fibrosis. *J Toxicol Sci* **32**, 311-9.

Mukhopadhyay, P., Rajesh, M., Pan, H., Patel, V., Mukhopadhyay, B., Batkai, S., Gao, B., Hasko, G., and Pacher, P. Cannabinoid-2 receptor limits inflammation, oxidative/nitrosative stress, and cell death in nephropathy. *Free radical biology & medicine* **48**, 457-67.

Negishi, K., Noiri, E., Doi, K., Maeda-Mamiya, R., Sugaya, T., Portilla, D., and Fujita, T. (2009). Monitoring of urinary L-type fatty acid-binding protein predicts histological severity of acute kidney injury. *Am J Pathol* **174**, 1154-9.

Norman, J. T., Lewis, M. P. (1996). Matrix metalloproteinases (MMPs) in renal fibrosis. *Kidney Int* **49**(54), S61–S63.

Norman, J. T., Gatti, L., Wilson, P. D., Lewis, M. (1999). Matrix metalloproteinases and tissue inhibitor of matrix metalloproteinases expression by tubular epithelia and interstitial fibroblasts in the normal kidney and in fibrosis. *Exp Nephrol* **3**, 88–89.

Owens, D. M., and Keyse, S. M. (2007). Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. *Oncogene* **26**, 3203-13.

Pabla, N., and Dong, Z. (2008). Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. *Kidney Int* **73**, 994-1007.

Pabla, N., Murphy, R. F., Liu, K., and Dong, Z. (2009). The copper transporter Ctr1 contributes to cisplatin uptake by renal tubular cells during cisplatin nephrotoxicity. *Am J Physiol Renal Physiol* **296**, F505-11.

Pedersen, P. S., Procida, K., Larsen, P. L., Holstein-Rathlou, N. H., and Frederiksen, O. (2005). Water permeability in human airway epithelium. *Pflugers Arch* **451**, 464-73.

Peyrou, M., Hanna, P. E., and Cribb, A. E. (2007). Cisplatin, gentamicin, and p-aminophenol induce markers of endoplasmic reticulum stress in the rat kidneys. *Toxicol Sci* **99**, 346-53.

Ramesh, G. and Reeves, W. B. (2003) TNFR2-mediated apoptosis and necrosis in cisplatin-induced acute renal failure. *Am J Physiol Renal Physiol* **285**, F610-618.

Ramesh, G., and Reeves, W. B. (2002). TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *The Journal of clinical investigation* **110**, 835-42.

Ramesh, G., and Reeves, W. B. (2004). Inflammatory cytokines in acute renal failure. *Kidney international*, S56-61.

Rozencweig, M., Von Hoff, D., D., Slavik, M., Muggia, F. M. (1977). Cis-diamminechloroplatinium (II): a new anticancer drug. *Ann Intern Med*. 86:803.

Saad, S. Y., Arafah, M. M., and Najjar, T. A. (2007). Effects of mycophenolate mofetil on cisplatin-induced renal dysfunction in rats. *Cancer Chemother Pharmacol* **59**, 455-60.

Safirstein, R., Winston, J., Goldstein, M., Moel, D., Dikman, S., and Guttenplan, J. (1986). Cisplatin nephrotoxicity. *Am J Kidney Dis* **8**, 356-67.

Saleh, S., and El-Demerdash, E. (2005). Protective effects of L-arginine against cisplatin-induced renal oxidative stress and toxicity: role of nitric

oxide. *Basic & clinical pharmacology & toxicology* **97**, 91-7.

Sanchez-Gonzalez, P. D., Lopez-Hernandez, F. J., Lopez-Novoa, J. M., and Morales, A. I. An integrative view of the pathophysiological events leading to cisplatin nephrotoxicity. *Critical reviews in toxicology*.

Sanchez-Gonzalez, P. D., Lopez-Hernandez, F. J., Perez-Barriocanal, F., Morales, A. I., Lopez-Novoa, J. M. (2011). Quercetin reduces cisplatin nephrotoxicity in rats, without compromising its antitumour activity. *Nephrol Dial Transplant.* (Eupub ahead of print)

Santos, N. A., Bezerra, C. S., Martins, N. M., Curti, C., Bianchi, M. L., and Santos, A. C. (2008). Hydroxyl radical scavenger ameliorates cisplatin-induced nephrotoxicity by preventing oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. *Cancer Chemother Pharmacol* **61**, 145-55.

Schrier, R. W., Chen, Y. C., and Cadnapaphornchai, M. A. (2004). From finch to fish to man: role of aquaporins in body fluid and brain water regulation. *Neuroscience* **129**, 897-904.

Shirwaikar, A., Issac, D., and Malini, S. (2004). Effect of Aerva lanata on cisplatin and gentamicin models of acute renal failure. *J Ethnopharmacol* **90**, 81-6.

Skinner, R., Parry, A., Price, L., Cole, M., Craft, A. W., and Pearson, A. D. (2009). Persistent nephrotoxicity during 10-year follow-up after cisplatin or carboplatin treatment in childhood: relevance of age and dose as risk factors. *Eur J Cancer* **45**, 3213-9.

Sung, M. J., Kim, D. H., Jung, Y. J., Kang, K. P., Lee, A. S., Lee, S., Kim, W., Davaatseren, M., Hwang, J. T., Kim, H. J., Kim, M. S., Kwon, D. Y., and Park, S. K. (2008). Genistein protects the kidney from cisplatin-induced injury. *Kidney Int* **74**, 1538-47.

Tadagavadi, R. K., and Reeves, W. B. Endogenous IL-10 attenuates cisplatin nephrotoxicity: role of dendritic cells. *J Immunol* **185**, 4904-11.

Taguchi, T., Nazneen, A., Abid, M. R., and Razzaque, M. S. (2005). Cisplatin-associated nephrotoxicity and pathological events. *Contributions to nephrology* **148**, 107-21.

Thadhani, R., Pascual, M., and Bonventre, J. V. (1996). Acute renal failure. *The New England journal of medicine* **334**, 1448-60.

Townsend, D. M., Deng, M., Zhang, L., Lapus, M. G., and Hanigan, M. H. (2003). Metabolism of Cisplatin to a nephrotoxin in proximal tubule cells. *J Am Soc Nephrol* **14**, 1-10.

Tsang, R. Y., Al-Fayea, T., and Au, H. J. (2009). Cisplatin overdose: toxicities and management. *Drug Saf* **32**, 1109-22.

Tsuruya, K., Tokumoto, M., Ninomiya, T., Hirakawa, M., Masutani, K., Taniguchi, M., Fukuda, K., Kanai, H., Hirakata, H., and Iida, M. (2003). Antioxidant ameliorates cisplatin-induced renal tubular cell death through inhibition of death receptor-mediated pathways. *Am J Physiol Renal Physiol* **285**, F208-18.

Uehara, T., Watanabe, H., Itoh, F., Inoue, S., Koshida, H., Nakamura, M., Yamate, J., and Maruyama, T. (2005). Nephrotoxicity of a novel antineoplastic platinum complex, nedaplatin: a comparative study with cisplatin in rats. *Archives of toxicology* **79**, 451-60.

Vaidya, V. S., Ford, G. M., Waikar, S. S., Wang, Y., Clement, M. B., Ramirez, V., Glaab, W. E.,

Troth, S. P., Sistare, F. D., Prozialeck, W. C., Edwards, J. R., Bobadilla, N. A., Mefferd, S. C., and Bonventre, J. V. (2009). A rapid urine test for early detection of kidney injury. *Kidney Int* **76**, 108-14.

Vaidya, V. S., Ramirez, V., Ichimura, T., Bobadilla, N. A., and Bonventre, J. V. (2006). Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. *Am J Physiol Renal Physiol* **290**, F517-29.

Winston, J. A., and Safirstein, R. (1985). Reduced renal blood flow in early cisplatin-induced acute renal failure in the rat. *The American journal of physiology* **249**, F490-6.

Xu, E. Y., Perlina, A., Vu, H., Troth, S. P., Brennan, R. J., Aslamkhan, A. G., and Xu, Q. (2008). Integrated pathway analysis of rat urine metabolic profiles and kidney transcriptomic profiles to elucidate the systems toxicology of model nephrotoxicants. *Chem Res Toxicol* **21**, 1548-61.

Yatsu, T., Aoki, M., and Inagaki, O. (2003). Preventive effect of zelandopam, a dopamine D1 receptor agonist, on cisplatin-induced acute renal failure in rats. *European journal of pharmacology* **461**, 191-5.

Yokoo, K., Murakami, R., Matsuzaki, T., Yoshitome, K., Hamada, A., and Saito, H. (2009). Enhanced renal accumulation of cisplatin via renal organic cation transporter deteriorates acute kidney injury in hypomagnesemic rats. *Clinical and experimental nephrology* **13**, 578-84.

Yoshida, M., Iizuka, K., Terada, A., Hara, M., Nishijima, H., Akinori, Shimada, Nakada, K., Satoh, Y., and Akama, Y. (2000). Prevention of nephrotoxicity of cisplatin by repeated oral administration of ebselen in rats. *Tohoku J Exp Med* **191**, 209-20.

Yoshiki, N., Kubota, T., and Aso, T. (2000). Expression and localization of inducible nitric oxide synthase in human non-pregnant and early pregnant endometrium. *Molecular human reproduction* **6**, 283-7.

Zhang, B., Ramesh, G., Norbury, C. C., and Reeves, W. B. (2007). Cisplatin-induced nephrotoxicity is mediated by tumor necrosis factor-alpha produced by renal parenchymal cells. *Kidney Int* **72**, 37-44.

Zhou, H., Cheruvanky, A., Hu, X., Matsumoto, T., Hiramatsu, N., Cho, M. E., Berger, A., Leelahavanichkul, A., Doi, K., Chawla, L. S., Illei, G. G., Kopp, J. B., Balow, J. E., Austin, H. A., 3rd, Yuen, P. S., and Star, R. A. (2008). Urinary exosomal transcription factors, a new class of biomarkers for renal disease. *Kidney Int* **74**, 613-21.

ANEXO IV

NEPHROTOXICITY OF URANIUM: PATHOPHYSIOLOGICAL, DIAGNOSTIC AND TERAPEUTIC PERSPECTIVES.

Laura Vicente-Vicente, Yaremi Quiros, Fernando Perez-Barriocanal, José Miguel López-Novoa, Francisco J. López-Hernández, and Ana Isabel Morales.

Revista: Toxicological Sciences; 2010

doi: 10.1093/toxsci/kfq178

TOXICOLOGICAL SCIENCES **118(2)**, 324–347 (2010)
doi:10.1093/toxsci/kfq178
Advance Access publication June 16, 2010

REVIEW

Nephrotoxicity of Uranium: Pathophysiological, Diagnostic and Therapeutic Perspectives

Laura Vicente-Vicente,*,† Yaremi Quiros,† Fernando Pérez-Barriocanal,† José Miguel López-Novoa,† Francisco José López-Hernández,†,‡ and Ana Isabel Morales*,†,[1]

*Unidad de Toxicología and †Departamento de Fisiología y Farmacología, Universidad de Salamanca, 37007 Salamanca, Spain; and ‡Unidad de Investigación, Hospital Universitario de Salamanca, 37007 Salamanca, Spain

[1]To whom correspondence should be addressed at Unidad de Toxicología, Universidad de Salamanca, Edificio Departamental, S-19, Campus Miguel de Unamuno, 37007 Salamanca, Spain. Fax: +34-923-294-669. E-mail: amorales@usal.es.

Received April 12, 2010; accepted June 8, 2010

As in the case of other heavy metals, a considerable body of evidence suggests that overexposure to uranium may cause pathological alterations to the kidneys in both humans and animals. In the present work, our aim was to analyze the available data from a critical perspective that should provide a view of the real danger of the nephrotoxicity of this metal for human beings. A further aim was to elaborate a comparative compilation of the renal pathophysiological data obtained in humans and experimental animals with a view to gaining more insight into our knowledge of the mechanisms of action and renal damage. Finally, we address the existing perspectives for the improvement of diagnostic methods and the treatment of intoxications by uranium, performing an integrated analysis of all these aspects.

Key Words: **uranium; nephrotoxicity; chronic; acute; diagnosis; treatment.**

Human beings are constantly exposed to a certain amount of uranium because it is heterogeneously present in natural form in food, the air, the soil, and water. The repercussions of this natural exposure as regards human physiology and pathophysiology are not completely known. However, the evidence gathered so far suggests that overexposure to uranium may result in toxicity, which is derived from an excessive accumulation of the element in the organism. This accumulation, in turn, depends on the route of entry, the duration of the exposure, the dose and the chemical compound of which it forms part, and its absorption (Maynard and Hodge, 1949; Stokinger *et al*., 1953). Natural exposure, overexposure, and intoxication can occur by ingestion, inhalation, or skin contact (Fig. 1). In any case, a small portion of the uranium gains access to the circulation from which it distributes throughout the body. Uranium accumulates mainly in the bones (66%), kidneys (8%), and liver (16%) (ICRP, 1996), and it is eliminated with the urine, rapidly from the blood and slowly from organ depots (ICRP, 1996; La Touche *et al*., 1987).

Human beings may be subjected to pathological overexposure to the metal, both acutely and chronically, as a consequence of (1) contamination of the usual sources of normal exposure with high amounts of uranium arising from the anisotropy of the distribution of the metal in the earth's crust, as in ground veins or water masses in contact with them, or from human dumping and (2) direct contact with new sources of exposure originated by human activity and enriched in the element, such as in materiel and aeronautics or in the fields of mining and industry. The main industrial use of uranium is for fuel in nuclear reactors, which produce 17% of the world's electricity (Uranium Institute, 1996). Many countries are being driven into nuclear energy generation as a consequence of (1) the energy demand escalation, (2) the limited reserve of fossil fuels, (3) the scarce development of alternative sources, (4) the climate change, and (5) the regulation imposed by the Kyoto treaty. In this sense, uranium is one of the most useful fuels for nuclear energy production; it is reasonably inexpensive and complies with the Kyoto protocol. Other industrial uses of the element include the manufacture of aircraft stabilizers, in satellites, and in naval architecture (Wilkinson, 1962); in inertial orientation devices and gyroscopes (ATSDR/CDC, 1990); in green or yellow glasses (Peeks *et al*., 2002; Rossol, 1997); and in certain luminous devices, in highly penetrating gun ammunitions and in the production of high-energy x-rays (EPA, 1985). For years, it was used in the manufacture of dental porcelain (Thompson, 1976). The long half-life of the ^{238}U isotope (4.51×10^9 years) is a good proxy for estimating the age of igneous rocks and in other types of radiometric dating (ATSDR, 1999).

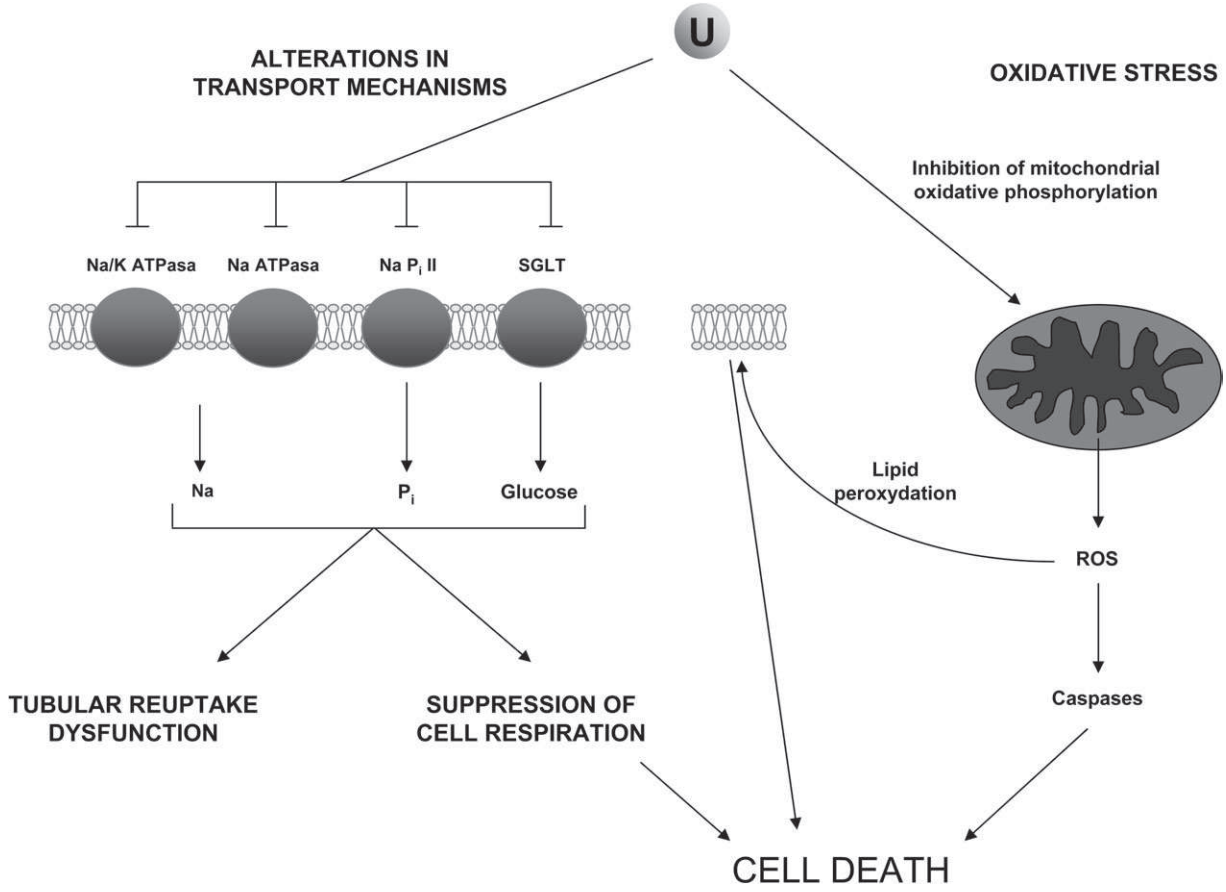


FIG. 1. Possible mechanisms involved in uranium nephrotoxicity. U, Uranium; Na/K ATPase, sodium-potassium pump; Na ATPase, sodium pump; Na, sodium; Pi, inorganic phosphate.

The toxicity of the metal depends on several factors, such as sex, age, the body mass index (Kurttio *et al.*, 2006), and species. Of all the mammals studied, humans seem to be the least sensitive to uranium (Kathren and Burklin, 2008). An interspecies order of sensitivity has been proposed: rabbit > rat > guinea pig > pig > mouse > dog > cat > human (Orcutt, 1949; Tannenbaum *et al.*, 1951). Uranium is responsible for both radiological and chemical toxicity. The radiological toxicity has been theoretically associated with the production of cancer. However, because the specific radioactivity of natural uranium is low, there seems to be no evident danger of cancer from radiological effects. The results of different studies carried out on animals and humans are consistent with this notion (Morris *et al.*, 1990; Muller *et al.*, 1967; Sanders, 1986; Stokinger *et al.*, 1953). On the other hand, the chemical toxicity has been associated with hepatic, lung, and renal injury (UNSCEAR, 1988). It has also been suggested that the net effects caused by this metal in the kidneys and lungs could be because of cooperation between the chemical and radiological properties through a complementary mechanism of action, although this relationship has not been demonstrated experimentally (Ballou *et al.*, 1986; Filippova *et al.*, 1978; Spiegel, 1949; Spoor and Hursh, 1973; Stokinger *et al.*, 1953).

No significant toxicity of uranium has been evidenced at the cardiovascular (Boice *et al.*, 2007; Dygert, 1949; Gilman *et al.*, 1998c), muscle-skeletal (Gilman *et al.*, 1998c), endocrine (Boice *et al.*, 2007; Dygert, 1949; Gilman *et al.*, 1998c; Maynard and Hodge, 1949; Stokinger *et al.*, 1953), gastrointestinal (Boice *et al.*, 2007; Gilman *et al.*, 1998c; Maynard and Hodge, 1949), and skin (Boice *et al.*, 2007; Spiegel, 1949) levels. No effects on reproduction have been reported in humans (Mays *et al.*, 1985; McDiarmid *et al.*, 2007). In contrast, in animal experimentation, relatively high doses of uranium have been reported to elicit reproductive abnormalities, manifested as a decrease in sperm counts (Llobet *et al.*, 1991), fetal toxicity (Domingo *et al.*, 1989), and testicular lesions (Maynard *et al.*, 1953). In this article, the available information on the nephrotoxicity of uranium upon acute and chronic intoxication is thoroughly analyzed. For this purpose, both data from human as well as animal studies are critically compared. Human studies are scarcer and less controlled than animal studies. As such, information from experimental animals can, to a certain extent, be processed

and extrapolated into the framework delineated by data obtained from human beings, in order to create a more complete picture of the pathophysiological aspects of the nephrotoxicity of uranium and the real risk posed by this metal to the human being.

NEPHROTOXICITY BECAUSE OF ACUTE OVEREXPOSURE

Acute Nephrotoxicity in Animal Models

Pathophysiological Studies

In animals, it has been possible to characterize the renal damage caused by the element in detail, under predetermined and controlled experimental conditions. Several studies have reported decreases in creatinine clearance (Banday *et al*., 2008; Haley, 1982; Sanchez *et al*., 2001; Shim *et al*., 2009), which is indicative of a reduction in the glomerular filtration rate (GFR). Congruently, with the decrease in the GFR, a significant increase in the plasma concentration of creatinine and blood ureic nitrogen (BUN) has been reported after uranium administration (Banday *et al*., 2008; Fukuda *et al*., 2005b; Sanchez *et al*., 2001; Shim *et al*., 2009; Taulan *et al*., 2006; Yapar *et al*., 2010; Zimmerman *et al*., 2007). It is unknown whether such a decrease in the GFR is because of (1) the glomerular effects of uranium, (2) the tubuloglomerular feedback brought about after tubular insult in order to prevent an uncontrolled loss of water and electrolytes, or (3) a combination of both. In this sense, in animals acutely intoxicated with uranium, functional tubular alterations have been observed. These are reflected in a significant increase in electrolyte excretion (sodium, potassium, magnesium, calcium, and inorganic phosphate) (Banday *et al*., 2008; Haley, 1982), proteins (Haley, 1982; Sanchez *et al*., 2001), β-2-microglobulin (Fukuda *et al*., 2008), and glucose (Nomiyama *et al*., 1974; Taulan *et al*., 2006). Increases in the urinary activity of several enzymes indicating tissue lesion have also been reported. At least in part, this could be explained by the functional tubular alterations. Still, it is not possible to rule out a direct effect of the metal on transport mechanisms or tubular cell functions, independently of cell viability. Among the increased urinary enzymes are N-acetyl glucosaminidase (NAG) (Diamond *et al*., 1989; Fukuda *et al*., 2005b, 2008; Sanchez *et al*., 2001) and alkaline phosphatase (ALP) (Banday *et al*., 2008; Nomiyama *et al*., 1974). The increase in ALP in urine has been linked to the loss of microvilli in the proximal tubules, where this enzyme is mainly located (Yuile, 1973). Increases in the activities of other enzymes in the urine, such as gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), and acid phosphatase (Banday *et al*., 2008; Diamond *et al*., 1989; Sanchez *et al*., 2001; Taulan *et al*., 2006), have been described, pointing to the presence of tissue lesions. LDH is a nonspecific marker of renal tissue lesion, but NAG, ALP, and GGT are mainly markers of proximal tubule insult (Emeigh Hart, 2005).

Other effects of uranium on the kidneys possibly related to the function and viability of renal structures include (1) alterations in the activities of several enzymes associated with glycolysis (aldolase and phosphoglycerokinase), the tricarboxylic acid cycle (isocitrate, succinate, and malate dehydrogenases), and gluconeogenesis (FBPase and G6Pase) (Banday *et al*., 2008); (2) disturbances in the oxidative balance (Schramm *et al*., 2002), potentially related to increases in the activity of superoxide dismutase (SOD) (Banday *et al*., 2008; Schramm *et al*., 2002); and (3) increases in the plasma renin levels, associated with increases in blood pressure (Kato *et al*., 1994; Mendelsohn and Smith, 1980).

Table 1 summarizes the most relevant data obtained from the acute intoxications with uranium carried out in experimental animals, with regard to the alterations in renal function and structure observed; the animal species; and the dose, route, and duration of the exposure. The diversity of route of administration, uranium type, and other factors introduces some complexity when comparing the results from different studies. Most of them have been done with rats, a species very sensitive to uranium's toxicity. Regardless of strain and route of administration, single doses of a few (> 2) milligram per kilogram are consistently and overtly nephrotoxic, as demonstrated by alterations in classical parameters of renal function (serum creatinine, BUN, etc.) and renal tissue status (e.g., urinary excretion of NAG), during the immediate days after exposure. This is also true for other less sensitive species, such as mice and dogs. There are fewer studies using lower doses, which were all conducted in rats. However, it is evident that, at least when uranium is administered ip, the toxic threshold for single-dose exposures is set around 0.5 mg/kg. In the case of the im administration, at least doses of or over 1 mg/kg induce a renal injury that is still detectable 28 days after exposure (Fukuda *et al*., 2005b). Because this is the only study monitoring renal parameters so late after an acute exposure, there is not enough evidence to know whether this is rather specific of the im route or it can also be observed upon intoxication by other routes.

All together, these data indicate that several species acutely intoxicated with uranium undergo some degree of renal damage in a dose-dependent and route-independent manner. Doses of 5 mg/kg U (or higher) are overtly nephrotoxic for rats, mice, and dogs. Doses higher than 0.5 mg/kg U are nephrotoxic at least for rats. "Documented Human Cases" section presents the evidence on acute intoxication of humans gathered in documented cases and compares the information with that obtained in animals.

Morphological Renal Modifications

As far as we are aware, there are no histological studies about the acute effects of uranium on kidney structure in

TABLE 1
Studies of Acute Intoxication in Experimental Animals

Reference	Species	Uranium type	Dose	Route	Exposure	Time of analysis	Observations		
							Serum parameters	Urine parameters	Others
Zimmerman *et al.* (2007)	SD rats	DU	0.1 mg/kg	ip	1 dose	3 and 7 days	_ Crs, BUN, albumin		
Fukuda *et al.* (2005b)	Wistar rats	DU	0.2 mg/kg	im	1 dose	28 days	_ GOT, GPT, ALP, Prot., Ca, BUN, Crs, erythrocyte, hematocrit < hemoglobin (slightly)		
Zimmerman *et al.* (2007)	SD rats	DU	0.3 mg/kg	ip	1 dose	3 and 7 days	_ Albumin > Crs, BUN (slightly)		
Banday *et al.* (2008)	Wistar rats	UN	0.5 mg/kg	ip	1 dose	5 days	> Cr, BUN, cholesterol, phospholipids	> UF, Glc, Na, K, Ca, Mg, Pi, GGT, ALP, LDH	< Clcr
Shim *et al.* (2009)	SD rats	UN	0.5 mg/kg	ip	1 dose	6 days before dose	> Crs, BUN		< Clcr
Diamond *et al.* (1989)	Long-Evans rats	UO2F2	0.66 mg/kg	ip	5 doses	6 days before last dose		_ UF, NAG, Gluc. > LDH, AST, Prot., albumin, alfa-amino nitrogenuria	_ Kidney weights, body weights
Fukuda *et al.* (2005b)	Wistar rats	DU	1 mg/kg	im	1 dose	28 days	_ GOT, GPT, protein, Ca > BUN, Crs, ALP < erythrocyte, hemoglobin _ hematocrit		
Zimmerman *et al.* (2007)	SD rats	DU	1 mg/kg	ip	1 dose	3 and 7 days	> Crs BUN, albumin		
Diamond *et al.* (1989)	Long-Evans rats	UO2F2	1.32 mg/kg	ip	5 doses	6 days before last dose		_ UF > LDH, AST, NAG, Prot., albumin, alfa-amino nitrogenuria, Glc.	_ Kidney weights, body weights
Fukuda *et al.* (2005a)	Wistar rats	DU	2 mg/kg	im	1 dose	28 days	_ GOT, GPT, ALP Crs, BUN, Ca, Prot., erytrhocyte, hemoglobin, hematocrit		
Fukuda *et al.* (2005b)	Wistar rats	DU	2 mg/kg	im	1 dose	28 days	> BUN, Crs	> NAG	< Body weight
Sanchez *et al.* (2001)	SD rats	UA	2.5 mg/kg	ip	1 dose	1 day	_ protein, uric acid > Crs, BUN, LDH	_Cr, urea > UF, Prot., LDH, GGT, NAG	< Body weight < Clcr
Fukuda *et al.* (2008)	Wistar rats	DU	4 mg/kg	sc	1 dose	1 day	_ GOT, ALP, Glc., Ca, BUN > Crs GPT	_ NAG	
Kato *et al.* (1994)	SD rats	UA	5 mg/kg	iv	1 dose	2 days	> Crs, BUN, FENa, plasma renin activity		< Body weight
Sanchez *et al.* (2001)	SD rats	UA	5 mg/kg	ip	1 dose	1 day	> Crs, BUN, LDH _ Prot., uric acid < Clcr	> UF, Prot., LDH, GGT, NAG _Cr, urea	< Body weight, Clcr
Tolson *et al.* (2005)	SD rats	UA	5 + 10 mg/kg	ip	2 doses	5 days before the dose	> Crs, BUN		
Haley *et al.* (1982)	SD rats	UN	10 mg/kg	ip	1 dose	5 days		> Na, Prot.	< GFR
Tolson *et al.* (2005)	SD rats	UA	10 mg/kg	ip	1 dose	5 days	> Crs BUN		

TABLE 1—*Continued*

Reference	Species	Uranium type	Dose	Route	Exposure	Time of analysis	Observations		
							Serum parameters	Urine parameters	Others
Fukuda *et al.* (2008)	Wistar rats	DU	16 mg/kg	sc	1 dose	1 day	_ GOT, ALP, Glc, Ca > Crs, BUN, GPT	> NAG	
Fukuda *et al.* (2005a)	Wistar rats	DU	7.9; 15.8; 31.5; 63; and 126 mg/kg	im	1 dose	3–7 days			< Body weight (rats die at 3–7 days)
Taulan *et al.* (2006)	C57 Bl/6J mice	UN	5 mg/kg	ip	1 dose	2 days	> Crs, BUN	> GGT, Glc.	
Yapar *et al.* (2010)	Albino Swiss mice	UA	5 mg/kg	ip	1 dose	5 days	> Crs, BUN, AST, ALT		
Martinez *et al.* (2003)	Balb-C mice	UN	350 mg/kg bw	Oral	3 days	2 days	> Crs BUN		Died day 3 postintoxication
Nomiyama *et al.* (1972)	Rabbits	UA	0.2 mg/kg	iv	1 dose	2 days	> ALP, GOT, GPT, LDH	> ALP, GOT, GPT, Glc, LDH	
Stefanovic *et al.* (1987)	Dogs	UN	10 mg/kg	ip	1 dose		> Ca, Pi		

Note. >, increase; <, decrease; _ no changes; DU, depleted uranium; UN, uranyl nitrate; UO2F2, uranyl fluoride; UA, uranyl acetate; SD, Sprague-Dawley; Crs, serum creatinine; BUN, blood ureic nitrogen; GOT, glutamic oxalo transaminase; GPT, glutamic piruvic transaminase; Prot., protein; Ca, calcium; UF, urinary flow; Glc, glucose; Na, sodium; K, potasium; Mg, magnesium; Pi, inorganic phosphate; GGT, gamma-glutamil-transpeptidase; Clcr, creatinine clearance; NAG, N-acetyl-β-*D*-glucosaminidase; AST, aspartate aminotransferase; FENa, fractional excretion rate of sodium, bw, body weight.

human beings. In contrast, its effects on the kidney structures of laboratory animals are fairly well documented because uranyl nitrate has been widely used as an experimental nephrotoxic agent (Domingo *et al.*, 1989; Haley, 1982; Haley *et al.*, 1982; Kobayashi *et al.*, 1984; McDonald-Taylor *et al.*, 1997; Sun *et al.*, 2002). Modifications in the color and smoothness of the kidney surface have been described (Fukuda *et al.*, 2005a). At toxic doses (5–20 mg/kg), ip injected uranyl nitrate elicits specific damage to the S2 and S3 segments of the proximal tubule (Gilman *et al.*, 1998c; Haley, 1982; Haley *et al.*, 1982; Oliver, 1915), cell vacuolization (Gilman *et al.*, 1998c; Haley, 1982; Haley *et al.*, 1982; Martinez *et al.*, 2000; Taulan *et al.*, 2006), loss of the brush border membrane (Taulan *et al.*, 2006; Haley, 1982; Haley *et al.*, 1982; Schwartz and Flamenbaum, 1976), and in the S1 segment, an increase in lysosomal and vacuolar mass. In other studies, reports have also been made of variations in mitochondrial mass (Haley, 1982; McDonald-Taylor *et al.*, 1997). At very high doses (5–10 mg/kg body weight, ip), it is also possible to observe necrosis of the proximal tubules (Haley *et al.*, 1982; Hirsch, 1976; Taulan *et al.*, 2006), especially in the corticomedullary area (Fukuda *et al.*, 2005a; Shim *et al.*, 2009; Sun *et al.*, 2002). When the dose of uranyl nitrate administered is not very high (0.5 mg/kg), the glomerulus is apparently left intact (Taulan *et al.*, 2006). However, if the dose is very high (~10 mg/kg), adherences and congestion are seen in the glomerular epithelium (Haley, 1982) together with a decrease in the glomerular surface (Shim *et al.*, 2009).

Once exposure has ceased, the uranium bound to the tubular cells is eliminated in the urine (Leggett, 1989) and a process of tubular re-epithelization begins (Zager *et al.*, 1994). It is believed that interstitial myofibroblasts and macrophages could play an important role in regeneration after acute insult owing to their role in scarring (Leibovich and Ross, 1975; Powell *et al.*, 1999). The appearance of myofibroblasts and monocytes/macrophages in the renal interstitium of rats has been observed following injections of uranyl nitrate at toxic doses (Sun *et al.*, 2002). A network of myofibroblasts surrounding the tubular basal membranes has been observed; this persists until cellular re-epithelization has been fully completed (Sun *et al.*, 2002). It is thought that this formation may serve to provide contractile capacity and to prevent nephron collapse, as well as to reinforce the extracellular matrix and promote the production of cytokines that favor re-epithelization (Sun *et al.*, 2002). This regenerated tubular epithelium seems to be more resistant to the toxicity of uranium than the original one (Hodge *et al.*, 1973).

Documented Human Cases

Acute overexposure to uranium in humans is very rare and unlikely, such that few cases have been documented. Table 2 shows a summary of the main studies addressing acute

TABLE 2
Documented Cases of Acute Intoxication with Uranium in Humans

Study	Participants number	Exposure source	Uranium amount and type	Study moment	Observations
Bassett *et al.* (1948)	6 volunteers	iv	6.3–70.9 μg/kg UN (0.44–4.96 mg/kg)[a]	During exposure	> Urinary catalase > Prot. (for the highest dose used)
Butterworth (1955)	1 volunteers	Oral	1 g UN (14.3 mg/kg)[a]	During exposure	Vomiting, diarrhea > microalbuminury
Hursh and Spoor (1973)	4 patients	Oral	10.9 mg UN (0.16 mg/kg)[a]	During exposure	Without kidney damage
Pavlakis *et al.* (1996)	1 attempted suicide	Oral	15 g UN (214.3 mg/kg)[a]	After cessation	All renal parameters altered
Kathren and Moore (1986)	3 men	Inhalation	UF_6	Sortly after the accident	< Clcr
Fisher *et al.* (1990)	31 enrichment plant workers	Inhalation	0.47–24 mg/m^3 UF_6	After cessation	> U in urine
Lu and Zhao (1990)	1	Inhalation	NU	1 week after cessation	> Prot., NNP, aminoacidury > U in urine
Bijlsma *et al.* (2008)	2499 firefighters, police and airport workers	Inhalation	NU and DU	8.5 years after cessation	No > U in urine; No differences in the other renal parameters

Note. UN, uranyl nitrate; UF6, uranium hexafluoride; NU, natural uranium; DU, depleted uranium; Prot., proteinuria; Clcr, creatinine clearance; NNP, nonprotein nitrogen; U, uranium.

[a]Values between brackets represent the estimated dose (milligram per kilogram) for a 70-kg individual.

exposure to the metal. The information comes from a case of intended suicide (Pavlakis *et al.*, 1996), several controlled administration of uranium (via oral) with research purposes on volunteers (Bassett *et al.*, 1948; Butterworth, 1955; Hursh and Spoor, 1973), and professional accidents in which individuals were exposed through inhalation (Bijlsma *et al.*, 2008; Fisher *et al.*, 1990; Kathren and Moore, 1986; Lu and Zhao, 1990). In most of these cases of acute intoxication in humans, there is clear evidence of acute nephrotoxicity. A decrease in the GFR (as assessed by the measurement of creatinine clearance) (Kathren and Moore, 1986; Tanigawara *et al.*, 1990), or consequences of this, such as increases in plasma creatinine levels (Pavlakis *et al.*, 1996), has been reported. Increases in the urinary excretion of proteins (Friberg *et al.*, 1986; Lu and Zhao, 1990), amino acids (Lu and Zhao, 1990), and urinary catalase (Bassett *et al.*, 1948; Friberg *et al.*, 1986) have also been reported. Other studies have described increases in the excretion of certain proteins, such as albumin (in amounts in the microalbuminuria range) (Butterworth, 1955), and β-2-microglobulin (Butterworth, 1955). In those studies, the origin of the proteinuria was not determined, such that it could reflect glomerular or tubular alterations (or both). In other cases, it has been reported that uranium affects both the reabsorption of filtered solutes and the excretion of other solutes. All the above findings suggest that, depending on the effective dose, acute intoxication with uranium may lead to kidney impairment of varying intensity, which is strongly dependent on the circumstances of the exposure.

There seems to be some divergence of results regarding the renal effects of exposure to uranium because the available information is extremely varied as regards the route of exposure, the dose (sometimes unknown), and the type of uranium used, the time of analysis, etc. None of the studies in which renal function was evaluated after several years has reported altered parameters. In analyses carried out during or immediately after acute intoxication, in addition to increased urinary uranium levels, an alteration of different renal parameters has been observed after both oral and inhalation intoxication. Through oral administration, only in one case of exposure to low doses, in the range of a few milligram of uranium (Hursh and Spoor, 1973) corresponding to an estimated dose of 0.16 mg/kg, was renal function apparently unaltered. For higher doses, more serious alterations were observed as the dose increased, ranging from simple microalbuminuria with a dose of 14.3 g/kg in Butterworth (1955) to alterations in all the renal parameters measured in Pavlakis *et al.* (1996), after an estimated dose of about 214.3 mg/kg. It therefore seems that acute exposure (at least through the oral route) to more than about 15 mg/kg is required for renal alterations to start appearing.

Along with ingestion, inhalation is the other most likely potential route through which humans can be overexposed to uranium. We have found four studies reporting overexposure to uranium though inhalation. One of them (Bijlsma *et al.*, 2008) studied renal function parameters 8.5 years after the exposure and found no alteration other than higher urinary excretion of uranium. The other three studied renal function immediately after the exposure. Kathren and Moore (1986) found reduced creatinine clearance, Lu and Zhao (1990) proteinuria and increased serum non protein nitrogen (NPN),

and Fisher *et al.* (1990) only higher excretion of uranium. Except in Fisher *et al.* (1990), the concentration of uranium in the air is not known (see Table 2). Accordingly, it is impossible to draw conclusions on toxic dosage or sensitivity through this route and much less to make comparisons with other exposure routes. However, it is clear that feasible accidents or sporadic circumstances can overexpose the human being to uranium through inhalation resulting in some degree of nephrotoxicity.

In conclusion, animal studies diverge from human studies in the exposure route, which is a key determinant of uranium bioavailability, and blood and tissue levels. However, it is clear that acute intoxication with uranium leads to nephrotoxicity in both animals and humans, in a dose-dependent manner. Yet one study in rats (Kato *et al.*, 1994) and another one in humans (Bassett *et al.*, 1948) of acute intoxication via iv with similar doses yield interesting information. Humans exposed to ~5 mg/kg U showed proteinuria as the most significant renal alteration, whereas both serum creatinine and BUN increased in rats subject to the same dose. This indicates that humans underwent some degree of renal alteration (probably in tubular reuptake) that did not end up in the renal dysfunction seen in rats. This highlights the lower sensitivity to U toxicity of humans when compared with rats. Through the iv route, uranium surpasses interspecies differences posed by different absorption and different influence of other physical barriers working upon exposure through other routes.

Finally, it should be noted that it is not known whether acute intoxication with uranium is able to trigger chronic renal lesions that will progress irreversibly and autonomously regardless of the presence of the metal, consistent with the well-known fact that the concurrence of several acute renal insults may drive the kidneys to enter an autonomous chronic degenerative process (Basile, 2008). The few data available concerning acute intoxications in human beings, together with the lack of this type of study in animals, make it impossible to draw conclusions. As a possible indication, the work of Bijlsma *et al.* (2008) (see Table 2), in which renal function was assessed years after the acute exposure, suggests that this would not be the case, although the intensity of those exposures is not known.

NEPHROTOXICITY BECAUSE OF CHRONIC OVEREXPOSURE

Under certain circumstances, humans are chronically overexposed to uranium. It remains largely unknown whether such exposure may elicit kidney damage and neither are the determinants of the possible nephrotoxicity known. It is also unknown whether the possible nephrotoxicity is triggered (1) as a subacute effect when a certain level of tissue accumulation after a more or less long exposure time has been surpassed or in contrast (2) as chronic renal damage that gradually develops into irreversible degeneration that is even independent of the presence of uranium, as occurs with most of the causes of chronic renal impairment (CRI) (Remuzzi *et al.*, 2006). In this section, we attempt to shed some light on these aspects.

Pathophysiological Studies with Animal Models

In general, studies carried out in laboratory animals have used higher doses of uranium than those found in human exposure, although the time of exposure was much shorter (months instead of years). Table 3 summarizes the most relevant data obtained from the chronic exposure to uranium in experimental animals, with regard to the alterations in renal function, the animal species, uranium type, the estimated dose (milligram per kilogram), and the route and duration of the exposure. As commented above, the toxicity of uranium depends on sex, age, the body mass index, and species. Data from Table 3 are not in agreement with the interspecies sensitivity classically reported (Orcutt, 1949; Tannenbaum *et al.*, 1951). Indeed, after chronic oral exposure, the rat seems to be more sensitive than the rabbit. Rabbits showed no biochemical changes in the estimated dose range 0.048–30 mg/kg during 3 months of exposure. However, rats subject to even milder conditions (exposure of 1 month and a lower dosage range [0.02–16 mg/kg]) showed renal alterations, including glucosuria and increased leucine aminopeptidase activity. Comparison with the mouse is more controversial because exposure time was longer (4 months), which may have induced a higher accumulation resulting in greater kidney damage. The importance of exposure time has been evidenced by the studies of Berradi *et al.* (2008) and Tissandie *et al.* (2008), which show major renal alterations owing to longer time of exposure.

Regarding inhalation exposure to uranium, renal damage has been reported in studies from 1 to 24 months mainly in dogs. Stokinger *et al.* (1953), in a study conducted at low doses (0.05 mg U/m^3 air), observed that NPN levels in plasma were normal, and there were no differences in the excretion of urinary proteins, whereas a decrease in creatinine clearance and an increase in urinary catalase were observed. At higher doses (0.13 mg U/m^3), an increase in protein excretion and NPN (Pozzani, 1949) was observed. In other studies conducted during a similar exposure time but with higher dose, changes in the usual markers of renal function (serum creatinine, BUN, and urinary proteins) were observed. Importantly, similar results were obtained in a wide range of doses (37.5–187 mg U/m^3) (Maynard and Hodge, 1949).

It is difficult to compare the results obtained in oral and inhalation exposure because, among many other factors, the absorption process in each route is different. Generally, when exposure occurs with high doses of uranium, the markers of renal damage, such as plasma creatinine and nonprotein nitrogen in plasma, are found to be altered, but when exposure

TABLE 3
Studies of Chronic Intoxication in Experimental Animals

Reference	Species	Uranium type	Dose	Estimated dose	Route	Exposure	Observations		
							Serum parameters	Urine parameters	Others
Gilman *et al.* (1998c)	SD rats	UN	0.96 mg/l	0.02 mg/kg[a]	Oral	3 months	_ hemoglobin, erythrocytes, Glc. > leucine aminopeptidase		_ Body weight
Gilman *et al.* (1998c)	SD rats	UN	4.8 mg/l	0.32 mg/kg[a]	Oral	3 months	_ hemoglobin, erythrocytes > leucine aminopeptidase, Glc.		_ Body weight
Ortega *et al.* (1989)	SD rats	UA	2 mg/kg	2 mg/kg	Oral	1 month		> Glc.	
Berradi *et al.* (2008)	SD rats	UN	40 mg/l	2.67 mg/kg[a]	Oral	9 months	_ Leucocytes < hemoglobin, hematocrit (sligthly) < RBC		
Tissandie *et al.* (2008)	SD rats	EU	40 mg/l	2.67 mg/kg[a]	Oral	9 months	_ ALT, AST, Crs, BUN, PTH, Pi > Ca		_ Body weight, kidney weight
Ortega *et al.* (1989)	SD rats	UA	4 mg/kg	4 mg/kg	Oral	1 month		> Glc.	
Ortega *et al.* (1989)	SD rats	UA	8 mg/kg	8 mg/kg	Oral	1 month		> Glc.	
Ortega *et al.* (1989)	SD rats	UA	16 mg/kg	16 mg/kg	Oral	1 month		> Glc.	
Gilman *et al.* (1998c)	SD rats	UN	600 mg/l	400 mg/kg[a]	Oral	3 months	_ hemoglobin, erythrocytes, Glc. > leucine aminopeptidase		_ Body weight
Taulan *et al.* (2004)	Mice	UN	80 mg/l	13.33 mg/kg[b]	Oral	4 months	_ BUN > Crs	_ Glc., GGT	_ Kidney weight
Taulan *et al.* (2004)	Mice	UN	160 mg/l	26.67 mg/kg[b]	Oral	4 months	_ BUN > Crs	_ Glc. > GGT	_ Kidney weight
Gilman *et al.* (1998b)	Rabbits	UN	0.96 mg/l	0.048 mg/kg[c]	Oral	3 months	No biochemical changes		
Gilman *et al.* (1998b)	Rabbits	UN	4.8 mg/l	0.24 mg/kg[c]	Oral	3 months	No biochemical changes		
Gilman *et al.* (1998a)	Rabbits	UN	24 mg/l	1.2 mg/kg[c]	Oral	3 months	No biochemical changes		
Gilmanm *et al.* (1998b)	Rabbits	UN	24 mg/l	1.2 mg/kg[c]	Oral	3 months	No biochemical changes		
Gilman *et al.* (1998a)	Rabbits	UN	600 mg/l	30 mg/kg[c]	Oral	3 months	No biochemical changes		
Gilman *et al.* (1998b)	Rabbits	UN	600 mg/l	30 mg/kg[c]	Oral	3 months	No biochemical changes		
Stokinger *et al.* (1953)	Dogs	UO2	0.05 mg U/m^3		Inhalation	12–24 months	_ NPN	_ Prot. > catalase	< Clcr
Pozzani (1949)	Dogs	UO2	0.13 mg U/m^3		Inhalation		> NPN	> Prot., catalase	< Clcr
Maynard and Hodge (1949)	Dogs	UO2F2	37.5 mg U/m^3		Inhalation	1–24 months	> Crs, BUN	> Prot.	
Maynard and Hodge (1949)	Dogs	UO2F2	187 mg U/m^3		Inhalation	1–24 months	> Crs, BUN	> Prot.	

Note. >, increase; <, decrease; _ no change; SD, Sprague-Dawley; UN, uranyl nitrate; UA, uranyl acetate; UO2, uranium dioxide; UO2F2, uranyl fluoride; RBC, red blood cells; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Crs, serum creatinine; BUN, blood ureic nitrogen; PTH, parathyroid hormone; Ca, calcium; NPN, nonproteic nitrogen; Prot., proteins; Glc., glucose; EU, enriched uranium.

[a]Estimated dose assuming that rat median weight of 300 g and daily intake of water 20 ml/day.

[b]Estimated dose assuming that mice median weight of 30 g and daily intake of water 5 ml/day.

[c]Estimated dose assuming rabbit median weight of 4 kg and daily intake of water 200 ml/day.

occurs at lower doses, these markers are not altered or at least their alteration is not dose dependent. In the case of oral exposure, an increase in glucosuria has also been observed in several studies, whereas in the case of the inhalation route, no alteration in glucose excretion has been described. It is also important to note that the work carried out on oral exposure has focused more on the nephrotoxicity of uranium than studies carried out on the inhalation route, in which many of the markers of renal damage were not analyzed. Accordingly, it is not possible to rule out that certain parameters, such as glucosuria, might be altered because of chronic uranium exposure through this route. These factors presumably determine the accumulation of the metal in the various compartments of the organism; hypothetically, such accumulation could be an important component of the renal toxicity of uranium.

Data Sources of Human Cases

Several epidemiological studies have attempted to link chronic exposure to uranium and renal damage, which is

TABLE 4
Documented Cases of Chronic Intoxication with Uranium in Humans

Study	Participants number	Male	Female	Ages	Exposure source	Exposure time	Uranium amount and type	Study moment	Observations
Shiraishi *et al.* (1992)	—; general population	—	—	—	Oral (water)	10 years approximately	1.07–42.6 ng/dm^3 NU	During exposure	No clinical effects
Zamora *et al.* (1998)	30; 20	10; 7	20; 13	13–87; 16–68	Oral (water); oral (water)	3 years approximately	High dose (2–780 μg/l); Low dose (< 1 μg/l) NU	During exposure	> U in urine (exposed); > LDH, ALP and GGT (slightly); > Glc.; No changes in Prot. and NAG
Kurttio *et al.* (2002)	325; general population	—	—	15–82	Oral (water)	1–34 years	High dose (> 100 μg/l); low dose (10–100 μg/l)	During exposure	> U in urine; > Ca, Phosphate and glc in urine; No changes in Clcr. Albumin, BMG, Crs.
Pinney *et al.* (2003)	—; residents near uranium plant	—	—	—	Oral (water)	Years	—; NU	During exposure	> U in urine; > microalbuminury; > red cells and hematocit in blood
Orloff *et al.* (2004)	105; general population	50	55	15–79	Oral (water)	Months	High dose (620 μg/l) NU	6–10 months after cessation	> U in urine
Karpas *et al.* (2005)	205; general population	102	103	18–81	Oral (water)	Years	0.03–2.775 μg/day NU	During exposure	> U in urine
Wyatt *et al.* (2008)	156; general population	—	—	—	Oral (water)	Years	> 30 μg/l NU	1 year after cessation	> Crs and BUN; > U in urine
Kurttio *et al.* (2006)	193; general population	95	98	18–81	Oral (water)	16 years approximately	25 μg/l NU	During exposure	> Glc and ALP in urine; > U in urine; No changes in NAG, LDH, GGT, Ca, Prot, phosphates, Glc, Crs
Magdo *et al.* (2007)	2 adults + 5 children; general population	5	2	3–37	Oral (water)	5 years approximately	866 and 1.160 μg/l NU	3 months after cessation	> BMG; > U in urine
Oeh *et al.* (2007)	—; workers during Balcans War	—	—	—	Oral	Years	17.7 μg/l NU	2–6 years after cessation	No differences in uranium excretion
Selden *et al.* (2009)	453; general population	227	226	18–74	Oral (water)	Years	6.7–25.2 μg/l NU	During exposure	> U in urine; < NAG (exposed); > (tendency) BMG, kappa chains, HC protein; No changes in urinary Glc, phosphates, calcium, Prot., Cr.
Zamora *et al.* (2009)	54	39	15	12–73	Oral (water)	Years	0,4–845 μg/l NU	During exposure	> GGT, ALP, LDH, NAG and BMG in urine; No changes in Prot., and glc.
Anderson *et al.* (2007)	581; gas plant workers	—	—		Inhalation	Years	73 μg/m^3 NU and EU	During exposure	> U in urine
Boice *et al.* (2007)	2161; workers and residents near uranium factory	1368	796	> 18	Inhalation	> 1 year	—; NU	During exposure	> U in urine
Parrish *et al.* (2008)	—; residents near uranium factory	—	—	—	Inhalation	Years	300 μg/ g U, DU	20 years after cessation	> U in urine
McDiarmid *et al.* (2001)	15; Gulf war veterans	—	—	—	Inhalation; dermal	Years	—; DU	8 years after cessation	> U in urine; > phosphates; < Clcr; No change in Crs, BUN, BMG, Cru y Prot.

TABLE 4—*Continued*

Study	Participants number	Male	Female	Ages	Exposure source	Exposure time	Uranium amount and type	Study moment	Observations
McDiarmid *et al.* (2006)	31; Gulf War veterans	—	—	—	Inhalation; dermal	Years	—; DU	12 years after cessation	> U in urine; No change in Crs, BUN, Ca, RBP, BMG, NAG, ALP, phosphates, Clcr
Squibb and McDiarmid (2006)	102; Gulf War veterans	—	—	—	Dermal	Years	—; DU	15 years after cessation	> U in urine; No change in Crs, BUN, Clcr, Ca, Glc Phosphates, BMG, Prot., RBP, ALP, NAG
Squibb *et al.* (2005)	16; Gulf War veterans	—	—	—	Dermal; oral; inhalation	6–10 years	25–190 μg DU (dermal)	6–10 years after cessation	> U in urine; > Prot., RBP;No change in Crs, ALP, NAG
McDiarmid *et al.* (2007)	108; Gulf War veterans	—	—	—	Dermal; oral; inhalation	Years	—; DU	Years after cessation	> U in urine; > Phosphates and Ca in urine; No change in Crs, BUN, Clcr, Ca, pot, BMG, RBP, ALP, NAG
Helmer *et al.* (2007)	56; Gulf War veterans	—	—	—	Oral; inhalation; dermal	Years	—; DU	Years after cessation	> U in urine

Note. NU, natural uranium; DU, depleted uranium; U, uranium; Glc, gucosuria; Crs, serum creatinine; BUN, blood urea nitrogen; BMG, β-2-microglobulin; Cru, urinary creatinine; Prot., proteinuria; Ca, calcium; Clcr, creatinine clearance; NAG, N-acetyl-β-*D*-glucosaminidase; GGT, gamma-glutamyl-transpeptidase; EU, enriched uranium.

usually determined through alterations in parameters, such as microalbuminuria, glucosuria, and β-2-microglobulinuria. There are few studies from which epidemiological information can be drawn and they are incomplete and biased (Table 4). This is because it is difficult to know (1) the number of people exposed, (2) whether they in fact underwent some degree of renal damage, (3) the characteristics of each episode of exposure are highly variable (duration, dose, route of exposure, etc.), and (4) the possible existence of other comorbility factors, known or not.

The first documented data concerning chronic exposure date back to the 19th century, before the discovery of insulin, because uranium was then used as a treatment for diabetes mellitus (Hodge *et al.*, 1973). Thus, the treated population could provide data about the chronic toxicity of uranium in humans. However, the information from this population should be taken with caution because it is now known that diabetes is the main cause of chronic kidney disease in developed countries (Molitch *et al.*, 2004). Another factor that further complicates the issue is the documented fact that hyperglycaemia *per se* reduces the renal damage caused by metals (Jin *et al.*, 1996; Shyh *et al.*, 1984), although diabetic nephropathy caused by chronic diabetes increases the susceptibility to metal nephrotoxicity (Jin *et al.*, 1994, 1999). Several independent studies have provided evidence of humans (totaling ~24 people) treated orally with soluble uranyl nitrate, at 3 doses of 2 g daily (6 g/day) over months or even years (Bond, 1898; Bradbury, 1896; Duncan, 1897; Wilcox, 1917). No kind of renal damage was reported in any of these cases (Wilcox, 1917). Nevertheless, it is not possible to rule out that the patients treated with these concentrations of uranium might have suffered some type of kidney lesion. In the 19th century, there was no pathological or diagnostic information available that would allow a slight or moderate degree of damage to be detected, unlike current diagnostic techniques that allow specific aspects of such damage to be determined.

The highest concentrations of natural uranium present in water are found in mountainous regions of countries, such as Finland (Kurttio *et al.*, 2002; Vesterbacka *et al.*, 2005), Norway (Frengstad *et al.*, 2000), Canada (Mao *et al.*, 1995; Zamora *et al.*, 1998, 2009), Sweden (Selden *et al.*, 2009), and the United States (Hakonson-Hayes *et al.*, 2002; Magdo *et al.*, 2007; Orloff *et al.*, 2004). Accordingly, in these zones, population epidemiological studies on oral exposure to uranium have been conducted. One important bias common to such studies is that it is extremely difficult to establish the number of years over which the people surveyed have been exposed. It has generally been assumed that this would be proportional to the time of residence in the place affected. Table 4 shows the most significant data of these and other studies addressing chronic exposure to uranium, specifying the available data concerning renal effects, which are evaluated in the next section.

Pathophysiological Picture from Human Studies

The documented cases of chronic intoxication in humans indicate that there are few situations in which uranium produces symptoms of a reduction in glomerular filtration and azotemia, such as a decrease in the GFR (McDiarmid *et al*., 2001), and an increase in plasma creatinine and urea concentrations (Wyatt *et al*., 2008). In most studies, no reports have been made of alterations in these parameters, although a few authors have described alterations in parameters related to the function and integrity of the kidney structures, especially the tubular compartment, although inconsistently among the different studies. However, it is necessary to take into account that such inconsistency could be because of strong biases among them as regards the dose, duration and route of exposure, the time of diagnosis, sex, age, and other determinant factors. Nonetheless, as discussed below and reflected in Figure 2, it is interesting to note that the same changes have also been observed in laboratory animals exposed chronically to the metal ("Pathological Studies with Animal Models" section) and that the pattern of damage is similar to that produced by acute overexposure (with much higher doses—"Nephrotoxicity because of Acute Overexposure" section).

In some studies, reports have been made of proteinuria or the excretion of certain specific proteins, such as albumin (retinol-binding protein [RBP] and β-2-microglobulin, Magdo *et al*., 2007; Pinney *et al*., 2003), after uranium exposure. These findings are inconsistent with those obtained by other authors (Kurttio *et al*., 2006; McDiarmid *et al*., 2001, 2006, 2007; Zamora *et al*., 1998). In part, this may be also because of differences in sampling, the analytical method used, the statistical analyses applied, or the level of exposure to uranium. It is not known whether the observed general or selective proteinuria is of glomerular or tubular origin.

As in the case of acute nephrotoxicity, studies have also measured a series of kidney enzymes used as markers of tissue damage, including ALP, GGT, LDH, and NAG (Kurttio *et al*., 2002, 2006; McDiarmid *et al*., 2006; Zamora *et al*., 1998). In these studies, it was not possible to relate alterations in these enzymes to the ingestion of uranium. Nevertheless, in rats, a decrease in the activity of GGT in urine has been observed following chronic ingestion of the metal (Niwa *et al*., 1993), possibly because of the fact that the activity of this enzyme is inhibited by uranium (Nechay *et al*., 1980). ALP activity seems to be related to the chronic ingestion of uranium because a tendency for it to increase in urine has been observed (Kurttio *et al*., 2006; Zamora *et al*., 1998, 2009), although in other studies, it seems to be unaltered (McDiarmid *et al*., 2006; Squibb and McDiarmid, 2006; Squibb *et al*., 2005). A tendency for LDH activity to increase after chronic exposure to uranium in humans has also been reported (Zamora *et al*., 1998, 2009). Some studies have reported alterations of other tubular functions, such as increases in the excretion of calcium (Kurttio *et al*., 2002; Squibb and McDiarmid, 2006), glucose (Kurttio *et al*., 2002, 2006; Zamora *et al*., 1998), and phosphate (Kurttio *et al*., 2002; McDiarmid *et al*., 2001). However, no increases in calcium or phosphate levels have been observed in other studies (McDiarmid *et al*., 2006; Selden *et al*., 2009). However, no clear relation between exposure level, duration of exposure, and observed renal effects can be drawn from the available studies in humans (Table 3). Just as an example, whereas Wyatt *et al*. (2008) found increased serum creatinine in 156 people 1 year after the cessation of a chronic overexposure of years to drinking water containing over 30 μg U/l, Kurttio *et al*. (2002) found no alterations in this parameter in 325 people during an overexposure of years to water containing over 100 μg U/l. Together with the results obtained in animal models ("Pathological Studies with Animal Models" section), the available information indicates that chronic exposure to uranium may lead to a variable degree of renal damage, which in general terms ranges from no detectable alterations to a mild injury mostly of tubular origin. However, undetermined comorbidity factors seem to play an important role at determining the final effect of uranium in the kidneys.

Recent studies suggest that chronic exposure to uranium would be associated with an increase in plasma renin concentrations, which would result in an elevation of blood pressure and hence a predisposition to hypertension in subjects exposed to uranium (Kurttio *et al*., 2006). As commented above ("Nephrotoxicity because of Chronic Overdose" section), this effect has also been reported in acute overexposure to uranium. A new hypothesis has associated the kidney damage produced by chronic ingestion of uranium with the induction of renal anemia (anemia because of renal disease), which has been described as an early symptom in the progression of chronic renal disease (Berradi *et al*., 2008). This conclusion was reached after the discovery, in laboratory animals, of low red blood cell levels following chronic exposure to the metal (Berradi *et al*., 2008). However, this has not been corroborated in uranium workers (Shawky *et al*., 2002), although it has been observed in people living close to nuclear power plants (Pinney *et al*., 2003) and in soldiers exposed to uranium (Squibb and McDiarmid, 2006).

Finally, studies have been carried out to determine whether age influences renal damage. This aspect is of special relevance because children may be at a greater risk of developing renal damage after uranium exposure because they drink more water and food per kilogram of body weight than adults (Ershow and Cantor, 1989). In one study, it was found that chronic ingestion of uranium by a 3-year-old boy caused a much greater increase in the urinary excretion of β-2-microglobulin than the other individuals exposed to the same amount of the metal (Magdo *et al*., 2007). Moreover, adults with impaired renal function may also be at greater risk. In these, a decrease in creatinine clearance together with an increase in serum cystatin C levels has been reported (Kurttio *et al*., 2006). Regarding the possible increase in

		HUMAN	ANIMAL
SERUM	Serum creatinine	▲ Wyatt *et al.*, 2008 — Kurttio *et al.*, 2002; 2006; McDiarmid *et al.*, 2001; 2006; 2007; Squibb and McDiarmid 2006; Squibb *et al*,, 2005	▲ Maynard and Hodge, 1949; Taulan *et al.*, 2004 — Berradi *et al.*, 2008; Gilman *et al.*, 1998a; 1998b; Tissandié *et al.*, 2008
SERUM	BUN	▲ Wyatt *et al.*, 2005 — McDiarmid *et al.*, 2001; 2006; 2007; Squibb and McDiarmid, 2006	▲ Maynard and Hodgue, 1949 — Gilman *et al.*, 1998a; 1998b;Taulan *et al*,, 2004; Tissandié *et al.*, 2008
SERUM	Creatinine clearance	▼ McDiarmid *et al.*, 2001 — Kurttio *et al*,, 2002; McDiarmid *et al.*, 2006; 2007; Squibb and McDiarmid, 2006	▼ Pozzani, 1949; Stockinger *et al.*, 1953
URINE	Proteinuria	▲ Magdo *et al.*, 2007; Pinney *et al.*, 2003; Squibb *et al.*, 2005 — Kurttio *et al.*, 2002; 2006; McDiarmid *et al.*, 2001; 2006; 2007; Selden *et al*,, 2009; Squibb and McDiarmid, 2006; Zamora *et al.*, 1998; 2009	▲ Maynard and Hodgue, 1949; Pozanni, 1949; Stockinger *et al*,, 1953
URINE	Glucose	▲ Kurttio *et al.*, 2002; Zamora *et al.*, 1998; McDiarmid *et al.*, 2007 — Kurttio *et al*,, 2006; Selden *et al*,, 2009; Zamora *et al.*, 2009	▲ Ortega *et al.*, 1989 — Taulan *et al.* 2004
URINE	Ca^{++}	▲ Kurttio *et al.*, 2002 — Kurttio *et al.*, 2006;McDiarmid *et al.*, 2006; 2007 Selden *et al*,, 2009; Squibb and McDiarmid, 2006	— Gilman *et al.*, 1998a; 1998b
URINE	Phosphates	▲ Kurttio *et al.*, 2002; McDiarmid *et al.*, 2001 — Kurttio *et al.*, 2006; McDiarmid *et al.*, 2006; 2007; Selden *et al*,, 2009; Squibb and McDiarmid, 2006	— Gilman *et al.*, 1998a, 1998b
URINE	ALP	▲ Zamora *et al.*, 1998; 2009 — Kurttio *et al*,, 2006; McDiarmid *et al.*, 2006; 2007; Squibb and McDiarmid, 2006; Squibb *et al.*, 2005	
URINE	GGT	▲ Zamora *et al.*, 1998; 2009 — Kurttio *et al.*, 2006	▲ Taulan *et al.*, 2004 ▼ Niwa *et al.*, 1993 — Gilman *et al.*, 1998a, 1998b
URINE	LDH	▲ Zamora *et al.*, 1998; 2009 — Kurttio *et al.*, 2006	— Gilman *et al.*, 1998a; 1998b
URINE	NAG	▲ Zamora *et al.*, 1998; 2009 — Kurttio *et al.*, 2006; McDiarmid *et al.*, 2006; 2007; Squibb *et al.*, 2005;Squibb and McDiarmid, 2006; Zamora *et al.*, 1998;	— Gilman *et al.*, 1998a; 1998b

FIG. 2. Simptomatology after Chronic Exposure to Uranium. Comparison between Humans and Animals. BUN, blood urea nitrogen; Ca, calcium; ALP, alkaline phosphatase; GGT, gamma-glutamyl-transpeptidase; NAG, N-actetyl-β-*D*-glucosaminidase, ▲, increase; ▼, decrease; —, no change.

blood pressure because of increases in plasma renin concentrations, a correlation has been found between exposure to uranium and increased blood pressure (Kurttio *et al*., 2006).

Histological Alterations

There is little histological information in humans following chronic uranium exposure, and in a review of the literature, we have only been able to find a few studies performed on uranium workers. In one case, an autopsy was performed on a miner who had been exposed to uranium for years. The mean uranium content in was 2 ng U/g kidney, and his annual uranium excretion calculated with several data was 14.3 mg U/year (0.04 mg U/day). Sclerotic zones were observed in the glomeruli, together with lymphocyte infiltration and zones of arteriosclerosis (Russell and Kathren, 2004). In another work, the authors performed an autopsy on seven uranium workers together with another six people not known to have been

exposed to the element. In this case, no histological differences were observed between both groups (Russell *et al*., 1996). Studies have been carried out on chronic exposure in laboratory animals and the third segment of the proximal tubule (S3) has been established as the site mainly affected (Gilman *et al*., 1998a,b,c; Mao *et al*., 1995). Apical nuclear displacement, cytoplasmic vacuolization, and tubular dilation were observed, although a certain degree of glomerular damage, such as adherences and focal sclerosis, was also found (Gilman *et al*., 1998c).

The histological data obtained to date in human beings do not allow a clear idea to be gained of the type of damage caused by uranium through chronic exposure nor in which part of the kidney such damage is caused. However, several investigations carried out in animals suggest that the proximal tubule is the one most affected by exposure to uranium, a result that has been reproduced in both acute and chronic intoxications. In both cases, tubular dilation, cytoplasmic vacuolization, and apical nuclear displacement have been reported. However, the corticomedullary necrosis observed after acute intoxications is not observed after a chronic exposure to uranium. This metal has also been linked to the production of glomerular damage, although in this case, the evidence is not as clear because this kind of damage has only been observed in a few studies.

As in the case of acute exposure, renal tissue tends to regenerate after exposure to high and repeated doses of the metal, pointing to the development of resistance to the toxic effects of uranium (Bentley *et al*., 1985; Durbin *et al*., 1997; Dygert, 1949; Maynard and Hodge, 1949; Pozzani, 1949; Yuile, 1973). The mechanism through which this tolerance is acquired is based on the morphological effects observed in regenerated cells of the proximal tubule (Leggett, 1989; MacNider, 1929), which appear swollen, without microvilli on the luminal surface and with a reduced number of mitochondria. It has been suggested that this reduction in microvilli could give rise to a decrease in the binding of uranium to the renal cell surface and hence reduce its toxic action at this site (Gilman *et al*., 1998c).

Another possible tolerance mechanism is related to the increase in heat-shock proteins (HSPs) (Ciocca *et al*., 1992; Elliott *et al*., 1982; Honda and Sudo, 1982; Salminen *et al*., 1997). In *in vitro* studies, it has been observed that renal tubular cells express high levels of HSPs in response to uranyl nitrate (Goering *et al*., 2000; Mizuno *et al*., 1997). In particular, the HSP25 and HSP70i proteins have been associated with cytoprotection against other renal toxic agents, among which are mercury and gentamicin (Elliott *et al*., 1982; Goering *et al*., 2000; Zager *et al*., 1994). HSP induction seems to be different in the case of uranium (Goering *et al*., 2000) because exposure to this metal has mainly been linked to an increase in HSP73 expression in kidney cells (Mizuno *et al*., 1997) as well as increases in the levels of HSP25, HSP32, and HSP70i. In contrast, in *in vivo* studies, no increases in the levels of these proteins have been reported (Ananthan *et al*., 1986), such that it has not been possible to corroborate this hypothesis.

Conclusions and Perspectives

All the above suggests that chronic exposure to uranium cannot be easily linked to the occurrence of nephrotoxicity and that, in the event of the metal being responsible for it, it may revert with time. In studies on exposure performed only a short time after the ingestion or inhalation of uranium, some urinary markers of renal damage have been found to be altered, such as urinary β-2-microglobulin, although some years after exposure to the metal has ceased, the renal parameters studied seem to return to normal values and only an increased urinary excretion of uranium is observed. It has been proposed that such excretion could be a marker of exposure to the metal. However, it is difficult to associate uranium excretion with nephrotoxicity because despite the excretion of the metal in the urine, in most cases, no nephrotoxicity is observed. This therefore indicates that (1) the risk of renal damage in humans because of chronic exposure to uranium is at most uncertain and variable and (2) even if in some cases chronic exposure produces an undetermined level of renal injury, it reverts with time, suggesting that people exposed chronically to uranium do not develop a typical chronic renal disease.

Our impression is that a very long period of overexposure (many years) would be necessary for uranium to accumulate in target organs (in this case, the kidneys) at levels above the toxicity threshold and to cause tangible deleterious effects. Studies with an exposure time of months carried out on animals require higher doses to produce similar effects to those detected in humans subjected to years of low-dose exposure. Figure 2 shows a comparison of the changes observed in different markers following chronic exposure in humans and animals. In sum, by integrating the information concerning acute and chronic overexposure, it may be deduced that uranium nephrotoxicity probably derives from tissue accumulation above certain levels, which can be attained with different combinations of exposure time and dose, such that the greater the dose, the shorter the time and *vice versa*. However, further information is needed together with new studies to determine correctly the profile of uranium nephrotoxicity because of both chronic and acute overexposure.

An emerging issue to be considered is the possibility that chronic exposure to uranium, in stages in which it still does not cause any renal alteration, might be able to predispose subjects to develop acute renal impairment (including acute renal failure) because of exposure to other potentially nephrotoxic environmental or therapeutic agents that under normal conditions would not cause renal damage. In this sense, data from our own laboratory (unpublished) indicate that chronic exposure of rats to high doses of the metal over some months, without eliciting symptoms of nephrotoxicity by itself, reduces

the threshold of nephrotoxicity and enhances the nephrotoxic effects of certain drugs, such as the aminoglycoside antibiotic gentamicin. Were it to be confirmed, this situation would be of huge clinical relevance because, in an occult and nondiagnosable way, chronic overexposure could render the sector of the population in contact with the metal more susceptible to renal failure. We believe that this is an issue requiring further research effort in the near future, especially within the context of the detection of this potential situation.

MECHANISMS OF NEPHROTOXIC ACTION

Although uranium is widely used as an experimental nephrotoxic agent, the underlying physiological mechanism responsible for renal damage has not been fully elucidated. One limitation to our knowledge about this issue is that most of the information has been obtained in acute studies with animals (Diamond *et al*., 1989; Haley, 1982, Haley *et al*., 1982; Morrow *et al*., 1982; Rothstein, 1949; Stokinger *et al*., 1953; Taylor and Taylor, 1997; Thun *et al*., 1985).

The first issue that has not been suitably clarified is whether uranium needs to penetrate cells to exert its toxic effect. Some authors have proposed that this would not be necessary because its effects (or a large part of them) derive from binding to certain components of the cell membrane (Leggett, 1989; Muller *et al*., 2006). According to those authors, such effects would be based on interference with the reabsorption of glucose, sodium, amino acids, proteins, water, and other substances, which would lead to a slow cell death because of the suppression of cell respiration (Hori *et al*., 1985; Leggett, 1989; Nechay *et al*., 1980). However, others have proposed that the metal does need to enter cells to exert its toxic effects. This has been observed in LLC-PK_1 cells of the proximal tubules in studies aimed at determining whether there are differences in the toxicity of the U-bicarbonate and U-citrate complexes (L'Azou *et al*., 2002; Mirto *et al*., 1999). In these studies, it was possible to correlate the presence of the uranium complex inside the cells and the toxic effect. Thus, it was observed that the U-citrate complex entered the cells and exerted a significant toxic effect in them, whereas the U-bicarbonate complex, which did not enter the cells, exerted a much lower toxic effect (Mirto *et al*., 1999). Accordingly, the authors concluded that as regards its toxic effects, the entry of uranium into cells is very important. Below, we detail some of the aspects related to the mechanism involved in uranium nephrotoxicity.

Alterations in Solute Transport

In studies of brush border membrane vesicles from rat renal tubular cells, it has been observed that uranyl acetate produces a decrease in glucose transport because of a reduction in the number of sodium-glucose transporters (SGLT) (Goldman *et al*., 2006). Hori *et al*. (1985) also reported a decrease in the sodium-dependent glucose gradient that led them to suspect that the enzymatic activity of Na^+ K^+, adenosine triphosphatase (Na^+, K^+ ATPase, or sodium pump) could be inhibited by uranyl nitrate. Similar results were obtained by Brady *et al*. (1989) in rabbit kidney cells. Those authors suggested that uranyl nitrate would inhibit both the sodium-dependent and the sodium-independent ATP utilization and mitochondrial oxidative phosphorylation. Muller *et al*. (2006) reported that the cytotoxicity of uranium for LLC-PK_1 cells would depend on the extracellular concentration of phosphate. High concentrations of phosphate in the medium gave rise to the formation of uranium-phosphate complexes, which inhibited the Na/P_i II transporter participating in the reabsorption of organic phosphate.

Oxidative Stress

Oxidative stress has been proposed as a possible mediator of renal damage because of exposure to uranium. In the rat renal proximal tubular cell line (NRK-52E), uranium stimulates the production of reactive oxygen species (ROS), caspases 9 and 3, and cell death because of apoptosis (Thiebault *et al*., 2007). In studies carried out on rat renal tissue (Linares *et al*., 2006), an increase was observed in ROS levels, in oxidized glutathione (GSSG), and in the activity of SOD. An increase in thiobarbituric acid–reactive substances, indicative of lipid peroxidation and oxidative stress, was also observed. It has also been suggested that uranium could act as a catalyst in the Fenton/Haber-Weiss reaction (Kovacic and Jacintho, 2001), which would facilitate the conversion of the superoxide anion and hydrogen peroxide into hydroxyl radicals, believed to be responsible for initiating lipid peroxidation (Linares *et al*., 2006; Stohs and Bagchi, 1995; Taulan *et al*., 2004). In other studies, the pro-oxidant action of uranium has been related to a disturbance in the activity of acetyl cholinesterase and of monoamine metabolism (Kurttio *et al*., 2002; Linares *et al*., 2006; Sanchez *et al*., 2001) and to an increase in mitochondrial oxidative phosphorylation in the proximal tubule (Brady *et al*., 1989). These results indicate that uranium could produce oxidative stress, inducing, at least in part, the death of target cells (Sabolic, 2006). Figure 3 shows the possible mechanism responsible for renal damage caused by uranium.

Alterations in Gene Expression

Chronic exposure to uranium in animals elicits changes in the profiles of the renal expression of genes related to oxidative stress, cellular metabolism, solute transport, and signal transduction, among other processes (Taulan *et al*., 2004, 2006). As seen in Table 5, some of these changes in renal gene expression are correlated with the appearance of pathological effects, such as inflammation, apoptosis, oxidative stress, and alterations in cellular homeostasis. However, the role of these genes in the

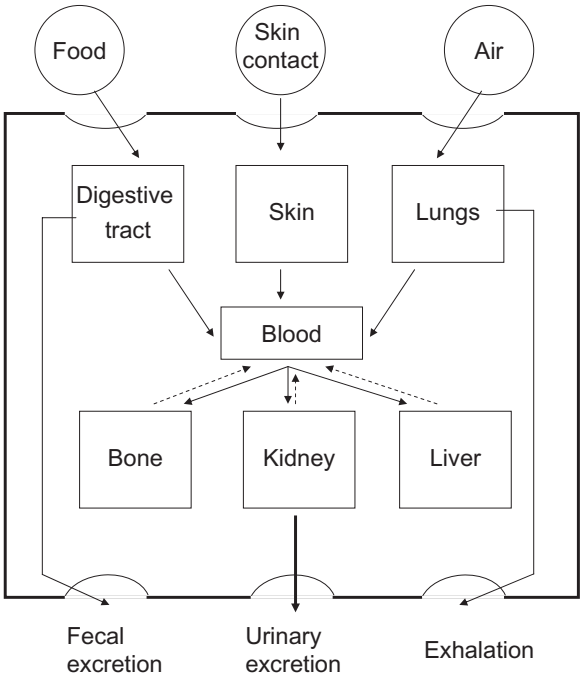


FIG. 3. Absorption, distribution, accumulation, and excretion of uranium in the human body.

initiation and development of such effects, as well as their causal relationships with them, have not yet been clarified.

Diagnosis of Intoxication by Uranium

The diagnosis of nephrotoxicity because of uranium requires two components: (1) the detection of overexposure to the metal and (2) determination of the renal toxic effects. Detection of the latter indicates that the former has occurred at a specific moment. It is also possible that subnephrotoxic chronic exposure could lead to a hidden predisposition to acute renal failure because of other agents or that it might in some way cooperate or increase the effect of other causes of CRI. As commented in "Conclusions and Perspectives" section under "Nephrotoxicity because of Chronic Overexposure" section, some results obtained by us indicate that chronic treatment with uranyl nitrate predisposes individuals to the acute renal failure elicited by other potentially nephrotoxic drugs, such as gentamicin.

Detection of Overexposure

To date, the most efficient way of diagnosing exposure to uranium is its detection in urine (Ballou *et al.*, 1986; Cooper *et al.*, 1982; Downs *et al.*, 1967; Morrow *et al.*, 1982; Stradling *et al.*, 1981, 1991). According to the United States Nuclear Regulatory Commission guide, acceptable methods for quantifying uranium in urine should have a limit of 5 μg/kg and a precision of 30% (Kressin, 1984). Different methods for detecting the metal are available, such as kinetic fluorescence analysis (Hooper *et al.*, 1999; Price, 1989), alpha spectrometry (Beyer *et al.*, 1993; Chalabreysse *et al.*, 1989; Harduin *et al.*, 1994; Sachett *et al.*, 1984; Spencer *et al.*, 1990; Wreen *et al.*, 1992), inductively coupled plasma mass spectrometry (Baglan *et al.*, 1999; Ejnik *et al.*, 2000; Krystek and Ritsema, 2009; Lorber *et al.*, 1996; Paquet *et al.*, 2006; Zamora *et al.*, 1998), neutron activation (Sansone *et al.*, 2001), and atomic absorption (Wessman, 1984).

Besides uranium determination, of all the other markers studied only an increase in renal glucose excretion has been linked to exposure to uranium in the human being (Kurttio

TABLE 5
Modifications in Renal Gene Expression Profiling (ARNm) after an Acute and Chronic Exposure to Uranium in Mice

Gen	Physiological process	Acute exposure	Chronic exposure	Observed effect
Na-Pi II	Solute transporters	<	—	Hypophosphatemia
Na/K ATPase	Solute transporters	—	<	Decreased sodium reabsorption
SOD	Oxidative stress	<	>	Induction of ROS
GPx	Oxidative stress	>	>	Induction of ROS
Fau	Ribosomal protein synthesis	>	>	Perturbation in protein synthesis
Odc	Cellular metabolism	<	<	Arrest of the cell cycle
Umod	Tamm Horsfall protein synthesis	>	<	Inflammation
Tctp	Apoptosis, inflammation	>	>	Apoptosis, inflammation
Gal-3	Apoptosis, inflammation	>	—	Apoptosis, inflammation
Opn	Inflammation, proximal tubules regeneration	>	—	Inflammation, tissular regeneration
MT-2	Metallothionein synthesis	>	—	Uranium detoxification
Igfbp7	Cellular proliferation	>	—	Not established
Rps 29	Apoptosis	>	—	Not established
Octs 2	Organic cation transport	<	—	Decreased of organic cation transport

Note. This table was performed with data from Taulan *et al.* (2004) (chronic) and Taulan *et al.* (2006). <, decreased; >, increased; – unchanged. Modified genes: Fau, Finkel-Biskis-Reilly murine sarcoma virus; Odc, ornithine decarboxylase; Umod, uromoduline; Tctp, translationally regulated transcript; Gal-3, galactose binding, soluble 3; Opn, osteopontin; MT-2, metallothionein 2; Igfbp7, insulin-like growth factor binding protein 7; Octs 2, organic cation transporter 2.

et al., 2002, 2006; Zamora *et al*., 1998), in agreement with experimental studies carried out on different animal species (Gilman *et al*., 1998c; Martinez *et al*., 2003; Ortega *et al*., 1989).

Detection of Nephrotoxicity

For the detection of renal toxicity, there are no specific biomarkers for diagnosing that it has been produced by uranium; thus, general markers of nephrotoxicity are used when a person is suspected to have been exposed to the metal (Saccomanno *et al*., 1982; Thun *et al*., 1985; Zamora *et al*., 1998). As in any situation in which there is suspicion of renal damage, the most common diagnosis involves measurement of the plasma creatinine concentration, which increases with the decrease in the GFR. From the plasma creatinine concentration, together with certain common anthropometric data (weight, sex, age, etc.), by using established algorithms such as the Modification in Diet in Renal Disease equation or the Cockroft-Gault equation (Snively and Gutierrez, 2004; Snyder and Pendergraph, 2005), it is possible to obtain an estimation of the GFR. A slight proteinuria and amino aciduria are also indicative of this damage (Saccomanno *et al*., 1982; Thun *et al*., 1985), together with other markers of tubular damage, such as urinary glucose, ALP, and β-2-microglobulin (Zamora *et al*., 1998).

Presently, the most serious problem in the diagnosis of renal impairment, both chronic and acute, is that it is based on the detection of the consequences of renal dysfunction, which only occur when the kidney damage is in a very advanced stage. At that time, both the possibility of intervening and the prognosis are poor (Vaidya *et al*., 2008). The future of the diagnosis of these kidney diseases will require the identification of markers, preferentially urinary markers, able to detect damage in the early stages (Vaidya *et al*., 2008). In the case of acute renal impairment, some urinary markers that appear in the urine a few hours after the start of the damage have been identified. Among them is neutrophil gelatinase–associated lipocalin (lipocalin 2), Kidney Injury Molecule 1 (KIM-1), interleukin 18, and cystatin C, to mention but a few, that are in an advanced stage of validation (reviewed in Vaidya *et al*., 2008). The next step in the refinement of the capacity to diagnose acute renal damage will be the discovery of markers (or sets of markers—fingerprints) that as well as being detected early are able to discern the cause of the damage. This, then, is also a matter pending in the diagnosis of acute renal impairment because of uranium exposure.

Regarding chronic renal disease, there are not even any good early markers of the disease. In the case of some chronic renal conditions with a given etiology, such as diabetes, the possible role of microalbuminuria in the early diagnosis and prognosis of the evolution of the disease is currently being discussed (Parving *et al*., 2002; Schena and Gesualdo, 2005). In diabetic nephropathy, microalbuminuria occurs prior to the appearance of signs of kidney dysfunction, such as the increase in plasma creatinine concentrations. However, its value as a marker is debatable and it probably does not occur early enough to be of use (Parving *et al*., 2002). In the case of chronic intoxication with uranium, it remains to be seen whether the gradual accumulation of the metal will lead to a subacute kidney lesion. This difference is not trivial in the diagnosis of nephrotoxicity because of chronic exposure because CRI is a disease that, when the no-return point has been passed, enters a vicious degenerative cycle and progresses irreversibly and independently of the initial cause of the damage (Compton, 2004).

Conclusions and Perspectives

Accordingly, the perspectives for improving the diagnosis of this condition are necessarily based on a better understanding of the renal pathology associated with chronic intoxication with the metal. In the case of chronic uranium exposure producing CRI, it would be necessary to identify very early general and specific markers of this type of lesion. In the case of chronic exposure inducing a subacute lesion, improvements in diagnosis will probably involve the identification of urinary markers that afford information about the level of uranium accumulation in the kidney. In this sense, it will be necessary to determine whether the urinary excretion of the element could fulfill this function or whether it would be necessary to identify other markers that are more tightly correlated with the physiopathological outcomes of increased levels of accumulation of the metal in renal structures.

TREATMENT OF INTOXICATION BY URANIUM

Therapy for intoxication with uranium has a dual mission. On one hand, it must attempt to palliate the intoxication by halting the absorption, distribution, or the action of the metal or by accelerating its excretion, and—on the other—it must take into account the repair of the toxic damage caused.

Prevention of Intoxication

Prevention of intoxication is attempted at two nonexcluding levels aimed at preventing absorption and, if this has already occurred, at preventing uranium action on target organs.

Prevention of Absorption

The main objective in treating patients overexposed to uranium is to prevent or minimize absorption from the site of entry and distribution and to increase its removal from the blood or target organs by favoring its excretion (Cronin and Heinrich, 2000). When acute intoxication occurs through the oral route, the usual measures stipulated for the treatment of the

ingestion of toxic substances must be brought into play with a view to lowering intestinal absorption. Among such measures are (1) stomach gastric lavage; (2) the use of emetics, such as oral ipecacuana; (3) laxatives; (4) ion exchange agents; and (5) the administration of antacids containing salts of aluminium, barium sulfate, sodium phytate, and salts of glucuronic and maluronic acid (ICRP, 1991). When intoxication has occurred through the inhalation route, the therapeutic agents include pancreatic dornase, Triton, or Tween-90, which decrease the viscosity of the endobronchial mucosa and act on the mucopolysaccharides and nucleoproteins of the respiratory tree, favor the elimination of uranium through coughing, and prevent its pulmonary absorption (ICRP, 1991).

Prevention of Action

If it is suspected or known that the uranium has reached the blood stream, complex-forming agents are employed, such as bicarbonate, citrate, lactate, and fumarate. Of these, bicarbonate is the one reported to have the highest elimination efficiency (Neuman *et al*., 1948) because it binds to uranium to form ring-shaped complexes, which are excreted in the urine. Additionally, bicarbonate alkalinizes the urine, which improves renal uranium excretion. This treatment must be applied as soon as possible after exposure, before the uranium has a chance to be incorporated into the target organs. Generally, it is administered 24 h after exposure (Domingo *et al*., 1992; Ortega *et al*., 1989), but its use is limited because of the possible adverse effects of hypocalcaemia and alkalosis (Bhattacharyya *et al*., 1992; NCRP, 1980; WHO, 1984).

Classic chelating agents. If uranium has already entered the target organs, therapy with chelating agents is initiated. In clinical practice, these agents are usually used as antidotes in intoxications, both acute and chronic, with metals. These compounds bind to the metal and improve its excretion. Moreover, in some cases, they decrease the toxicity of the metal because they prevent it from binding to its target cells. To obtain the best effects in therapy with chelating agents, it is necessary to adjust the length of the treatment (Basinger and Jones, 1981; Domingo *et al*., 1990; Henge-Napoli *et al*., 1995, 1999) and the doses of the chelating agents (Henge-Napoli *et al*., 1999) and to take into account the route of entry and the chemical form of the uranium involved (Houpert *et al*., 2003).

In humans, the chelating agents used are EDTA and diethylene triamine pentaacetic acid (DTPA) (Basinger and Jones 1981; Dagimanjian *et al*., 1956; Domingo *et al*., 1989, 1990; Durbin *et al*., 1997; Henge-Napoli *et al*., 1995, 1998, 1999; Houpert *et al*., 2001; Martinez *et al*., 2000, 2003; Stradling *et al*., 1991; Ubios *et al*., 1994).

EDTA has been used both in human medicine and in experimentation with animals for the treatment of intoxications by inorganic substances (Hammond and Beliles, 1980). In intoxications with uranium, both acute and chronic, it is administered iv dissolved in 5% glucose or in physiological saline. It is crucial to assess kidney function before starting treatment because the use of EDTA is contraindicated in patients with established renal disease. EDTA is also used in combination with sodium (Na-EDTA), although this may give rise to hypocalcaemia, and thus, the use of EDTA associated with calcium is preferred (Ca-EDTA).

DTPA is a chelating agent belonging to the polyaminocarboxylate series that forms highly stable water-soluble complexes that are excreted by the kidney. The U.S. Food and Drug Administration approves the use of calcium and zinc salts with DTPA in cases of human contamination with transuranic elements. Ca-DTPA provides efficient treatment of contamination with actinides (Rosen *et al*., 1989). The therapeutic efficiency of both (Ca-DTPA and Zn-DTPA) depends on the chemical form and solubility of the transuranic element. Both agents are useful for the elimination of soluble uranium salts, such as nitrates and chlorides, but their efficiency with sparingly soluble salts such as oxides is somewhat weaker (Catsch, 1959). They are injected or infused iv, injected im, or are administered in aerosol form for inhalation. The route of administration depends on the circumstances of the intoxication by uranium, its chemical form, and the route of contamination. Ca-DTPA is more efficient than Zn-DTPA if used early on after the contamination has occurred (Lloyd *et al*., 1977), but the efficiency of both is the same if they are administered later. The injection of 1 g of Ca-DTPA per week in long-term treatments does not elicit toxic effects in patients contaminated with actinides (Ballou, 1962). In contrast, a constant infusion of Ca-DTPA did cause severe toxic effects in animals, which led to death after a few days (Taylor and Mays, 1979). The toxicity of Zn-DTPA is 30-fold lower than that of its Ca-DTPA counterpart in fractionated doses (Lushbaugh and Washburn, 1979). Although DTPA is the recommended treatment after accidental exposures to uranium, derivatives, it is very risky to use this substance because at high doses, it is nephrotoxic (Diamond *et al*., 1989; Doolan *et al*., 1967). In addition, (1) in certain studies with laboratory animals, the use of DTPA has not proved to be useful (Archimbaud *et al*., 1994; Domingo *et al*., 1989, 1990; Ubios *et al*., 1994); and (2) more recently, *in vitro*, it has been observed that the administration of DTPA increases the cytotoxicity of uranium in LLC-PK1 cells (Houpert *et al*., 2003).

DTPA together with EDTA were used in the treatment of a patient who ingested a large amount of uranium. This treatment was inefficient at increasing the excretion of the metal (Pavlakis *et al*., 1996).

New chelating agents. Apart from the classic chelating agents, others have also been studied in animal experimentation, among which is sodium-4,5-dihydroxybenzene-1,3-disulphonate (Tiron), which has been seen to decrease uranium toxicity in mice (Domingo *et al*., 1989, 1990, 1992; Gomez *et al*., 1991; Ortega *et al*., 1989). In this species, Tiron has been

found to be useful at doses of 1500 mg/kg/day (Bosque *et al*., 1993; Ortega *et al*., 1991), whereas in rats, it has not been reported to be so efficient (Zalups, 1991). For treatment with Tiron to be effective, early administration is crucial, such that its use would only be of value in acute intoxications. Another chelating agent used in experimental rodents is ethane-1-hydroxy-1,1-biphosphonate. This is a drug used in the treatment of osteoporosis and when administered ip is useful in the treatment of uranium intoxication (Henge-Napoli *et al*., 1999; Houpert *et al*., 2001; Martinez *et al*., 2000, 2003; Ubios *et al*., 1994). Catechol-3, 6-bismethylaminodiacetic acid (CBMIDA) has also been found to be effective in the experimental setting (Fukuda *et al*., 2001). Studies have been performed to determine whether the administration of this chelating agent together with bicarbonate might improve the toxic effects. The results obtained to date suggest that this is not the case, and additionally, bicarbonate has adverse effects when coadministered with ethane-1-hydroxy-1, 1-bisphosphonate (EHBP) (Fukuda *et al*., 2005a). Furthermore, a series of chelating agents with which no beneficial effects have been obtained has been removed from the therapeutic arsenal, among which are triethylene tetraamino hexaacetic acid (TTHA) and diamine diethylthioether tetraacetic acid (DDTA) (Ivannikov, 1966).

The affinity of uranium for phosphoric acid molecules is known, and for this reason, the efficacy of polyaminophosphoric acids, bisphosphonates, and phosphoalkylpolyphosphates has been investigated (Bailly *et al*., 1994; Bulman, 1987; Dagimanjian *et al*., 1956; Ebetino and Jamieson, 1990; Ebetino *et al*., 1990; Gillard *et al*., 1989) because these substances complex uranium (Bulman, 1987; Ebetino and Jamieson, 1990; Ebetino *et al*., 1990). In some cases, a strong reduction in uranium contents in kidney and bone was observed when administration was performed rapidly after exposure to the metal (Gray *et al*., 1992; Henge-Napoli *et al*., 1998; Ubios *et al*., 1994). The problem with polyphosphates is that although they reduce the mortality caused by poisoning with uranium, they also elicit metabolic acidosis and hypocalcaemia, which makes their use impractical in the treatment of uranium contamination (Dagimanjian *et al*., 1956).

In the same sense, studies have been conducted with hydroxyaspartate, citrate (Rajan and Martell, 1964), and catechol disulphonate (Lusky and Braun, 1950). Of these, multidentate catecholate and ligands of hydroxyaspartate administered rapidly after iv or ip injection of uranium decrease the renal content of the metal to a considerable extent (Henge-Napoli *et al*., 1995, 1998). However, the treatment is not successful if administered more than 30 min after the initial exposure to this substance (Durbin *et al*., 1997; Henge-Napoli *et al*., 1995), such that its behavior can be said to be similar to that of phosphonates and hence of no use in clinical practice (Gray *et al*., 1992; Henge-Napoli *et al*., 1998; Ubios *et al*., 1994).

Several attempts have been made to produce a lipophilic chelating agent that will allow better access to the intracellular medium through the membrane lipid layer. Among such compounds, a lipophilic compound called Puchel, produced at Harwell (United Kingdom), was observed to be more effective than DTPA when administered through the inhalation route (Stradling *et al*., 1981), with better therapeutic effects when used in combination with DTPA, but this combination has not proved useful in clinical practice. For the treatment of chronic intoxication with uranium, studies have also been carried out regarding the possibility of inducing the mobilization of uranium from bone structures by means of parathyroid hormone. This has been studied in several experimental models, but this technique does not seem to offer a practical alternative for decreasing contamination by uranium in the organism (Durakovic *et al*., 1973).

Repair of Renal Damage

As mentioned above, another important therapeutic aspect is the repair of the damage produced, involving the regeneration of kidney tissues. Thus, cessation of exposure to the metal should favor the process of tissue regeneration discussed in "Treatment of Intoxication by Uranium" section. Because to date no treatments that favor renal tissue regeneration after damage because of any etiology are available, this remains a challenge for the future. In this sense, although still in an experimental context, some regenerative therapeutic strategies that could possibly be applied in uranium-induced nephrotoxicity are currently being tested. On one hand, investigators are developing treatment with growth factors that regulate the viability, proliferation, and migration of cells, among which hepatic growth factor (reviewed in Matsumoto *et al*., 2000, and Nigam and Lieberthal, 2000), but also insulin-like growth factor and epidermal growth factor (reviewed in Nigam and Lieberthal, 2000), are of interest. Exogenous administration of these factors to laboratory animals has demonstrated their ability to improve the repair of acute and chronic renal damage.

A new cell-based repair strategy consists of (1) stimulation of resident renal stem cells (Oliver *et al*., 2004) and (2) exogenous injection of kidney stem cells or bone marrow stem cells with transdifferentiation capacity (e.g., mesenchymal cells or haematopoietic stem cells, Anglani *et al*., 2004) to achieve the re-epithelialization of lost tissues, especially damaged tubules. In one experimental study, mesenchymal cells from bone marrow were used (reviewed in Brodie and Humes, 2005) owing to their known ability to differentiate into epithelial tubular cells (Herrera *et al*., 2004; Kale *et al*., 2003) and mesangial (Masuya *et al*., 2003) and endothelial cells for neovascularization (Patschan *et al*., 2006; Takahashi *et al*., 1999). This type of cell has been used to repair the damage caused by drugs or ischemia (Herrera *et al*., 2004), although its efficacy has also been questioned (reviewed in Brodie and Humes, 2005). Finally, other systems are being developed

including (1) a bioartificial kidney consisting of a dialysis system formed by a tubular structure containing ~1 billion tubular cells (Humes *et al*., 2004) and (2) implants of encapsulated cells with a view to removing uraemic toxins and for drug administration (see Brodie and Humes, 2005).

ACKNOWLEDGMENTS

We thank Nicholas S. Skinner from the Servicio de Idiomas, Universidad de Salamanca (Spain), for his assistance on the English version of the manuscript.

REFERENCES

Agency for Toxic Substances and Disease Registry, Centers for Disease Control and Prevention (ATSDR/CDC). (1990). *Agency for Toxic Substances and Disease Registry, Centers for Disease Control and Prevention*. Subcommittee Report on Biological Indicators of Organ Damage, Atlanta, GA. Available at: http://www.atsdr.cdc.gov/. Updated August 28, 2008. Accessed July 7, 2010.

Agency for Toxic Substances and Disease Research (ATSDR). (1999). *Agency for Toxic Substances and Disease Registry. Toxicological Profile for Uranium*. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Ananthan, J., Goldberg, A. L., and Voellmy, R. (1986). Abnormal proteins serve as eukaryotic stress signals and trigger the activation of heat shock genes. *Science* **232,** 522–524.

Anderson, J. L., Spitz, H. B., and Yiin, J. H. (2007). Estimating active bone marrow dose from occupational exposure to uranium at a former gaseous diffusion plant. *Health. Phys*. **93,** 113–119.

Anglani, F., Forino, M., Del Prete, D., Tosetto, E., Torregrossa, R., and D'Angelo, A. (2004). In search of adult renal stem cells. *J. Cell. Mol. Med*. **8,** 474–487.

Archimbaud, M., Henge-Napoli, M. H., Lilienbaum, D., Desloges, M., and Montagne, C. (1994). Application of calixarenes for the decorporation of uranium: present limitations and further trends. *Radiat. Prot. Dosimetry*. **53,** 327–330.

Baglan, N., Cossonnet, C., Trompier, F., Ritt, J., and Berard, P. (1999). Implementation of ICP-MS protocols for uranium urinary measurements in worker monitoring. *Health Phys*. **77,** 455–461.

Bailly, T., Burgada, R., Stradling, G. N., and Gray, S. A. (1994). In *Synthesis of new ligands for uranium decorporation*. (J. R. Maisin, Ed.), Vol. 75, p. 37. European Late Effects Project Group (EULEP) Newsletter, Mol, Belgium.

Ballou, J. E. (1962). Preliminary evaluation of several chelating agents for plutonium removal. *Health Phys*. **8,** 731–734.

Ballou, J. E., Gies, R. A., Case, A. C., Haggard, D. L., Buschbom, R. L., and Ryan, J. L. (1986). Deposition and early disposition of inhaled 233UO2(NO3)2 and 232UO2(NO3)2 in the rat. *Health Phys*. **51,** 755–771.

Banday, A. A., Priyamvada, S., Farooq, N., Yusufi, A. N., and Khan, F. (2008). Effect of uranyl nitrate on enzymes of carbohydrate metabolism and brush border membrane in different kidney tissues. *Food Chem. Toxicol*. **46,** 2080–2088.

Basile, C. (2008). The long-term prognosis of acute kidney injury: acute renal failure as a cause of chronic kidney disease. *J. Nephrol*. **21,** 657–662.

Basinger, M. A., and Jones, M. M. (1981). Tiron (sodium 4,5-dihydroxybenzene-1,3-disulfonate) as an antidote for acute uranium intoxication in mice. *Res. Commun. Chem. Pathol. Pharmacol*. **34,** 351–358.

Bassett, S. H., Frenkel, A., Cedars, N., Van Alstine, H., Waterhouse, C., and Cusson, K. (1948). *The Excretion of Hexavalent Uranium Following Intravenous Administration. II. Studies on Human Subjects*. U.S. Rochester: The University of Rochester, URB37, Washington, DC.

Bentley, K. W., Stockwell, D. R., Britt, K. A., and Kerr, C. B. (1985). Transient proteinuria and aminoaciduria in rodents following uranium intoxication. *Bull. Environ. Contam. Toxicol*. **34,** 407–416.

Berradi, H., Bertho, J. M., Dudoignon, N., Mazur, A., Grandcolas, L., Baudelin, C., Grison, S., Voisin, P., Gourmelon, P., and Dublineau, I. (2008). Renal anemia induced by chronic ingestion of depleted uranium in rats. *Toxicol. Sci*. **103,** 397–408.

Beyer, D., Biehl, R., and Pilwat, G. (1993). Normal concentration of uranium in urine. *Health Phys*. **64,** 321.

Bhattacharyya, M. H., Breitenstein, B. D., Metivier, H., Muggenburg, B. A., Stradling, G. N., and Volf, V. (1992). Guidebook for the treatment of accidental internal contamination of workers. *Radiat. Prot. Dosimetry*. **41,** 1.

Bijlsma, J. A., Slottje, P., Huizink, A. C., Twisk, J. W., van der Voet, G. B., de Wolff, F. A., Vanhaecke, F., Moens, L., and Smid, T. (2008). Urinary uranium and kidney function parameters in professional assistance workers in the Epidemiological Study Air Disaster in Amsterdam (ESADA). *Nephrol. Dial. Transplant*. **23,** 249–255.

Boice, J. D., Jr., Cohen, S. S., Mumma, M. T., Chadda, B., and Blot, W. J. (2007). Mortality among residents of Uravan, Colorado who lived near a uranium mill, 1936–84. *J. Radiol. Prot*. **27,** 299–319.

Bond, C. H. (1898). Remarks upon the value of uranium nitrate in the control of glycosuria. *Practitioner* **8,** 257–264.

Bosque, M. A., Domingo, J. L., Llobet, J. M., and Corbella, J. (1993). Effectiveness of sodium 4,5-dihydroxybenzene-1,3-disulfonate (Tiron) in protecting against uranium-induced developmental toxicity in mice. *Toxicology* **79,** 149–156.

Bradbury, J. (1896). Case of diabetes mellitus treated by uranium nitrate. *Brit. Med. J*. **2,** 847.

Brady, H. R., Kone, B. C., Brenner, R. M., and Gullans, S. R. (1989). Early effects of uranyl nitrate on respiration and K+ transport in rabbit proximal tubule. *Kidney Int*. **36,** 27–34.

Brodie, J. C., and Humes, H. D. (2005). Stem cell approaches for the treatment of renal failure. *Pharmacol. Rev*. **57,** 299–313.

Bulman, R. (1987). The chemistry of chelating agents in medical sciences. *Struct. Bonding* **67,** 91–141.

Butterworth, A. (1955). The significance and value of uranium in urine analysis. *Assoc. Ind. Med. Offrs*. **5,** 30–43.

Catsch, A. (1959). Die wirkung einiger chelatbildner auf die akute toxcitat von uranilonitrat. *Klin. Wochenschrift*. **37,** 657–660.

Chalabreysse, J., Beau, P., Chevalier, C., Jeanmaire, L., Bataller, G., and Berard, P. (1989). French experience with uranium compounds. *Radiat. Prot. Dosimetry*. **26,** 49–56.

Ciocca, D. R., Fuqua, S. A., Lock-Lim, S., Toft, D. O., Welch, W. J., and McGuire, W. L. (1992). Response of human breast cancer cells to heat shock and chemotherapeutic drugs. *Cancer Res*. **52,** 3648–3654.

Compton, A. (2004). Chronic kidney disease. Avoiding the point of no return. *Adv. Nurse Pract*. **12,** 75–78.

Cooper, J. R., Stradling, G. N., Smith, H., and Ham, S. E. (1982). The behaviour of uranium-233 oxide and uranyl-233 nitrate in rats. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med*. **41,** 421–433.

Cronin, R., and Heinrich, W. (2000). Toxic nephropaties. In *The Kidney*, 6th ed. (B. M. Brenner, Ed.), pp. 1563–1596. W.B. Saunders Company, Philadelphia.

Dagimanjian, R., Maynard, E., and Hodge, H. C. (1956). The effect of calcium disodium ethylene diamine tetraacetate on uranium poisoning in rats. *J. Pharmacol. Exp. Ther*. **117,** 20–28.

Diamond, G. L., Morrow, P. E., Panner, B. J., Gelein, R. M., and Baggs, R. B. (1989). Reversible uranyl fluoride nephrotoxicity in the Long Evans rat. *Fundam. Appl. Toxicol.* **13,** 65–78.

Domingo, J. L., Colomina, M. T., Llobet, J. M., Jones, M. M., Singh, P. K., and Campbell, R. A. (1992). The action of chelating agents in experimental uranium intoxication in mice: variations with structure and time of administration. *Fundam. Appl. Toxicol.* **19,** 350–357.

Domingo, J. L., Ortega, A., Llobet, J. M., and Corbella, J. (1990). Effectiveness of chelation therapy with time after acute uranium intoxication. *Fundam. Appl. Toxicol.* **14,** 88–95.

Domingo, J. L., Ortega, A., Llobet, J. M., Paternain, J. L., and Corbella, J. (1989). The effects of repeated parenteral administration of chelating agents on the distribution and excretion of uranium. *Res. Commun. Chem. Pathol. Pharmacol.* **64,** 161–164.

Doolan, P. D., Schwartz, S. L., Hayes, J. R., Mullen, J. C., and Cummings, N. B. (1967). An evaluation of the nephrotoxicity of ethylenediaminetetraacetate and diethylenetriaminepentaacetate in the rat. *Toxicol. Appl. Pharmacol.* **10,** 481–500.

Downs, W. L., Wilson, H. B., Sylvester, G. E., Leach, L. J., and Maynard, E. A. (1967). Excretion of uranium by rats following inhalation of uranium dioxide. *Health Phys.* **13,** 445–453.

Duncan, E. (1897). The treatment of diabetes mellitus by nitrate of uranium. *Brit. Med. J.* **2,** 1044–1047.

Durakovic, A. B., Hollins, J. G., and Storr, M. C. (1973). The influence of age and sex on the metabolism of americium by rats. *Health Phys.* **24,** 541–546.

Durbin, P. W., Kullgren, B., Xu, J., and Raymond, K. N. (1997). New agents for in vivo chelation of uranium(VI): efficacy and toxicity in mice of multidentate catecholate and hydroxypyridinonate ligands. *Health Phys.* **72,** 865–879.

Dygert, H. (1949). Concepts in inhalation toxicology. In *Pharmacology and Toxicology of Uranium Compounds*, 1st ed. (H. Hodge and C. Voegtlin, Eds.), pp. 603–700. McGraw-Hill, New York.

Ebetino, F., Degenhardt, C., Jamieson, L. A., and Burdsall, D. (1990). Recent work on the synthesis of phosphonatecontaining bone active heterocycles. *Heterocycles* **30,** 855–862.

Ebetino, F., and Jamieson, L. (1990). The design and synthesis of bone-active phosphinic acid analogues 1. The pyridylaminomethanephosphonoalkylphosphinates. *Phosphorus Sulfur Silicon Relat. Elem.* **51,** 23–26.

Ejnik, J. W., Carmichael, A. J., Hamilton, M. M., McDiarmid, M., Squibb, K., Boyd, P., and Tardiff, W. (2000). Determination of the isotopic composition of uranium in urine by inductively coupled plasma mass spectrometry. *Health Phys.* **78,** 143–146.

Elliott, W. C., Houghton, D. C., Gilbert, D. N., Baines-Hunter, J., and Bennett, W. M. (1982). Gentamicin nephrotoxicity. II. Definition of conditions necessary to induce acquired insensitivity. *J. Lab. Clin. Med.* **100,** 513– 525. 1.

Emeigh Hart, S. G. (2005). Assessment of renal injury in vivo. *J. Pharmacol. Toxicol. Methods.* **52,** 30–45.

Environmental Protection Agency (EPA). (1985). *Environmental Protection Agency. Code of Federal Regulation Low-Level Radioactive Waste Policy Amendments Act.* Public Law 96–573, as amended, 42 USC 2021b et seq. Environmental Protection Agency, Washington, DC. Available at: http://www.epa.gov/rpdweb00/laws/laws_sum.html. Updated September 19, 1985. Accessed July 7, 2010.

Ershow, A. B., and Cantor, K. P. (1989). *Total Water and Tapwater Intake in the United States: Population-based Estimates of Quantities and Sources.* Research Office, Federation of American Societies for Experimental Biology, Rockville, WA.

Filippova, L. G., Nifativ, A., and Lyubchanskii, E. R. (1978). Some of the long-term sequelae of giving rats enriched uranium. *Radiobiology* **18,** 400–405.

Fisher, D. R., Swint, M. J., and Kathren, R. L. (1990). *Evaluation of Health Effects in Sequoyah Fuels Corporation Workers from Accidental Exposure to Uranium Hexafluoride.* Nuclear Regulatory Commission, Washington, DC.

Frengstad, B., Skrede, A. K., Banks, D., Krog, J. R., and Siewers, U. (2000). The chemistry of Norwegian groundwaters: III. The distribution of trace elements in 476 crystalline bedrock groundwaters, as analysed by ICP-MS techniques. *Sci. Total Environ.* **246,** 21–40.

Friberg, L., Nordberg, G. F., and Vouk, V. B. (1986). General aspects. In *Handbook on the Toxicology of Metals* (G.F.B. N, M. Nordberg, and L. Friberg, Eds.), Vol. 1, pp. 623–637. Elsevier Science Publishing, Amsterdam.

Fukuda, S., Iida, H., Ikeda, M., Yan, X., and Xie, Y. (2005a). Toxicity of uranium and the removal effects of CBMIDA and EHBP in simulated wounds of rats. *Health Phys.* **89,** 81–88.

Fukuda, S., Iida, H., Yan, X., and Xie, X. (2001). Effects of CBMIDA on removal of uranium in rats. *Biomark Environ* **4,** 35–37.

Fukuda, S., Ikeda, M., Chiba, M., and Kaneko, K. (2005b). Clinical diagnostic indicator of renal and bone damage in rats intramuscularly injected with depleted uranium. *Radiat. Prot. Dosimetry.* **118,** 307–314.

Fukuda, S., Ikeda, M., Nakamura, M., Katoh, A., Yan, X., Xie, Y., and Kontoghiorghes, G. J. (2008). The effects of bicarbonate and its combination with chelating agents used for the removal of depleted uranium in rats. *Hemoglobin* **32,** 191–198.

Gillard, R. D., Newman, P. D., and Collins, J. D. (1989). Speciation in aqueous solutions of diethylenetriaminepentamethylenephosphonic acid and some metal complexes. *Polyhedron* **8,** 2077–2086.

Gilman, A. P., Moss, M. A., Villeneuve, D. C., Secours, V. E., Yagminas, A. P., Tracy, B. L., Quinn, J. M., Long, G., and Valli, V. E. (1998a). Uranyl nitrate: 91-day exposure and recovery studies in the male New Zealand white rabbit. *Toxicol. Sci.* **41,** 138–151.

Gilman, A. P., Villeneuve, D. C., Secours, V. E., Yagminas, A. P., Tracy, B. L., Quinn, J. M., Valli, V. E., and Moss, M. A. (1998b). Uranyl nitrate: 91-day toxicity studies in the New Zealand white rabbit. *Toxicol. Sci.* **41,** 129–137.

Gilman, A. P., Villeneuve, D. C., Secours, V. E., Yagminas, A. P., Tracy, B. L., Quinn, J. M., Valli, V. E., Willes, R. J., and Moss, M. A. (1998c). Uranyl nitrate: 28-day and 91-day toxicity studies in the Sprague-Dawley rat. *Toxicol. Sci.* **41,** 117–128.

Goering, P. L., Fisher, B. R., Noren, B. T., Papaconstantinou, A., Rojko, J. L., and Marler, R. J. (2000). Mercury induces regional and cell-specific stress protein expression in rat kidney. *Toxicol. Sci.* **53,** 447–457.

Goldman, M., Yaari, A., Doshnitzki, Z., Cohen-Luria, R., and Moran, A. (2006). Nephrotoxicity of uranyl acetate: effect on rat kidney brush border membrane vesicles. *Arch. Toxicol.* **80,** 387–393.

Gomez, M., Domingo, J. L., Llobet, J. M., and Corbella, J. (1991). Evaluation of the efficacy of various chelating agents on urinary excretion and tissue distribution of vanadium in rats. *Toxicol. Lett.* **57,** 227–234.

Gray, S., Stradling, G., Pearce, M., Moody, J., and Ebetino, F. (1992). *Efficacy of Some Phosphonic Acid Derivatives for Enhancing the Excretion of Uranium from the Rat.* NRPB-M-339 National Radiological Protection Board, Chilton, WI.

Hakonson-Hayes, A. C., Fresquez, P. R., and Whicker, F. W. (2002). Assesing potential risks from exposure to nautal uranium in well water. *J. Environ. Radioact.* **59,** 29–40.

Haley, D. P. (1982). Morphologic changes in uranyl nitrate-induced acute renal failure in saline- and water-drinking rats. *Lab. Invest.* **46,** 196–208.

Haley, D. P., Bulger, R. E., and Dobyan, D. C. (1982). The long-term effects of uranyl nitrate on the structure and function of the rat kidney. *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.* **41,** 181–192.

Hammond, P. B., and Beliles, R. P. (1980). Metals. In *Toxicology. The Basic Science of Poisons*, 2nd ed. (U. J, and L. J. Casarett, Eds.), pp. 409–467. MacMillan Publishing Company, New York.

Harduin, J. C., Royer, P., and Piechowski, J. (1994). Uptake and urinary excretion of uranium after oral administration in man. *Radiat. Prot. Dosimetry*. **53,** 245–248.

Helmer, D. A., Rossignol, M., Blatt, M., Agarwal, R., Teichman, R., and Lange, G. (2007). Health and exposure concerns of veterans deployed to Iraq and Afghanistan. *J. Occup. Environ. Med.* **49,** 475–480.

Henge-Napoli, M. H., Ansoborlo, E., Chazel, V., Houpert, P., Paquet, F., and Gourmelon, P. (1999). Efficacy of ethane-1-hydroxy-1,1-bisphosphonate (EHBP) for the decorporation of uranium after intramuscular contamination in rats. *Int. J. Radiat. Biol.* **75,** 1473–1477.

Henge-Napoli, M. H., Ansoborlo, E., Houpert, P., Mirto, H., Paquest, F., Burgada, R., Hodgson, S., and Stradling, G. N. (1998). Progress and trends in in vivo chelation of uranium. *Radiat. Protect. Dosimetry.* **79,** 449–452.

Henge-Napoli, M. H., Archimbaud, M., Ansoborlo, E., Metivier, H., and Gourmelon, P. (1995). Efficacy of 3,4,3-LIHOPO for reducing the retention of uranium in rat after acute administration. *Int. J. Radiat. Biol.* **68,** 389–393.

Herrera, M. B., Bussolati, B., Bruno, S., Fonsato, V., Romanazzi, G. M., and Camussi, G. (2004). Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. *Int. J. Mol. Med.* **14,** 1035–1041.

Hirsch, G. H. (1976). Differential effects of nephrotoxic agents on renal transport and metabolism by use of in vitro techniques. *Environ. Health Perspect.* **15,** 89–99.

Hodge, H. C., Stannard, J. N., and Hursh, J. B. (1973). Uranium, plutonium, transplutonic elements. In *Handbook of Experimental Pharmacology*, 1st ed. (H. C. Hodge, J. N. Stannard, and J. B. Hursh, Eds.), Vol. 36, pp. 165–195. Springer-Verlag, New York.

Honda, N., and Sudo, M. (1982). Resistance to uranyl acetate-induced acute renal failure in rabbits: renal function and morphology. In *Acute Renal Failure* (H. E. Eliahou, Ed.), p. 105. John Libbey, London.

Hooper, F. J., Squibb, K. S., Siegel, E. L., McPhaul, K., and Keogh, J. P. (1999). Elevated urine uranium excretion by soldiers with retained uranium shrapnel. *Health Phys.* **77,** 512–519.

Hori, R., Takano, M., Okano, T., and Inui, K. (1985). Transport of p-aminohippurate, tetraethylammonium and D-glucose in renal brush border membranes from rats with acute renal failure. *J. Pharmacol. Exp. Ther.* **233,** 776–781.

Houpert, P., Chazel, V., Paquet, F., Bailly, T., Burgada, R., and Henge-Napoli, M. H. (2001). Reduction of uranium transfer by local chelation in simulated wounds in rats. *Hum. Exp. Toxicol.* **20,** 237–241.

Houpert, P., Muller, D., Chazel, V., Claraz, M., and Paquet, F. (2003). Effect of DTPA on the nephrotoxicity induced by uranium in the rat. *Radiat. Prot. Dosimetry*. **105,** 517–520.

Humes, H. D., Weitzel, W. F., and Fissell, W. H. (2004). Renal cell therapy in the treatment of patients with acute and chronic renal failure. *Blood Purif.* **22,** 60–72.

Hursh, J. B., and Spoor, N. H. (1973). Data on man. In *Uranium Plutonium Transplutonic Elements. Handbook of Experimental Pharmacology*, 1st ed. (H. C. Hodge, J. N. Stannard, and J. B. Hursh, Eds.), Vol. 36, pp. 197–239. Springer-Verlag, New York.

International Commission on Radiological Protection (ICRP). (1991). *International Commission on Radiological Protection. Recommendations of the International Commission on Radiological Protection*. Publication 60, Annals of the ICRP, Pergamon Press, Oxford, UK.

International Commission for Radiation Protection (ICRP). (1996). *International Commission for Radiation Protection. Age-Dependent Doses to Members of the Public from Intake of Radionuclides: Part 4, Inhalation Dose Coefficients*. Publication 72, Annals of the ICRP, Pergamon Press, Oxford, UK.

Ivannikov, A. (1966). In *Physiochemical Approaches to the Selection of Organic Compounds Designed to Eliminate Radioactive Substances from the Organism*. Atomic Energy Commission, Washington, DC.

Jin, T., Nordberg, G., Sehlin, J., and Vesterberg, O. (1996). Protection against cadmium-metallothionein nephrotoxicity in streptozotocin-induced diabetic rats: role of increased metallothionein synthesis induced by streptozotocin. *Toxicology* **106,** 55–63.

Jin, T., Nordberg, G., Sehlin, J., Wallin, H., and Sandberg, S. (1999). The susceptibility to nephrotoxicity of streptozotocin-induced diabetic rats subchronically exposed to cadmium chloride in drinking water. *Toxicology* **142,** 69–75.

Jin, T., Nordberg, G. F., Sehlin, J., Leffler, P., and Wu, J. (1994). The susceptibility of spontaneously diabetic mice to cadmium-metallothionein nephrotoxicity. *Toxicology* **89,** 81–90.

Kale, S., Karihaloo, A., Clark, P. R., Kashgarian, M., Krause, D. S., and Cantley, L. G. (2003). Bone marrow stem cells contribute to repair of the ischemically injured renal tubule. *J. Clin. Invest.* **112,** 42–49.

Karpas, Z., Lorber, A., Sela, H., Paz-Tal, O., Hagag, Y., Kurttio, P., and Salonen, L. (2005). Measurement of the 234U/238U ratio by MC-ICPMS in drinking water, hair, nails, and urine as an indicator of uranium exposure source. *Health. Phys.* **89,** 315–321.

Kathren, R. L., and Burklin, R. K. (2008). Acute chemical toxicity of uranium. *Health Phys.* **94,** 170–179.

Kathren, R. L., and Moore, R. H. (1986). Acute accidental inhalation of U: a 38-year follow-up. *Health Phys.* **51,** 609–619.

Kato, A., Hishida, A., and Nakajima, T. (1994). Effects of oxygen free radical scavengers on uranium-induced acute renal failure in rats. *Free Radic. Biol. Med.* **16,** 855–859.

Kobayashi, S., Nagase, M., Honda, N., and Hishida, A. (1984). Glomerular alterations in uranyl acetate-induced acute renal failure in rabbits. *Kidney Int.* **26,** 808–815.

Kovacic, P., and Jacintho, J. D. (2001). Reproductive toxins: pervasive theme of oxidative stress and electron transfer. *Curr. Med. Chem.* **8,** 863–892.

Kressin, I. K. (1984). Spectrophotometric method for the determination of uranium in urine. *Anal. Chem.* **56,** 2269–2271.

Krystek, P., and Ritsema, R. (2009). An incident study about acute and chronic human exposure to uranium by high-resolution inductively coupled plasma mass spectrometry (HR-ICPMS). *Int. J. Hyg. Environ. Health.* **212,** 76–81.

Kurttio, P., Auvinen, A., Salonen, L., Saha, H., Pekkanen, J., Makelainen, I., Vaisanen, S. B., Penttila, I. M., and Komulainen, H. (2002). Renal effects of uranium in drinking water. *Environ. Health Perspect.* **110,** 337–342.

Kurttio, P., Harmoinen, A., Saha, H., Salonen, L., Karpas, Z., Komulainen, H., and Auvinen, A. (2006). Kidney toxicity of ingested uranium from drinking water. *Am J Kidney Dis* **47,** 972–982.

L'Azou, B., Henge-Napoli, M. H., Minaro, L., Mirto, H., Barrouillet, M. P., and Cambar, J. (2002). Effects of cadmium and uranium on some in vitro renal targets. *Cell Biol. Toxicol.* **18,** 329–340.

La Touche, Y. D., Willis, D. L., and Dawydiak, O. I. (1987). Absorption and biokinetics of U in rats following an oral administration of uranyl nitrate solution. *Health Phys.* **53,** 147–162.

Leggett, R. W. (1989). The behavior and chemical toxicity of U in the kidney: a reassessment. *Health Phys.* **57,** 365–383.

Leibovich, S. J., and Ross, R. (1975). The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. *Am. J. Pathol.* **78,** 71–100.

Linares, V., Belles, M., Albina, M. L., Sirvent, J. J., Sanchez, D. J., and Domingo, J. L. (2006). Assessment of the pro-oxidant activity of uranium in kidney and testis of rats. *Toxicol. Lett.* **167,** 152–161.

Llobet, J. M., Sirvent, J. J., Ortega, A., and Domingo, J. L. (1991). Influence of chronic exposure to uranium on male reproduction in mice. *Fundam. Appl. Toxicol.* **16,** 821–829.

Lloyd, R. D., Taylor, G. N., Boseman, J. J., Mays, C. W., and Atherton, D. R. (1977). Further comparison of Ca-DTPA and Zn-DTPA for removal of 241Am from beagles. *Health Phys.* **33,** 92–94.

Lorber, A., Karpas, Z., and Halicz, L. (1996). Flor injection method for determination of uranium in urine and serum by inductively coupled plasma mass spectrometry. *Anal. Chem. Acta* **334,** 295–301.

Lu, S., and Zhao, F. Y. (1990). Nephrotoxic limit and annual limit on intake for natural U. *Health Phys.* **58,** 619–623.

Lushbaugh, C. C., and Washburn, L. C. (1979). FDA IND approval for Zn-DTPA, new clinical agent for decorporation therapy of actinides. *Health Phys.* **36,** 472.

Lusky, L., and Braun, H. (1950). Sodium catechol disulphonate protection in experimental uranium nitrated poisoning. *Fed. Proc.* **9,** 297–299.

MacNider, W. (1929). The functional and pathological response of the dogs subjected to a second subcutaneous injection of uranium nitrate. *J. Exp. Med.* **49,** 411–431.

Magdo, H. S., Forman, J., Graber, N., Newman, B., Klein, K., Satlin, L., Amler, R. W., Winston, J. A., and Landrigan, P. J. (2007). Grand rounds: nephrotoxicity in a young child exposed to uranium from contaminated well water. *Environ. Health Perspect.* **115,** 1237–1241.

Mao, Y., Desmeules, M., Schaubel, D., Berube, D., Dyck, R., Brule, D., and Thomas, B. (1995). Inorganic components of drinking water and micro-albuminuria. *Environ. Res.* **71,** 135–140.

Martinez, A. B., Cabrini, R. L., and Ubios, A. M. (2000). Orally administered ethane-1-hydroxy-1,1-biphosphonate reduces the lethal effect of oral uranium poisoning. *Health Phys.* **78,** 668–671.

Martinez, A. B., Mandalunis, P. M., Bozal, C. B., Cabrini, R. L., and Ubios, A. M. (2003). Renal function in mice poisoned with oral uranium and treated with ethane-1-hydroxy-1,1-bisphosphonate (EHBP). *Health Phys.* **85,** 343–347.

Masuya, M., Drake, C. J., Fleming, P. A., Reilly, C. M., Zeng, H., Hill, W. D., Martin-Studdard, A., Hess, D. C., and Ogawa, M. (2003). Hematopoietic origin of glomerular mesangial cells. *Blood* **101,** 2215–2218.

Matsumoto, K., Mizuno, S., and Nakamura, T. (2000). Hepatocyte growth factor in renal regeneration, renal disease and potential therapeutics. *Curr. Opin. Nephrol. Hypertens.* **9,** 395–402.

Maynard, E. A., Down, W. L., and Hodge, H. C. (1953). Oral toxicity of uranium compounds. In *Pharmacology and Toxicology of Uranium Compounds* (C. Voegtlin and H. C. Hodge, Eds.), Vol. 2, pp. 309–376. McGraw-Hill, New York.

Maynard, E. A., and Hodge, H. C. (1949). Studies of the toxicity of various uranium compounds when fed to experimental animals. In *Pharmacology and Toxicology of Uranium Compounds* (C. Voegtlin and H. C. Hodge, Eds.), pp. 309–376. McGraw-Hill, New York.

Mays, C. W., Rowland, R. E., and Stehney, A. F. (1985). Cancer risk from the lifetime intake of Ra and U isotopes. *Health Phys.* **48,** 635–647.

McDiarmid, M. A., Engelhardt, S. M., Oliver, M., Gucer, P., Wilson, P. D., Kane, R., Cernich, A., Kaup, B., Anderson, L., Hoover, D., *et al.* (2007). Health surveillance of Gulf War I veterans exposed to depleted uranium: updating the cohort. *Health Phys.* **93,** 60–73.

McDiarmid, M. A., Engelhardt, S. M., Oliver, M., Gucer, P., Wilson, P. D., Kane, R., Kabat, M., Kaup, B., Anderson, L., Hoover, D., *et al.* (2006). Biological monitoring and surveillance results of Gulf War I veterans exposed to depleted uranium. *Int. Arch. Occup. Environ. Health* **79,** 11–21.

McDiarmid, M. A., Squibb, K., Engelhardt, S., Oliver, M., Gucer, P., Wilson, P. D., Kane, R., Kabat, M., Kaup, B., Anderson, L., *et al.* (2001). Surveillance of depleted uranium exposed Gulf War veterans: health effects observed in an enlarged "friendly fire" cohort. *J. Occup. Environ. Med.* **43,** 991–1000.

McDonald-Taylor, C. K., Singh, A., and Gilman, A. (1997). Uranyl nitrate-induced proximal tubule alterations in rabbits: a quantitative analysis. *Toxicol. Pathol.* **25,** 381–389.

Mendelsohn, F. A., and Smith, E. A. (1980). Intrarenal renin, angiotensin II, and plasma renin in rats with uranyl nitrate-induced and glycerol-induced acute renal failure. *Kidney Int.* **17,** 465–472.

Mirto, H., Henge-Napoli, M. H., Gibert, R., Ansoborlo, E., Fournier, M., and Cambar, J. (1999). Intracellular behaviour of uranium(VI) on renal epithelial cell in culture (LLC-PK1): influence of uranium speciation. *Toxicol. Lett.* **104,** 249–256.

Mizuno, S., Fujita, K., Furuy, R., Hishid, A., Ito, H., Tashim, Y., and Kumagai, H. (1997). Association of HSP73 with the acquired resistance to uranyl acetate-induced acute renal failure. *Toxicology* **117,** 183–191.

Molitch, M. E., DeFronzo, R. A., Franz, M. J., Keane, W. F., Mogensen, C. E., Parving, H. H., and Steffes, M. W. (2004). Nephropathy in diabetes. *Diabetes Care* **27**(Suppl. 1), 79–83.

Morris, K. J., Khanna, P., and Batchelor, A. L. (1990). Long-term clearance of inhaled UO2 particles from the pulmonary region of the rat. *Health Phys.* **58,** 477–485.

Morrow, P. E., Leach, L. J., Smith, F. A., Gelein, R. M., Scott, J. B., Beiter, H. D., Amato, F. J., Picano, J. J., Yuile, C. L., and Consler, T. G. (1982). In *Metabolic Fate and Evaluation of Injury in Rats and Dogs following Exposure to the Hydrolysis Products of Uranium Hexafluoride. Implications for a Bioassay Program Related to Potential Releases of Uranium Hexafluoride* Govt Rep Announce Ind. Reported Period: July 1979–October 1981. Rochester University, New York, Department of Radiation Biology and Biophysics, Rochester, WA.

Muller, C., Ruzicka, L., and Bakstein, J. (1967). The sex ratio in the offsprings of uranium miners. *Acta Univ. Carol. Med. (Praha)* **13,** 599–603.

Muller, D., Houpert, P., Cambar, J., and Henge-Napoli, M. H. (2006). Role of the sodium-dependent phosphate co-transporters and of the phosphate complexes of uranyl in the cytotoxicity of uranium in LLC-PK1 cells. *Toxicol. Appl. Pharmacol.* **214,** 166–177.

National Council on Radiation Protection (NCRP). (1980). *National Council on Radiation. Management of Persons Accidentally Contaminated with Radionuclides Protection and Measurements*. (NCRP Report No. 65). NCRP Publications, Washington, DC. Available at: http://www.ncrponline.org /Publications/65press.html. Updated April 15, 1980. Accessed July 7, 2010.

Nechay, B. R., Thompson, J. D., and Saunders, J. P. (1980). Inhibition by uranyl nitrate of adenosine triphosphatases derived from animal and human tissues. *Toxicol. Appl. Pharmacol.* **53,** 410–419.

Neuman, W. F., Fleming, R. W., Dounce, A. L., Carlson, A. B., O'Leary, J., and Mulryan, B. (1948). The distribution and excretion of injected uranium. *J. Biol. Chem.* **173,** 737–748.

Nigam, S., and Lieberthal, W. (2000). Acute renal failure. III. The role of growth factors in the process of renal regeneration and repair. *Am. J. Physiol. Renal Physiol.* **279,** 3–11.

Niwa, T., Katsuzaki, T., Yazawa, T., Tatemichi, N., Emoto, Y., Miyazaki, T., and Maeda, K. (1993). Urinary trehalase activity in chronic glomerulonephritis. *Nephron* **63,** 423–428.

Nomiyama, K., Yamamoto, A., and Sato, C. (1974). Assay of urinary enzymes in toxic nephropathy. *Toxicol. Appl. Pharmacol.* **27,** 484–490.

Oeh, U., Li, W. B., Hollriegl, V., Giussani, A., Schramel, P., Roth, P., and Paretzke, H. G. (2007). Daily uranium excretion in German peacekeeping personnel serving on the Balkans compared to ICRP model prediction. *Radiat. Prot. Dosimetry* **127,** 329–332.

Oliver, J. (1915). The histogenesis of chronic uranium nephritis with special reference to epithelial regeneration. *J. Exp. Med.* **21,** 4.

Oliver, J. A., Maarouf, O., Cheema, F. H., Martens, T. P., and Al-Awqati, Q. (2004). The renal papilla is a niche for adult kidney stem cells. *J. Clin. Invest.* **114,** 795–804.

Orcutt, J. (1949). The toxicology of compounds of uranium following application to the skin. In *Pharmacology and Toxicology of Uranium*

Compounds, 1st ed. (H. Hodge and C. Voegtlin, Eds.), Vol. 3, pp. 377–414. McGraw Hill, New York.

Orloff, K. G., Mistry, K., Charp, P., Metcalf, S., Marino, R., Shelly, T., Melaro, E., Donohoe, A. M., and Jones, R. L. (2004). Human exposure to uranium in groundwater. *Environ. Res.* **94,** 319–326.

Ortega, A., Domingo, J. L., Llobet, J. M., Tomas, J. M., and Paternain, J. L. (1989). Evaluation of the oral toxicity of uranium in a 4-week drinking-water study in rats. *Bull. Environ. Contam. Toxicol.* **42,** 935–941.

Ortega, A., Sanchez, D. J., Domingo, J. L., Llobet, J. M., and Corbella, J. (1991). Developmental toxicity evaluation of tiron (sodium 4,5-dihydroxybenzene-1,3-disulfonate) in mice. *Res Commun. Chem. Pathol. Pharmacol.* **73,** 97–106.

Paquet, F., Houpert, P., Blanchardon, E., Delissen, O., Maubert, C., Dhieux, B., Moreels, A. M., Frelon, S., and Gourmelon, P. (2006). Accumulation and distribution of uranium in rats after chronic exposure by ingestion. *Health Phys.* **90,** 139–147.

Parrish, R. R., Horstwood, M., Arnason, J. G., Chenery, S., Brewer, T., Lloyd, N. S., and Carpenter, D. O. (2008). Depleted uranium contamination by inhalation exposure and its detection after approximately 20 years: implications for human health assessment. *Sci. Total Environ.* **390,** 58–68.

Parving, H. H., Chaturvedi, N., Viberti, G., and Mogensen, C. E. (2002). Does microalbuminuria predict diabetic nephropathy? *Diabetes Care* **5,** 406–407.

Patschan, D., Plotkin, M., and Goligorsky, M. S. (2006). Therapeutic use of stem and endothelial progenitor cells in acute renal injury: Ca ira. *Curr. Opin. Pharmacol.* **6,** 176–183.

Pavlakis, N., Pollock, C. A., McLean, G., and Bartrop, R. (1996). Deliberate overdose of uranium: toxicity and treatment. *Nephron* **72,** 313–317.

Peeks, M., Walter, T., and Walter, S. (2002). Uranium, uranium alloys, and uranium compounds. In *Ullmann's Encyclopedia of Industrial Chemistry,* 6th ed. (W.-V. Verlag. Gmbh. and Co, Eds.), pp. 270–281. Wiley-VCH, Weinheim, Germany.

Pinney, S. M., Freyberg, R. W., Levine, G. E., Brannen, D. E., Mark, L. S., Nasuta, J. M., Tebbe, C. D., Buckholz, J. M., and Wones, R. (2003). Health effects in community residents near a uranium plant at Fernald, Ohio, USA. *Int. J. Occup. Med. Environ. Health* **16,** 139–153.

Powell, D. W., Mifflin, R. C., Valentich, J. D., Crowe, S. E., Saada, J. I., and West, A. B. (1999). Myofibroblasts. I. Paracrine cells important in health and disease. *Am. J. Physiol.* **277,** 1–9.

Pozzani, U. C. (1949). Toxicity following inhalation. In *Pharmacology and Toxicology of Uranium Compounds* (H. Hodge and C. Voegtlin, Eds.), Vol. 2, pp. 622–635. McGraw-Hill, New York.

Price, A. (1989). Review of methods for the assessment of intake of uranium by workers at BNGL springfields. *Radiat. Prot. Dosimetry.* **26,** 35–42.

Rajan, K., and Martell, A. (1964). Equilibrium studies of uranilo complexes. Interaction of uranilo ion with citric acid. *Inorg. Chem.* **26,** 1927–1944.

Remuzzi, G., Benigni, A., and Remuzzi, A. (2006). Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J. Clin. Invest.* **116,** 288–296.

Rosen, L. C., Gur, D., Pan, S. I., and Wals, N. (1989). Long term removal of Am-241 using Ca-DTPA. *Health Phys.* **39,** 101–106.

Rossol, M. (1997). *Ceramic Glazes, Frit Metal Enamels, and Glass; Personal Communication and Supporting Materials.* Arts, Crafts and Theater Safety, New York.

Rothstein, A. (1949). Toxicity following inhalation: uranium trioxide. In *Pharmacology and Toxicology of Uranium Compounds*, 1st ed. (H. Hodge and C. Voegtlin, Eds.), Vol. 2, pp. 635–648. McGraw-Hill, New York.

Russell, J. J., and Kathren, R. L. (2004). Uranium deposition and retention in a USTUR whole body case. *Health Phys.* **86,** 273–284.

Russell, J. J., Kathren, R. L., and Dietert, S. E. (1996). A histological kidney study of uranium and non-uranium workers. *Health Phys.* **70,** 466–472.

Sabolic, I. (2006). Common mechanisms in nephropathy induced by toxic metals. *Nephron Physiol.* **104,** 107–114.

Saccomanno, G., Thun, M. J., Baker, D. B., Steenland, K., Smith, A. B., Halperin, W., and Berl, T. (1982). The contribution of uranium miners to lung cancer histogenesis renal toxicity in uranium mill workers. *Cancer Res.* **82,** 43–52.

Sachett, I. A., Nobrega, A. W., and Lauria, D. C. (1984). Determination of uranium isotopes by chemical stripping and alpha-spectrometry. *Health Phys.* **46,** 133–139.

Salminen, W. F., Jr., Voellmy, R., and Roberts, S. M. (1997). Protection against hepatotoxicity by a single dose of amphetamine: the potential role of heat shock protein induction. *Toxicol. Appl. Pharmacol.* **147,** 247–258.

Sanchez, D. J., Belles, M., Albina, M. L., Sirvent, J. J., and Domingo, J. L. (2001). Nephrotoxicity of simultaneous exposure to mercury and uranium in comparison to individual effects of these metals in rats. *Biol. Trace Elem. Res.* **84,** 139–154.

Sanders, C. L. (1986). *Radiological Health. Toxicological Aspects of Energy Production.* Battelle Press, Columbus OH, Canada.

Sansone, U., Stellato, L., Jia, G., Rosamilia, S., Gaudino, S., Barbizzi, S., and Belli, M. (2001). Levels of depleted uranium in Kosovo soils. *Radiat. Prot. Dosimetry.* **97,** 317–320.

Schena, F. P., and Gesualdo, L. (2005). Pathogenetic mechanisms of diabetic nephropathy. *J. Am. Soc. Nephrol.* **16**(Suppl. 1), 30–33.

Schramm, L., La, M., Heidbreder, E., Hecker, M., Beckman, J. S., Lopau, K., Zimmermann, J., Rendl, J., Reiners, C., Winderl, S., *et al*. (2002). L-arginine deficiency and supplementation in experimental acute renal failure and in human kidney transplantation. *Kidney Int.* **61,** 1423–1432.

Schwartz, J. H., and Flamenbaum, W. (1976). Uranyl nitrate and HgCl2-induced alterations in ion transport. *Kidney Int.* **6**(Suppl.), 123–127.

Selden, A. I., Lundholm, C., Edlund, B., Hogdahl, C., Ek, B. M., Bergstrom, B. E., and Ohlson, C. G. (2009). Nephrotoxicity of uranium in drinking water from private drilled wells. *Environ. Res.* **109,** 486–494.

Shawky, S., Amer, H. A., Hussein, M. I., el-Mahdy, Z., and Mustafa, M. (2002). Uranium bioassay and radioactive dust measurements at some uranium processing sites in Egypt—health effects. *J. Environ. Monit.* **4,** 588–591.

Shim, W. S., Park, J. H., Ahn, S. J., Han, L., Jin, Q. R., Li, H., Choi, M. K., Kim, D. D., Chung, S. J., and Shim, C. K. (2009). Testosterone-independent down-regulation of Oct2 in the kidney medulla from a uranyl nitrate-induced rat model of acute renal failure: effects on distribution of a model organic cation, tetraethylammonium. *J. Pharm. Sci.* **98,** 739–747.

Shiraishi, K., Igarashi, Y., Takaku, Y., Masuda, K., Yoshimizu, K., Nishimura, Y., Hongo, S., and Yamaguchi, H. (1992). Daily intakes of 232Th and 238U in Japanese males. *Health. Phys.* **63,** 187–191.

Shyh, T. P., Beyer, M. M., and Friedman, E. A. (1984). Induced hyperglycemia protects against mercury nephrotoxicity in the rat. *Trans. Am. Soc. Artif. Intern. Organs* **30,** 260–263.

Snively, C. S., and Gutierrez, C. (2004). Chronic kidney disease: prevention and treatment of common complications. *Am. Fam. Physician* **70,** 1921–1928.

Snyder, S., and Pendergraph, B. (2005). Detection and evaluation of chronic kidney disease. *Am. Fam. Physician* **72,** 1723–1732.

Spencer, H., Osis, D., Fisenne, I. M., Perry, P. M., and Harley, N. H. (1990). Measured intake and excretion patterns of naturally occurring 234U, 238U, and calcium in humans. *Radiat. Res.* **124,** 90–95.

Spiegel, C. (1949). Uranium hexafluoride. In *Pharmacology and Toxicology of Uranium Compounds*, 1st ed. (H. Hodge and C. Voegtlin, Eds.), Vol. 2, pp. 532–547. McGraw-Hill, New York.

Spoor, N., and Hursh, J. (1973). Protection criteria. In *Handbook of Experimental Pharmacology* (J. N. Stannard and J. B. Hursh, Eds.), pp. 241–270. Springer- Verlag, Berlin.

Squibb, K. S., Leggett, R. W., and McDiarmid, M. A. (2005). Prediction of renal concentrations of depleted uranium and radiation dose in Gulf War veterans with embedded shrapnel. *Health Phys.* **89,** 267–273.

Squibb, K. S., and McDiarmid, M. A. (2006). Depleted uranium exposure and health effects in Gulf War veterans. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **361,** 639–648.

Stefanovic, V., Ivic, M. A., and Strahinjic, S. (1987). Calcium and phosphate metabolism in uranyl nitrate-induced acute renal failure. *Arch. Int. Physiol. Biochim.* **95,** 223–228.

Stohs, S., and Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med.* **18,** 321–336.

Stokinger, H. E., Baxter, R. C., and Dygent, H. (1953). Toxicity following inhalation for 1 and 2 years. In *Pharmacology and Toxicology of Uranium Compounds*, 1st ed. (H. Hodge and C. Voegtlin, Eds.), Vol. 2, pp. 1522–1553. Mcgraw-Hill, New York.

Stradling, G. N., Gray, S. A., Moody, J. C., and Ellender, M. (1991). Efficacy of tiron for enhancing the excretion of uranium from the rat. *Hum. Exp. Toxicol.* **10,** 195–198.

Stradling, G. N., Stather, J., Ham, S. E., and Sumner, S. A. (1981). The use of puchel and DTPA for removing Pu-239 from the lungs of hamsters. *Health Phys.* **41,** 387–391.

Sun, D. F., Fujigaki, Y., Fujimoto, T., Goto, T., Yonemura, K., and Hishida, A. (2002). Relation of distal nephron changes to proximal tubular damage in uranyl acetate-induced acute renal failure in rats. *Am. J. Nephrol.* **22,** 405–416.

Takahashi, T., Kalka, C., Masuda, H., Chen, D., Silver, M., Kearney, M., Magner, M., Isner, J. M., and Asahara, T. (1999). Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat. Med.* **5,** 434–438.

Tanigawara, Y., Saito, Y., Aiba, T., Ohoka, K., Kamiya, A., and Hori, R. (1990). Moment analysis of drug disposition in kidney. III: transport of p-aminohippurate and tetraethylammonium in the perfused kidney isolated from uranyl nitrate-induced acute renal failure rats. *J. Pharm. Sci.* **79,** 249–256.

Tannenbaum, A., Silverstone, H., and Koziol, J. (1951). The distribution and excretion of uranium in mice, rats and dogs. In *Toxicology of Uranium Compounds* (A. Tannenbaum, Ed.), pp. 128–181. McGraw-Hill, New York.

Taulan, M., Paquet, F., Argiles, A., Demaille, J., and Romey, M. C. (2006). Comprehensive analysis of the renal transcriptional response to acute uranyl nitrate exposure. *BMC Genomics* **7,** 2.

Taulan, M., Paquet, F., Maubert, C., Delissen, O., Demaille, J., and Romey, M. C. (2004). Renal toxicogenomic response to chronic uranyl nitrate insult in mice. *Environ. Health Perspect.* **112,** 1628–1635.

Taylor, D. M., and Taylor, S. K. (1997). Environmental uranium and human health. *Rev. Environ. Health* **12,** 147–157.

Taylor, G., and Mays, C. (1979). Fatal injury induced by Ca-DTPA in dogs. *Health Phys.* **35,** 858–860.

Thiebault, C., Carriere, M., Milgram, S., Simon, A., Avoscan, L., and Gouget, B. (2007). Uranium induces apoptosis and is genotoxic to normal rat kidney (NRK-52E) proximal cells. *Toxicol. Sci.* **98,** 479–487.

Thompson, D. (1976). In *Uranium in Dental Porcelain*. IN US Department of Health, Education and Welfare, HEW Publication (FDA) 76-8061, Rockville, WA.

Thun, M. J., Baker, D. B., Steenland, K., Smith, A. B., Halperin, W., and Berl, T. (1985). Renal toxicity in uranium mill workers. *Scand. J. Work Environ. Health* **11,** 83–90.

Tissandie, E., Gueguen, Y., Lobaccaro, J. M., Grandcolas, L., Aigueperse, J., Gourmelon, P., and Souidi, M. (2008). Enriched uranium affects the expression of vitamin D receptor and retinoid X receptor in rat kidney. *J. Steroid Biochem. Mol. Biol.* **110,** 263–268.

Tolson, J. K., Roberts, S. M., Jortner, B., Pomeroy, M., and Barber, D. S. (2005). Heat shock proteins and acquired resistance to uranium nephrotoxicity. *Toxicology* **206,** 59–73.

Ubios, A. M., Braun, E. M., and Cabrini, R. L. (1994). Lethality due to uranium poisoning is prevented by ethane-1-hydroxy-1,1-biphosphonate (EHBP). *Health Phys.* **66,** 540–544.

United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). (1988). *United Nations Scientific Committee on the Effects of Atomic Radiation. Sources, Effects and Risks of Ionizing Radiation.* Report to the General Assembly, with annexes, New York.

Uranium Institute. (1996). *The Nuclear Fuel Cycle.* WNA, London. Available at: http://www.world-nuclear.org. Updated October, 1996. Accessed July 7, 2010.

Vaidya, V. S., Ferguson, M. A., and Bonventre, J. V. (2008). Biomarkers of acute kidney injury. *Annu. Rev. Pharmacol. Toxicol.* **48,** 463–493.

Vesterbacka, P., Makelainen, I., and Arvela, H. (2005). Natural radioactivity in drinking water in private wells in Finland. *Radiat. Prot. Dosimetry* **113,** 223–232.

Wessman, R. (1984). An overview of the radiochemical analysis of uranium. In *Biokinetics and Analysis of Uranium in Man. Proceedings of a Colloquium* (W. Richland, Ed.), pp. 1–57. Prepared by the Hanford Environmental Health Foundation for the Department of Energy, Richland, WA.

Wilcox, R. W. (1917). The theraputics of uranium nitrate. *Med. Rec. (NY)* **92,** 361–364.

Wilkinson, W. (1962). Uranium metallurgy. In *Uranium Process Metallurgy* (R. Harper, Ed.), Vol. 1, pp. 722–745. Interscience Publishing, New York.

World Health Organization (WHO). (1984). *World Health Organization. Guidelines for Drinking Water Quality,* WHO, Geneva, Switzerland.

Wrenn, M., Ruth, H., Burleigh, D., and Singh, N. P. (1992). Background levels of uranium in human urine. *J. Radioanal. Nucl. Chem.* **156,** 407–412.

Wyatt, S. A., Reitz, L. V., Croley, T. R., Hawkins, D., Barrett, E., McKeown, A., Powell, N., West, A., Hamner, T., and Royster, M. O. (2008). Biological monitoring of uranium exposure in south central Virginia. *J. Expo. Sci. Environ. Epidemiol.* **18,** 59–75.

Yapar, K., Cavusoglu, K., Oruc, E., and Yalcin, E. (2010). Protective role of Ginkgo biloba against hepatotoxicity and nephrotoxicity in uranium-treated mice. *J. Med. Food* **13,** 179–188.

Yuile, C. (1973). Animal experiments. In *Uranium, Plutonium, Transplutonium Elements* (H. C. Hodge, J. N. Stannard, and J. B. Hursh, Eds.), Vol. 36, pp. 165–196. Springer-Verlag, Berlin.

Zager, R. A., Iwata, M., Burkhart, K. M., and Schimpf, B. A. (1994). Post-ischemic acute renal failure protects proximal tubules from O2 deprivation injury, possibly by inducing uremia. *Kidney Int.* **45,** 1760–1768.

Zalups, R. K. (1991). An evaluation of Tiron (sodium 4,5-dihydroxybenzene-1,3-disulfonate) as a rescue agent for the nephropathy induced by uranyl fluoride (UO2F2) in rats. *Res. Commun. Chem. Pathol. Pharmacol.* **72,** 125–128.

Zamora, M. L., Tracy, B. L., Zielinski, J. M., Meyerhof, D. P., and Moss, M. A. (1998). Chronic ingestion of uranium in drinking water: a study of kidney bioeffects in humans. *Toxicol. Sci.* **43,** 68–77.

Zamora, M. L., Zielinski, J. M., Moodie, G. B., Falcomer, R. A., Hunt, W. C., and Capello, K. (2009). Uranium in drinking water: renal effects of long-term ingestion by an aboriginal community. *Arch. Environ. Occup. Health* **64,** 228–241.

Zimmerman, K. L., Barber, D. S., Ehrich, M. F., Tobias, L., Hancock, S., Hinckley, J., Binder, E. M., and Jortner, B. S. (2007). Temporal clinical chemistry and microscopic renal effects following acute uranyl acetate exposure. *Toxicol. Pathol.* **35,** 1000–1009.

OBJETIVOS

Con objeto de mejorar el diagnóstico del daño renal agudo, nos propusimos identificar marcadores potencialmente útiles en la práctica clínica para nuevas facetas diagnósticas, dirigidas a detectar, de forma anticipada, el riesgo adquirido de padecer esta enfermedad como consecuencia de tratamientos farmacológicos aparentemente inocuos, pero que predisponen a la acción nefrotóxica de otros fármacos o tóxicos ambientales. Para ello planteamos los siguientes objetivos concretos:

OBJETIVO 1. Caracterizar la predisposición al fracaso renal agudo de dos modelos experimentales, uno de predisposición aguda y otro de predisposición crónica.

OBJETIVO 2. Identificar marcadores urinarios relacionados con la predisposición aguda y crónica que sirvan para detectar dicha condición.

RESVLTADOS METODOLOGíA Y DiSCVSiÓN

GENERALIDADES

Los resultados de esta tesis doctoral se exponen en dos artículos.

El primero de ellos, se titula: **"Urinary albumin, transferrin and fumarylacetoacetase as potential markers of the predisposition to acute kidney injury induced by sub-nephrotoxic cisplatin administration.".** En este artículo se describe la identificación de biomarcadores urinarios (albúmina, transferrina y fumarilacetoacetasa) de predisposición al daño renal agudo por la administración subnefrotóxica de cisplatino y la administración de un segundo fármaco, a dosis también subtóxicas, la gentamicina.

El segundo se adjunta acompañado de la presentación de una patente y se ha titulado: **"Rats chronically predisposed to nephrotoxicity have increased urinary excretion of albumin, hemopexin, transferrin and VDBP: potential diagnostic applications."** en el mismo se describe la identificación de marcadores urinarios (albúmina, transferrina, hemopexina y VDBP) de predisposición al daño renal agudo por la exposición crónica a dosis sub-nefrotoxicas de nitrato de uranilo y la administración de un segundo sub-nefrotoxico, la gentamicina.

ARTÍCVLO i

URINARY ALBUMIN, TRANSFERRIN AND FUMARYLACETOACETASE AS POTENTIAL MARKERS OF PREDISPOSITION TO ACUTE KIDNEY INJURY INDUCED BY SUB-NEPHROTOXIC CISPLATIN ADMINISTRATION.

Laura Vicente-Vicente, Laura Ferreira L, José Manuel González-Buitrago, Francisco J. López-Hernández, José Miguel López-Novoa and Ana Isabel Morales.

Revista: Toxicological Science; 2010 (Enviado, pendiente de revision)

Urinary albumin, transferrin and fumarylacetoacetase as potential markers of the predisposition to acute kidney injury induced by sub-nephrotoxic cisplatin administration.

Laura Vicente-Vicente [*,§], Laura Ferreira L[‡], José Manuel González-Buitrago [‡,¶], Francisco J. López-Hernández [*,†,‡,§], José Miguel López-Novoa [*,§] and Ana Isabel Morales [*,§]

(*) Unidad de Toxicología and Unidad de Fisiopatología Renal y Cardiovascular, Departamento de Fisiología y Farmacología, Universidad de Salamanca, Spain. (†) Instituto de Estudios de Ciencias de la Salud de Castilla y León (IECSCYL), Soria, Spain. (‡) Unidad de Investigación, Hospital Universitario de Salamanca, Salamanca, Spain. (§) Fundación Renal Íñigo Álvarez de Toledo, Instituto Reina Sofía de Investigación Nefrológica, Madrid, Spain. (¶) Departamento de Bioquímica y Biología Molecular, Universidad de Salamanca, Salamanca, Spain.

The treatment with the antineoplasic drug, cisplatin, is limited, in a large percentage of cases, by its secondary adverse effect, the nephrotoxicity. But the kidney damage may appear at non-toxic dosage, when subsequently administration of another nephrotoxicant is necessary. This condition could be called predisposition to acquire kidney injury. Common markers used in clinical practice can no detect the predisposition, so it is necessary the search of new markers that appear before the damage occurs. With this propose rats were treated with sub-toxic dose of cisplatin (3mg/Kg b.w., i.p.) and after two days with a sub-toxic regime of gentamicin (50 mg/Kg b.w./day, during 6 days, i.p.). Throughout the experiment renal function and renal histology studies were carried out. Cisplatin treatment did not produce any renal alteration but predisposes to acute kindey injury (AKI). Tree proteins, albumin, transferrin and fumarylacetoacetase were detected increased in urine of animals treated with cisplatin, at day 2 before gentamicin administration. These proteins could detect individuals at risk of AKI and stratifying them with quantitative parameters according to their individual risk.

Key words: acute kidney injury, acquired predisposition, cisplatin, urinary biomarkers.

INTRODUCTION

Cisplatin is an antineoplasic drug widely used in the treatment of solid tumours with high efficiency. However, its use is limited by its most common adverse effect: the nephrotoxicity. Inicial experience reported that approximately 25-30% of treated patients develop a nephrotoxicity even with a single dose of cisplatin (Ries and Klastersky, 1986; Kovach et al., 1973). The nephrotoxic effect is dose-dependent and therefore the amount of cisplatin administered is limited, being this drug treatment less effective as desired. The most common effect observed after cisplatin administration is a reduction in the glomerular filtration rate in a dose -dependent manner (Winston and Safirstein, 1985) even with a single dose treatment.

The uptake of cisplatin in the kidney ocurrs mainly in the S3 segment of the proximal tubule, wich leads in tubular injury in this segment, but also in the thick ascending limb of the loop of Henle (Dobyan et al., 1980). Clinically the manifestations of kidney damage produced by hig amounts of cisplatin administration consist in increases in serum parameters as creatinine and urea as well as decrease in creatinine clearance. Also an increased excretion of enzymes as N-acetyl-beta-glucosaminidase (NAG) and electrolyte disturbances have been shown in acute cases (Goldstein and Mayor, 1983).

Several strategies have been used to avoid cisplatin nephrotoxicity, some of them quite effective, as is the

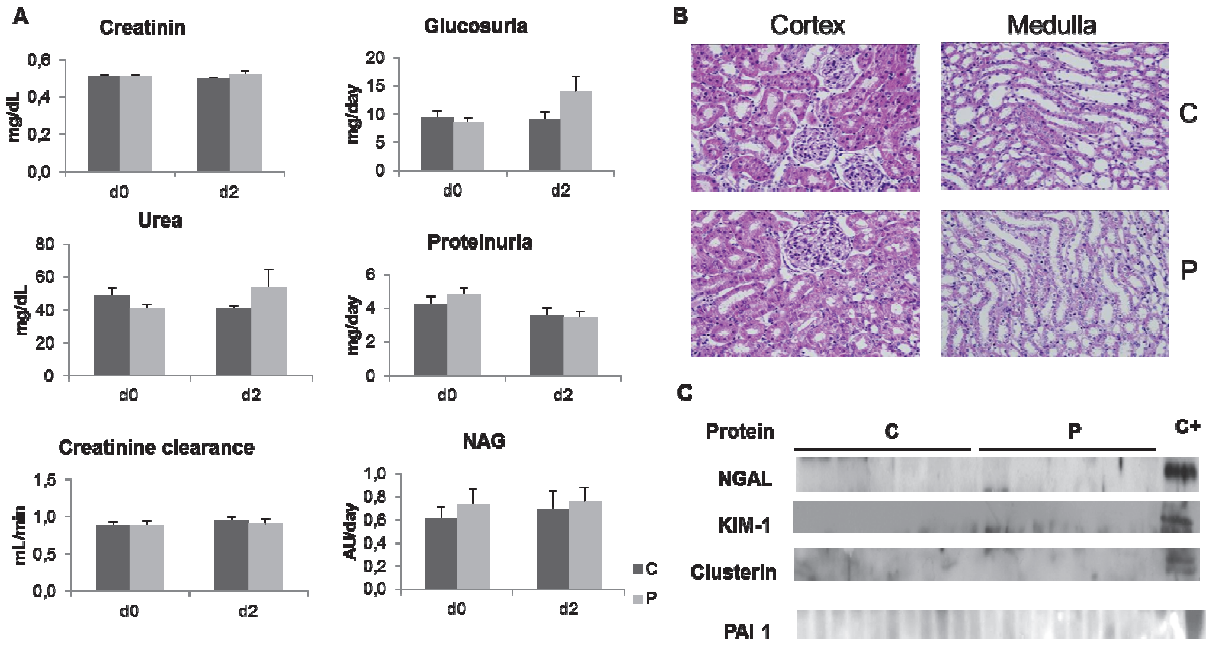


Figure 1. Sub-toxic cisplatin administration does not produce kidney damage. A) Plasma creatinine and urea concentration, creatinin clearance, glucosuria, proteinuria and N-acetyl-beta-D-glucosaminidase (NAG) activity at times basal (d0) and two days before gentamicin administration (d2) in control (C), and cisplatin treated animals (P). B) Representative photographs of the cortical and medullary areas of renal slices staned with hematoxilyn and eosin from control and cisplatin treated rats, 2 days after cisplatin administration. C) Western blot images of the urinary excretion of the proteins lipocalin 2 (NGAL), kidney injury molecule 1 (KIM 1), Clusterin and plasminogen activator inhibitor-1 (PAI-1) at day 2.

case of patient hydration before and during treatment (Arany and Safirstein, 2003), while others are under study, mainly the administration of substances which compte for platinium binding (Bodenner et al., 1986) or directly try to prevent renal failure (Hartman et al., 2000), with more or less success.

Despite all this, therapeutic doses of cisplatin in humans yet still triggering different types of kidney damage. Also must consider that the incidence of cisplatin nephrotoxicity is not well estimated because, in most cases, renal function is only measured by serum creatinine, which have been seeing that it is not the best marker for this condition (Skinner et al., 1991; Womer et al., 1985). Therefore, a number of early biomarkers are being studied to detect kidney damage in its earliest stages (Gautier et al., 2010; McDuffie et al., 2010).

But, the early detection is not only important to prevent AKI produced by cisplatin administration. Some studies have related cases of patients treated with cisplatin and without evidences of kidney damage which suffered febrile neutropenia and needs to be treated with aminoglycosides, these patients develop acute kidney injury (Milovic et al., 2010; Salem et al., 1982). It means that these patients may be at risk if they are subsequently exposed to another neprotoxic agent. This concept could be called as predisposition to adquire AKI and it has been recently demonstrated by other authors with different nephrotoxins (Quiros et al., 2010), and even with cisplatin in pig, suffering for a second insult, i.e. unilateral nephrectomy (Robbins et al, 1990; 1992).

Taking into account these situations, we decided to study if the sub-toxic cisplatin administration in rats, apparentely without renal disfunction symptoms, may

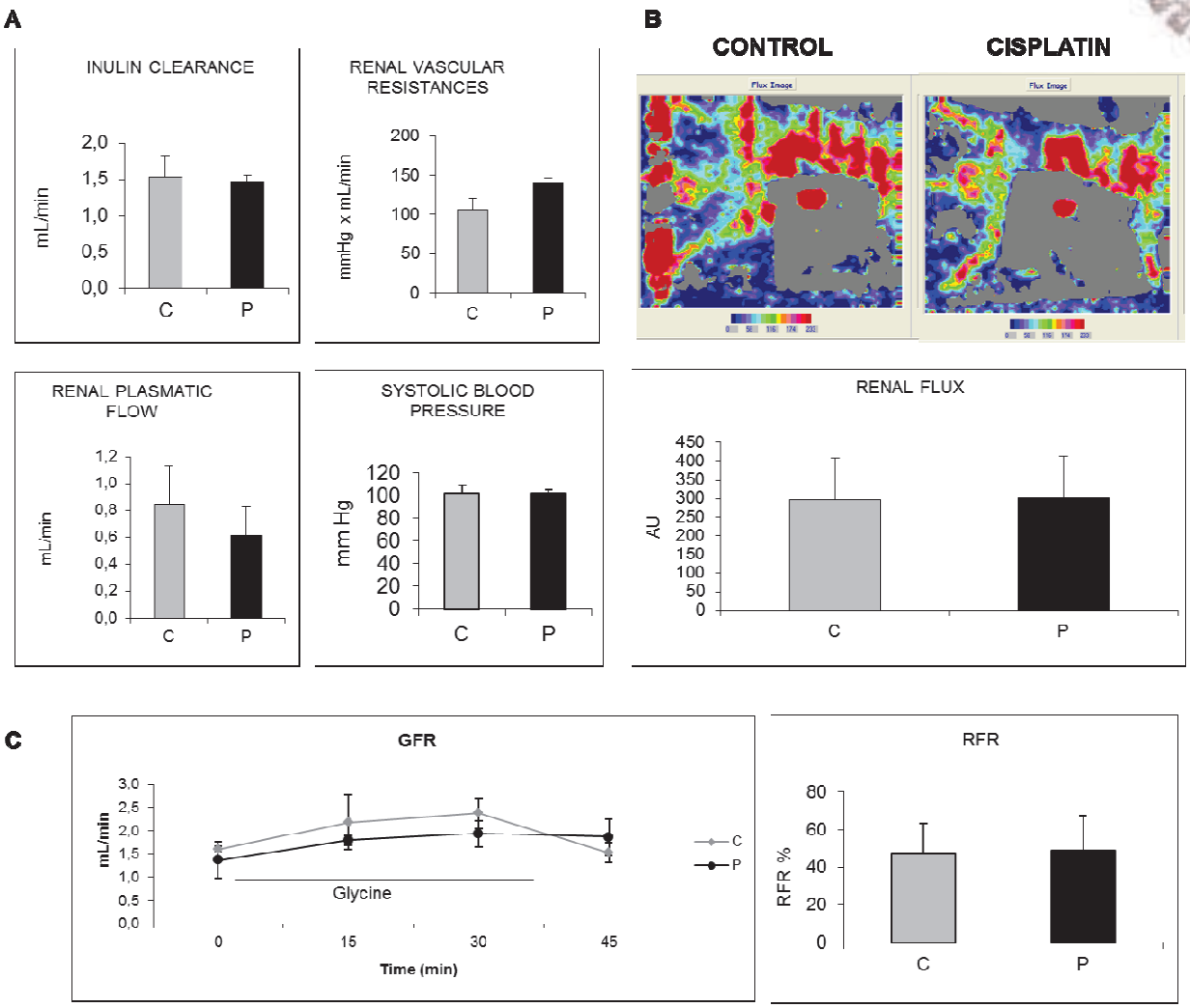


Figure 2. Sub-nephrotoxic cisplatin administration does not alter renal hemodynamic. A) Glomerular fitration rate (measured as inulin clearance), renal plasma flow (measured as p-aminohipuric acid), renal vascual resistance and systolic blodd pressure in control (C) and cisplatin treated rats (P). B) Representative images of renal blood flow and quantification with Moor LSLD software. C) Glomerular filtration rate progression during glycine perfusión and renal functional reserve, calculated as percentage, in control (C) and cisplatin (P) groups. AU, arbitrary units.

lead in AKI when these animals are exposed to a second sub-toxic nephrotoxicant (in this case, the antibiotic, gentamicin). Our results indicate that, subtoxic administration of cisplatin does not produce renal damage or renal dysfunction, but sensitizes animals to developing AKI. This sensitization correlates with the alteration of the urinary proteome resulting in increased excretion of albumin, transferrin and fumarylacetoacetase which are presented as potential markers of this condition.

METHODS

Animals and drugs

Male wistar rats (200-250g initally) were housed under controlled environmental conditions in metabolic cages and had free acces to standard rat chow and drinking water. Animals were divided into four groups (n=12 rats per group) (i)control group (C) wich received no treatment, (ii) Cisplatin group (P): rats were given a single i.p. injection of cisplatin a low dose (3 mg/Kg) on day 0, (iii) Gentamicin group (G): rats were treated with a low dosage regime of

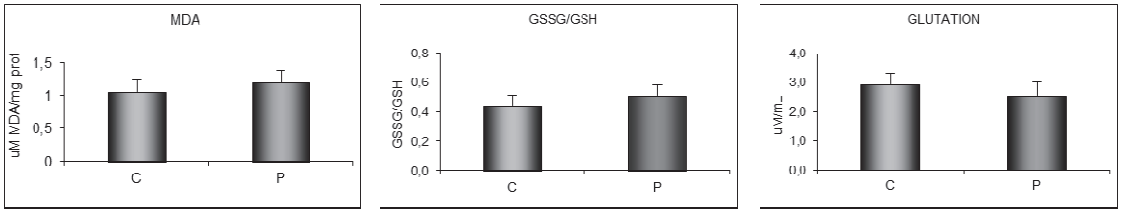


Figure 3. Sub-nephrotoxic cisplatin administration does not alter oxidative status. Malondyaldehide (MDA) content, relation between oxidized and reduced glutathione (GSSG/GSH) an total glutatión in control (C) and cisplatin treated (P) animals in kidney homogenate samples, at day 2.

gentamicin (50 mg/Kg) i.p. during six days starting on day 2, and (iv) Cisplatin + Gentamicin (PG) group: this animals were treated first with cisplatin (3 mg/Kg) on day 0 and after with 6 doses of gentamicin (50 mg/Kg day) starting on day 2. At day 2, two animals of each group were sacrificed and the remaining animals at day 8. Urine was collected at days 0, 2, 4, 6, 8, and cleared by centrifugation and finally stored at -80ºC. At these times, blood samples were also obtained in heparinized capillaries from a small incision in the tail tip. Plasma was separated by centrifugation and kept at -80ºC. At the time of sacrifice, rats were anesthetized, and the kidneys dissected. One half of each organ was fixed in 3.7% para-formaldehyde and further used for histological studies. The remaining renal tissue was kept at -80 ºC for oxidative stress determinations.

Biochemical analysis

Plasma samples were analyzed for urea and creatinine (Crp), and urine samples for creatinine (Cru) concentration with an automatic analyzer (Reflotron plus®; Roche Diagnostics; lower detection limit 0.5 mg/dL). Creatinine clearance (Clcr) was calculated using the equation Clcr = (Cru X UF)/Crp (were UF means urinary flow). Urine was also assayed for protein concentration using the Bradford technique (Bradford, 1976), for glucose using the o-toluidine method (Passey et al., 1982) and for the NAG activity using a commercial kit based on colorimetric (Roche Diagnostics) following the manufacturers' instructions.

Western Blot.

Urine samples were separated by polyacrylamide gel electrophoresis (PAGE; Mini Protean II system, Bio-Rad). Proteins were electro-transferred to Immobilon P membranes (Millipore), which were then incubated with goat polyclonal antibodies against kidney injury molecule-1(KIM-1; RD Systems, USA) Fumarylacetoacetase and Transferrin (Santa Cruz Biotechnology CA, USA), with chiken polyclonal antibodies against albumin, with mouse polyclonal antibody against plasminogen activator inhibitor-1 (PAI-1; Santa Cruz Biotechnology CA, USA), and with rabbit polyclonal antibodies against Lipocaline-2 (NGAL; MBL international, USA) and clusterin (Santa Cruz Biotechnology CA, USA). Membranes were then incubated with horseradish peroxidase (HRP)-coupled secondary antibodies, subsequently incubated with the quimioluminiscent HRP-substrate (ECL; Millipore), and exposed to photographic films (Kodak, Rochester, NY, USA).

Histological studies

Paraffin blocks were made with para-formaldehyde fixed tissue and 5-µm tissue sections were stained with hematoxilin and eosin. Photographs were taken under an Olympus BX51 microscope connected to an Olympus DP70 colour, digital camera.

Flowmetry measured by Laser Doppler

Renal flow was measured by laser Doppler flowmetry by applying the beam of light directly on the kidney surface. For this purpose, an incision in the abdomen of the anesthetized rat was made, and its left kidney was placed on a black surface. As moving erythrocytes displace the light frequency (Doppler shift), blood flow within the capillary network can be derived by analyzing the power spectra from Doppler frequencies of backscattered laser light. Flow is then

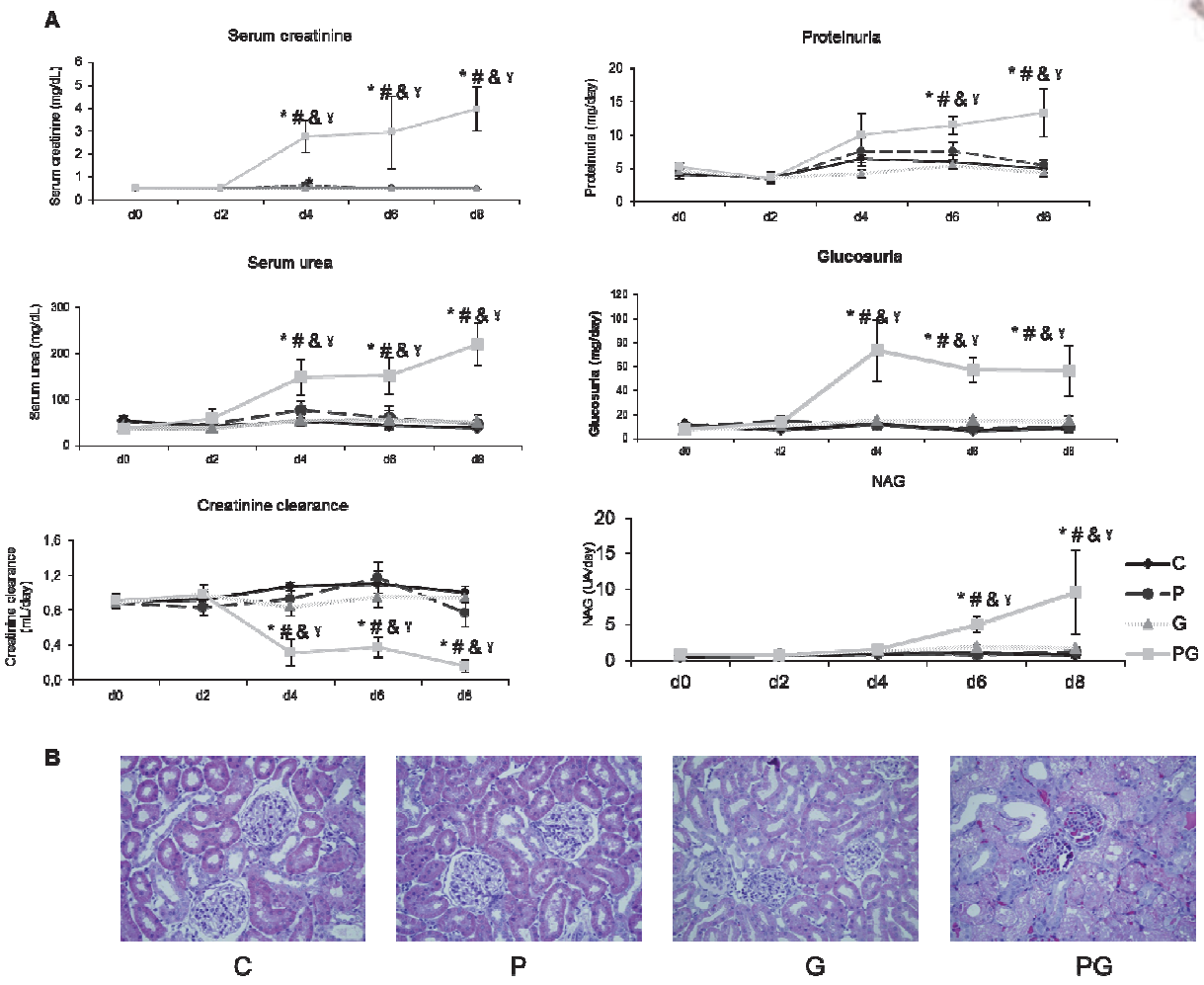
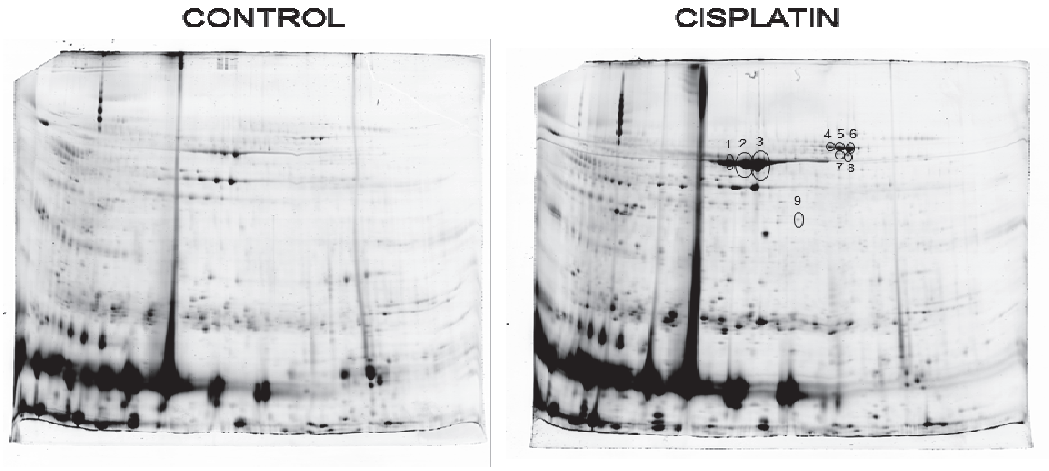


Figure 4. Sub-toxic cisplatin administration predisposes animals to AKI. A) Plasma creatinine and urea concentration, creatinin clearance, glucosuria, proteinuria and N-acetyl-beta-D-glucosaminidase (NAG) activity at times basal (d0), days 2, 4, 6 and 8 (d2, d4, d6 and d8). Data expressed as mean ± standard error of the mean, #p< 0.05 vs basal in the same group; *p<0.05 vs control at the same time point; &p<0.05 vs cisplatin at the same time point; γp<0.05 vs gentamicin at the same time point. B) Representative photographs of the cortical area of renal slices staned with hematoxilyn and eosin from control (C), cisplatin (P), gentamicin (G) and cisplatin + gentamicin (PG) treated rats in day 8.

related to the velocity multiplied by the number of moving erythrocytes and this information is transformed in colours code, low or no perfusion is displayed as dark blue, whereas the highest perfusion interval is displayed as red. The stored perfusion values behind the colour-coded pixels remain available for data analysis. The Moor LSLD software analyses this information by an analytic quantification of the selected area. Plasmatic flow was expressed as flux units which are arbitrary units (AU; Schreen et al., 2011; González-Escalada et al., 1999).

Inulin and para amminohipuric acid (PAH) clearance.

After two days of treatment with cisplatin a subset of animals were anesthetized (sodium pentobarbital, 80 mg/kg, i.p.) and their carotid artery, jugular vein and urinary bladder were cannulated in order to perform renal clearance studies as previously described (Valdivielso et al., 1997). Carotid artery catheter was connected to a pressure transducer (Beckman R511A, USA) and arterial pressure was registered along the experiment (Mac Lab, AD Instruments, Australia).



Spot	Acronym	Protein name	Sc
1	ALBU-RAT	Serum albumin	176
2	ALBU-RAT	Serum albumin	233
3	ALBU-RAT	Serum albumin	235
4	TRFE-RAT	Serotransferrin	99
5	TRFE-RAT	Serotransferrin	140
6	TRFE-RAT	Serotransferrin	170
7	TRFE-RAT	Serotransferrin	116
8	TRFE-RAT	Serotransferrin	116
9	FAAA_RAT	Fumarylacetoacetase	43

Figure 5. Differential proteomic profiling of the urine from control and cisplatin treated rats. Representative images of 2-DE gels. The marked spots correspond to proteins significantly increased in the urine of cisplatin treated rats (P) as compared with control rats (C). The table below reports the protein identification parameters obtained by MALDI-TOF analysis and data base blast for the selected spots. Sc, score, MW, molecular weigth (in kDa), pl iselectric point.

Tritium-labeled inulin (ARC, St. Louis, USA) and carbon-14-labeled PAH (PerkinElmer, MA, USA) were infused throughout the jugular vein at a rate of 3 mL/h, and 3 consecutive (30 min) urine samples were collected from the urinary bladder. Every half hour, blood was collected from the carotid artery into heparinized capillary tubes. Inulin and PAH were measured in plasma and urine using a two-channel Liquid Scintillation Counter (Wallac 1409 DSA, Turku, Finland). Glomerular filtration rate (GFR) was measured by clearance of ^{3}H-inulin and renal plasma flow (RPF) was measured by clearance of ^{14}C-para-aminohipuric acid. The calculation of GFR and RPF was performed by standard formulae. Renal blood flow (RBF) was calculated from RPF and packed cell volume. Filtration fraction (FF) was calculated as GFR/RPF. Renal vascular resistance was calculated as RBF/Mean arterial pressure.

Renal functional reserve (RFR)

Another subset of animals was used for this study, also at day 2 of study (before gentamicina administration). The surgical method was the same than described in PAH-inulin clearance, but in this case two

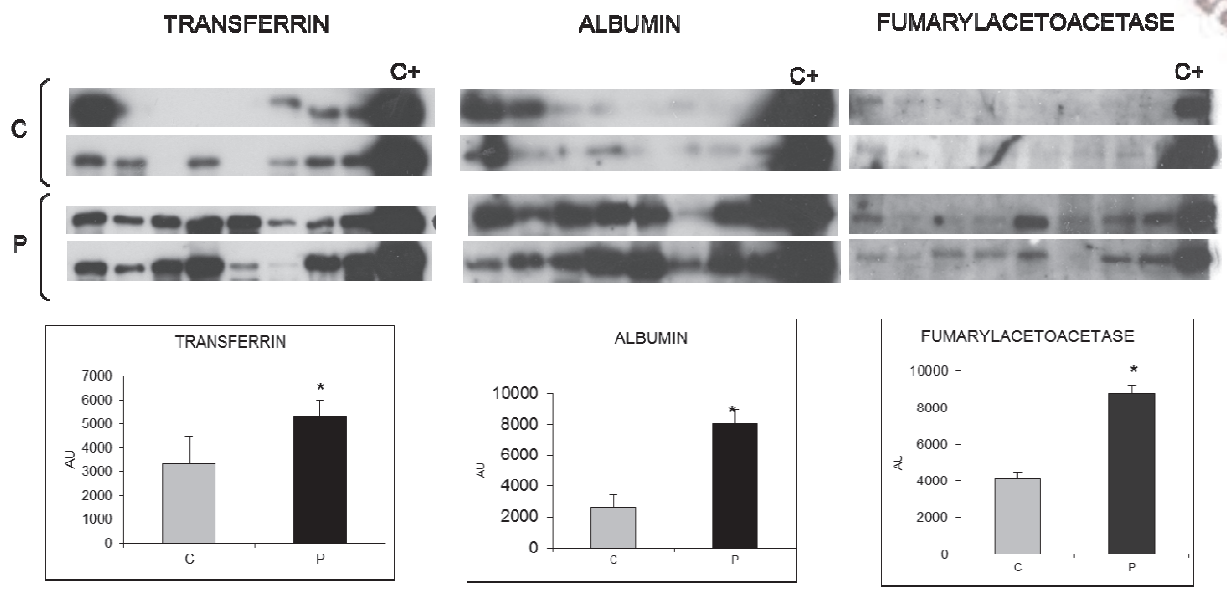


Figure 6. Urinary biomarker validation by Western blot. Confirmation by western blot of the urinary excretion of the proteins transferrin, albumin and fumarylacetoacetas identified by proteomics at day 2 in control (C) and cisplatin treated animals (P). Bands were quantified with the Scion Image software. Each bar represents the mean strandard error of the mean (* $p<0.05$ vs control). AU, arbitrary units.

period studies were conducted in each rat as previously described Slomowitz et al. (2001) but with modifications. GFR were measured in a control period and after glycine infusion. During control period rats were given a 1 mL bolus and then infunded intravenously the mixture I-PAH at approximately 3 mL/h and in the second period animals were infuded with the same mixture wich contains 200 mg/mL glycine and at rate of 1.5 mL/h. After 30 minut stabilization time in each case, urine and blood samples were obtained each 15 minutes. RVR was calculated by the difference between GFR induced by glycine less GFR obtained in control period, and expressed as a percentage.

Stress oxidative studies

Lipid peroxidation

The malondialdehyde (MDA) content, a marker of lipid peroxidation, was assayed in the form of thiobarbituric acid-reactive substances (TBARS) by the colorimetric method of Recknagel and Glende, 1984. The reaction mixture (20% trichloroacetic acid and 0.67% thiobarbituric acid) was added to kidney tissue homogenated, heated at 90ºC and centrifuged. The absorbance was measured at 532 nm.

Glutathione GSH-GSSG assay

The reduced and oxidized glutathione (GSH and GSSG) were assayed by the fluorometric method described by Hissin and Hilf, 1976. *GSH assay:* kidney tissue homgenate were incubated with *buffer* (100 mM EDTA-5 mM phosphate buffer, pH 8.0) followed by 0.1% *o*-phthaldehyde solution (OPT). The fluorescence was read at 350/420 nm (excitation/emission). *GSSG assay:* kidney tissue homogenate were incubated with 0.04M *N*-ethylmaleimide (NEM) to interact with GSH present in the sample. This mixture was incubated with 0.1 N NaOH followed by 0.1% OPT solution. The fluorescence was read at 350/425nm (emission/excitation).

Proteomics

The proteome of urine samples from day 2 from animals without gentamicin (C, P) and animals receiving the aminoglycoside (G, PG) was analyzed by two-dimensional electrophoresis (2-DE), basically as described (Quiros et al., 2010; Ferreira et al., 2010). In short, urine proteins (200 µg) were precipitated and isoelectrically focused (500-8,000 V) through 18-cm long immobilized pH gradient (IPG) strips, pH 3-

11NL (GE Healthcare, Madrid, Spain). Then, proteins in IPG strips were separated by 12% SDS-polyacrylamide gels, fixed and stained with Sypro Ruby (Molecular Probes). The spots of interest were analyzed with the Image Master Platinum software (GE Healthcare) and in-gel digested with porcine trypsin (Promega). Tryptic peptides were analyzed by MALDI-TOF on an Autoflex III instrument (Bruker Daltonics). One microlitre of each sample was deposited on Prespotted AnchorChip targets (Bruker Daltonics), which are prespotted with α-cyano-4-hydroxycinnamic acid (HCCA) matrix. Spectra were acquired with the FlexControl 3.0 software (Bruker Daltonics) and then processed using the Flex Analysis 3.0 software (Bruker Daltonics). The equipment was first externally calibrated employing protonated mass signals from calibrants prespotted in the target, covering the 1000–4000 m/z range. All known contaminants (trypsin-derived peptides and keratins) were excluded during the process. The resulting final peak list was used for the identification of the proteins by peptide mass fingerprint (PMF). Protein identification was performed with the MASCOT software (www.matrixscience.com) against non redundant protein sequence database Swiss Prot (containing 516603 sequences and 181919312 residues). The mass tolerance was set as 50 ppm, and the taxonomic category was Rattus (7509 sequences). Only one missed cleavage per peptide was allowed and cysteine residues were assumed to be carbamidomethylated with acrylamide adducts, and methionine residues were in oxidized form. In all protein identifications, the probability scores were greater than the score fixed by MASCOT as significant with a p-value lower than 0.05.

Statistical analysis.

Experimental groups were compared using a two-way analysis of variance (ANOVA) followed by Sceffe's test when the data were normally distributed and by the Kruskal-Wallis test when they were not normal distributed. Values were considered significantl when $p<0.05$. Data were analyzed using the Number Cruncher Statistical System (NCSS) software, version 6.0.10 for Windows. Data are shown as mean ± standard error of the mean.

RESULTS

Sub- toxic administration of cisplatin did not alter renal function

As sown in figure 1, at day 2, sub-toxic cisplatin administration of 3mg/Kg b.w. did not produce any alteration in the plasmatic biomarkers of kidney injury urea and creatinine. Values of GFR measured by Clcr did also not show differences between animals receiving cisplatin and animals without the chemotherapeutic agent. In the same way, urinary biomarkers of proteinuria, glucosuria and enzymatic NAG activity were not altered at this time point.

Some novel biomarkers were tested: NGAL, KIM (Tonomura et al., 2010; Vaidya et al., 2010), Clusterin (Tonomura et al., 2010), and PAI1 (Vaidya et al., 2008). These proteins were chosen because they had been useful in the kidney injury early detection in different models of nephrotoxicity. None of them appeared in the urine of any of the studied groups. This situation highlights the need to find new biomarkers able to detect hidden conditions.

The sub-toxic administration of cisplatin did not produce any morphologic alteration as shown renal histology. These results indicate that cisplatin administration at dose of 3 mg/Kg apparently produce neither functional kidney damage measured by different biochemical methods (both plasmatic and urinary), nor structural changes as reveal the histology.

With the purpose of make a deeper study of renal function on day two, renal homeostasis was studied, using three different techniques: inulin-PAH clearance, flowmetry by laser Doppler and RFR (figure 2). GFR measured by inulin clearance did not show any difference between treated and no treated animals, data confirming Clcr results. PAH clearance provides data of RVR and FSR (or FPR), cisplatin administration induce al slight increase in RVR and decrease in FSR but not differences statically significants were shown. Renal flux also was measured using the laser Doppler technique. In this case, there was no difference between groups probably due to saturation in the colour measurement by the high flux in both groups.

As homeostasic mechanisms appeared not to be altered, the RFR was studied. FR is defined as the ability of the kidney to respond to an aggression by increasing their GFR. Animals treated and non treated with cisplatin responded in the same way, indicating that cytostatic treatment did not alter this kidney ability.

It is well known that cisplatin exerts its nephrotoxic activity by an alteration in oxidative stress. Studies of oxidative stress in kidney samples at day 2 were made. The TBARs, and the glutation route were measured. As shown in figure 3, any of these parameters were altered by cisplatin administration at day 2 (before gentamicin administration).

Sub-toxic cisplatin predisposes rats to AKI

Gentamicin administration at day 2, produced a development of AKI in the animals previously treated with low dose of cisplatin, as shown in figure 4, by a significant increase in all the biochemical parameters in the PG group, evident at d4, which it is not observed in the rest of the groups, only the P group showed a small increase in serum creatinine at d4 but returns to baseline levels at d6. Clcr in PG group presented a decrease following the same trend than plasmatic biomarkers and it is not altered in the rest of groups.

But this second nephrotoxicant administration did not only impaired the kidney functionality in PG rats, renal histology revealed an extensive and aggressive damage in rats co treated with cisplatin and gentamicin as showed renal sections stained with hematoxilin and eosin (figure 4). The analysis of this histology showed a gross renal failure characterized by tubular necrosis in the renal cortex, loss of brush border, cytoplasmic vacuolization, tubular obstruction with hyaline material. But glomerular structures were also damaged.

In the other side, kidneys from control animals (C) or treated with only one drug (P and G groups) did no show any gross morphological alteration.

Search for new biomarkers of susceptibility to kidney damage.

Proteomic study was conducted to find different patterns of urinary protein excretion between animals treated with cisplatin and not treated at day 2 (before gentamicin administration). Urinary excretion of proteins seems to be very similar between groups (figure 5), but subtle differences were observed. Treated animals excreted medium molecular weight (MMV) proteins in higher proportion than control rats. Using a specific satatistical analysis of urinary proteomes between groups, three proteins were found to be significantly increased in urine by cisplatin treatment: albumin, transferrin and fumarylacetoacetase. The urinary increased excretions of these proteins were reconfirmed by western blot analysis, as shown in figure 6, the amount of protein excreted is statically different in animals treated with cisplatin when compared with controls.

DISCUSIÓN

Clinical evidences showed that chemotherapeutic drugs are myelosupressive, since that cisplatin patients usually suffer from infections, mainly febrile episodes. Broad expectrum antibiotics are necessary in the treatment of urinary and pulmorary infections in patients with metastatic lesions. The bacteria responsible from these infections are mainly gram-negative bacilli, which made necessary the use of antibiotics as aminoglycosides. Some studies had reported that, in rats, the combination of cisplatin with aminoglycoside terapy produced a potentiation in the cisplatin nephrotoxicity (Jonguejan et al., 1988;1989; Kawamura et al., 1980; Engineer et al., 1987). Different dosages of cisplatin and different aminoglycosides are used in these articles, for example, Jonguejan et al., 1989 and 1998 used amikacin, Engineer et al., 1987 used gentamicin and Kawamura et al., 1980 used tobramicin in all these cases, cisplatin doses were lightly nephrotoxic or even with evident nephrotoxicity, and they showed that concurrent aminoglycoside administration increases nephrotoxicity incidence. The biomarkers most common used to measure kidney damage are blood urea nitrogen (BUN) and glomerular filtration rate (GFR). They do not use better kidney damage markers or more specifics.

Engineer et al. (1987) reports that cisplatin reduces

gentamicin excretion because they observed a decrease in gentamicin clearance in the animals treated previously with cisplatin which results in an elevated concentration of gentamicin in plasma and tissues. Jonguejan et al., 1998, discusses that this toxicity may be due for a pontentitation by amikacin of the cisplatin nephrotoxicity or as an increase in the aminoglycoside sensitivity to cisplatin because the kidney was predammaged.

In humans treated with cisplatin and gentamicin therapy (Salem et al., 1982; Milovic et al., 2010, Dentino et al., 1978 Gonzalez-Vitale 1978) the chemotherapeutic agent produced a potentiation in the nephrotoxic effect of the aminoglycoside as compared with patients treated only with cisplatin. Increases in serum creatinine, BUN and decreases in GFR have been reported. In one case, the severity of the damage was as extreme as the patient died of renal failure after treatment with these two drugs (Salem et al., 1982). The final conclusion of this articles said that administration of aminoglycosides in patients previously treated with cisplatin should be avoided or, at less, given separated by several days.

Our opinion is that cisplatin produces a silent acquired sensitivity to AKI wich is unmasked when the second nephrotoxicant, gentamicin, was administered and this condition could be called as predisposition to acquired AKI.

Drug-induced predisposition to AKI has been recently demonstrated (Quiros et al., 2010). Indeed, animals exposed to a subnephrotoxic regime of gentamicin are predisposed to AKI. This predisposition is manifested as a higher sensibility to the action of other renal insults. Indeed, predisposed rats undergo an overt ARF when subject to subnephrotoxic doses of other drugs, contrast media or environmental metals, which cause no harm to non-predisposed animals.

The results obtained in this work differ from others obtained in animals or humans since the cisplatin dose used is sub-toxic and apparently without kidney injury lesions. Cisplatin administration alone or gentamicin regimen alone did not produce nephrotoxicity measured by different biomarkers. However when this two therapies were given in combination, the rats developed acute kidney injury, evident since two gentamicin doses. It means that cisplatin predisposes to acquire acute kidney injury when a second insult occurs. This work uses the common creatinine and BUN as well as GFR measured by different techniques, but also specific biomarkers as proteinuria, glucosuria, NAG activity and novel early biomarkers (KIM, NGAL), none of them altered before gentamicin administration. However, the relative composition of urinary proteins is altered by cisplatin. As depicted in picture 5 were more medium molecular weight proteins (MMW; i.e. 35-65 KDa) and less low molecular weight proteins (LMW; i.e. <35 KDa) were detected in urine samples of rats treated with cisplatin. The explanation of this event may be due an alteration on the glomerular filtration barrier (GFB) which let that some proteins, that in normal conditions should not pass the glomerular barrier, are found in the glomerular filtrate. However, proteinuria was not observed, probably due to equilibrium of reabsorption into the tabules was produced and thus compensate this increase in the glomerular permissively. So this, final composition of urine is different in treated animals with cisplatin than those in control animals. Aparition in urine of the proteins albumin, transferrin and fumarylacetoacetase make these three proteins able to be candidates to predisposition kidney injury biomarkers induced by subtoxic administration of cisplatin.

Urinary excretion of albumin and transferrin had been detected in other studies as usefull parameters for detection of cisplatin nephrotoxicity in early stages in childrens, but this increase was associated with a decrease in GFR (Erdlenbruch et al., 2001) as in our work, serum creatinine was not modified when this proteins appear in urine.

An increase in urine excretion of both proteins also were reported by other authors (Rossi et al., 1994) but

in this work cisplatin was administered combined with ifosfamide, 6% of patients treated with both drugs excreted higher amounts of transferrin in urine and 30% albumin, but this patients also had increased the NAG, and also suffer from hiperaminoaciduria. In our work the presence of both proteins is not related with other biomarkers, neither new early biomarkers.

Previous results in our laboratory had detected these proteins as probable markers of predisposition to AKI in a chronic model of exposure to an environmental nephrotoxicant, uranium. The relation between chronic uranium model and sub-acute cisplatin model is that, in both cases, the nephrotoxic substance is a hard metal which once reach blood circulation needs to be bound to albumin and transferrin to be transported.

By other side, the protein fumarylacetoacetase (also known as fumarylacetate hydrolase) have not been used as marker of nephrotoxicity by cisplatin nor another nephrotoxic substance. Only the work of Bandara et al. (2003) relate the enzyme fumarlyacetoacetate hydrolase as possible biomarker of kidney toxicity after treating Fischer rats with toxic doses of d-serine, but this enzyme appears increased in plasma and is not measured in urine. In fact, the results of this paper show for first time the detection of fumarylacetoacetase in urine, and the possibility to use it as a marker of predisposition to kidney injury by sub-toxic administration of cisplatin.

In conclusion, the clinical investigation of this tree biomarkers might be very useful for detecting treated individuals at risk, in order to improve their clinical handling and to reduce the incidence of drug-related ARF.

REFERENCES

Arany, I. and Safirstein, R. L. (2003) Nephrotoxicity and hydration management for cisplatin, carboplatin, and ormaplatin. *Seminars in Neprology* Vol 23, **No 5**: pp 460-464.

Bandara, L. R., Kelli, M. D., Lock, E. A., and Kennedy S. (2003). A potencial biomarker of kidney damage identified by proteomics: preliminary findings. *Biomarkers* **8**(3-4), 272-286.

Bodenner, D., L., Dedon, P. C., Keng P., C. and Borch, R. F. (1986). Effect of diethyldithiocarbamate on cis-diamminedichloroplatinum(II)-induced cytotoxicity, DNA cross-linking and gamma-glutamil transpeptidase inhibition. *Cancer Res* **46**, 2745-50.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72,** 248-254.

Dentino, M., Luft, F. C., Yum, M. N., Williams, S. D. and Einhorn, E. H. (1987). Long term effect of cis-diamminedichloride platinium (CDDP) on renal function and structure in man. *Cancer* **41** (4), 1274-81.

Dobyan, D. C., Levi, J., Jacobs, C., Kosek, J. and Weiner, M. W. (1980) Mechanism of cisplatinum nephrotoxicity: II. Morphologic observations. *J Pharmacol Exp Ther* **213**(3), 551 –556.

Engineer, M. S., Bodey, G. P., Newman, R. A. and Ho, D. H. (1987). Effects of cisplatin-induced nephrotoxicity on gentamicin pharmacokinetics in rats. *Drug Metab Dispos* **15**(3), 329-34.

Erdlenbruch, B., Pekrum, A., Roth, C., Grunewald, R. W., Kern, W. and Lakomek, M. (2001). Cisplatin nephrotoxicity in children after continuous 72-h and 3X1-h infusions. *Pediatr Nephrol* **16**(7), 586-93.

Ferreira, L., Quiros, Y., Sancho-Martínez, S. M., García-Sánchez, O., Raposo, C., López-Novoa, J. M., González-Buitrago, J. M., López-Hernández, F. J. (2010). Urinary levels of re-

generating islet-derived protein III β and gelsolin differentiate gentamicin from cisplatin-induced acute kidney injury in rats. *Kidney Int* **79**(5), 518-28.

Gautier, J. C., Riefke, B., Walter, J., Kurth, P., Mylecraine, L., Guilpin, V., Barlow, N., Gury, T., Hoffman, D., Ennulat, D., Schuster, K., Harpur, E. and Pettit, S. (2010). Evaluation of novel biomarkers of nephrotoxicity in two strains of rat treated with cisplatin. *Toxicol Pathol* **38**(6), 943-56.

Goldstein, R. S. and Mayor, G. H. (1983) Minireview. The neprotoxicity of cisplatin. *Life Sciencences* **32,** 685-90.

González-Escalada, J. R., de la Calle, J. L. and Per u c h o, A. (1999). Flujometría campimétrica por láser doppler. Un nuevo procedimiento diagnóstico y evaluativo del dolor. *Rev Soc Esp Dol* **6**:187-198.

Gonzalez-Vitale, J. C., Hayes, D. M., Cvitkovic, E. and Sternberg, S. S. (1978). Acute renal failure after cis-dichlorodiammineplatinum(II) and gentamicin-cephalothin therapies. *Cancer Treat Rep* **62**(5), 693-8.

Hartman, J. T., Knop, S., Fels. L. M., van Vangerow, A., Stolte, H., Kanz, L. and Bokemeyer, C. (2000). The use of reduced doses of amifostine to ameliorate nephrotoxicity of cisplatin ifosfamide- based chemotherapy in patients with solid tumors. *Anticancer Drugs* **11**, 1-6.

Hisin, P. J. and Hilf, R. (1976). A fluorimetic method for determination of oxidized and reduced glutathione in tissues. *Anal Biochem* **74**:214-226.

Jonguejan, H. T., Provoost, A. P. and Molenaar, J. C. (1988). Potentiation of cis-diamminedichloroplatinum nephrotoxicity by amikacin in rats. *Cancer Chemoter Pharmacol* **22**(2), 178-80.

Jonguejan, H. T., Provoost, A. P. and Molenaar, J. C. (1989). Potentitated nephrotoxicity of cisplatin when combined with amikacin comparing young and adult rats. *Pediatr Nephrol* **3**(3), 290-5.

Kawamura, J., Soeda, A. and Yoshida, O. (1980). Nephrotoxicity of cis-diamminedichloroplatinium (II) (cis-platinum) and the additive effect of antibiotics: Morphological and functional observation in rats. Toxicol Appl Pharmacol 58(3), 475-82.

Kovach, J. S., Moertel, G. C., Schutt, A. J., Reitermeier, R. G. and Hahn, R. G. (1973). Phase II study of cis-diamminedichloroplatinium (NSC-119875) in advanced carcinoma of the large bowel. *Cancer Chemoter Rep* **57**, 357-59.

McDuffie, J. E., Sablad, M., Ma, J. and Snook, S. (2010). Urinary parameters predictive of cisplatin-induced acute renal injury in dogs. *Cytokine* **52**(3), 156-62.

Milovic, M., Popov, I., Jezdic, S., Stojanovic, S., Stankovic, V. and Radic, S. (2010). Monitoring levels of nephrotoxicity of different aminoglycosides during febrile neutropenia caused by nephrotoxic chemotherapy: a single centre study. *J Buon* **15**(2), 297-302.

Passey, R. B. (1983). Glucosa method comparision criticized. *Clin Chem* **29**(6), 1320-1321.

Quiros, Y., Ferreira, L., Sancho-Martínez, S. M., González-Buitrago, J. M., López-Novoa, J. M., López-Hernández, F. J. (2010). Sub-nephrotoxic doses of gentamicin predispose animals to developing acute kidney injury and

to excrete ganglioside M2 activator protein. Kidney Int. Nov;78(10):1006-15.

Recknagel, R. O. and Glende, E. A. (1984). Spectrophotometric detection of lipid conjugated dienes. *Methods Enzymol* **105**, 331-37.

Ries, F. and Klastersky, J. (1986). Nephrotoxicity induced by cancer chemotherapy with special emphasis on cisplatin toxicity. *Am. J. Kidney Dis* **8,** 368-379.

Robbins, M. E., Campling, D., Whitehouse, E., Hopewell, J. M. and Michalowsky, A. (1990). Cisplatin-induced reductions in renal functional reserve uncovered by unilateral nephrectomy: an experimental study in the pig. *Cancer Chemoter Phharmacol* **27**(3), 211-8.

Robbins, M. E., Bywaters, T. B., Jaenke, R. S., Hopewell, J. W., Matheson, L. M., Tothill, P. and Whitehouse, E. (1992). Long-term studies of cisplatin-induced reductions in porcine renal functional reserve. *Cancer Chemoter Phharmacol* 29(5), 309-15.

Rossi, R. M., Kist, C., Wuster, U., Kulpman, W. R. and Ehrich, J. H. (1994). Estimation of ifosfamide/cisplatinium-induced renal toxicity by urinary protein análisis. *Pediatr Nephrol* **8** (2), 151-6.

Salem, P. A., Jabboury, K.W. and Khalil, M. F. (1982). Severe nephrotoxicity: a probable complication of cisdichloreamineplatinium (II) and cephalotin-gentamicin therapy. *Oncology* **39** (1), 31-2.

Scheeren, T. W., Martin, K., Maruschke, M. and Hakenberg, O. W. (2011). Prognostic value of intraoperative renal tissue oxygenation measurement on early renal transplant function. *Transpl Int*. Epub, ahead of print.

Skinner, R., Pearson, A. D., Coulthard, M. G., Skillen, A. W., Hodson, A. W., Goldfinch, M. E., Gibb, I. and Craft, A. W. (1991). Assesment of chemotherapy-associated nephrotoxicity in children with cancer. *Cancer Chemoter Pharmacol* **28**(2), 81-92.

Slomowitz, L. A., Deng, A., Hammes, J. S., Gabbai, F. and Thomson, S. C. (2002). Glomerular balance, dietary protein, and the renal response to glycine in diabetic rats. *Am J Physiol Regul Integr Comp Physuik* **282,** 1096-1103.

Tonomura, Y., Tsuchiya, N., Torii, M. and Uheara, T. (2010). Evaluation of the usefulness of urinary biomarkers for nephrotoxicity in rats. *Toxicology* **273**(1-3), 53-59.

Vaidya, V. S., Ferguson, M. A. and Bonventre, J. V. (2008). Biomarkers of acute kidney injury. *Annu Rev Pharmacol Toxicol* **48**, 463-93.

Vaidya, V. S., Ozer, J. S., Dieterle, F., Collings, F. B., Ramirez, V., Troth, S., Muniappa, N., Thudium, D., Gerhold, D., Holder, D. J., Bobadilla, N. A., Marrer, E., Perentes, E., Cordier, A., Vonderscher, J., Maurer, G., Goering, P. L., Sistare, F. D. and Bonventre, J. V. (2010). Kidney Injury Molecule-1 outperforms traditional biomarkers of kidney injury in multi-site preclinical biomarker qualification studies.*Nat Biotechnol* **28**(5), 478-85.

Valdivieso, J. M., Rivas-Cabañero, L., Pérez-Barriocanal, F., López-Novoa, J. M. (1997). Effect of nitric oxide sintesis modification on renal function in gentamicin-induced nephrotoxicity. *Environ Toxicol Pharmacol* **3** (2), 123-128.

Winston, J. A. and Safirstein, R. (1985). Reduced renal blood flow in early cisplatin-induced acute renal failure in the rat. *Am J Physiol* **249**, F490.

Womer, R. B., Pitchard, J. and Barratt, E. M. (1985). Renal toxicity of cisplatin in children. *J Pediatr* **106**(4), 659-63.

ARTÍCULO II

RATS CHRONICALLY PREDISPOSED TO NEPHROTOXICITY HAVE INCREASED URINARY EXCRETION OF ALBUMIN, HEMOPEXIN, TRANSFERRIN AND VDBP: POTENTIAL DIAGNOSTIC APPLICATIONS.

Laura Vicente-Vicente, Laura Ferreira L, José Manuel González-Buitrago, Francisco J. López-Hernández, José Miguel López-Novoa and Ana Isabel Morales.

Revista: Kidney International; 2010 (Enviado, pendiente de revision)

Rats chronically predisposed to nephrotoxicity have increased urinary excretion of albumin, hemopexin, transferrin and VDBP: potential diagnostic applications

Laura Vicente-Vicente *,§, Laura Ferreira L‡, José Manuel González-Buitrago ‡,¶, Francisco J. López-Hernández *,†,‡,§, José Miguel López-Novoa *,§ and Ana Isabel Morales *,§

(*) Unidad de Toxicología and Unidad de Fisiopatología Renal y Cardiovascular, Departamento de Fisiología y Farmacología, Universidad de Salamanca, Spain. (†) Instituto de Estudios de Ciencias de la Salud de Castilla y León (IECSCYL), Soria, Spain. (‡) Unidad de Investigación, Hospital Universitario de Salamanca, Salamanca, Spain. (§) Fundación Renal Íñigo Álvarez de Toledo, Instituto Reina Sofía de Investigación Nefrológica, Madrid, Spain. (¶) Departamento de Bioquímica y Biología Molecular, Universidad de Salamanca, Salamanca, Spain.

Rats may become transitorily predisposed to acute renal failure (ARF) by acute, subnephrotoxic exposure to potentially nephrotoxic drugs. Impaired renal function is another risk factor for drug-induced ARF. However, it is not known whether animals might become chronically predisposed to ARF by potentially nephrotoxic insults under long-term subnephrotoxic circumstances. To this end, rats were treated with a subtoxic dosage of the experimental nephrototoxin uranyl nitrate (UN) in the drinking water for 21 weeks, or plain water (as control), and then with low-dose gentamicin for 7 days. Renal function and renal tissue damage were evaluated through the experiment. The renal damage caused by gentamicin was markedly magnified in rats having received UN chronically, which was evident both at the functional and histological level. Four proteins, namely albumin, hemopexin, transferrin and vitamin D binding protein were increased in the urine in temporal association with the appearance of chronic predisposition. Our results suggest that these proteins might be potentially used as markers of hidden predisposition to ARF, in order to appropriately and pre-emptively stratify and handle individuals according to their specific risk in the long term, as regards their life conditions or additional clinical procedures or treatments that might trigger the disease. This might reduce ARF incidence and severity and the associated costs.

Key words: acute kidney injury, acquired predisposition, chronic risk, urinary biomarkers, preventive medicine, personalized medicine.

INTRODUCTION

Acute kidney injury (AKI) defines a rapid functional or structural alteration in the kidneys resulting from an abrupt insult of diverse aetiology (1). AKI may range from mild, reversible alterations to severe injuries leading to acute renal failure (ARF) and death, or even progress towards progressive and irreversible chronic kidney disease (CKD) (2). ARF is a very serious and life-threatening condition in which renal excretory function rapidly declines in a few hours or days. It is estimated that approximately 1% of hospital admissions are associated to some degree of AKI, and that 2-7% of hospitalized patients eventually develop AKI (3, 4). Mortality due to ARF is extremely high, especially if multi-organ damage occurs and also within the population of critically ill patients, in which case it may reach to 50-80% of patients (5-8). Even mild episodes of AKI, with transient episodes of renal dysfunction and repairable parenchymal injury are associated to higher short and long term mortality rates (9-11). A number of AKI patients progress towards progressive and irreversible chronic kidney disease, keep chronically a certain degree of renal dysfunction (10, 12-14) or need long or permanent dyalisis (15). Drug nephrotoxicity poses one of the leading causes of AKI and a considerable health and economic burden worldwide. In fact, nephrotoxicity causes 10-20% of the ARF cases (16). Nearly 25% of the top 100, most used drugs in intensive care units are potentially nephrotoxic (17). AKI and AKI severity are most commonly diagnosed and classified according to the "Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease" (RIFLE) cri-

	T0		T11		T21	
	Control	UN	Control	UN	Control	UN
Weight (g)	257±25.8	241±3.2	433±20.5	331±14.1*	506±21.3	367±18.9*
Water intake (mL/day)	30.3	17.6	32.5	14.9	32.6	15.6
Urinary flow (mL/day)	9.17±1.15	9.00±1.26	9.35±1.21	2.98±0.56*	12.67±2.44	2.90±0.75*
UN ingestion (mg/day)	0	0	0	80.64±11.66*	0	84.25±14.42*
Urinary excretion of uranium (µg/day/100 g b.w.)	N/D	N/D	0.16±0.1	9.86±2.86*	0.05±0.02	6.37±1.5*
Uranium in kidney (g U/kg kidney)	N/D	N/D	0	24±1.88*	0	22±2.13*

Table 1. Body weight (b.w.), water intake, urinary output, uranium ingestion, renal accumulation and excretion in control and UN-exposed rats at 0, 11 and 21 week of treatment. Data are expressed as the mean ± standard error of the mean of n = 12 animals per group. * $p \leq 0.05$ vs. control. N/D, not determined.

teria (18), which are based on alterations in the plasmatic concentration of creatinine (Cr_{pl}), glomerular filtration rate (GFR) and urinary output alterations. The first stage or severity grade of AKI ("risk") is achieved when Cr_{pl} increases 1.5 fold, GFR decreases over a 25%, or urine output decreases at a rate of 0.5 mL/kg/h in a period of 6 hours (19). At this point, when renal function is only mildly impaired, renal tissue damage may be extensive. This is because, due to renal functional reserve, the equivalent in function of a large fraction of nephrons must be void for Cr_{pl} to increase or overall GFR to decrease. Because of this, we have recently put forward a diagnostic concept different from the detection of mild damage or mild dysfunction, which we have named "acquired predisposition to ARF" (20). This concept states that experimental animals subject to an absolutely sub-nephrotoxic, acute treatment with certain drugs (e.g. aminoglycoside antibiotics) according to the finest clinical and academic criteria, are transitorily more sensitive to a second potentially nephrotoxic insult than non-predisposed animals. As such, doses of a second insult that have no effect on non-predisposed animals cause an overt ARF on predisposed animals. Most interestingly, predisposed animals show increased urinary excretion of specific markers, despite showing no gross alteration in the composition of the urine. These markers might have a potential application for the detection of acquired predisposition to AKI in order to stratify and handle patients conveniently, pre-emptively and individually according to quantitative parameters of individual risk.

It is well known that compromised renal function is a risk factor for AKI (10). As such, CKD patients are more prone to drug-induced AKI than those with normal renal function. On these grounds, we decided to step a little further by studying whether animals with absolutely normal renal function and no signs of kidney injury might also become chronically predisposed or sensitized to ARF. This condition would gain special importance for its silent course, especially in those people with no signs of kidney injury or renal dysfunction in the long term. For these reason, we also aimed at identifying markers of chronically acquired predisposition to ARF which might serve to better monitor the population over longer periods of time. Prevention of AKI has also significant socioeconomic consequences. AKI episodes, regardless of prognosis or future impact on health, extend hospitalization time and often require dialysis. In 2005, it was estimated that, in the United States, costs derived from an AKI episode were directly proportional to the increment in plasma creatinine, ranging from 8,902 to 33,162 dollars for creatinine increments of 0.3 to 2.0 mg/dL, respectively (21). The cost associated to AKI was 5% of all-admission costs.

RESULTS

UN rats experienced a lower body weight gain compared to control rats, which was significant by week 11 (Table 1). Water ingestion was lower in UN rats through the experiment, which correlated with a lower urinary flow (Table 1). Uranium consumption was calculated weekly from water intake data. UN animals

A

	T11		T21	
	Control	UN	Control	UN
Plasma creatinine (mg/dL)	0.51 ± 0.01	0.54 ± 0.06	= 0.5	0.54 ± 0.02
Plasma urea (mg/dL)	34.79 ± 2.38	47.5 ± 5.37	37.7 ± 1.7	47.9 ± 3.02
Proteinuria (mg/day/100 g b.w.)	1.25 ± 0.12	1.05 ± 0.2	0.96 ± 0.28	1.36 ± 0.14
Glucosuria (mg/day/100 g b.w.)	2.98 ± 0.19	3,6 ± 0,5	3.31 ± 0.26	3.08 ± 0.11
Systolic pressure (mmHg)	115.5 ± 6.0	135.7 ± 23.2*	103.6 ± 3.3	144.8 ± 24.5*

B

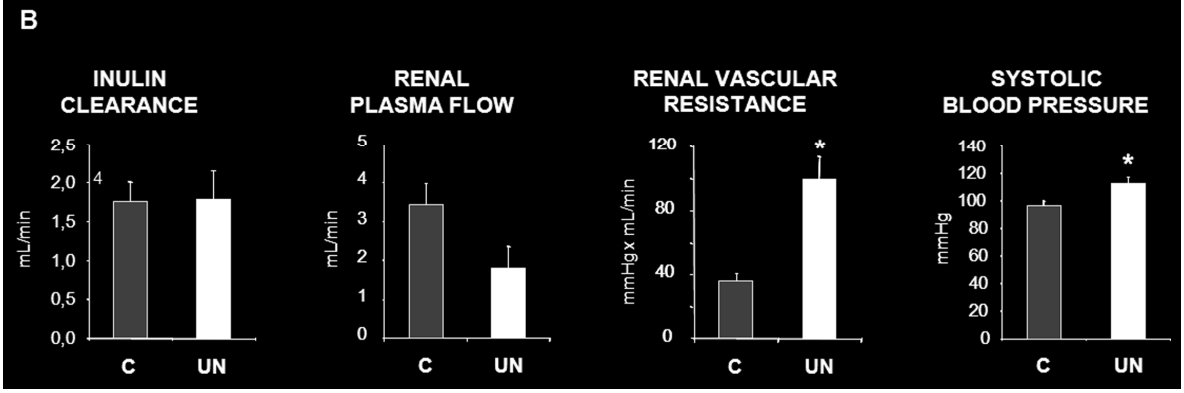


Figure 1. Overexposure to UN during 21 weeks does not induce renal dysfuction. A) Plasma creatinine and urea concentration; urinary protein and glucose excretion; and systolic pressure after 11 (T11) and 21 weeks (T21) of exposure to uranyl nitrate (UN). Data are expressed as the mean ± standard error of the mean of n = 12 animals per group (* $p<0.05$ vs control). B) Glomerular filtration rate (measured as inulin clearance); renal plasma flow (mesured as p-aminohipuric acid clearance), renal vascular resistance and systolic blood pressure in control and UN-treated rats after 21 weeks. Data are expressed as the mean ± standard error of the mean of n = 6 animals per group (* $p<0.05$ vs control).

showed an elevated intake throughout the study ranging between 80-85 mg UN/day (Table 1). Uranium excretion in the urine and uranium concentration in the kidney were measured at eleven weeks (T11) and twenty-one weeks (T21). The UN group showed a high uranium concentration in the kidneys at T11 which remained elevated at T21. Urinary excretion of uranium also appears elevated at T11 but decreases to some extent at T21 (Table 1). Because previous studies have associated uranium intoxication with increases in arterial pressure (22), we also measured this parameter. Systolic pressure measured by the tail cuff method was higher in UN than in control rats at 11 weeks of exposure, and remained elevated at 21 weeks (Figure 1A). At 21 weeks, systolic pressure was also measured directly from the carotid artery, in anesthetized animals. By this method, systolic pressure was also higher in UN than in control rats (Figure 1B).

Chronic exposure to UN does not alter renal function or renal tissue integrity.

Chronic administration of UN in drinking water during 21 weeks did not modify renal function or renal tissue integrity, as revealed by serum and urine biochemical parameters, and renal histology. In fact, the analysis of renal sections stained with hematoxilin and eosin after 21 weeks of treatment do not reveal any gross morphological alterations (Figure 2-A). In addition, the urinary level of sensitive markers of renal injury, such as KIM-1, NGAL, NAG and LDH showed no differences between both groups, further supporting the absence of tissue damage (Figure 2-B-C). With respect to renal excretory function, glomerular filtration appears to be normal in UN-treated rats, as indicated by inulin clearance experiments (Figure 1 -B), which agrees with the unaltered levels of plasma creatinine and urea concentration (Figure 1-A). Furthermore, proteinuria and glycosuria are also normal

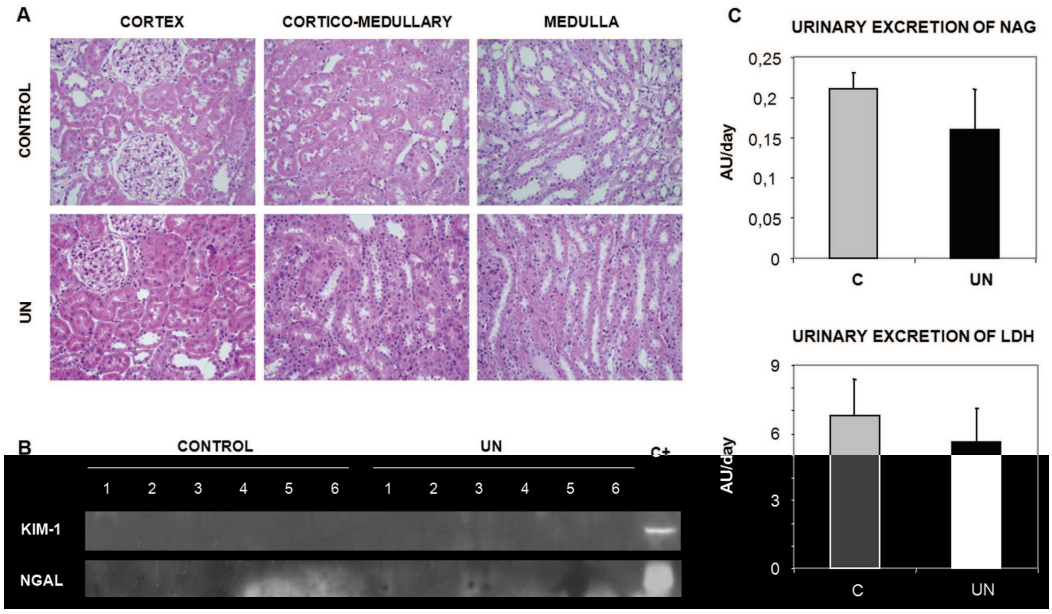


Figure 2. Overexposure to UN during 21 weeks does not cause renal tissue injury. A) Representative images of renal slices stained with hematoxylin and eosine from control and UN-treated rats during 21 weeks (magnification = 400x). Panel show the cortical, cortico-medullary and medullary areas. B) Western blot analysis of the sensitive urinary markers of kidney tissue injury kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) after 21 weeks. Possitive control (C+). C) Urinary excretion of N-acetyl-beta-D-glucosaminidase (NAG) and lactate dehydrogenase (LDH) in control and UN groups at 21 weeks. Data are expressed as the mean ± standard error of the mean of n = 12 animals per group.

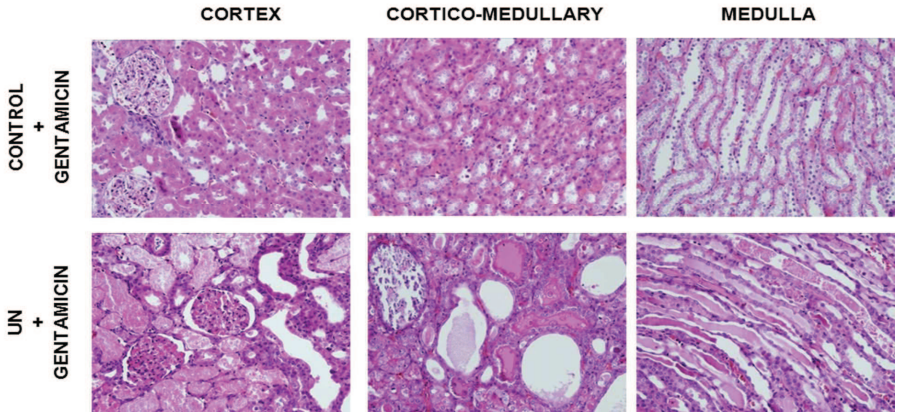


Figure 3. Overexposure to UN during 21 weeks sensitizes to renal damage: histological evidence. Representative photographs of the cortical, cortico-medullary and medullary areas of renal slices stained with hematoxylin and eoxin from control and UN-treated rats, 7 days after gentamicin administration. n = 6 animals per group.

in UN rats through the experiment (Figure 1-A). The only parameter altered in UN rats is the reduced renal blood flow (RBF) due to increased renal vascular resistance (RVR), despite systolic pressure being slightly increased (Figure 1-B). These data indicate that, in our model, chronic administration of UN causes no demonstrable renal injury; and that both glomerular and tubular function are apparently normal, despite a pronounced readjustment in renal hemodynamics.

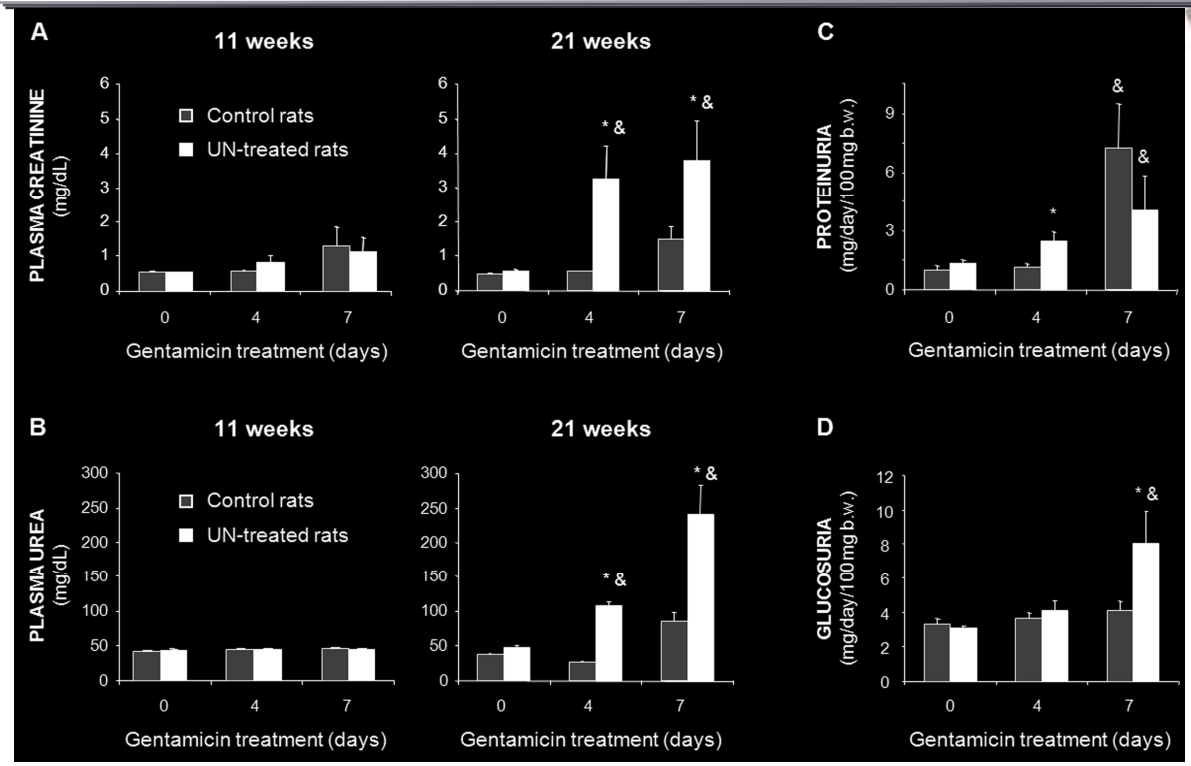


Figure 4. Overexposure to UN during 21 weeks sensitizes to renal damage: functional evidence. (A) Plasma creatinine and urea right before gentamicin administration (0) and 4 and 7 days after the inception of the gentamicin regime, in control and UN-traeted rats during 11 or 21 weeks. In the urine of these rats, proteinuria (C) and gluosuria (D) were also measured. Data expressed as mean ± standard error of the mean. n = 12 animals per group. (* $p<0.05$ vs control at the same time point; & $p<0.05$ vs basal in the same group).

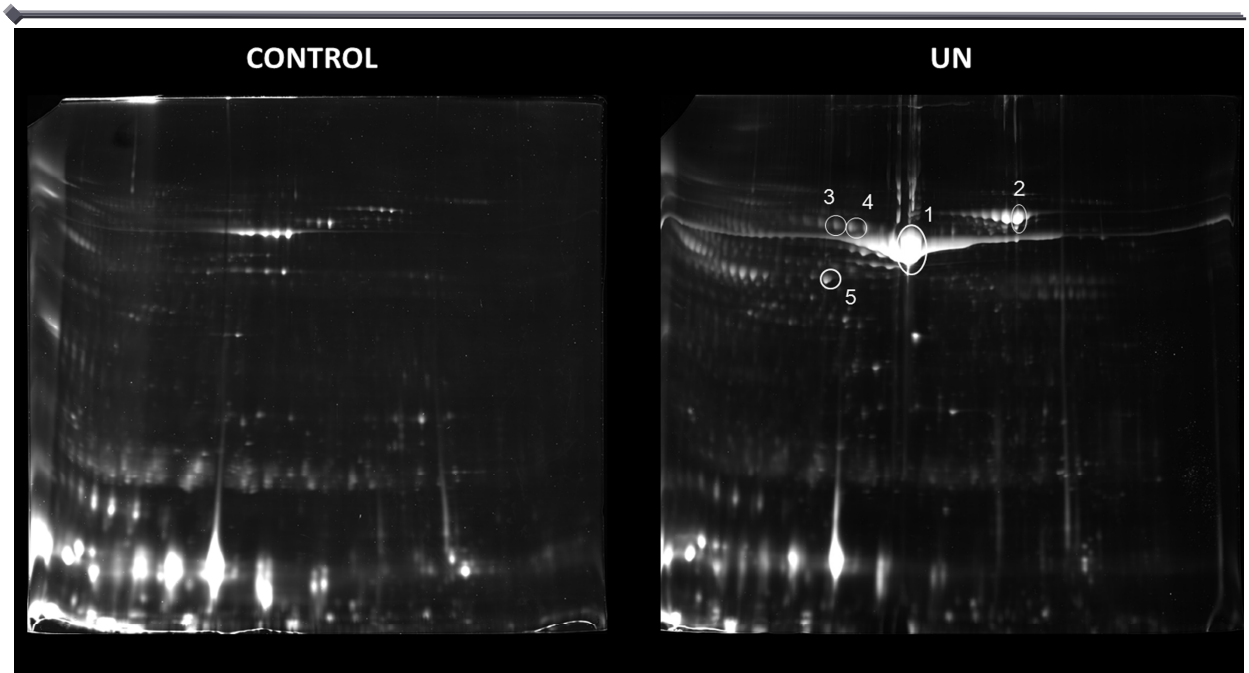
Chronic exposure to UN sensitizes rats to acute renal failure.

However, when rats exposed to UN during 21 weeks where challenged with low doses of a second potentially nephrotoxic insult, namely the aminoglycoside antibiotic gentamicin, they developed an overt renal failure characterized by massive tubular necrosis in the renal cortex, extensive tubular obstruction with hyaline material in the cortico-medullary and medullary areas, and significant tubular dilation in the cortico-medullary region (Figure 3); whereas control rats subject to the same regime of gentamicin suffered only a mild renal dysfunction with no gross parenchymal injury, indicating that chronic UN exposure magnified the effect of gentamicin. At the functional level, gentamicin induced a much higher elevation of serum creatinine and urea concentration in UN compared to control rats (Figure 4-A-B); whereas proteinuria was similarly elevated by gentamicin in both groups (Figure 4-C). Glycosuria was increased only in rats preconditioned with UN and subsequently treated with gentamicin (Figure 4-D).

Interestingly, this sensitization to ARF does not occur after only 11 weeks of UN exposure, as revealed by similar levels of serum creatinine and urea concentration in control and UN (11 weeks) rats treated with gentamicin (Figure 4-A-B). This occurs despite renal accumulation of uranium being similar to those observed after 21 weeks, and uranium excretion being even higher at 11 weeks than at 21 weeks (Table 1).

Chronic exposure to UN alters the urinary proteomic profile

With the aim of identifying urinary markers of UN over-exposure related to ARF sensitization, we compared the urinary proteome of rats exposed during 21 weeks to UN and of control rats. As shown in Figure 5, a general glimpse reveals that the urine of UN-treated rats contains a higher proportion of medium molecular weight (MMW) proteins, and a lower proportion of low molecular weight proteins than the urine of control rats. This is despite the total urinary



Spot	Acronym	Protein name	Sc	Nº of peptides	% sequence coverage	MW / pI
1	ALBU_RAT	Serum albumin	217	19	12	70,6/6,09
2	TRFE_RAT	Serotransferrin	175	12	18	78,5 / 7,14
3	HEMO_RAT	Hemopexin	98	12	30	51,4 / 7.33
4	HEMO_RAT	Hemopexin	274	15	35	51,4 / 7,33
5	VTDB_RAT	VDBP	270	9	27	55,1/5,6

Figure 5. Differential proteomic profiling of the urine from control and UN-treated rats. Representative images of 2-D gels (n = 6 animals per group) obtained with the urine of control rats and rats treated during 21 weeks with UN. The marked spots correspond to proteins significantly increased in the urine of UN-rats. The table below reports the protein identification parameters obtained by MALDI-TOF analysis and data base blast for the selected spots. Sc, score. MW, molecular weight (in kDa). pI, isoelectric point. VDBP, vitamin D binding protein

excretion of proteins being unaltered in UN-exposed rats (Figure 1-A). This indicates that (i) the permissiveness or selectivity of the filtration barrier might be altered by uranium; (ii) tubular handling of proteins might also be altered; or (iii) a combination of both. Statistical analysis of the urinary proteomes from control and UN rats revealed that four proteins, namely albumin, hemopexin, transferring and vitamin D binding protein (VDBP), were significantly increased in the urine of UN-treated rats (Figure 5). The urinary level of these proteins was further analyzed by Western blot. Figure 6 shows that the excretion of albumin, hemopexin, transferring and VDBP is increased by 21 weeks of UN exposure, whereas it is indistinguishable from that in control rats by week 11. This closely correlates with the appearance of the predisposition to renal damage by week 21 and not by week 11 (Figure 4 A-B).

DISCUSSION

Drug nephrotoxicity is a very serious health and economic problem worldwide. As such, strategies aimed at preventing drug-induced AKI may result in lower incidence and high economic savings for health systems. In this line, the investment in new diagnostic procedures may largely compensate for AKI-derived expenditure. We have previously proposed (20) a preemptive strategy to identify individuals at risk before subjecting them to potentially kidney-injuring procedures, such as certain pharmacological treatments, contrast radiographies, etc. In this line, we have demonstrated that an acute, sub-nephrotoxic treatment with gentamicin transitorily sensitizes rats to ARF (20). This correlates with the excretion of new urinary

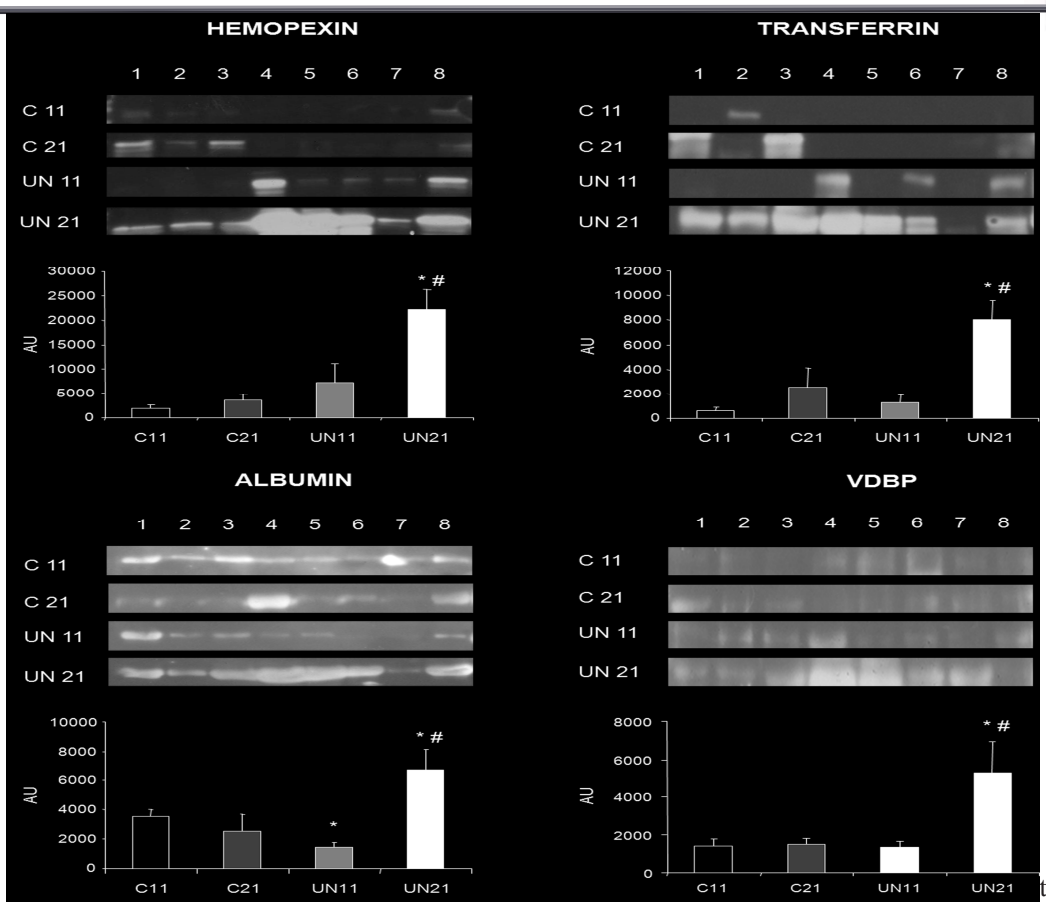


Figure 6. Urinary biomarker validation by Western blot. Confirmation by western blot of the urinary excretion of the proteins identified by proteomics (i.e. hemopexin, transferrin, albumin and vitamin D binding protein (VDBP)), both at 11 weeks (11) and 21 weeks (21) in control (C) and UN groups. Bands were quantified with the Scion Image software. Each bar represents the mean ± standard error of the mean (* $p<0.005$ vs control at same time point; # $p<0.005$ vs T11 in the same group). AU, arbitrary units.

markers, which might serve to detect this condition. Our present results demonstrate that rats may also become chronically predisposed to ARF, in this case by an experimental manoeuvre [e.g. chronic treatment with the potential nephrotoxin uranyl nitrate; (23)]

The results obtained in our model indicate that chronic over-exposure of rats to the toxin during some weeks, without eliciting symptoms of nephrotoxicity by itself, reduces the threshold of nephrotoxicity and enhances the nephrotoxic effects of a second renal insult, in this case a treatment with low-dose gentamicin. This chronically acquired sensitivity to ARF might be especially significant for its silent clinical course. Translated to a potential clinical application, this might be one, among many other predisposing circumstances that would stratify patients according to their risk, which would gain outstanding relevance in patients under critical conditions. As such, markers of chronic predisposition would allow us to detect and classify humans unaware of their risk, which cannot be associated to acute risk factors. For this purpose, we focused on the urine to search for markers of this condition, because it is one of the most suitable body samples for population-scale screening in a non-traumatic manner.

In our model, the daily urinary protein excretion was not modified by chronic uranium overexposure. This suggests that the overall balance between glomerular filtration and tubular reabsorption of proteins is not altered. However, as depicted in figure 5, the relative composition of urinary proteins is altered by uranium. In fact, more medium molecular weight (MMW; i.e. 35-65 KDa) proteins and fewer low molecular weight (LMW; i.e. < 35 KDa) proteins are excreted. This

probably indicates that the sieving properties of the glomerular filtration barrier (GFB) are affected by chronic uranium overexposure. As a consequence, some MMW proteins mostly excluded from filtration in normal circumstances by size or electrical reasons are now capable of spanning the GFB. However, this increased filtration does not translate into proteinuria, likely because the moderate excess of filtered proteins is reabsorbed in the tubuli. Still, the different protein composition of the ultrafiltrate alters the equilibrium of competition among proteins for the tubular transporters and endocytic systems, leading to a different composition of the final urine (24-29). The urinary excretion of four proteins was significantly increased in predisposed rats, compared to control rats, namely the blood-borne proteins albumin, hemopexin, transferring and VDBP. About 20% of uranyl ions in serum are bound to the protein pool (30). Uranium binds some major plasma proteins, including ceruloplasmin, hemopexin and transferrin (30-33). It might be speculated that the increased urinary excretion of some of these proteins would be the result of their being swept along with uranium to the final urine. However, this does not seem to be the case, because in our model, by week 11 the excretion of uranium is almost identical to that at week 21, and no significant increase in the urinary excretion of these proteins is detected by that time. Accordingly, their increased excretion is more closely associated to the predisposition than to other conditions such as uranium exposure or uranium accumulation itself, because the intake and renal accumulation of uranium are by week 11 is as high as by week 21. Rather, other chronic events derived from subtoxic accumulation are needed to render animals more sensitive to ARF.

Hypertension has also been reported to increase the urinary excretion of determined proteins, including albumin and transferrin (34-37). However, in our model, the moderate hypertension caused by UN does not correlate either with the increased excretion of albumin, hemopexin, transferring or VDBP. In fact, hypertension is already installed by week 11. Independently from systemic arterial pressure, selective increased excretion of these proteins has been observed not only in hypertensive animals and patients, but also in pre-microalbuminuric (and microalbuminuric) diabetic patients (38-46). In these latter, the increased, selective protein excretion has been associated to increased intraglomerular pressure (45). In our model, by week 21 predisposed rats have normal glomerular fitration (i.e. inulin clearance), despite reduced RBF (figure 1). This can be theoretically explained by an increased intraglomerular pressure, which would also explain the increased filtration of MMW proteins. Albumin (47), hemopexin (48), transferrin (49) and VDBP (48) are serum-borne proteins whose increased urinary excretion has been widely reported in renal diseases coursing with alterations in the sieving properties of the glomerular filtration barrier. Microalbuminuria and microtransferrinuria have also been reported in early stages of diabetic nephropathy (42, 47). A new use of the increased urinary excretion of these proteins is proposed by our results. Especially in non-diabetic patients (but also in them), increased urinary excretion of albumin, transferrin, hepomexin or VDBP (or combinations thereof), besides indicating a possible incipient stage of nephropathy (in the absence of markers of renal dysfunction or injury) might alert health professionals on an increased risk of acute nephropathy. Clinical studies are necessary to explore the incidence of AKI in patients with increased urinary excretion of these proteins. In conclusion, the future development of this new diagnostic capability might enable the preemptive and personalized handling of patients by allowing us to stratify them with quantitative parameters according to their individually acquired risk or sensibility to AKI, when they need to be subject to acute, potentially nephrotoxic treatments, diagnostic procedures (e.g. contrast radiography) or clinical procedures (e.g. cardiovascular surgery). This new diagnostic strategy might help to reduce AKI incidence and severity, and also the associated sanitary and socioeconomic costs.

MATERIALS AND METHODS

Except where otherwise indicated, all reagents were purchased from Sigma-Aldrich (Madrid, Spain).

Animal handling and sample collection.

Experiments were performed with male Sprague-Dawley rats weighing 220-250 g initially. Rats were housed under controlled environmental conditions and had free access to standard rat chow and drinking water. Animals were divided into two groups: (i) control group (n=24), which received no treatment; and (ii) uranyl nitrate (UN)-treated group (n=24), which received 5.4 g/L UN in the drinking water during up to 22 weeks, as the experimental, chronically predisposing factor. The dose of UN was determined, for this experimental purpose, in pilot studies based on the information from previous reports (50, 51). After 11 or 21 weeks, some animals were sacrificed and others were treated with a low dosage regime of gentamicin (50 mg/kg/day, 7 days, i.p.), and then sacrificed. Water intake consumption was recorded weekly to estimate uranium intake. At determined time points rats were transitorily moved to individual metabolic cages for urine collection. Urine was cleared by centrifugation and stored at -80ºC. Coinciding with urine collection, blood samples were also obtained in heparinized capillaries from a small incision in the tail tip. Plasma was separated by centrifugation and kept at -80ºC. At the time of sacrifice, rats were anesthetized, and the kidneys dissected. One half of each organ was fixed in 3.7% para-formaldehyde and further used for histological studies. The remaining renal tissue was kept at -80 ºC and eventually used for uranium content analysis.

Renal tissue, urine and serum biochemical analysis.

Plasma samples were analyzed for urea and creatinine concentration with an automatic analyzer (Reflotron plus®; Roche Diagnostics, Barcelona, Spain; lower detection limit 0.5 mg/dL). Urine was assayed for protein concentration using the Bradford technique (52) and for glucose using the o-toluidine method (53). Commercial kits based on colorimetric or fluorimetric methods were also used for the determination of the urinary enzymes N-acetyl-β-D-glucosaminidase (NAG; Roche Diagnostics, Barcelona, Spain) and lactate deshidrogenase (LDH; Ana Spec INC, San Jose, USA), following the manufacturers' instructions. Uranium content was measured in kidney tissue and urine samples using inductively coupled plasma mass spectrometry (*ICP-MS; Perkin-Elmer ELAN-6000, Madrid, Spain).*

Inulin and PAH clearance.

Inulin and para-aminohipuric acid (PAH) renal clearance was studied in a subset of animals at the end of the 21-week treatment, basically as previously described (54). Briefly, animals were anesthetized and the left carotid artery, the right jugular vein and the bladder were cannulated. The carotid artery was connected to a pressure transducer (Beckman R511A, Miami. FL, USA) through the process. The jugular vein was connected to an infusion pump containing an isotonic saline infusion of [metoxy-^{14}C] inulin (ARC Inc., Sant Louis, MO, USA) and [^{3}H] PAH (Perkin-Elmer, Madrid, Spain). Initially, a 1 mL bolus of mixture was injected intravenously and then the pump was connected in a continuous infusion (3 mL/h). After a 30 minute stabilization period, blood was collected through the artery every 30 minutes for 3 times. Urine samples were obtained directly from the bladder. Blood and urine samples were centrifuged and their [^{14}C] and [^{3}H] activities were measured in a liquid scintillation counter (Wallac 1409 DSA, Turku, Finland). Packed cell volume (PCV) was determined by the microcapillary method. Inulin and PAH clearances were calculated to measure GFR and RPF, respectively. RBF was calculated from RPF and PCV. RVR was calculated from RBF and MAP.

Western Blot.

Urine samples were separated by polyacrylamide gel electrophoresis (PAGE; Mini Protean II system, Bio-Rad, Madrid, Spain). Proteins were electro-transferred to Immobilon P membranes (Millipore, Madrid, SPain), which were then incubated with goat polyclonal antibodies against kidney injury molecule 1 (KIM1/TIM1) (RD Systems, Minneapolis, MN, USA), transferrin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and VDBP (Everest Biotech, Upper Heyford, UK), and with rabbit polyclonal antibodies

against lipocaline-2 (NGAL; MBL international, Woburn, MA USA) and hemopexin (Biovision INC, Mountain View, California, USA) and with chiken polyclonal against human serum albumin (Abcam, Cambridge, UK). Membranes were then incubated with horseradish peroxidase (HRP)-coupled secondary antibodies, subsequently incubated with the quimioluminiscent HRP-substrate (ECL; Millipore, Madrid, Spain), and exposed to photographic films (Kodak, Rochester, NY, USA). Bands were quantified with the Scion Image software (Scion Corporation; Frederick, MD, USA).

Histological studies.

Paraffin blocks were made with para-formaldehyde fixed tissue and 5-µm tissue sections were stained with hematoxilin and eosin. Photographs were taken under an Olympus BX51 microscope connected to an Olympus DP70 colour, digital camera (Olympus, Madrid, Spain).

Urinary proteomics.

The proteome of urine samples from week 21 from control and UN-treated rats was analyzed by two-dimensional electrophoresis (2-DE), basically as described (20, 55). In short, urine proteins (200 µg) were precipitated and isoelectrically focused (500-8,000 V) through 18-cm long immobilized pH gradient (IPG) strips, pH 3-11NL (GE Healthcare, Madrid, Spain). Then, proteins in IPG strips were separated by 12% SDS-polyacrylamide gels, fixed and stained with Sypro Ruby (Molecular Probes, Barcelona, Spain). The spots of interest were analyzed with the Image Master Platinum software (GE Healthcare, Madrid, Spain) and in-gel digested with porcine trypsin (Promega, Barcelona, Spain). Tryptic peptides were analyzed by MALDI-TOF on an Autoflex III instrument (Bruker Daltonics, Madrid, Spain). One microlitre of each sample was deposited on Prespotted AnchorChip targets (Bruker Daltonics, Madrid, Spain), which are prespotted with α-cyano-4-hydroxycinnamic acid (HCCA) matrix. Spectra were acquired with the FlexControl 3.0 software (Bruker Daltonics, Madrid, Spain) and then processed using the Flex Analysis 3.0 software (Bruker Daltonics, Madrid, Spain). The equipment was first externally calibrated employing protonated mass signals from calibrants prespotted in the target, covering the 1000–4000 m/z range. All known contaminants (trypsin-derived peptides and keratins) were excluded during the process. The resulting final peak list was used for the identification of the proteins by peptide mass fingerprint (PMF). Protein identification was performed with the MASCOT software (www.matrixscience.com) against non redundant protein sequence database Swiss Prot (containing 516603 sequences and 181919312 residues). The mass tolerance was set as 50 ppm, and the taxonomic category was Rattus (7509 sequences). Only one missed cleavage per peptide was allowed and cysteine residues were assumed to be carbamidomethylated with acrylamide adducts, and methionine residues were in oxidized form. In all protein identifications, the probability scores were greater than the score fixed by MASCOT as significant with a p-value lower than 0.05.

STATICAL ANALISYS

Experimental groups were compared using a two-way analysis of variance (ANOVA) followed by Scheffé's test when the data were normally distributed and by the Kruskal-Wallis test when they were not normally distributed. P-values < 0.05 were considered significant. Statistical tests were performed using the Number Cruncher Statistical System (NCSS) software, version 6.0.10 for Windows. Data are shown as mean ± standard error of the mean.

DISCLOSURE

JMLN and FJLH are minority share-holders of BioinRen, S.L., a biotech company in the field of diagnostic markers of renal diseases.

REFERENCES

1. Molitoris BA, Levin A, Warnock DG, Joannidis M, *et al.* Improving outcomes from acute kidney injury. *J Am Soc Nephrol* 2007; **18:** 1992-1994.

2. Chawla LS, Amdur RL, Amodeo S, Kimmel PL, *et al.* The severity of acute kidney injury predicts progression to chronic kidney disease. *Kidney Int* **79:** 1361-1369.

5. Neild GH. Multi-organ renal failure in the elderly. *Int Urol Nephrol* 2001; **32:** 559-565.

6. Block CA, Schoolwerth AC. The epidemiology and outcome of acute renal failure and the impact on chronic kidney disease. *Semin Dial* 2006; **19:** 450-454.

7. Kellum JA, Hoste EA. Acute kidney injury: epidemiology and assessment. *Scandinavian journal of clinical and laboratory investigation* 2008; **241:** 6-11.

8. Waikar SS, Liu KD, Chertow GM. Diagnosis, epidemiology and outcomes of acute kidney injury. *Clin J Am Soc Nephrol* 2008; **3:** 844-861.

9. Gupta R, Gurm HS, Bhatt DL, Chew DP, *et al.* Renal failure after percutaneous coronary intervention is associated with high mortality. *Catheter Cardiovasc Interv* 2005; **64:** 442-448.

10. Basile C. The long-term prognosis of acute kidney injury: acute renal failure as a cause of chronic kidney disease. *J Nephrol* 2008; **21:** 657-662.

11. Sinning JM, Ghanem A, Steinhauser H, Adenauer V, *et al.* Renal function as predictor of mortality in patients after percutaneous transcatheter aortic valve implantation. *JACC Cardiovasc Interv* **3:** 1141-1149.

12. Bell M. Acute kidney injury: new concepts, renal recovery. *Nephron Clin Pract* 2008; **109:** c224-228.

13. Macedo E, Bouchard J, Mehta RL. Renal recovery following acute kidney injury. *Curr Opin Crit Care* 2008; **14:** 660-665.

14. Yang L, Besschetnova TY, Brooks CR, Shah JV, *et al.* Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury. *Nat Med* **16:** 535-543, 531p following 143.

15. Aspelin P, Aubry P, Fransson SG, Strasser R, *et al.* Nephrotoxic effects in high-risk patients undergoing angiography. *N Engl J Med* 2003; **348:** 491-499.

16. Brivet FG, Kleinknecht DJ, Loirat P, Landais PJ. Acute renal failure in intensive care units--causes, outcome, and prognostic factors of hospital mortality; a prospective, multicenter study. French Study Group on Acute Renal Failure. *Crit Care Med* 1996; **24:** 192-198.

17. Taber SS, Mueller BA. Drug-associated renal dysfunction. *Crit Care Clin* 2006; **22:** 357-374, viii.

18. Ricci Z, Cruz DN, Ronco C. Classification and staging of acute kidney injury: beyond the RIFLE and AKIN criteria. *Nat Rev Nephrol* **7:** 201-208.

19. Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. *Kidney Int* 2008; **73:** 538-546.

20. Quiros Y, Ferreira L, Sancho-Martinez SM, Gonzalez-Buitrago JM, *et al.* Sub-nephrotoxic doses of gentamicin predispose animals to developing acute kidney injury and to excrete ganglioside M2 activator protein. *Kidney Int* **78:** 1006-1015.

21. Chertow GM, Burdick E, Honour M, Bonventre JV, *et al.* Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol* 2005; **16:** 3365-3370.

22. Kurttio P, Harmoinen A, Saha H, Salonen L, *et al.* Kidney toxicity of ingested uranium from drinking water. *Am J Kidney Dis* 2006; **47:** 972-982.

23. Vicente-Vicente L, Quiros Y, Perez-Barriocanal F, Lopez-Novoa JM, *et al.* Nephrotoxicity of uranium: pathophysiological, diagnostic and therapeutic perspectives. *Toxicol Sci* **118:** 324-347.

24. Mendel D. Tubular reabsorption of protein in rats with experimental proteinuria. *J Physiol* 1961; **156:** 544-554.

25. Ratcliffe PJ, Esnouf MP, Ledingham JG. Tubular reabsorption rates for myoglobin in the isolated perfused rat kidney. *Clin Sci (Lond)* 1986; **70:** 595-599.

26. Kassab AS, Makeen A, Wardle EN. Albumin infusion increases the urinary excretion of lysozyme in diabetics. *Nephron* 1991; **59:** 321.

27. Branten AJ, Wetzels JF. Influence of albumin infusion on the urinary excretion of beta2-microglobulin in patients with proteinuria. *Nephron* 1999; **81:** 329-333.

28. ten Dam MA, Branten AJ, Klasen IS, Wetzels JF. The gelatin-derived plasma substitute Gelofusine causes low-molecular-weight proteinuria by decreasing tubular protein reabsorption. *J Crit Care* 2001; **16:** 115-120.

29. Veldman BA, Schepkens HL, Vervoort G, Klasen I, *et al.* Low concentrations of intravenous polygelines promote low-molecular weight proteinuria. *Eur J Clin Invest* 2003; **33:** 962-968.

30. Vidaud C, Dedieu A, Basset C, Plantevin S, *et al.* Screening of human serum proteins for uranium binding. *Chem Res Toxicol* 2005; **18:** 946-953.

31. Taylor DM, Taylor SK. Environmental uranium and human health. *Rev Environ Health* 1997; **12:** 147-157.

32. Scapolan SEA, Moulin C, Madic C. Uranium-(VI)-transferrin system studied by time-resolved laser induced fluorescence. *Radiat Prot Dosimetry* 1998; **79:** 505-508.

33. Taylor DM. The bioinorganic chemistry of actinides in blood. *J Alloys Compd* 1998; **271:** 6-10.

34. Alli C, Lombardo M, Zanni D, Agrati AM, *et al.* Albuminuria and transferrinuria in essential hypertension. Effects of antihypertensive therapy. *Am J Hypertens* 1996; **9:** 1068-1076.

35. Bang LE, Holm J, Svendsen TL. Retinol-binding protein and transferrin in urine. New markers of renal function in essential hypertension and white coat hypertension? *Am J Hypertens* 1996; **9:** 1024-1028.

36. Zeller A, Haehner T, Battegay E, Martina B. Diagnostic significance of transferrinuria and albumin-specific dipstick testing in primary care patients with elevated office blood pressure. *J Hum Hypertens* 2005; **19:** 205-209.

37. Hosoba M, Fujita H, Miura T, Morii T, *et al.* Diurnal changes in urinary excretion of IgG, transferrin, and ceruloplasmin depend on diurnal changes in systemic blood pressure in normotensive, normoalbuminuric type 2 diabetic patients. *Horm Metab Res* 2009; **41:** 910-915.

38. Bernard AM, Amor AA, Goemaere-Vanneste J, Antoine JL, *et al.* Microtransferrinuria is a more sensitive indicator of early glomerular damage in diabetes than microalbuminuria. *Clin Chem* 1988; **34:** 1920-1921.

39. Bernard A, Amor AO, Goemare-Vanneste J, Antoine JL, *et al.*

Urinary proteins and red blood cell membrane negative charges in diabetes mellitus. *Clin Chim Acta* 1990; **190:** 249-262.

40. McCormick CP, Konen JC, Shihabi ZK. Microtransferrinuria and microalbuminuria. I. In the diabetic human. *Clin Physiol Biochem* 1990; **8:** 53-58.

41. McCormick CP, Shihabi ZK, Konen JC, Goodman HO*, et al.* Microtransferrinuria and microalbuminuria. II. In the rat. *Clin Physiol Biochem* 1990; **8:** 59-63.

42. Kanauchi M, Nishioka H, Hashimoto T, Dohi K. Diagnostic significance of urinary transferrin in diabetic nephropathy. *Nippon Jinzo Gakkai Shi* 1995; **37:** 649-654.

43. Hong CY, Chia KS. Markers of diabetic nephropathy. *J Diabetes Complications* 1998; **12:** 43-60.

44. Kazumi T, Hozumi T, Ishida Y, Ikeda Y*, et al.* Increased urinary transferrin excretion predicts microalbuminuria in patients with type 2 diabetes. *Diabetes care* 1999; **22:** 1176-1180.

45. Narita T, Sasaki H, Hosoba M, Miura T*, et al.* Parallel increase in urinary excretion rates of immunoglobulin G, ceruloplasmin, transferrin, and orosomucoid in normoalbuminuric type 2 diabetic patients. *Diabetes care* 2004; **27:** 1176-1181.

46. Narita T, Hosoba M, Kakei M, Ito S. Increased urinary excretions of immunoglobulin g, ceruloplasmin, and transferrin predict development of microalbuminuria in patients with type 2 diabetes. *Diabetes care* 2006; **29:** 142-144.

47. Basi S, Fesler P, Mimran A, Lewis JB. Microalbuminuria in type 2 diabetes and hypertension: a marker, treatment target, or innocent bystander? *Diabetes care* 2008; **31 Suppl 2:** S194-201.

48. Varghese SA, Powell TB, Budisavljevic MN, Oates JC*, et al.* Urine biomarkers predict the cause of glomerular disease. *J Am Soc Nephrol* 2007; **18:** 913-922.

49. Mackinnon B, Shakerdi L, Deighan CJ, Fox JG*, et al.* Urinary transferrin, high molecular weight proteinuria and the progression of renal disease. *Clinical nephrology* 2003; **59:** 252-258.

50. Gilman AP, Moss MA, Villeneuve DC, Secours VE*, et al.* Uranyl nitrate: 91-day exposure and recovery studies in the male New Zealand white rabbit. *Toxicol Sci* 1998; **41:** 138-151.

51. Rouas C, Stefani J, Grison S, Grandcolas L*, et al.* Effect of nephrotoxic treatment with gentamicin on rats chronically exposed to uranium. *Toxicology* **279:** 27-35.

52. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72:** 248-254.

53. Passey RB. Glucosa method comparision criticized. *Clin Chem* 1983; **29(6):** 1320-1321.

54. Valdivieso JM, Rivas-Cabañero L, Pérez-Barriocanal F, López -Novoa JM. Effect of nitric oxide sintesis modification on renal function in gentamicin-induced nephrotoxicity. *Environ Toxicol Pharmacol* 1997; **3(2):** 123-128.

55. Ferreira L, Quiros Y, Sancho-Martinez SM, Garcia-Sanchez O*, et al.* Urinary levels of regenerating islet-derived protein III beta and gelsolin differentiate gentamicin from cisplatin-induced acute kidney injury in rats. *Kidney Int* **79:** 518-528.

PATENTE

PATENTE

MÉTODO PARA LA DETECCIÓN DE LA PREDISPOSICIÓN CRÓNICA AL DAÑO RENAL AGUDO.

Laura Vicente-Vicente, Laura Ferreira, José M. González-Buitrago, Francisco J. López-Hernández, José M. López-Novoa y Ana Isabel Morales.

Oficina Española de Patentes y Marcas

MINISTERIO DE INDUSTRIA, TURISMO Y COMERCIO

Oficina Española de Patentes y Marcas

Justificante de presentación electrónica de solicitud de patente

Este documento es un justificante de que se ha recibido una solicitud española de patente por vía electrónica, utilizando la conexión segura de la O.E.P.M. Asimismo, se le ha asignado de forma automática un número de solicitud y una fecha de recepción, conforme al artículo 14.3 del Reglamento para la ejecución de la Ley 11/1986, de 20 de marzo, de Patentes. La fecha de presentación de la solicitud de acuerdo con el art. 22 de la Ley de Patentes, le será comunicada posteriormente.

Número de solicitud:	P201130914	
Fecha de recepción:	02 junio 2011, 14:31 (CEST)	
Oficina receptora:	OEPM Madrid	
Su referencia:	ES1367.45	
Solicitante:	UNIVERSIDAD DE SALAMANCA	
Número de solicitantes:	1	
País:	ES	
Título:	MÉTODO PARA LA DETECCIÓN DE LA PREDISPOSICIÓN CRÓNICA AL DAÑO RENAL AGUDO	
Documentos enviados:	Descripcion-1.pdf (41 p.) Reivindicaciones-1.pdf (5 p.) Resumen-1.pdf (1 p.) Dibujos-1.pdf (6 p.) OLF-ARCHIVE.zip SEQLPDF.pdf (9 p.) SEQLTXT.txt	package-data.xml es-request.xml application-body.xml es-fee-sheet.xml feesheet.pdf request.pdf
Enviados por:	CN=ENTIDAD PONS PATENTES Y MARCAS INTERNACIONAL SL - CIF B84921709 - NOMBRE PONS ARIÑO ANGEL - NIF 50534279J,OU=703015345,OU=fnmt clase 2 ca,O=FNMT,C=es	
Fecha y hora de recepción:	02 junio 2011, 14:32 (CEST)	
Codificación del envío:	3E:23:00:86:5D:B8:9B:93:CC:AC:ED:6B:27:A1:CA:A9:6C:3D:48:6F	

/Madrid, Oficina Receptora/

MINISTERIO
DE INDUSTRIA, TURISMO
Y COMERCIO

Oficina Española
de Patentes y Marcas

(1) MODALIDAD: **PATENTE DE INVENCIÓN** **MODELO DE UTILIDAD**	[✓] [ ]
(2) TIPO DE SOLICITUD: PRIMERA PRESENTACIÓN ADICIÓN A LA PATENTE EUROPEA ADICIÓN A LA PATENTE ESPAÑOLA SOLICITUD DIVISIONAL CAMBIO DE MODALIDAD TRANSFORMACIÓN SOLICITUD PATENTE EUROPEA PCT: ENTRADA FASE NACIONAL	 [✓] [ ] [ ] [ ] [ ] [ ] [ ]
(3) EXP. PRINCIPAL O DE ORIGEN: MODALIDAD: N.º SOLICITUD: FECHA SOLICITUD:	
4) LUGAR DE PRESENTACIÓN:	OEPM, Presentación Electrónica
(5) DIRECCIÓN ELECTRÓNICA HABILITADA (DEH):	
(6-1) SOLICITANTE 1: DENOMINACIÓN SOCIAL: NACIONALIDAD: CÓDIGO PAÍS: DNI/CIF/PASAPORTE: CNAE: PYME: DOMICILIO: LOCALIDAD: PROVINCIA: CÓDIGO POSTAL: PAÍS RESIDENCIA: CÓDIGO PAÍS: TELÉFONO: FAX: PERSONA DE CONTACTO: MODO DE OBTENCIÓN DEL DERECHO: INVENCIÓN LABORAL: CONTRATO: SUCESIÓN:	 UNIVERSIDAD DE SALAMANCA España ES Q3718001E PATIO DE ESCUELAS, 1 SALAMANCA 37 Salamanca 37008 España ES [✓] [ ] [ ]
(7-1) INVENTOR 1: APELLIDOS: NOMBRE: NACIONALIDAD: CÓDIGO PAÍS: DNI/PASAPORTE: (7-2) INVENTOR 2: APELLIDOS: NOMBRE: NACIONALIDAD: CÓDIGO PAÍS: DNI/PASAPORTE: (7-3) INVENTOR 3: APELLIDOS: NOMBRE: NACIONALIDAD: CÓDIGO PAÍS: DNI/PASAPORTE:	 VICENTE VICENTE LAURA España ES FERREIRA REDONDO LAURA España ES GONZALEZ DE BUITRAGO ARRIERO JOSE MANUEL España ES

(7-4) INVENTOR 4:	
APELLIDOS:	LOPEZ HERNANDEZ
NOMBRE:	FRANCISCO JOSE
NACIONALIDAD:	España
CÓDIGO PAÍS:	ES
DNI/PASAPORTE:	
(7-5) INVENTOR 5:	
APELLIDOS:	LOPEZ NOVOA
NOMBRE:	JOSE MIGUEL
NACIONALIDAD:	España
CÓDIGO PAÍS:	ES
DNI/PASAPORTE:	
(7-6) INVENTOR 6:	
APELLIDOS:	MORALES MARTÍN
NOMBRE:	ANA ISABEL
NACIONALIDAD:	España
CÓDIGO PAÍS:	ES
DNI/PASAPORTE:	
(8) TÍTULO DE LA INVENCIÓN:	MÉTODO PARA LA DETECCIÓN DE LA PREDISPOSICIÓN CRÓNICA AL DAÑO RENAL AGUDO
(9) PETICIÓN DE INFORME SOBRE EL ESTADO DE LA TÉCNICA:	
SI	[ ]
NO	[✓]
(10) SOLICITA LA INCLUSIÓN EN EL PROCEDIMIENTO ACELERADO DE CONCESIÓN	
SI	[ ]
NO	[✓]
(11) EFECTUADO DEPÓSITO DE MATERÍA BIOLÓGICA:	
SI	[ ]
NO	[✓]
(12) DEPÓSITO:	
REFERENCIA DE IDENTIFICACIÓN:	
INSTITUCIÓN DE DEPÓSITO:	
NÚMERO DE DEPÓSITO:	
ACCESIBILIDAD RESTRINGIDA A UN EXPERTO (ART. 45.1. B):	
(13) DECLARACIONES RELATIVAS A LA LISTA DE SECUENCIAS:	
LA LISTA DE SECUENCIAS NO VA MÁS ALLÁ DEL CONTENIDO DE LA SOLICITUD	[✓]
LA LISTA DE SECUENCIAS EN FORMATO PDF Y ASCII SON IDENTICOS	[✓]
(14) EXPOSICIONES OFICIALES:	
LUGAR:	
FECHA:	
(15) DECLARACIONES DE PRIORIDAD:	
PAÍS DE ORIGEN:	
CÓDIGO PAÍS:	
NÚMERO:	
FECHA:	
(16) AGENTE/REPRESENTANTE:	
APELLIDOS:	PONS ARIÑO
NOMBRE:	ANGEL
CÓDIGO DE AGENTE:	0499/5
NACIONALIDAD:	España
CÓDIGO PAÍS:	ES
DNI/CIF/PASAPORTE:	50534279-J
DOMICILIO:	GLORIETA DE RUBÉN DARIO, 4
LOCALIDAD:	MADRID
PROVINCIA:	28 Madrid
CÓDIGO POSTAL:	28010
PAÍS RESIDENCIA:	España

CÓDIGO PAÍS:	ES
TELÉFONO:	
FAX:	
CORREO ELECTRÓNICO:	
NÚMERO DE PODER:	
(17) RELACIÓN DE DOCUMENTOS QUE SE ACOMPAÑAN:	
DESCRIPCIÓN:	[✓] N.º de páginas: 41
REIVINDICACIONES:	[✓] N.º de reivindicaciones: 27
DIBUJOS:	[✓] N.º de dibujos: 6
RESUMEN:	[✓] N.º de páginas: 1
FIGURA(S) A PUBLICAR CON EL RESUMEN:	[ ] N.º de figura(s):
ARCHIVO DE PRECONVERSION:	[✓]
DOCUMENTO DE REPRESENTACIÓN:	[ ] N.º de páginas:
LISTA DE SECUENCIAS PDF:	[✓] N.º de páginas: 9
ARCHIVO PARA LA BUSQUEDA DE LS:	[✓]
OTROS (Aparecerán detallados):	
(18) EL SOLICITANTE SE ACOGE AL APLAZAMIENTO DE PAGO DE TASA PREVISTO EN EL ART. 162 DE LA LEY 11/1986 DE PATENTES, DECLARA: BAJO JURAMIENTO O PROMESA SER CIERTOS TODOS LOS DATOS QUE FIGURAN EN LA DOCUMENTACIÓN ADJUNTA:	[ ]
DOC COPIA DNI:	[ ] N.º de páginas:
DOC COPIA DECLARACIÓN DE CARENCIA DE MEDIOS:	[ ] N.º de páginas:
DOC COPIA CERTIFICACIÓN DE HABERES:	[ ] N.º de páginas:
DOC COPIA ÚLTIMA DECLARACIÓN DE LA RENTA:	[ ] N.º de páginas:
DOC COPIA LIBRO DE FAMILIA:	[ ] N.º de páginas:
DOC COPIA OTROS:	[ ] N.º de páginas:
(19) NOTAS:	
(20) FIRMA:	
FIRMA DEL SOLICITANTE O REPRESENTANTE:	ENTIDAD PONS PATENTES Y MARCAS INTERNACIONAL SL - CIF B84921709 - NOMBRE PONS ARIÑO ANGEL - NIF 50534279J
LUGAR DE FIRMA:	MADRID
FECHA DE FIRMA:	02 Junio 2011

DISCVSIÓN GENERAL

La nefrotoxicidad producida por los tratamientos farmacológicos o incluso la toxicidad derivada de una sobreexposición a sustancias ambientales suponen un problema sanitario y socioeconómico.

Varios son los fármacos que presentan daño renal entre sus efectos adversos más problemáticos, se estima que aproximadamente 1 de cada 4 medicamentos utilizados en la unidad de cuidados intensivos es nefrotóxico. La aparición de nefrotoxicidad durante el tratamiento con diversos fármacos provoca el abandono del tratamiento o la necesidad de disminuir la dosis administrada. Estas estrategias preventivas suponen una disminución en la eficacia terapeútica de los fármacos, pues se ven limitadas las dosis que pueden ser administradas.

Otra causa importante a tener en cuenta a la hora de tratar las causas de nefrotoxicidad, es la exposición crónica a sustancias ambientales. Este es el caso de los metales pesados entre los que se encuentran, p.e., el mercurio, el plomo y el urnanio. La exposición a estas sustancias puede en sí no producir aparentemente ningún daño, pero predisponer a los individuos expuestos a sufrir un daño renal cuando tienen que someterse a un tratamiento con un fármaco nefrotóxico.

Una mejora sustancial en el manejo de la nefrotoxicidad de las diferentes sustancias, sería el uso de marcadores capaces de detectar el daño renal en sus fases más iniciales. El estado actual del diagnóstico del daño renal está experimentando grandes cambios. Se ha pasado del uso de marcadores que detectan fases ya avanzadas de daño renal, a la búsqueda de marcadores más tempranos y más específicos. Pero, a pesar de que en los últimos años se está realizando una búsqueda extensiva y varios son los marcadores ya propuestos como marcadores tempranos, aún queda mucho camino por recorrer.

Los resultados de esta tesis doctoral, junto con las publicaciones obtenidas con los mismos, ponen de manifiesto la necesidad de seguir investigando en el diagnóstico del fracaso renal agudo a fin de prevenir su aparición, además de ser capaces de identificarla y controlarla de una forma más precisa.

Mediante la labor de investigación llevada a cabo en este trabajo de tesis doctoral, hemos identificado marcadores capaces de detectar la predisposición al daño renal que puede producir la exposición a diferentes nefrotóxicos a dosis no tóxicas. Esta predisposición se ha puesto de manifiesto tanto tras la administración de un fármaco de forma subaguda como por una exposición crónica a un tóxico ambiental.

En primer lugar hemos demostrado que la administración de un fármaco (cisplatino), a dosis aparentemente no tóxicas, que son inocuas tanto funcional como estructuralmente para la estructura de los riñones, hace más sensible a los animales de sufrir un fracaso renal agudo cuando los animales tienen que someterse a un segundo tratamiento con otro nefrotóxico (gentamicina) a dosis no tóxicas.

Pero la novodead que muestra este trabajo, es que no solamente presentamos un nuevo modelo de predisposición aguda al daño renal. En los resultados expuestos reproducimos experimentalmente, en rata, un nuevo modelo de predisposición al daño renal por exposición crónica a un contaminante medioambiental, el uranio

(estudiado mediante el uso de su derivado, el nitrato de uranilo). Al igual que en el caso anterior, los animales que entraban en contacto con el primer nefrotóxico eran más susceptibles a padecer daño renal que los que no habían estado expuestos previamente. Estos resultados sugieren que la exposición crónica a uranio disminuye el umbral de toxicidad de otro agente nefrotóxico. Por lo tanto, los animales expuestos a este metal son más susceptibles a padecer un fracaso renal agudo cuando han de someterse a un tratamiento con un fármaco nefrotóxico, a dosis que prácticamente no producen daño renal.

Tanto en el modelo de predisposición aguda como en modelo de predisposición crónica, se ha observado un diferente perfil proteico de excreción urinaria entre animales tratados con uno de los nefrotóxicos y animales control. Los animales predispuetos presentaban mayor excreción de proteínas de medio peso molecular y una menor excreción de las proteínas de bajo peso molecular. Estos resultados nos sugieren que las propiedades de filtración de la barrera glomerular podrían estar alteradas, pero de una forma sub-clínica que no se traduce en un incremento de excreción urinaria de proteínas. Además las técnicas más precisas de estudio de la función glomerular, como es el aclaramiento de inulina, no son capaces de detectar alteraciones en dicha función.

La procedencia de las proteínas propuestas como potenciales marcadores de daño renal también es incierta. Su aparición probablemente no está relacionada con el daño a las estructuras, ya que nuestros resultados indican que las dosis usadas de cisplatino o de nitrato de uranilo que predisponen al FRA no causan ningún daño detectable (por marcadores bioquímicos y por estudio histopatológico) en ninguna de las muestras biológicas analizadas (orina y sangre). Más bien pensamos que su acumulación (incluso subtóxica) en el interior de las células diana puede alterar rutas de señalización o procesos celulares de exocitosis, endocitosis, transporte transmembrana, etc., que resulten en la modificación de los niveles de alguna sustancia normalmente presente o ausente en la orina, que puedan asociarse a la predisposición al FRA.

Un posible mecanismo implicado en la producción de predisposición por cisplatino y por uranio podría estar relacionado con alteraciones en la hemodinámica renal. En el modelo agudo y en el crónico se han observado una disminución en el flujo plásmático renal de animales predispuestos y por consecuencia, una aumento de las resistencias vasculares renales (diferencias estadísticamente significativas en el caso de la exposición crónica a uranio). Pero estos resultados son solo una aproximación. Es necesario un estudio más intenso en el que poder determinar, con mayor precisión, cuales son los mecanismos responsables de predisponer a los animales al fracaso renal agudo cuando han sido previamente tratados con nefrotóxicos a dosis aparentemente no tóxicas.

Independientemente del mecanismo por el que se produce la predisposición, la identificación en orina de proteínas nos proporicionan un nuevo concepto "teranóstico" permitiéndonos el estudio de nuevos marcadores capaces de detectar una fase anterior a la aparición de los efectos tóxicos renales. El desarrollo de estos marcadores podría permitirnos realizar un diagnóstico previo a la administración de un segundo tratamiento farmacológico en pacientes ya tratados con otro nefrotóxico o en personas potencialmente expuestas a toxicos ambientales. Estos nuevos marcadores podrían informarnos, de forma anticipada, del posible riesgo de padecer una enfermedad de origen tóxico.

El diagnóstico temprano supondría un gran avance a la hora de elegir un correcto tratamiento clínico y además podría contribuir significativamente

a la disminución de la incidencia del FRA derivado de la acción de los fármacos.

El objetivo inmediatamente posterior sería comprobar la validez en el ser humano de los marcadores encontrados en estos modelos experimentales. La orientación de este proyecto es la aplicación clínica útil de los resultados en un futuro cercano.

CONCLVSIONES

De los estudios expuestos en este trabajo de Tesis Doctoral podemos extraer las siguientes conclusiones:

1. La administración subtóxica de cisplatino produce una predisposición al FRA en la rata, que se pone de manifiesto por la disminución del umbral de toxicidad de otro agente nefrotóxico (la gentamicina) administrado en un régimen no toxico.

2. Los marcadores de daño renal, utilizados comúnmente en la práctica clínica, no son capaces de detectar la predisposición al daño renal producido por cisplatino. Tampoco son útiles para el diagnóstico de esta condición los novedosos marcadores de daño temprano como las proteínas KIM o NGAL entre otros.

3. El tratamiento subtóxico con el cisplatino no alteró sustancialmente el perfíl de excreción urinaria de proteínas. Sin embargo, se incremento la excreción urinaria de albúmina, transferrina y fumarilacetoacetasa, que fueron identificadas como potenciales biomarcadores de predisposición aguda al daño renal causado por el cisplatino.

4. La exposición crónica a nitrato de uranilo a dosis que aparentemente no alteran la función renal, produce una predisposición al daño renal agudo que no puede detectarse con los marcadores disponibles en la práctica clínica.

5. La exposición crónica a nitrato de uranilo indujo una alteración del perfil de excreción proteico, que sugiere una sutil alteración en la barrera de filtración glomerular que no da lugar a proteinuria. Concretamente, cuatro proteínas fueron identificadas como posibles marcadores de predisposición crónica al daño renal agudo: la albúmina, la transferrina, la hemopexina y la VDBP.

6. La aparición en orina de estas proteínas se correlaciona temporalmente con la aparición de la predisposición, por lo constituyen marcadores potenciales de esta condición.

7. En perspectiva, estos candidatos podrían ser útiles para detectar de forma preventiva a los individuos en riesgo de sufrir un daño renal agudo y de estratificarlos con parámetros cuantitativos de acuerdo con su riesgo individual