UNIVERSIDAD DE SALAMANCA

FACULTAD DE FARMACIA
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TESIS DOCTORAL

Terapia Personalizada en la Infección por el VIH: Aplicación de Criterios Farmacocinéticos y Farmacogenéticos

Almudena Sánchez Martín

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Terapia Personalizada en la Infección por el VIH: Aplicación de Criterios Farmacocinéticos y Farmacogenéticos

Trabajo presentado por Almudena Sánchez Martín para obtener el grado de Doctor en Farmacia.

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La Doctora Dña. MARÍA JOSÉ GARCÍA SÁNCHEZ, Catedrática del Departamento de Farmacia y Tecnología Farmacéutica de la Universidad de Salamanca, la doctora Dña. DOLORES SANTOS BUELGA, Profesora Titular del Departamento de Farmacia y Tecnología Farmacéutica de la Universidad de Salamanca y el doctor D. SALVADOR CABRERA FIGUEROA, Profesor Auxiliar del Instituto de Farmacia de la Universidad Austral de Chile.

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En calidad de directores de la Tesis cuyo título es **"Terapia Personalizada en la Infección por el VIH: Aplicación de Criterios Farmacocinéticos y Farmacogenéticos"** realizada por la Licenciada en Farmacia Dña. Almudena Sánchez Martín, consideran concluido el trabajo y autorizan su presentación a fin de que pueda ser juzgado por el Tribunal correspondiente.

Y para que así conste, firman el presente certificado en Salamanca, a veinticinco de Noviembre de dos mil diez.

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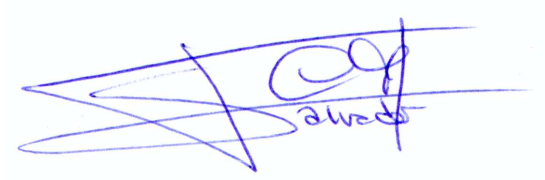

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Índice

Prefacio

Esta tesis se ha redactado en forma de artículos para obtener una mayor difusión del trabajo realizado.

El primer artículo consiste en un trabajo de revisión bibliográfica que nos acerca al tema a tratar desde lo más general a lo más concreto.

El segundo artículo consiste en la descripción de un caso clínico que expone la realidad del tema a investigar.

El tercer artículo es un extenso trabajo en el campo de la investigación del tema a tratar.

El cuarto artículo es un trabajo en el que se intenta aplicar los resultados de la investigación anterior en la práctica clínica.

Abreviaturas y términos en inglés

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

ATV: Antiretroviral

AUC: Área bajo la curva

BMI: Body mass index

CD_{4+}: Linfocitos CD_{4+}

CL: Aclaramiento

$C_{max\ ss}$: Concentración máxima en plasma en el estado estacionario

$C_{min\ ss}$: Concentración mínima en plasma en el estado estacionario

CV: Coeficiente de variación

CVP: Carga viral plasmática

CYP: Citocromo P450

DE: Desviación estándar

EFV: Efavirenz

ETV: Etravirina

F: Biodisponibilidad

GGT: Glutamyltranspeptidase

HPLC: Cromatografía líquida de alta eficacia

IP: Inhibidores de la proteasa

ITIAN: Inhibidores de la transcriptasa inversa análogos de nucleótidos.

ITINN: Inhibidores de la transcriptasa inversa no análogos de nucleósidos.

K_a: Constante de absorción

KGGT: Association for Quality Assessment in TDM and Clinical Toxicology

LC: Límite de cuantificación

LD: Límite de detección

LDL: Low-density lipoprotein

MDR-1: Proteínas resistentes a "multidrugs"

MS: Espectrofotometría de masas

NVP: Nevirapina

PG: Farmagenética

P-gp: P-glicoproteína

PK: Farmacocinético

PLT: Platelets

R^2: Coeficiente de determinación

SCR: Serum creatinine

SIDA: Síndrome de inmunodeficiencia humana

SMAQ: Simplified Medication Adherence Questionnaire

SNC: Sistema Nervioso Central

TARGA: Tratamiento antirretroviral de gran actividad

TB: Total bilirubin

TBW: Total body weigh

TC: Total cholesterol

TDM: Therapeutic drug monitoring

TG: Triglycerides

UV: Ultravioleta

Vd: Volumen de distribución

VHB: Virus de la hepatitis B

VHC: Virus de la hepatitis C

VIH: Virus de la inmunodeficiencia humana

Objetivos generales

- Conocer todos los aspectos relacionados con efavirenz, principalmente farmacogenéticos y farmacocinéticos, para que nos permita contribuir al desarrollo de una terapia personalizada en la infección por el VIH al individualizar el tratamiento con este fármaco.

- Evaluar la utilidad clínica de la monitorización de niveles plasmáticos y del análisis farmacogenético para dirigir el ajuste individualizado de las dosis de efavirenz y optimizar su tratamiento respecto a eficacia y seguridad.

- Identificar y caracterizar los polimorfismos genéticos en genes que codifican las principales enzimas metabolizadoras (citocromo P450) y proteínas transportadoras (p-glicoproteína y proteinas de multirrestistencia) de fármacos, que mayor relevancia tienen en el metabolismo y transporte de efavirenz.

- Analizar la influencia que tienen estos polimorfismos genéticos sobre los parámetros farmacocinéticos de efavirenz, para poder así determinar la variabilidad interindividual a la respuesta al tratamiento con este fármaco.

- Construir un modelo farmacocinético/farmacogenético poblacional de efectos mixtos que evalúe el comportamiento cinético de efavirenz, sus variabilidades inter e intraindividuales así como la identificación y cuantificación de la influencia de los factores demográficos, farmacogenéticos, clínicos y de tratamiento que pueden explicar parte de estas variabilidades.

Capítulo I

Terapia Personalizada con Efavirenz: Farmacocinética y Farmacogenética

Revisión bibliográfica

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Enfermedades InfecciosasMicrobiología Clínica

Resumen

Hace más de una década desde que efavirenz (EFV) forma parte del tratamiento antirretroviral, siendo un componente esencial que tiene una adecuada eficacia y seguridad según numerosos ensayos clínicos. Sin embargo, la experiencia clínica nos muestra una elevada variabilidad interindividual en la respuesta, lo cual se traduce en un importante número de cambios de tratamiento por fracasos virológicos y efectos adversos. La individualización del tratamiento parece ser la clave para anticiparse a estas consecuencias. En este sentido y teniendo en cuenta que los factores genéticos pueden influir en la farmacocinética de EFV y ser la causa de estas diferencias entre pacientes, la monitorización de niveles plasmáticos y el análisis farmacogenético se perfilan como dos nuevas estrategias para cumplir este objetivo. En esta revisión se analizan todos estos aspectos, principalmente farmacocinéticos y farmacogenéticos, que podrían contribuir al desarrollo de una terapia personalizada con este fármaco.

Palabras clave: Efavirenz. Farmacogenética. Monitorización terapéutica de fármacos.

Introducción

El virus de la inmunodeficiencia humana (VIH) es el agente causal de una enfermedad caracterizada por el deterioro progresivo del sistema inmunológico, especialmente de los linfocitos CD_{4+}, cuya expresión clínica final es el síndrome de inmunodeficiencia adquirida (SIDA).

Hace más de dos décadas, cuando se detectaron los primeros casos de infección por el VIH, era difícil predecir cómo podría evolucionar esta enfermedad y la repercusión que tendría a nivel mundial. Lamentablemente la epidemia creció de forma exponencial en muchos países, a la vez que se extendió a muchos otros que en un principio no se habían visto afectados, por lo que llegó a adquirir la categoría de pandemia. Además, debido a su elevada mortalidad, se convirtió en una de las causas más importantes de muerte en todo el mundo, sobre todo en países en vías de desarrollo como África subsahariana.

Actualmente, podemos decir que la epidemia de VIH continúa siendo una importante prioridad sanitaria en el mundo, ya que son más de 33 millones de personas las que viven con el virus. Sin embargo, en los últimos años se han logrado importantes avances en cuanto a prevención y acceso a la medicación antirretroviral. Así en el año 2008, el número estimado de nuevas infecciones (2,7 millones) refleja un descenso del 17% en los últimos ocho años y el número de personas de países de ingresos medios y bajos que reciben tratamiento antirretroviral (4 millones) supone un aumento diez veces mayor en los últimos cinco años[1].

En España, según estudios realizados por diferentes organismos, la prevalencia de infección por VIH en la población general podría estar en una cifra alrededor de 1,5/1000. El número de notificaciones totales desde el inicio de la epidemia alcanzan un total de 77.953 casos de SIDA [2].

Desde el inicio del tratamiento antirretroviral de gran actividad (TARGA) se ha observado una reducción progresiva de los casos de SIDA. No obstante, el porcentaje de casos en la población inmigrante ha experimentado un crecimiento. Es así como hasta 1997 la proporción de casos de SIDA en personas

cuyo país de origen no era España estuvo por debajo del 3%, pero a partir de 1998 esta cifra subió progresivamente hasta alcanzar el 26,5% en 2008, año en el que el 41,3% de estas personas extranjeras procedía de Latinoamérica, y el 33,9% de países de África [2].

A pesar del marcado descenso de la incidencia de SIDA en España desde la extensión de los nuevos tratamientos antirretrovirales, España sigue siendo uno de los países con mayor incidencia de SIDA en Europa Occidental. Durante muchos años el mecanismo de transmisión habitualmente implicado en los nuevos diagnósticos de VIH fue el uso de drogas por vía parenteral (ADVP), en la actualidad son las relaciones sexuales (55,2% de los nuevos casos de sida en 2008)[2].

Sin duda, el desarrollo del TARGA, conocida al día de hoy como TAR, ha cambiado la historia natural de la infección VIH/SIDA, al retardar la evolución de la enfermedad y mejorar la calidad de vida de los individuos infectados[3,4].

El primer ensayo clínico realizado con un medicamento con actividad antirretroviral (la zidovudina, un nucleósido análogo de la timidina que inhibe la transcriptasa inversa) se completó en 1986[5]. A partir de entonces, se han desarrollado en un corto plazo de tiempo un elevado número de medicamentos con actividad frente al VIH-1. Así, en el momento actual, se dispone de seis familias de antirretrovirales (ART) que engloban un total de 25 medicamentos activos. Entre ellas destacan las familias que inhiben a las principales enzimas implicadas en la replicación del virus: inhibidores de la proteasa (IP) o de la transcriptasa inversa (ITI) análogos o no análogos de los nucleósidos, según su estructura química. Últimamente también han adquirido gran importancia aquéllas que inhiben la replicación del VIH-1 interviniendo en los complejos procesos de adherencia viral y fusión con las células del hospedador o la integración del ADN proviral en el ADN celular.

El tratamiento inicial de elección de la infección por el VIH en el momento actual consiste en la combinación de al menos tres fármacos que incluyan dos inhibidores de la transcriptasa inversa análogos de nucleósidos (ITIAN) y un inhibidor de la proteasa potenciado con ritonavir (IP/r) o un inhibidor de la transcriptasa inversa no análogo (ITINN). Con la mayoría de estas

combinaciones se puede conseguir CV< 50 copias/mL en más del 70% de los casos en aproximadamente unas 48 semanas[4,6-13].

Actualmente, las pautas basadas en ITINN presentan varias ventajas frente a los IP/r. Entre ellas destaca un menor número de interacciones farmacocinéticas, un perfil metabólico más favorable y un menor coste. Además, debido a la baja barrera genética de los ITINN, el inicio de tratamiento es el momento idóneo para su uso, ya que en tratamientos de rescate tienen menor actividad[10-13]. En España disponemos en este momento de tres ITINN: nevirapina (NVP), efavirenz (EFV) y el recientemente comercializado etravirina (ETV). La elección de uno u otro depende principalmente de las características individuales de cada paciente, ya que todos estos fármacos presentan una serie de ventajas e inconvenientes que hay que tener en cuenta. Pero si es importante destacar que EFV ha sido un componente esencial del tratamiento de la infección por el VIH y ha contribuido de manera muy significativa a la evolución del HAART. Actualmente, EFV ha mostrado en numerosos estudios clínicos una adecuada eficacia y seguridad en el tratamiento de la infección por el VIH-1, por lo que numerosas guías clínicas tanto nacionales como internacionales recomiendan la utilización de este fármaco como primera línea en el tratamiento antirretroviral[4,6-9].

Sin embargo, a pesar de estos grandes avances, aún son necesarias más herramientas que permitan optimizar el tratamiento con estos fármacos. Así, al no poder erradicar el VIH-1, estos tratamientos, que en la mayoría de los casos son complejos, deben administrarse de forma crónica, lo que conlleva una serie de importantes consecuencias en cuanto a cumplimiento, toxicidad a largo plazo, resistencias, etc. Además, existe una elevada variabilidad interindividual en la respuesta farmacológica por lo que es necesario buscar nuevas estrategias terapéuticas dirigidas a la individualización de estos tratamientos.

El objetivo de este trabajo ha sido realizar una extensa revisión de todos los aspectos relacionados con EFV (principalmente farmacocinéticos y farmacogenéticos) que permita la actualización y orientación hacia el desarrollo de una terapia personalizada con este fármaco.

Características generales de efavirenz

Formulación y composición química

Hace más de una década desde que EFV forma parte del tratamiento antirretroviral. Su aprobación en la Union Europea (UE) fue en el año 1999, con el nombre comercial de Sustiva® (Bristol, Mayers) y conocido químicamente como (S)-6-cloro-4-ciclopropiletinil-4-trifluorometil-1,4-dihidro-2H-3,1-benzoxazin-2-ona[14]. Su fórmula y peso molecular son $C_{14}H_9ClF_3NO_2$ y 315,67, respectivamente. Y en cuanto a sus propiedades fisico-químicas, se describe como un polvo cristalino de color blanco a ligeramente rosado y prácticamente insoluble en el agua (<10 μg/mL)[14].

Actualmente en España, EFV (Sustiva®) está disponible como cápsulas duras de 50, 100 y 200 mg y como comprimidos recubiertos con película de 600 mg. Además recientemente, se ha comercializado en combinación con otros dos antirretrovirales en un único comprimido conocido con el nombre de Atripla®, que contiene 600 mg de EFV, 200 mg de emtricitabina y 300 mg de tenofovir [14].

Mecanismo de acción e indicaciones terapéuticas

EFV pertenece a la familia de los ITINN, por lo que su mecanismo de acción consiste en inhibir la actividad de la transcriptasa inversa (TR). Pero, sólo es eficaz inhibiendo a la TR del VIH-1[15], por lo que no es activo frente al VIH-2 o las polimerasas de ADN celular (α, β, γ ó δ). Además, se desconoce como actúa sobre otros virus. Por ello, sólo está indicado en el tratamiento antiviral combinado del VIH-1 en adultos infectados, adolescentes y niños de 3 años de edad y mayores[14].

Farmacocinética: Proceso del ADME

Absorción

La dosis recomendada en adultos es una dosis fija de 600 mg por vía oral cada 24 horas. Su biodisponibilidad es superior al 66% y puede aumentar hasta en un 22% si se administra junto con comida normal o con alto contenido en grasas, respectivamente[14]. Por ello, se recomienda administrarlo con el estómago vacío, ya que se ha observado que un aumento de las concentraciones plasmáticas podría producir un aumento en la frecuencia de las reacciones adversas.

Después de la administración de dosis múltiples de EFV, se requiere un tiempo de 3 a 5 horas para alcanzar concentraciones plasmáticas máximas y aproximadamente 6-7 días para que dichas concentraciones se encuentren en estado estacionario. Los valores medios estimados de concentración máxima en el estado estacionario ($C_{ss\ máx}$), concentración mínima en el estado estacionario ($C_{ss\ mín}$) y el área bajo la curva (AUC) [media ± D.S. (% C.V.)] son de 12,9 ± 3,7 µM (29%), 5,6 ± 3,2 µM (57%), y 184 ± 73 µM·h (40%). Sin embargo, aunque estos valores son lineales con dosis diarias de 200, 400 y 600 mg, a dosis mayores estos aumentos no son proporcionales sino inferiores a lo esperable, lo que sugiere que hay una absorción reducida a dosis más elevadas[14].

Distribución

EFV se distribuye por todo el organismo unido en un alto porcentaje (> 99%) a las proteínas plasmáticas humanas, principalmente a albúmina. Es destacable señalar que tiene fácil acceso al sistema nervioso central (SNC), ya que las concentraciones que alcanza en el líquido cefalorraquídeo (LCR) son aproximadamente tres veces superiores a la fracción libre de EFV en plasma[14,16].

Metabolismo

Su metabolismo es principalmente hepático a través del sistema del citocromo P450 (CYP), según han mostrado varios estudios clínicos en humanos y estudios in vitro en los que se utilizaron microsomas hepáticos humanos. Los estudios in vitro sugieren que CYP2B6 y CYP3A4 son las principales isoenzimas responsables del metabolismo del EFV[17], que lo transforman en metabolitos hidroxilados que posteriormente sufren un proceso de glucuronidación. Estos metabolitos son esencialmente inactivos frente al VIH-1. Aunque no hay que descartar que otras isoenzimas de este citocromo (CYP2A6, CYP2D6, CYP2C9, CYP2C19, etc.), puedan también intervenir en este proceso. Por otra parte, EFV inhibe las isoenzimas 2C9, 2C19 y 3A4 de P450 e induce algunas enzimas P450, por lo que produce también la inducción de su propio metabolismo[14].

Eliminación

En cuanto a su eliminación, EFV fue el primer antirretroviral en administrarse una vez al día, debido a presentar una vida media relativamente larga de al menos 52 horas después de la administración de dosis únicas y 40–55 horas tras dosis múltiples. Aproximadamente el 14-34% de una dosis de EFV marcada radiactivamente se recupera en la orina y menos de un 1% de la dosis se excreta en la orina como EFV sin alterar[14].

Eficacia, seguridad y tolerancia

La eficacia de EFV ha sido establecida en numerosos ensayos clínicos tanto en pacientes näive como en pacientes pretratados. Así, varios estudios han comparado la eficacia de EFV frente a diferentes IPs (indinavir, atazanavir, lopinavir/ritonavir)[18-21], otros ITINNs como NVP [22-23], regimenes de triple terapia con ITINs[10] e incluso frente a nuevos antirretrovirales como maraviroc y raltegravir [24-25], en los cuales ha quedado demostrada su buena eficacia clínica, ya que su capacidad para suprimir la carga viral es igual o superior a ellos.

En cuanto a la seguridad y tolerancia, EFV ha sido generalmente bien tolerado en los ensayos clínicos y sólo de un 4 a un 16% de los pacientes tratados con EFV tuvieron que suspender el tratamiento debido a efectos adversos[26]. Entre los más importantes asociados al tratamiento con EFV destacan la las alteraciones en el SNC y erupción cutánea[14].

Respecto a esta última, se han descrito casos de erupción de leve a moderada en aproximadamente un 18% de los pacientes y generalmente se resuelven durante la terapia continuada, aunque la administración de los antihistamínicos y/o corticosteroides apropiados pueden mejorar la tolerancia y acelerar la resolución de la erupción. Además, en menos del 1% de los pacientes tratados con EFV, se han descrito casos de erupción grave asociada con ampollas, descamación húmeda o úlceras[14].

Por otra parte, los efectos adversos relacionados con el SNC, han sido descritos en aproximadamente un 25-70% de los pacientes. Estos síntomas suelen aparecer en los primeros días o semanas de tratamiento y en la mayoría de los casos consisten en mareos, insomnio, pesadillas, inestabilidad, irritabilidad, somnolencia y alteración en la capacidad de concentración, que tienden a mejorar progresivamente en unas pocas semanas. Sin embargo, algunos estudios recientes muestran que estos desórdenes neuropsiquiátricos pueden persistir durante más tiempo en más de la mitad de los pacientes, afectando de forma importante a su calidad de vida[27-29].

Otros efectos adversos, tales como la aparición de lipodistrofia y alteraciones metabólicas (hipertriglicidemia, hipercolesterolemia, etc.), son mucho menos frecuentes respecto a aquellos tratamientos que llevan una triple terapia de ITIANs[14].

También es importante señalar que el uso de EFV está contraindicado en embarazo debido a su capacidad teratogénica (sobre todo en el primer trimestre del embarazo) y que su baja barrera genética le confiere resistencia de familia si aparece la mutación K103N[14].

Nuevas estrategias en la individualización del tratamiento con efavirenz

A pesar de estos buenos resultados en cuanto a eficacia y seguridad, la experiencia clínica nos demuestra que la respuesta al tratamiento con EFV varía mucho de unos pacientes a otros. Así, día a día observamos que un elevado porcentaje de pacientes presentan fracaso virológico nada más empezar el tratamiento, o bien experimentan importantes reacciones adversas que requieren la suspensión del fármaco. En ambos casos, es necesario cambiar de tratamiento ART, lo que supone importantes repercusiones en todos los niveles: clínicos, sociales, económicos, etc.

Por esta razón, necesitamos encontrar nuevas herramientas que nos permitan anticiparnos a estos cambios de tratamiento y conseguir que EFV sea eficaz y tolerado por todos y por cada uno de los pacientes. La individualización del tratamiento de EFV parece ser la única manera de lograr este objetivo. En este sentido, la monitorización de niveles plasmáticos (TDM) y el análisis farmacogenético se perfilan como dos nuevas estrategias que pueden ser aplicadas en la práctica clínica para optimizar los tratamientos con EFV.

MONITORIZACIÓN DE NIVELES PLASMÁTICOS

Las importantes diferencias encontradas en la respuesta al tratamiento con EFV han sido relacionadas con una elevada variabilidad interindividual en las concentraciones plasmáticas, lo cual ha sido observado al medir estas concentraciones en pacientes que tomaban la misma dosis.

La TDM permite ajustar las dosis en función de las concentraciones plasmáticas y consigue que estas concentraciones se mantengan dentro unos límites terapéuticos previamente definidos, mejorando así tanto la eficacia terapéutica como la toxicidad. Por ello podría considerarse como una buena herramienta para la individualización de los tratamientos.

Requisitos para la monitorización terapéutica:

Los requisitos que debe cumplir un fármaco para ser candidato a la TDM son:

Disponibilidad de técnicas analíticas adecuadas.

Las técnicas analíticas empleadas deben tener una exactitud y precisión aceptable con una alta especificidad para la determinación de la concentración del fármaco, y a su vez tienen que ser métodos accesibles para su utilización en la práctica clínica habitual[30].

En la actualidad, la técnica de elección para la TDM de la mayoría de ART, incluido EFV, es la cromatografía líquida de alta eficiencia (HPLC), cuyo método de detección puede ser la absorción ultravioleta (UV) o la espectrofotometría de masas (MS) y que permite analizar uno o varios fármacos simultáneamente [31,32]. Sin embargo, debido a la infraestructura que esta técnica conlleva, en estos momentos también se están evaluando otros métodos como los inmunoensayos enzimáticos (ELISA) con buenos resultados[33], aunque presentan el inconveniente de solo poder analizar un fármaco a la vez.

Correlación entre la concentración plasmática del fármaco y la eficacia o toxicidad.

El efecto farmacológico tiene que estar más estrechamente correlacionado con su concentración plasmática que con su dosis.

En el caso de los ART, existe evidencia clínica sobre la relación entre sus concentraciones plasmáticas y su eficacia o toxicidad. Así, en concreto con EFV, varios estudios han establecido una relación significativa entre concentraciones y la aparición de toxicidad o falta de eficacia[34-39].

Estrecho margen terapéutico.

El margen terapéutico de un medicamento es el intervalo de concentraciones del fármaco, habitualmente en suero o plasma, en el que la mayoría de los pacientes experimentará un efecto terapéutico y pocos experimentarán efectos adversos[40]. Se establece para la mayoría de los grupos farmacológicos a través de estudios farmacocinéticos/farmacodinámicos (PK/PD); y en general los fármacos con estrecho margen terapéutico son en los que la TDM resulta más útil.

En el caso de los ART, establecer un margen terapéutico adecuado ha sido un objetivo difícil, principalmente porque el tratamiento ART consiste en una combinación de varios fármacos y es necesario tener en cuenta el efecto sinérgico o aditivo entre ellos, lo cual puede dificultar el establecer la concentración mínima en el estado estacionario (C^{ss}_{min}) eficaz para un ART en concreto. Por otra parte, las mutaciones ocurridas durante la replicación del VIH pueden hacer que la concentración diana pueda no ser un valor fijo.

En el caso del EFV, el margen terapéutico establecido para las C^{ss}_{min} se encuentra entre 1-4 mg/L[41], lo cual han puesto de manifiesto por diversos estudios. Así, se ha observado que la incidencia de fracaso virológico parece estar incrementada cuando las concentraciones son inferiores a 1 mg/L, mientras que el riesgo de toxicidad a nivel del SNC es mayor en aquellos pacientes que presentan concentraciones plasmáticas superiores a 4 mg/L[34-39].

Alta variabilidad interindividual y baja variabilidad intraindividual en los índices nivel/dosis.

Existe una elevada variabilidad interindividual en las concentraciones plasmáticas de ART, lo cual se observa cuando se administran la misma dosis a distintos pacientes. Las causas de esta elevada variabilidad farmacocinética pueden ser múltiples (fisiológicas, clínicas, patológicas, genéticas) e intervenir en los diferentes procesos (absorción, distribución, metabolismo y eliminación) que sufre un fármaco cuando entra en el organismo. En el caso del EFV, esta variabilidad ha sido estimada en un 118% por Marzolini y col.[37], lo que justifica la monitorización para optimizar el tratamiento.

En cuanto a la variabilidad intraindividual, ésta debe ser baja o constante para tener un valor significativo. En el caso de los ITINN, los estudios realizados han observado que esta variabilidad es relativamente constante, aproximadamente del orden del 30%, de forma que una o dos determinaciones podrían proporcionar una buena información de las concentraciones que mantiene el paciente[42].

Parámetros utilizados en la interpretación de las concentraciones plasmáticas:

Los parámetros farmacocinéticos habitualmente utilizados en monitorización de concentraciones plasmáticas han sido el área bajo la curva (AUC) y la concentración máxima (C^{ss}_{max}) o C^{ss}_{min}. Cada uno de estos parámetros nos proporciona una información farmacocinética distinta[43-45]. Así, el AUC nos informa sobre la exposición total al fármaco, mientras que la C^{ss}_{max} y la C^{ss}_{min} nos indican únicamente la concentración máxima o mínima alcanzada tras la administración de múltiples dosis.

En la TDM de ART, la elección de qué parámetro es el mejor predictor de la respuesta viral ha sido ampliamente discutida. Por una parte, debido a la elevada tasa de replicación del VIH, la estrategia que parece más adecuada es mantener la concentración plasmática del ART por encima de un determinado umbral o diana, lo que apoya la hipótesis de un efecto terapéutico dependiente del tiempo para este grupo de fármacos. En este sentido, el parámetro farmacocinético que mejor se correlaciona con la eficacia virológica es la $C^{ss}_{min,}$ lo cual ha sido demostrado en diferentes estudios y propuesto por distintos documentos de consenso. No obstante, los parámetros AUC y C^{ss}_{max} también han presentado correlaciones aceptables con la respuesta clínica, de tal manera que cuando se evalúa la toxicidad, existe mayor probabilidad de que la C^{ss}_{max} esté más relacionada con la presencia de efectos adversos.

En relación al EFV, la utilización de la C^{ss}_{min} también parece ser lo más adecuado[34-37]. Sin embargo, existen dificultades prácticas para su determinación, ya que es un fármaco que se administra preferiblemente en horario nocturno. En este caso, la manera más precisa de determinar este

parámetro es mediante métodos bayesianos. Así, la aplicación del teorema de Bayes, en el contexto de la farmacocinética clínica, nos permite describir la relación cuantitativa entre la probabilidad a *priori* de presentar determinados valores de parámetros farmacocinéticos y la subsiguiente probabilidad a posteriori, una vez que las concentraciones del fármaco son conocidas[46]. La aplicación de esta metodología exige la selección correcta de un modelo poblacional (adaptado a los diferentes tipos de pacientes: niños, obesos, insuficienia renal, etc.) y de los parámetros que se utilizan como información previa, para evitar errores de dosificación, particularmente cuando se aplica para la estimación "a *priori*". Además, permite, controlar diversas variables que influyen en el perfil farmacocinético de un fármaco, como por ejemplo las interacciones farmacológicas. Actualmente, disponemos de varios modelos poblacionales de EFV que incluyen distintas variables y que predicen de forma fiable los diferentes parámetros farmacocinéticos[47-51].

Aplicación clínica

Actualmente la monitorización terapéutica de ART no forma parte de la práctica clínica habitual y el valor de ajustar la dosis para mantener unas concentraciones eficaces todavía es controvertido, por lo que EFV sigue siendo administrado a una dosis fija de 600 mg una vez al día.

Pero hay que señalar que en los últimos años la TDM ha cobrado un especial interés y que el papel que puede desempeñar en la optimización del tratamiento con estos fármacos ha sido evaluado[52-54]. De tal manera que varios documentos de consenso recomiendan su utilización en determinadas situaciones:

Control de la adherencia:

Debido a la baja barrera genética que presenta este fármaco es imprescindible tener una buena adherencia al tratamiento, para evitar el desarrollo de resistencias y llegar al fracaso virológico. Sin embargo, alcanzar elevadas tasas de adherencia durante largos periodos de tiempo es difícil de conseguir. La TDM, al ser un método directo de evaluación de la adherencia[53,55], puede resultar muy útil porque la detección de concentraciones plasmáticas bajas y/o

una variabilidad en las mismas superior al 100% podrían alertar de la existencia de un problema de adherencia o de cualquier otro tipo, y de algún modo pronosticar un posible fallo al tratamiento.

Identificación y control de interacciones:

Las posibilidades de interacciones farmacológicas durante el tratamiento antirretroviral son elevadas, ya que al hecho de ser una triterapia hay que sumar la posible co-medicación con otros fármacos, productos naturales y/o alimentos. Además, en el caso de los ITNN, y en concreto del EFV, las principales interacciones se producen como consecuencia de la inhibición o la inducción de las isoenzimas del CYP, ya que es un fármaco ampliamente metabolizado por esta vía. En estas situaciones, la TDM puede desempeñar un importante papel para establecer la relevancia clínica que puede tener en cada paciente.

Toxicidad:

A pesar de la buena eficacia que puede tener el tratamiento ART, algunos pacientes presentan efectos adversos que reducen su calidad de vida e incluso en algunas ocasiones necesitan cambiar de tratamiento por intolerancia. En el caso del EFV, estos efectos adversos están claramente relacionados con concentraciones plasmáticas elevadas. Por ello, la TDM puede ser de utilidad para realizar ajustes de dosis hasta conseguir concentraciones del fármaco igual de efectivas pero más tolerables.

Inicios y cambios de tratamiento:

Cuando se selecciona un nuevo tratamiento en un paciente (bien sea un inicio o un cambio de tratamiento), es una excelente oportunidad de evaluar, una vez alcanzado el estado de equilibrio, si las concentraciones alcanzadas en ese paciente se corresponden con las recomendadas en bibliografía. Una precoz detección de anormalidades permitirá su estudio y corrección, evitando el desarrollo de resistencias o de sintomatología adversa que provoque una disminución de la satisfacción del paciente con su tratamiento.

Pediatría:

Se trata de un grupo muy heterogéneo que además, difiere de la población adulta en su comportamiento farmacocinético, por lo que la extrapolación de las recomendaciones posológicas de un adulto (dosificación en mg/Kg o mg/m^2) puede no asegurar la misma exposición al fármaco y ser en consecuencia inapropiada.

Pacientes con pesos extremos:

La monitorización está recomendada en pacientes con pesos muy bajos y obesos, debido a los consecuentes riesgos de toxicidad e ineficacia clínica respectivamente derivados de cambios en el volumen de distribución. Además, las mujeres pueden tener mayores concentraciones probablemente debido al menor peso corporal, por lo que también se recomienda la monitorización en este grupo de población.

Daño hepático o renal:

Los pacientes con daño hepático parecen ser candidatos ideales para la TDM de EFV, debido a los cambios que se producen en su metabolismo, a la perspectiva de una toxicidad severa provocada por una sobreexposición, y al mayor potencial de cambios farmacocinéticos ante interacciones fármaco-fármaco.

En caso de insuficiencia renal, y debido a que ITINN se metabolizan principalmente por vía hepática, no sería preciso realizar ajustes posológicos, no obstante, en casos de insuficiencia renal avanzada es recomendable monitorizar las concentraciones para asegurar que no se exceden los límites recomendados y evitar una posible toxicidad.

Experiencia previa en monitorización terapéutica de efavirenz

Según estudios recientes, se calcula que la proporción de pacientes en los que no se consiguen concentraciones de EFV adecuadas cuando se utilizan dosis estándar puede llegar a ser entre un 35 y un 61%[56-58]. Esto pone de manifiesto una importante tendencia a la infra o sobredosificación, la cual podría

corregirse optimizando la posología de acuerdo al comportamiento cinético de EFV en cada paciente en particular.

Ajustes de dosis guiados por la TDM

Varios estudios han demostrado la utilidad de la TDM para realizar ajustes de dosis de EFV en algunas situaciones clínicas como son las interacciones farmacológicas o la toxicidad a nivel del SNC.

Respecto a las interacciones farmacológicas, la administración concomitante de EFV con rifampicina ha sido la más estudiada. La rifampicina, componente fundamental de la terapia de la tuberculosis, es un potente inductor de la actividad del CYP, por lo que según los datos farmacocinéticos disponibles puede reducir las concentraciones plasmáticas de EFV hasta un 40%. Además, su efecto inductor sobre el EFV es de tipo reversible y es necesario un tiempo para que desparezca totalmente. Algunos autores como Cabrera y col.[59] han confirmado la validez de la TDM al realizar aumentos de dosis a 800 mg que han permitido alcanzar concentraciones terapéuticas durante la co-administración con rifampicina.

Los pacientes que presentan efectos adversos relacionados con EFV, también se han beneficiado de emplear la TDM como estrategia para individualizar el tratamiento, así en varios trabajos se han obtenido buenos resultados clínicos, ya que se ha conseguido disminuir de manera considerable estos efectos adversos sin afectar la eficacia del tratamiento. Así, Gatanaga y cols. disminuyeron la dosis recomendada de EFV de 600 mg una vez al día a 400 mg e incluso a 200 mg en un total de 18 pacientes, los cuales presentaban concentraciones elevadas de EFV obteniendo buenos resultados clínicos. La carga vírica se mantuvo indetectable y los efectos adversos a nivel del SNC en 10 de los 14 que refirieron síntomas con la dosis estándar, aunque el resto de pacientes a pesar de no haber referido síntomas después de la disminución de dosis comentaron que su calidad de vida había aumentado. También otros autores[60-61] han descrito casos clínicos de pacientes de diferentes razas, a los que se le disminuyó la dosis a 400 mg y 200 mg una vez al día con una buena respuesta clínica.

ANALISIS FARMACOGENÉTICO

La elevada variabilidad interindividual de las concentraciones de EFV, condiciona importantes diferencias en cuanto a eficacia y toxicidad en los diferentes pacientes. Sin embargo, el conocer las consecuencias no es suficiente para poder seguir avanzando en la individualización del tratamiento, sino que es necesario también conocer las causas. En este sentido, los estudios farmacogenéticos tienen mucho que decir, ya que entre los múltiples factores que están involucrados en esta variabilidad, los factores genéticos están adquiriendo cada vez más importancia. Así, la farmacogenética, que es la ciencia que estudia la influencia que tienen las variaciones genéticas individuales en la respuesta a los fármacos, podría permitir seleccionar a aquellos pacientes con mayor riesgo de efectos adversos o ineficacia y por tanto desde un principio poder intervenir en la optimización del tratamiento.

Factores a tener en cuenta en un estudio farmacogenético.

Antes de realizar un estudio farmacogenético sobre un fármaco en concreto, es necesario tener en cuenta una serie de factores relacionados tanto con las variaciones genéticas a identificar, las características del fármaco y las técnicas empleadas para la determinación. A continuación se describen algunos de ellos:

Características del fármaco:

Es necesario que el fármaco tenga una serie de características que justifiquen la necesidad de realizar un estudio farmacogenético.

EFV es un buen candidato ya que cumple varios requisitos, es un fármaco que desempeña un papel esencial en el tratamiento antirretroviral y además debido a la elevada variabilidad interindividual en su respuesta, se producen importantes consecuencias tanto a nivel de toxicidad como de eficacia.

Selección de genes candidatos:

Los estudios farmacogenéticos deben tienen que estar bien diseñados, de tal forma que se haya realizado una adecuada selección de los genes candidatos a estudiar, es decir, es que los genes investigados tengan un vínculo de interacción con el fármaco previamente establecido. Por ello, es muy importante conocer todos los procesos (ADME) implicados en la variabilidad farmacocinética del fármaco para así poder identificar correctamente los genes que están involucrados en ellos.

En el caso del EFV, los genes candidatos, y por tanto los que han sido más estudiados por los diferentes estudios han sido los codificadores de las principales proteínas implicadas en el transporte (MDR1, MRP1, MRP2, MRP3, etc.) y metabolismo (CYP2B6, CYP2A6, CYP3A4, CYP3A5, CYP2D6, etc.) de EFV.

Tipos de variaciones genéticas:

La principal fuente de variabilidad en los genomas de los seres humanos son las variaciones en un sólo nucleótido, conocidas como SNP (Single Nucleotide Polimorphisms).

En la actualidad, se estima que hay alrededor de 10 millones de SNP en el genoma humano y que su frecuencia es al menos de uno por cada 1.000 pares de bases. Los SNP se clasifican en "no sinónimos" (nsSNP), cuando se modifica el aminóacido que formará la proteína y "sinónimos" cuando no se modifica. Los SNP también se denominan "funcionales", cuando alteran la expresión del gen o la función de la proteína, o "no funcionales" cuando no tienen ningún efecto. Hasta el momento, la mayoría de estudios farmacogenéticos analizan este tipo de variaciones genéticas.

Sin embargo, estudios recientes señalan que las variaciones en el número de copias (CNV), las cuales son extensas regiones en el código genético de un individuo determinado que están duplicadas o suprimidas, también pueden tener una importante contribución en la variación genética entre individuos.

Técnicas de genotipado:

Es necesario disponer de técnicas de genotipado rápidas, sensibles y altamente coste-efectivas, que permitan su aplicación en la práctica clínica habitual.

En la actualidad, varias técnicas cumplen estos requisitos (SNaPshot®, SNPlex®, Sequenom®, etc.), las cuales permiten analizar en un corto periodo de tiempo un elevado número de variaciones genéticas.

Aplicación clínica

Actualmente, la aplicación de la farmacogenética en la práctica clinica habitual no parece fácil. La mayoría de los estudios se han centrado en el efecto de polimorfismos de un solo gen, y sin embargo, la respuesta farmacológica es mucho más compleja, con la participación de múltiples genes relacionados entre ellos y con factores no genéticos.

Sin embargo, las investigaciones realizadas en este campo están avanzando rápidamente, y cada vez hay más estudios que confirman las significativas implicaciones que los polimorfismos genéticos tienen en la cinética de ART. Esto esta permitiendo que la farmacogenética en un futuro próximo pueda ser utilizada para:

Correlacionar el genotipo con el fenotipo clínico.

Identificar los pacientes con mayor riesgo de sufrir determinadas efectos adversos o tener diferente respuesta al tratamiento.

Mejorar la eficacia y disminuir los efectos adversos.

Individualizar el tratamiento.

Experiencia previa de estudios farmacogenéticos en EFV

Diversos estudios han puesto de manifiesto que la existencia de variaciones genéticas en genes que codifican ciertas proteínas implicadas en el transporte (P- glicoproteína, MRP1, MRP2, etc.) o en el metabolismo (CYP2B6, CYP3A4, CYP3A5, CYP2D6, etc.) de EFV[62] pueden influir en el comportamiento farmacocinético de este fármaco, condicionando a su vez, su eficacia y toxicidad.

Polimorfismos en enzimas metabolizadoras

CYP2B6

Hasta el momento, uno de los polimorfismos más relevantes que se han descrito tiene relación con alteraciones en el metabolismo de EFV, el cual se realiza principalmente por medio de la isoenzima 2B6 del sistema del CYP450 (CYP2B6), responsable del aclaramiento del 90% del EFV circulante[17]. Se ha demostrado que el gen que codifica esta isoenzima es extraordinariamente polimórfico (28 alelos descritos), sobre todo en la raza negra y que existe una gran variabilidad interindividual en la cantidad y en la actividad catalítica de esta isoenzima en el hígado humano.

Los alelos que contienen los siguientes polimorfismos 415 A>G, 516 G>T, 136 A>G, 296 G>A, 785 A>G, 419 G>A y 1172 T>A, han sido asociados con diferencias en la expresión de esta proteína. Sin embargo, la variante alélica del gen que parece afectar más a la expresión del CYP2B6 en el hígado y que altera más el metabolismo del EFV es un cambio de G a T en el codón 516 (polimorfismo 516 G>T). La consecuencia de este cambio se traduce en un descenso del nivel de actividad de la proteína codificada, con el consiguiente aumento en las concentraciones plasmáticas de EFV. La estrecha asociación existente entre el polimorfismo CYP2B6 G516T y las concentraciones plasmáticas de EFV se ha confirmado en varias investigaciones[34-35,63-68]. De igual manera, este polimorfismo y concentraciones plasmáticas elevadas de EFV se han correlacionado positivamente con los síntomas neuropsiquiátricos[34-37].

Así en un estudio realizado Haas y cols[35], que incluyó pacientes de diversas razas se encontró una asociación entre el genotipo CYP2B6 516T/T y una reducción del aclaramiento plasmático de EFV. En consecuencia, elevadas concentraciones de EFV están fuertemente influenciadas por el genotipo del CYP2B6.

Además, se ha observado que la toxicidad a nivel del SNC es más frecuente en pacientes que llevan el genotipo G/T o T/T en comparación con el G/G. Así, Nolan y cols.[66] sugirieron el uso de una combinación de genotipado del CYP2B6 y de la monitorización de fármacos (TDM) como estrategia para minimizar la toxicidad y la resistencia viral. De esta manera, genotipos CYP2B6 "de alto riesgo" podrían ser detectados, identificando por tanto a aquellos individuos que podrían beneficiarse de una TDM temprana, ya sea para optimizar la dosis o para elegir otra terapia apropiada.

Rodríguez-Novoa y cols.[69] también sugirieron la prescripción de dosis más bajas de EFV en sujetos con genotipo T/T del CYP2B6 con objeto de minimizar los AAM sin comprometer la eficacia del fármaco. Al igual que, Nyakutira C y cols.[67], los cuales observaron el polimorfismo CYP2B6*6 es muy frecuente en población africana y que está asociado con elevadas concentraciones de efavirenz, por lo que a priori podría realizarse una reducción de dosis en pacientes con genotipo T/T de hasta un 35%.

Recientemente también se han realizado más estudios en poblaciones diversas razas (Caucasiana, Hispánica, etc), no sólo de la africana, confirmando también la correlación del genotipo T/T del CYP2B6*6 con concentraciones plasmáticas elevadas y mayor riesgo de presentar efectos adversos[62].

Por otro lado, es interesante destacar que Nuñez y cols.[34] encontraron un porcentaje más elevado de concentraciones subterapéuticas de EFV en pacientes con genotipo común que en aquellos con el polimorfismo (19% en G/G frente a 2% en T/T). Estas diferencias podrían tener repercusión sobre la aparición de resistencias al EFV, debido a la diferente exposición plasmática observada en sujetos con los diferentes genotipos.

Además de las variantes alélicas más conocidas, actualmente también se están describiendo nuevos polimorfismos asociados con pérdida o disminución de la actividad enzimática del CYP2B6 como: 983T>C, 785A>G, 593T>C y 1132C>T que, especialmente en individuos homocigotos, representan un riesgo elevado de desarrollar concentraciones plasmáticas excesivas de EFV[62].

CYP3A4/A5

El CYP3A4 Y CYP3A5 son las isoenzimas que se encargan de aproximadamente del 50% del metabolismo del los fármacos. Su papel en el metabolismo hepático de EFV aún no está totalmente definido, aunque varios estudios sugieren una menor influencia en este proceso[70-72]. Las dos isoenzimas presentan varios polimorfismos genéticos, 20 alelos diferentes han sido descritos para CYP3A4 y 11 para CYP3A5. Así, varios de ellos entre los que se encuentran el CYP3A4*18 (878T>C), CYP3A4*1B (-392A>G), CYP3A5*3 (6986A>G) y el CYP3A5*6(14690G>A) han sido estudiados para analizar su influencia en la cinética de EFV.

Únicamente, en un análisis detallado de estas isoenzimas realizado por Arab-Alamedine y col.[47] se llegó a la conclusión de que el alelo CYP3A4*1B podría tener influencia en el aclaramiento de EFV. Sin embargo, esta influencia solo se observó en los pacientes que tenían el genotipo CYP2B6 516 T/T. Por lo que son necesarios más estudios para confirmar estos resultados.

CYP2A6

Recientemente se ha demostrado que la isoenzima CYP2A6 puede desempeñar un papel importante en el metabolismo de EFV. Esta isoenzima también es muy polimórfica, ya que se han descrito hasta el momento más de 30 alelos diferentes, algunos de los cuales están relacionados con una disminución en su actividad enzimática. Varios estudios han evaluado su influencia con la disminución del metabolismo de EFV encontrándose diferentes resultados. Así, Kwara y cols.[56] afirma que las variaciones genéticas (CYP2A9*9B y CYP2A6*17) pueden ser predictores independientes de las concentraciones plasmáticas de EFV, mientras que Arab-alamedine y col.[47] sólo encuentra una influencia

significativa con los niveles plasmáticos de EFV cuando los pacientes tienen genotipos metabolizadores lentos para el CYP2B6.

OTROS CYPs

En relación con los SNPs de isoenzimas CYP2C19, CYP2C9, CYP2C8 y CYP2D6, hay pocos estudios que hayan analizado estos polimorfismos, probablemente debido a su baja o desconocida contribución en el metabolismo de EFV. Sin embargo, para algunos polimorfismos del CYP2D6 (CYP2D6*3 (2549A>del), CYP2D6*4 (1846G>A) y CYP2D6*6 (1707T>del), Fellay y cols.[71] encontraron que los niveles plasmáticos de EFV tienden a aumentar.

UGT2B7

Las UDP-glucuroniltransferasas son unas enzimas encargadas de catalizar la conjugación de un amplio grupo de sustratos. Normalmente esta reacción se considera detoxificante. Dentro de este grupo, la UGT1A1 es una enzima específica que se encarga de catalizar la conjugación de la bilirrubina. Se han descrito más de 30 polimorfismos genéticos que pueden anular o reducir la actividad de esta enzima, causando enfermedades hepáticas más o menos graves.

Recientemente, también han sido estudiados los polimorfismos genéticos de la isoenzima UGT2B7, ya que se ha observado que es la principal enzima implicada en la N-glucuronidación del EFV. Los polimorfismos que se han estudiado son el UGT2B7*1c (735 A>G) y UGT2B7*2 (802 C>T), este último ha sido relacionado con niveles más elevados de EFV[73] por lo que también debería ser considerado a la hora de optimizar el tratamiento con este fármaco.

Polimorfismos en proteínas transportadoras

MDR1

La P-glicoproteína (P-gp) es una proteína transportadora de membrana que pertenece a la familia de transportadores ABC, subfamilia MDR/TAP. Se encuentra distribuida ampliamente por todo el organismo (hígado, páncreas, riñón, etc.), aunque presenta un alto nivel de expresión en el intestino y en la barrera hematoencefálica. Su función es expulsar de la célula a una gran variedad de sustratos, entre ellos fármacos, por lo que puede disminuir su concentración intracelular. Los polimorfismos genéticos de esta proteína han sido ampliamente estudiados y se han descrito un total de 50 polimorfismos y 3 deleciones/inserciones. Entre los más importantes destacan el 3435 C>T y 2677 G>T/A, que están asociados a una disminución en la expresión de la proteína. Varios estudios han sugerido que estos polimorfismos podían estar relacionados con bajas concentraciones de EFV, sin embargo los resultados no han sido concluyentes.

PROTEÍNAS DE MULTIRRESISTENCIAS (MRP)

Las proteínas de multirresistencias (MRP) están codificadas por los genes ABCC1, ABCC2, ABCC3 y ABCC4; y desempeñan también un papel importante en el transporte de ART.

Numerosos polimorfismos genéticos han sido descritos en estas proteínas transportadoras. Sin embargo se han realizado pocos estudios para evaluar su relación con la farmacocinética de EFV, además en ellos no se ha encontrado ninguna influencia[71].

Tabla 1. *Resumen de los principales polimorfismos genéticos implicados en el metabolismo y transporte de efavirenz.*

GEN	**Alelo /SNP**	**Efecto en la respuesta**
CYP2B6	CYP2B6*6 (516 G>T) CYP2B6*16 (983 T>C)	Aumento de las concentraciones plasmáticas.
CYP2A6	CYP2A9*9B CYP2A6*17	Posible aumento de las concentraciones plasmáticas.
CYP3A4	CYP3A4*1B (-392 A>G)	Posible aumento de las concentraciones plasmáticas.
CYP2D6	CYP2D6*3 (2549 delA) CYP2D6*4 (1846 G>A) CYP2D6*6 (1707delT)	Posible aumento de las concentraciones plasmáticas.
ABCB1 (MDR1)	3435 C>T 2677 G>T	Disminución de la expresión de la proteína.
UGT2B7	UGT2B7*1c (735 A>G) UGT2B7*2 (802 C>T)	N.D.

* N.D. = No disponible

Conclusiones

EFV es un fármaco que ocupa un importante lugar en la terapia antirretroviral, cuya eficacia y seguridad ha sido ampliamente estudiada y demostrada. Sin embargo debido a la elevada variabilidad interindividual en su respuesta, es necesario encontrar nuevas herramientas que nos ayuden a optimizar el tratamiento con este fármaco. La TDM y el análisis farmacogenético parecen ser dos buenas estrategias para conseguir este objetivo.

Así, el reconocimiento de que determinados polimorfismos genéticos pueden influir en la farmacocinética de EFV y condicionar diferencias farmacocinéticas marcadas entre los individuos podría tener importantes implicaciones en la terapia antirretroviral.

En el momento actual este fármaco se administra a una dosis fija de 600 mg una vez al día. La posibilidad de que una dosis más baja pudiera reducir los efectos adversos manteniendo la eficacia en pacientes con variantes alélicas del CYP2B6 asociadas con una mayor exposición al fármaco, resulta muy atractiva, tanto es así que ya se ha empleado con éxito en casos aislados.

La genotipificación del CYP2B6 podría así, ser de utilidad como adyuvante para una estrategia de terapia personalizada, basada en la medición de las concentraciones plasmáticas de EFV, orientada a incrementar la seguridad y la tolerancia de este fármaco. No obstante, es probable que el alto grado de superposición entre los genotipos y la multiplicidad de factores que pueden influir en la exposición al fármaco limiten el valor de los polimorfismos individuales en la práctica clínica.

Por tanto, la decisión última de ajuste posológico debería pasar siempre a través de un estudio fenotípico por medio de la determinación de las concentraciones plasmáticas de EFV.

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Capítulo II

Long-Term Efficacy and Safety of Efavirenz Dose Reduction to 200 mg Once Daily in a Caucasian Patient with HIV

A Case Report

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Clinical Drug Investigation

Abstract

A 48-year-old Caucasian male patient presented with severe adverse drug events (ADEs) while being treated with a standard dose of efavirenz (EFV). The patient had good clinical evolution; however, he described the presence of intense nightmares, cramps in his legs and stress that caused him a high degree of irritability.

Determination of EFV plasma levels showed a mean minimum concentration (C_{min}) of 12.7 mg/l, much higher than that recommended for this drug. Accordingly, the first dose reduction took place (to 400 mg/day), which decreased the frequency of ADEs.

Genotype testing results showed that the patient was homozygous for both the CYP2B6-G516T (T/T) and CYP2B6-A785G (G/G) alleles. Because of this and the fact that the EFV C_{min} (4.6 mg/l) was still high, the second dose reduction took place (to 200 mg/day). At present, CD4+ levels remain stable, the viral load continues to be undetectable and the mean EFV C_{min} (2.7 mg/l) is within the therapeutic range.

Introduction

Efavirenz (EFV) is an antiretroviral recommended as a first-line treatment for human immunodeficiency virus (HIV) infection in numerous international guidelines, because of its efficacy and patient tolerance. Nevertheless, this drug has a wide range of neuropsychiatric effects (sleep disturbances, dizziness, sadness, mood changes, irritability, nervousness, impaired concentration, abnormal dreams and somnolence) in up to 40–70% of patients, according to some published reports.[1–3] These complaints usually last for the first two to four weeks of treatment.[4,5] However, some studies show that neuropsychiatric disorders may indeed persist in more than one-half of patients receiving long-term EFV therapy.[6]

Adverse drug events (ADEs) have been reported to be more frequent in patients with high EFV plasma concentrations,[7–9] although this association has not been supported by other studies,[6,10,11] which makes further research necessary.

Several studies have revealed that the existence of genetic variations in certain proteins involved in either the transportation (P-glycoprotein) or the metabolism (CYP2B6, CYP2A6, CYP3A4, CYP3A5, CYP2D6, etc.) of EFV[12] could explain high inter-patient variability.[13–18] On the other hand, these studies have demonstrated that high EFV plasma levels and genetic variants are associated with neuropsychiatric ADEs.[7,8,13,18–21] Thus, this relationship opens a door to evaluate whether reduced EFV dosage in 'real world' practice could diminish ADEs without compromising the drug's virologic efficacy.[22,23]

To our knowledge, limited information exists on the long-term efficacy and safety of EFV dose reduction. We report the case of a Caucasian male patient who presented with severe ADEs while being treated with a standard dose of EFV. An EFV dose reduction to 200 mg/day increased safety margins while maintaining the efficacy of long-term therapy.

Case Report

A 48-year-old Caucasian male patient had his HIV infection documented in 1989. A nadir CD4+ lymphocyte level (137/μl) and a peak viral load of 26,032 copies/ml was attained in early 2000 and antiretroviral treatment (ART) consisting of zidovudine, lamivudine and EFV was recommended. The patient was co-infected with hepatitis C virus (HCV) but without liver fibrosis.

The patient presented with severe ADEs while being treated with a standard dose of EFV (600 mg/day). He described in every clinical control the presence of intense nightmares (vivid dreams with progressively increasing anxiety, ultimately resulting in wakefulness), dizziness, anxiety disturbances (nervousness, irritability) and intense cramps in his legs. The patient did not have a previous history of mental disorders and was not taking psychiatric medication at the time of the study.

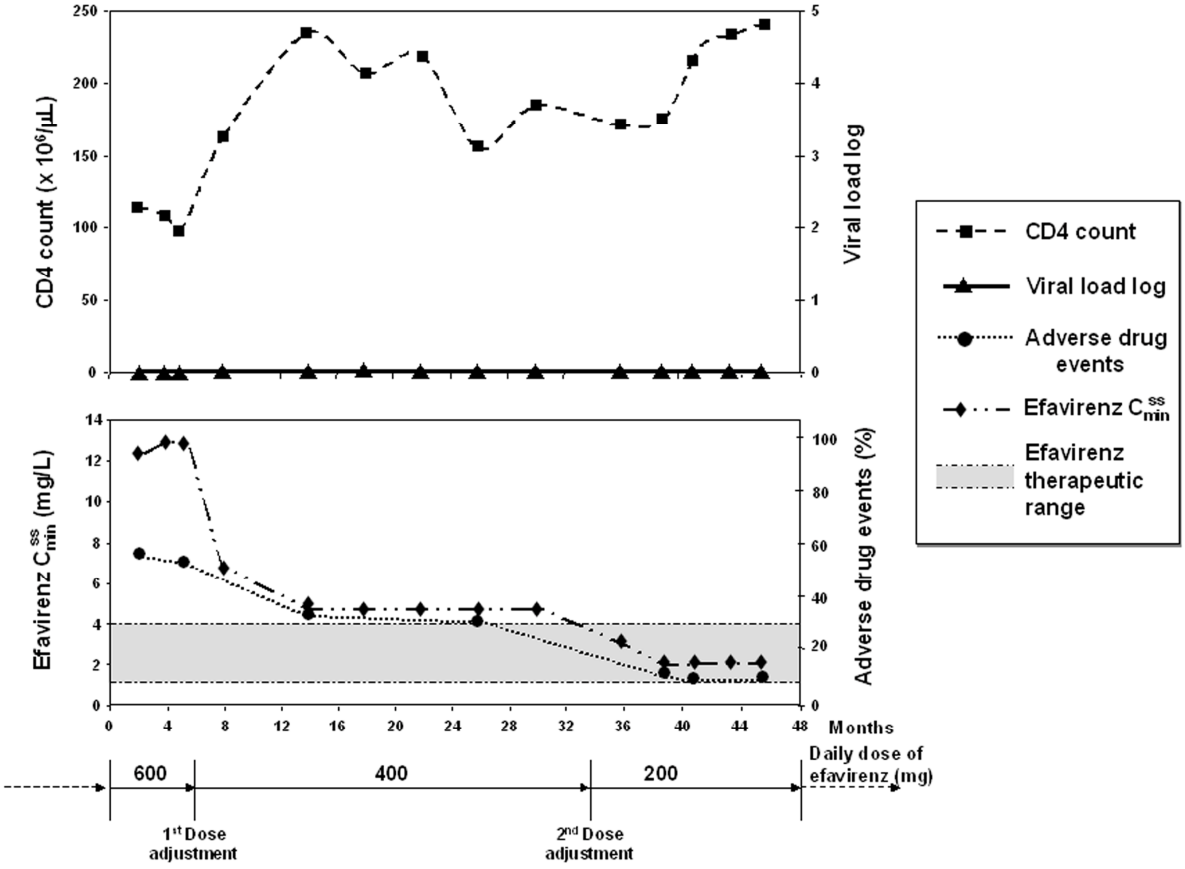
The patient was enrolled in a therapeutic drug monitoring (TDM) programme, which involved the taking of one blood sample during each visit to the hospital. Samples for the measurement of plasma drug concentrations were collected at steady-state (more than 4 weeks after the initiation of EFV treatment), usually at the mid-point of the dosage interval. EFV concentrations were assessed quantitatively by high-performance liquid chromatography (HPLC). In October 2005, the determination of EFV plasma levels showed a mean minimum concentration (C_{min}) of 12.7 mg/l, much higher than that recommended for this drug (EFV therapeutic range, 1–4 mg/l).[7]

Accordingly, in March 2006, the first dose reduction took place (to 400 mg/day). This posologic adjustment was done following pharmacokinetic analysis. For this analysis, assuming an open, one-compartment model with a fixed absorption constant and first-order elimination[18], the apparent oral clearance (CL/F) and apparent distribution volume (Vd/F) parameters were individually estimated using Bayesian algorithms. The population pharmacokinetic parameters were obtained from our own population and were incorporated into PKS® software. This software allows us to predict the dose adjustment and concentrations at different times, using the population

pharmacokinetic parameters and two or three EFV plasma levels obtained from the patient.

After this dose reduction, he reported a decrease in both the frequency of his nightmares and the intensity of his cramps. Our patient also commented that his irritability level had reduced. As anticipated, due to these improvements with regard to ADEs, our patient achieved a high level of satisfaction with the new dose.

Figure 1. Evolution of CD4 cell count, viral load, adverse drug events, and efavirenz plasma concentrations in the period analysis.



In May 2007, after having obtained informed consent from the patient and ethical approval from the Institutional Review Board of the University Hospital of Salamanca, Spain, genotype testing results (Table I) showed that the patient was homozygous for both the CYP2B6-G516T (T/T) and CYP2B6-A785G (G/G) alleles. This genotypic analysis was determined using PHARMAchip®

(Progenika Biopharma, Bilbao, Spain), a DNA-chip that analyses 91 polymorphisms present in 33 genes involved in phase I and II enzymatic metabolism, as well as transporters, neurotransmitter receptors and others.

Table I. Analysis of patient's genotype

Gene	Analyzed Allele	Genotype	Phenotype
CYP2B6	G516T	(T/T)	Reduced activity
CYP2B6	A785G	(G/G)	Reduced activity
CYP3A4	*1/*1B	(*1/*1)	Normal activity
CYP2D6	*1, *2, *3, *4, *5 (gene deletion), *6, *7, *8, *9, *10, *11, *14A, *14B, *15, *17, *19, *20, *25, *26, *29, *30, *31, *35, *36, *40, *41, gene duplication *1XN, *2XN, *4XN, *10XN, *17XN, *35XN, *41XN	(*2/*2)	Normal activity

Based on both genotypic results and the fact that the EFV C_{min} (4.6 mg/l) was still high, in July 2008 the second dose reduction took place (to 200 mg/day). One month after that he commented that his anxiety, nervousness and irritability had diminished considerably. Furthermore, before dose adjustment, he had also complained of numbness in the calves, which has since disappeared completely. Presently, in August 2009, CD4+ levels remain stable, the viral load remains undetectable and the mean EFV C_{min} (2.7 mg/l) has been within the therapeutic range in every clinical control (Figure 1). It is necessary to

emphasise that the patient's adherence to treatment was always optimal (100%) during the entire period of analysis, according to the records of dispensation of treatment and the simplified medication adherence questionnaire (SMAQ).[24] Likewise, during the entire period of the study, the absence of concomitant medication interfering with the EFV pharmacokinetic profile was assured.[25]

Discussion

To our knowledge, this is the first clinical case of EFV dose reduction to 200 mg/day that has demonstrated an increased safety margin while also maintaining the efficacy of long-term therapy in a patient of Caucasian extraction, given that until the present day such procedures have been described only in patients of African[26] and Japanese extraction.[22] At the same time, the duration of the follow-up is longer than in previously documented case reports, at 45 months.

Our patient experienced important ADEs with a standard dose (600 mg/day), in spite of the fact that the administration time was always before going to bed and that the effect of diet was minimised, since the patient's drug administration was performed at least 2 hours after the evening meal (in agreement with the recommendations of the pharmaceutical care programme), thereby eliminating any possible food–drug interactions.[4]

Accordingly, the evolution of ADEs (Figure 1) can be seen as having a direct proportional relationship between EFV plasma concentrations and the ADE score, expressed as a percentage of the maximum possible score (45 points). Thus, this leads to the conclusion that the ADEs described in our patient could be a consequence of elevated EFV plasma concentrations. To evaluate and quantify the evolution of ADEs, these were assessed in duplicate using a semi-structured interview at every posological interval. This interview included questions exploring common presumed EFV-related ADEs (cognitive, affective or mood, anxiety, psychotic and sleep disturbances), and it is based on two validated questionnaires (Pittsburgh Sleep Quality Index[27] and Hospital Anxiety and Depression Scale).[28]

These results show the opportunity that TDM offers in developing an optimal and individualised therapy for each patient. Also, they demonstrate that ADEs should not be always assumed to be an inherent consequence of ART, which can therefore be effective without causing significant toxicity. Also, these results open the door for investigation of the relationship between the ADEs of ART

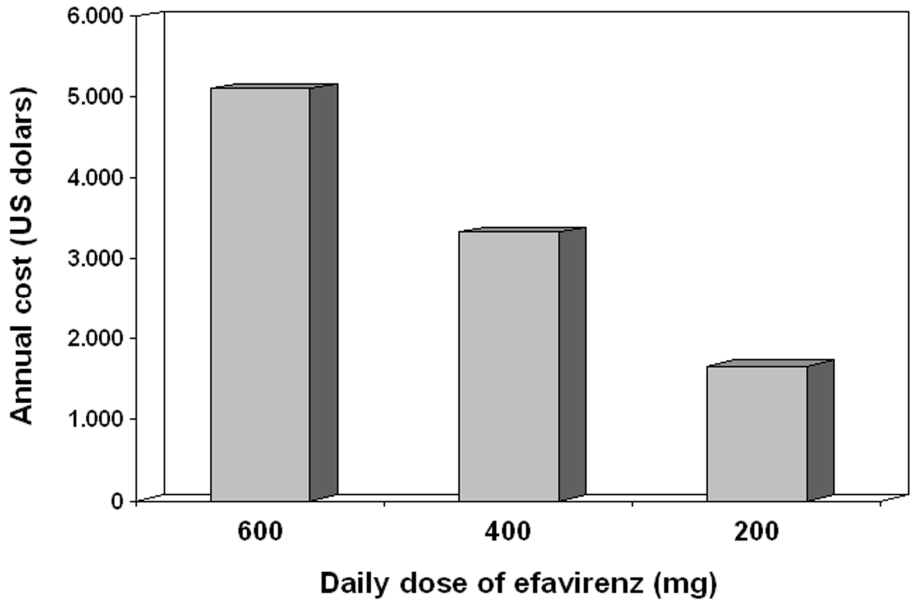
(lipodistrophy, dyslipidaemias, peripheral neuropathies, etc.) and high plasma concentrations, not only for EFV but also for other antiretrovirals.

There is no doubt that the main objective of ART is to achieve virological suppression, and thus restore the efficiency of the immune system to the greatest extent possible. The antiviral efficiency of EFV is high, but its high inter-individual pharmacokinetic variability complicates the achievement of long-term benefits.[29] Differences in the hepatic metabolism of EFV seem to explain much of this discrepancy. In our case, genotype testing results show that our patient is homozygous for the CYP2B6 G516T and A785G alleles. These polymorphisms are associated with a reduced enzymatic activity, which increases EFV plasma concentrations.[8,9,19–22,30–32] Thus, adequate interpretation of pharmacogenetic and pharmacokinetic data could be used to individualise treatment with this drug. However, in clinical practice, the pharmacogenetic data justify the information provided by TDM, even if the main advantage of the latter is that it reflects the phenotype through the pharmacokinetic behaviour of the drug in a particular patient, which depends mainly on the hepatic metabolism.[33] This, added to low intra-patient variability in EFV plasma concentrations and high inter-individual variability,[34] shows not only that TDM could be useful in the clinical management of HIV disease,[35] but also that in clinical practice TDM continues to be the best tool for optimizing the dosage regimen of EFV.[36]

It is important also to take the economic aspect into consideration, since dose reductions have relevant economic impact on this kind of treatment. The cost of treatment with EFV in this case decreases by approximately one-third with each dose reduction and, owing to the fact that the optimal dose is 200 mg/day, the annual savings would be US$3,446 per year (Figure 2). In addition, the costs associated with the determination of EFV plasma levels are minimal if we compare these with the savings generated by dose reduction. Thus, the average cost of testing plasma levels is about US$40, which includes personnel and technical expenses (technicians and HPLC equipment). Therefore, considering that we need three determinations of plasma levels to ensure that these values reflect the real exposure of the patient to the drug, the total cost of the "diagnosis of overdosage" is about US$120. This fact justifies the

implementation of TDM, which would induce more patients to access antiretroviral therapy because of the savings in treatment costs.

Figure 2. Comparison of annual cost and daily dose of efavirenz in the period analysis.



Conclusion

The factors that restrain dosage reduction in 'real world' practice when directed at the minimisation of ADEs are (1) the low genetic barrier of EFV; and (2) the fact that dose adjustment of antiretrovirals guided by TDM has not been extensively studied. However, based upon this case, the relationship between ADEs and EFV plasma levels confirms the usefulness of TDM in dosage individualization as one way of optimizing the management of long-term EFV therapy.

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Capítulo III

Extensive Genotyping Enzymes Metabolizing and Proteins Transport Genes for Predict Efavirenz Plasma Concentrations

Original Article

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Abstract

The administation of a fixed dose of 600 mg of efavirenz (EFV), an essential component of antiretroviral therapy, can result in significant differences in patient response to treatment, which can be attributed to high inter-individual variability in the disposition kinetics of EFV. The main objective of this study was to identify and analyze to what extent genetic factors are implicated in this variability. For this, this work performed a comprehensive pharmacogenetic analysis in a total of 125 HIV-infected Caucasian patients, getting to be one of the studies that a largest number of single nucleotide polimorphisms (SNPs) analyzed (total 90) in greater number of genes coding for proteins involved in the metabolism and transport of EFV.

Genotypes were mainly determined using Sequenom´s high-throughput matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), while plasma concentrations were assayed using quantitatively with high-performance liquid chromatography with ultraviolet detection (HPLC-UV) system. The estimation of pharmacokinetic (PK) parameters (the apparent oral clearance (CL/F), the maximum steady-state plasma concentration ($C_{max\ ss}$), the minimum steady-state plasma concentration ($C_{min\ ss}$)) from plasma concentration data for each patient was generated using Bayesian algorithms. On the other hand, relationships among genetic factors, PK parameters and demographic characteristics were examined by linear regression analysis. These data were also analysed by stepwise multiple regression to develop multivariate models, which might predict the variability of these PK parameters.

The obtained results show that about 50% of variability of PK parameters might be explained by genetic factors in final predictive models. Among the most significant SNPs are mainly those associated with the genes encoding CYP2B6 and CYP2A6 enzymes and MRP4 transport protein.The knowledge of influence of this genetic information on PK of EFV could be successfully utilized for optimization therapy of this drug.

Introduction

Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that has been accepted worldwide in association with two nucleoside analogue reverse transcriptase inhibitors (NRTIs) as a preferred first-line antiretroviral (ARV) therapy.[1-3] Despite the appropiate efficacy and safety demonstrated by EFV in many clinical trials,[4-6] current clinical experience has revealed important differences in patient response to treatment. Thus, when it is administered at a fixed dosage of 600 mg once daily, some patients suffer from central nervous system (CNS) toxicity or fail to achieve durable viral load suppression, which to a large extent can be attributed to high inter-individual variability in the disposition kinetics of EFV.[7] In addition, there is a concentration-effect relationship since this toxicity and inefficacy has been associated with high (> 4 mg/L) and low (< 1 mg/L) minimum EFV plasma concentrations ($C_{ss\ min}$), respectively.[8-13] Because of this, it is of substantial clinical importance to identify factors that contribute to pharmacokinetic (PK) variability of EFV for optimitazion of treatment.

As for most drugs, many factors are involved, including biological, environmental and genetic ones, by affecting the expression and/or function of proteins that interact with EFV at various levels. In the present, the role of genetic polymorphisms in genes encoding these proteins (metabolizing enzymes and transporters) is becoming very important, because they determine the process of absorption, distribution, metabolism, excretion (ADME) of EFV, and therefore, pharmacologic response.[14]

EFV is predominantly metabolised in the liver by the cytochrome P450 system (CYP) to hidroxylated metabolites (8-hidoxi and 7-hidroxyefavirenz), which are subsequently urinary and biliary excretion after conjugation (mainly glucuronidation) by uridine-glucuronyl-transferases.[15-17]

CYP2B6 is the main enzyme responsible for hidroxilation[15] with partial involvement of CYP3A4/3A5 and acording to recent studies of CYP2A6.[18-21] In adition, several other CYPs, including CYP2D6, CYP2C9, CYP2C19 and

CYP2C8, may also contribute, although their individual role in EFV metabolism is not clearly defined. [21-26]

Genetic polimorphisms in these enzymes genes may change their activity and therefore explain a large part of the high inter-individual variability. CYP2B6 genetic variants has been the most studied, in particular the single nucleotide polymorfism (SNP) CYP2B6 516 G>T (rs 3745274), which has been significantly associated with a pronounced reduced enzyme activity and consequently elevated EFV plasma concentrations in studies of different populations.[16,18,19-21,23-36] Most recently, the SNPs CYP2B6 983 T>G (rs 28399499) and 785 A>G (rs 2279343) have also been reported to affect EFV plasma concentrations.[26,34-37] These data have demostrated that CYP2B6 poor metabolizer genotypes can identify individuals at risk of high EFV plasma concentrations. Also, some genetic polymorphisms of others CYPs (CYP3A4/3A5, CYP2A6, CYP2D6, CYP2C9, CYP2C19, CYP2C8) have been analyzed in several studies, but their influence on EFV pharmacokinetics has not yet been well characterized. [21-26]

On the other hand, this variability may also be explained in part by polymorphisms of proteins transporter genes. There are two subfamily proteins particularly involved in the transport of antiretrovirals.These are the multidrug resistance (MDR)/TAP (subfamily B) and the multidrug resistance-associated proteins (MRP)/CFTR (subfamily C), which are members of the adenosine triphosphate ATP-binding cassette (ABC) superfamily of proteins. There is relatively limited information on the functional role of these ABC transporters in the disposition of EFV.[22,24]

Although MDR1 genetic variations has been the most studied,[22-24,27,31] there is now increasing evidence to suggest that genetic variations in others ABC transporters also can demonstrated high involvement in EFV plasma exposure. But it is yet not clearly defined and further studies are required to assess the clinical relevance.

Multiple polymorphisms in many genes may affect EFV response. However, despite numerous ongoing studies in this field, it is yet unknow which of them are specifically involved, because of this, requiring a genome-wide search for

the responsible genes. In adition, most previous studies have focused on individual polymorphisms, instead of accounting for combinations of SNPs.

Therefore, the aim of our study was to identify and characterize pharmacogenetic (PG) factors that influence inter-individual variability on EFV PK parameters to apply the results in clinical practice.

Material and methods

Study subjects and design

The present study enrolled HIV-positive adult patients treated with EFV at the outpatient unit of Pharmacy Service of the University Hospital of Salamanca (Spain). All patients were receiving, as initial dose, 600 mg oral EFV once a day in combination with two NRTIs as part of their ARV regimen.

All patients must meet the following criteria for entry into the study: confirmed HIV infection; treatment with EFV during at least 3 months (unchanged dosage for at least 1 month); age ≥ 18 years; adherence to the treatment regimen > 90% and no co-medication with known inducer or inhibitor drugs of EFV metabolism. The study was approved by the Ethics Committee of the University Hospital of Salamanca. Written informed consent from each patient and blood sample was obtained for genetic testing.

The patients were included in a therapeutic drug monitoring (TDM) program and plasma samples for EFV assay were drawn periodically at 3 to 6 month intervals on follow-up visits to the hospital, along with viral and biochemical tests. Individual information was carefully recorded at the time of collecting blood samples and included dose history, sampling time, time of last dose, ethnicity, gender, age, weight, height, concomitant pathologies (hepatitis C virus (VHC)), concomitant treatment (ARV and other drugs), life habits (tobacco consumption, alcohol intake) and adherence. Treatment adherence was assessed using ARV dispensation records, the Simplified Medication Adherence Questionnaire (SMAQ)[38] and a variation coefficient (CV) of the EFV concentration/dose ratio under 30% in each patient, according to the intra-patient variability observed previously.

Drug assays

Plasma samples for measuring drug concentrations were collected at steady-state (more than 4 weeks after the initiation of EFV treatment), usually at the mid-point of the dosage interval.

Blood samples (5 mL) were collected and plasma was isolated by centrifugation at 3000 g. Then samples were stored at −20 °C (previous virus inactivation in a water bath at 60 °C for 60 min) until analysis.

Concentrations of EFV were assessed quantitatively with high-performance liquid chromatography with ultraviolet detection (HPLC-UV) system (Waters, Milford, USA) with ultraviolet (UV) detection at 215 nm after previous solid phase extraction on the GX-271 ASPEC (Gilson, Villiers le Bel, France). This method was validated over the 0.5 to 10 mg/L range using 600 µL of plasma. The recovery of EFV from human plasma was 107.4%. Within and between-day precisions, expressed as CV, were always < 5.7% for all the internal quality controls (0.5, 2.0 and 10.0 mg/L). The limit of quantification was 0.25 mg/L and the specificity of the 21 drugs most used in HIV patients was tested. Our analysis laboratory participates in the International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring in HIV infection (Dutch association for Quality assessment in Therapeutic Drug Monitoring and Clinical Toxicology [KKGT]), and successful results have been obtained.

Estimates of pharmacokinetic parameters

The PK of EFV was characterized assuming an open one-compartment model with a fixed absorption constant (Ka=0.30 h^{-1}) and first-order elimination and using the nonlinear mixed effect modelling program (NONMEM® version VI; double precision, level 2.0).[39] The population PK parameters EFV obtained of our previous study [27] (CL/F=9.5 L/h; $CV_{CL/F:}$36.4%, Vd/F=311 L; $CV_{Vd/F:}$55.14%) were incorporated in clinical pharmacokinetic software (PKS® software, Abbot-Diagnostic, Chicago, USA). Estimated of the parameters (the apparent oral clearance (CL/F), apparent distribution volume (Vd/F), the maximum steady-state plasma concentration ($C_{max\ ss}$), the minimum steady-state plasma concentration ($C_{min\ ss}$), the elimination half-life ($t_{½}$) and the elimination constant (K_e)) from plasma concentration data for each individual were generated using Bayesian algorithms. These parameters were used for statistical analysis.

Genotyping analysis

Selection SNPs

SNPs were selected based on three main criteria: (1) SNP identified or presumed reports on functionality for enzymes and transporters of EFV, (2) the SNPs chosen are either functional SNPs (based on potential protein changes, or (3) SNPs which were reported by other groups from public databases (CYP alleles: http://www.cypalleles.ki.se and dbSNP: http://www.ncbi.nlm.nih.gov/SNP/).

Genotyping assays

All the genotyping experiments were conducted at the Spanish National Genotyping Centre (CeGen).
Genomic DNA was isolated automatically from 5 mL whole human blood using magnetic bead technology with the Chemagic Magnetic Separation Module I and the Chemagic DNA kit, according to the manufacturer's recommendations (Chemagen AG, Baesweiler, Germany).

Genotypes of metabolizing enzymes CYP and drug transporter genes (see table 1) were mainly determined using Sequenom´s high-throughput matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) in six multiplexes. Three control of *Centre d'Etude du polymorphisme Humain* (CEFP) and two non-template controls were included on each plate for sample and genotype, as well as 20% duplicate samples. In short, PCR amplification of the 80 AML and blood DNA samples was done using SNP–specific primers, followed by a base extension reaction using iPLEX chemistry (Sequenom). The PCR condition was 94°C for 15 min for hot start, followed by denaturation at 94°C for 20 s, annealing at 56°C for 30 s, extension at 72°C for 1 min for 45 cycles, and final incubation at 72°C for 3 min. The PCR products were then treated with 2 µL of shrimp alkaline phosphatase (Sequenom) for 20 min at 37°C, then ramped to 85°C for 5 min to remove excess deoxynucleotide triphosphates.

Table 1. Genotype date of analyzed SNPs.

			GENOTYPE SUBJECTS							QUALITY CONTROL		
			Wild-type		Heterozygous		Homozygous		Total	HWE	Genotyping Rate	MAF
rs NUMBER	GENE	GEN POSITION	(n, %)		(n, %)		(n, %)		(N)	(prob)	(%)	(%)
rs1801272	CYP2A6	479 T>A	118	94.40	7	5.60	0	0.00	125	0.7474	100.0	0.0280
rs28399435	CYP2A6	86 G>A	113	92.62	9	7.38	0	0.00	122	0.6723	97.60	0.0369
rs28399441	CYP2A6	459 G>A	123	98.40	2	1.60	0	0.00	125	0.9282	100.0	0.0080
rs28399454	CYP2A6	1093 G>A	124	99.20	1	0.80	0	0.00	125	0.9642	100.0	0.0040
rs72549433	CYP2A6	580 A>G	123	99.19	0	0.00	1	0.81	124	0.0000	99.20	0.0081
rs8192726	CYP2A6	1836 G>T	112	90.32	12	9.68	0	0.00	124	0.5712	99.20	0.0484
rs2279343	CYP2B6	785 A>G	73	58.40	44	35.20	8	6.40	125	0.6948	100.0	0.2400
rs28399499	CYP2B6	983 T>C	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
rs3211371	CYP2B6	1459 C>T	104	83.87	18	14.52	2	1.61	124	0.2552	99.20	0.0887
rs34097093	CYP2B6	1132 C>T	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
rs35303484	CYP2B6	136 A>G	118	95.93	5	4.07	0	0.00	123	0.8180	98.40	0.0203
rs36079186	CYP2B6	593 T>C	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
rs3745274	CYP2B6	516 G>T	76	60.80	41	32.80	8	6.40	125	0.4453	100.0	0.2280
rs8192709	CYP2B6	64 C>T	115	96.64	4	3.36	0	0.00	119	0.8521	95.20	0.0168
rs12248560	CYP2C19	-806 C>T	82	65.60	37	29.60	6	4.80	125	0.4965	100.0	0.1960
rs41291556	CYP2C19	358 T>C	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
rs4244285	CYP2C19	681 G>A	93	77.50	23	19.17	4	3.33	120	0.1049	96.00	0.1292
rs10509681	CYP2C8	1196 A>G	91	72.80	31	24.80	3	2.40	125	0.8526	100.0	0.1480
rs11572080	CYP2C8	416 G>A	91	72.80	31	24.80	3	2.40	125	0.8526	100.0	0.1480
rs11572103	CYP2C8	805 A>T	120	96.00	5	4.00	0	0.00	125	0.8195	100.0	0.0200
rs1057910_1	CYP2C9	1075 A>C	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
rs1057911	CYP2C9	1425 A>T	103	83.74	20	16.26	0	0.00	123	0.3264	98.40	0.0813
rs1799853_1	CYP2C9	430 C>T	121	100.0	0	0.00	0	0.00	121	-	96.80	0.0000
rs28371685	CYP2C9	1003 C>T	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
CYP2D6_1039	CYP2D6	1039 C>T	119	95.97	4	3.23	1	0.81	124	0.0004	99.20	0.0242
CYP2D6_124	CYP2D6	124 G>A	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
CYP2D6_1584	CYP2D6	1584 C>G	73	59.84	32	26.23	17	13.93	122	0.0002	97.60	0.2705
CYP2D6_1659	CYP2D6	1659 G>A	124	100.0	0	0.00	0	0.00	124	-	99.20	0.0000
CYP2D6_1661	CYP2D6	1661 G>C	42	34.43	46	37.70	34	27.87	122	0.0074	97.60	0.4672
CYP2D6_1758	CYP2D6	1758 G>A	124	100.0	0	0.00	0	0.00	124	-	99.20	0.0000
CYP2D6_1869	CYP2D6	1869 T>C	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
CYP2D6_1973i	CYP2D6	1973-1974insG	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
CYP2D6_2097	CYP2D6	2097 A>G	87	76.32	22	19.30	5	4.39	114	0.0325	91.20	0.1404
CYP2D6_2466	CYP2D6	2466 T>C	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
CYP2D6_2539d	CYP2D6	2539-2542del AACT	124	99.20	0	0.00	1	0.80	125	0.0000	100.0	0.0080
CYP2D6_2613d	CYP2D6	2613-2615delAGA	109	91.60	0	0.00	10	8.40	119	0.0000	95.20	0.0840
CYP2D6_2935	CYP2D6	2935A>C	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
CYP2D6_31	CYP2D6	31 G>A	104	83.20	20	16.00	1	0.80	125	0.9716	100.0	0.0880
CYP2D6_3183	CYP2D6	3183 G>A	122	99.19	1	0.81	0	0.00	123	0.9639	98.40	0.0041
CYP2D6_3198	CYP2D6	3198 C>G	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
CYP2D6_4042	CYP2D6	4042 G>A	109	99.09	1	0.91	0	0.00	110	0.9618	88.00	0.0045

Table 1. Continued

			GENOTYPE SUBJECTS							QUALITY CONTROL		
			Wild-type		Heterozygous		Homozygous		Total	HWE	Genotyping Rate	MAF
rs NUMBER	GENE	GEN POSITION	(n, %)		(n, %)		(n, %)		(N)	(prob)	(%)	(%)
CYP2D6_845_883	CYP2D6	CYP2D6_845_883#1	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
CYP2D6_883	CYP2D6	883 G>C	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
rs1065852	CYP2D6	100 C>T	86	72.27	27	22.69	6	5.04	119	0.0606	95.20	0.1639
rs16947	CYP2D6	2850 C>T	58	47.93	33	27.27	30	24.79	121	31.5392	96.80	0.3843
rs28371706	CYP2D6	1023 C>T	123	100.0	0	0.00	0	0.00	123	-	98.40	0.0000
rs3892097	CYP2D6	1846 G>A	88	72.73	27	22.31	6	4.96	121	0.0547	96.80	0.1612
rs4986774	CYP2D6	2549 A>del	120	96.77	0	0.00	4	3.23	124	0.0000	99.20	0.0323
rs5030655	CYP2D6	1707 T>del	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
rs2740574	CYP3A4	-392 A>G	115	92.00	8	6.40	2	1.60	125	0.0008	100.0	0.0480
rs28371759	CYP3A4	878 T>G	124	100.0	0	0.00	0	0.00	124	-	99.20	0.0000
rs10264272	CYP3A5	14690 G>A	121	96.80	4	3.20	0	0.00	125	0.8557	100.0	0.0160
rs28365085	CYP3A5	31551 T>C	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
rs28365095	CYP3A5	-86 G>A	124	99.20	1	0.80	0	0.00	125	0.9642	100.0	0.0040
rs28371764	CYP3A5	-74 C>T	117	93.60	8	6.40	0	0.00	125	0.7117	100.0	0.0320
rs41279854	CYP3A5	29753 T>C	124	100.0	0	0.00	0	0.00	124	-	99.20	0.0000
rs4646453	CYP3A5	17163 G>T	119	95.20	6	4.80	0	0.00	125	0.7834	100.0	0.0240
rs776746	CYP3A5	6986 A>G	104	83.20	19	15.20	2	1.60	125	0.3132	100.0	0.0920
rs1045642	MDR1	3435 C>T	39	31.45	55	44.35	30	24.19	124	0.2282	99.20	0.4637
rs1128503	MDR1	1236 C>T	46	37.10	56	45.16	22	17.74	124	0.4926	99.20	0.4032
rs2229109	MDR1	1199 G>A	114	91.94	10	8.06	0	0.00	124	0.6399	99.20	0.0403
rs2235046	MDR1	TAG6	48	38.40	55	44.00	22	17.60	125	0.3699	100.0	0.3960
rs9282564	MDR1	61 A>G	112	89.60	13	10.40	0	0.00	125	0.5397	100.0	0.0520
rs246221_1	MRP1	825 T>C	50	40.65	64	52.03	9	7.32	123	0.0583	98.40	0.3333
rs35587	MRP1	1062 T>C	54	43.20	62	49.60	9	7.20	125	0.1183	100.0	0.3200
rs45560437	MRP1	816 G>A	114	91.20	11	8.80	0	0.00	125	0.6068	100.0	0.0440
ABCC2_259	MRP2	+259 G>T	123	98.40	2	1.60	0	0.00	125	0.9282	100.0	0.0080
rs17222723	MRP2	3563 T>A	110	88.00	15	12.00	0	0.00	125	0.4755	100.0	0.0600
rs2273697	MRP2	1249 G>A	81	65.32	40	32.26	3	2.42	124	0.4517	99.20	0.1855
rs3740066	MRP2	3972 C>T	46	36.80	55	44.00	24	19.20	125	0.3044	100.0	0.4120
rs7080681	MRP2	1058G>A	124	100.0	0	0.00	0	0.00	124	-	99.20	0.0000
rs717620	MRP2	-24 C>T	75	60.00	41	32.80	9	7.20	125	0.3120	100.0	0.2360
rs8187707	MRP2	4488 C>T	109	87.90	15	12.10	0	0.00	124	0.4734	99.20	0.0605
rs8187710	MRP2	4544 G>A	109	87.90	15	12.10	0	0.00	124	0.4734	99.20	0.0605
rs11568658	MRP4	559 G>T	114	91.20	11	8.80	0	0.00	125	0.6068	100.0	0.0440
rs11568695	MRP4	3724 G>A	124	99.20	1	0.80	0	0.00	125	0.9642	100.0	0.0040
rs12875235	MRP4	3725 G>T	96	76.80	27	21.60	2	1.60	125	0.9488	100.0	0.1240
rs1557070	MRP4	1497 C>T	121	96.80	4	3.20	0	0.00	125	0.8557	100.0	0.0160
rs1751034	MRP4	3463 A>G	85	68.00	34	27.20	6	4.80	125	0.2922	100.0	0.1840
rs2274405	MRP4	969 G>A	56	44.80	53	42.40	16	12.80	125	0.5367	100.0	0.3400
rs2274406	MRP4	951 G>A	53	43.09	53	43.09	17	13.82	123	0.5239	98.40	0.3537
rs2274407	MRP4	912 G>T	100	80.00	24	19.20	1	0.80	125	0.7355	100.0	0.1040
rs3742106	MRP4	4131 T>G	44	35.48	62	50.00	18	14.52	124	0.6086	99.20	0.3952

Table 1. Continued

			GENOTYPE SUBJECTS							QUALITY CONTROL		
			Wild-type		Heterozygous		Homozygous		Total	HWE	Genotyping Rate	MAF
rs NUMBER	GENE	GEN POSITION	(n, %)		(n, %)		(n, %)		(N)	(prob)	(%)	(%)
rs45616431	MRP4	3310 T>C	124	99.20	1	0.80	0	0.00	125	0.9642	100.0	0.0040
rs899494	MRP4	669 C>T	87	69.60	35	28.00	3	2.40	125	0.8133	100.0	0.1640
rs7439366	UGTB7	*1C	40	32.00	66	52.80	19	15.20	125	0.3325	100.0	0.4160
rs4149313	ABCA1	2649 A>G	75	60.48	43	34.68	6	4.84	124	0.9590	99.20	0.2218
M376CT	BCRP	376 C>T	125	100.0	0	0.00	0	0.00	125	-	100.0	0.0000
rs2231137	BCRP	34 G>A	107	85.60	17	13.60	1	0.80	125	0.7233	100.0	0.0760
rs2231142	BCRP	421 C>A	107	85.60	16	12.80	2	1.60	125	0.1448	100.0	0.0800

Genotyping CYP2B6 and CYP2D6 genes

Before genotyping by Sequenom, two genes (CYP2B6 and CYP2D6) needed to be amplified and separated from their respective pseudogenes, which was done with different methodologies. Briefly, CYP2B6 gen was performed in 10 µL of reaction mix containing 5 µL of Taq PCR Master Mix (Qiagen, Hilden, Germany), 1 µM of each primer, 1-10 ng DNA sample template and 3 µL of water. This PCR was carried out in a thermocycler GenAmp PCR System 9700 (AB), with one cycle of 95°C for 15 min and then 35 cycles of 94°C for 30 s, 60°C for 60 s and 72°C for 50 s with a full extension cycle of 72°C for 10 min. After this reaction, PCR products and negative controls were checked in the Agilent 2100 Bioanalyzer. The primers used were shown in suplementary table 1. While the entire CYP2D6 gene (5.1kb) was amplified in long-PCR reaction using primers CYP2D6-F (5*-CCAGAAGGCTTTGCAGGCTTCA-3´) and CYP2D6-R (5´-ACTGAGCCCTGGGAGGTAGGTA-3´) to separate the gene from the flanking highly homologous CYP2D8P and CYP2D7 pseudogenes, as described.[40]

Suplementary Table 1. The primers used in multiplex PCR to genotyping CYP2B6.

Exon	*Forward*	*Reverse*
Exon 1	GGACCTCAGCGTCCTCCTCT	CCATTCGTCTGTGTCTTACC
Exon 4	TCGGTCTGCCCATCTATAAA	TGATTCTTCACATGTCTGCG
Exon 5	GGAAATTTACATCTGACTAT	TCTCTCTCTCCCTCTGTCTT
Exon 7	CCACCCACCTCAACCTCCAA	AACCCTCCACACACTCCACA
Exon 9	ACACTGGTGACCTTCTGTGT	CCTGCACTCACTTGCAATGT

Genotyping others SNPs

Furthermore, some SNPs of CYP2A6 (rs28399454, rs34816076) and CYP3A5 (rs28365095) could not be analyzed by Sequenom, so they were performed with Real time PCR Taqman drug Metabolism Genotyping assays according to the specifications of the manufacturer.

The SNPs rs35303484, rs8192709 were verified by sequencing that did not pass quality control with the following technique Sequenom. The PCR was performed in 10 µL of reaction mix containing 4 µL of Taq PCR Master Mix (Qiagen, Hilden, Germany), 0,5 µL 1 µM of each primer, 1 µL sample template and 4 µL of water. This PCR was carried out in a thermocycler GenAmp PCR System 9700 (AB), with one cycle of 95°C for 15 min and then 35 cycles of 94°C for 30 s, 58°C for 90 s and 72°C for 90 s with a full extension cycle of 72°C for 10 min. After this reaction, PCR products and negative controls were checked in agilent. The PCR product was purified with ExoSAP-IT (Amersham Biosciences); 2,15 µL of PCR product was incubated with 0,85 µL ExoSAP-IT for 20 min at 37°C followed by 15 min at 80°C for enzyme inactivation. Sequencing reaction was performed in 11.5 µL of reaction mixture, containing 2.5 µL of sequencing buffer (5X), 0,5 µL of BigDye Terminator v 3.1 Cycle sequencing Kit (AB), 1 µL of the corresponding primer (final concentration was 1 µM), 3 µL of the purified PCR product and water up to 11.5 µL. Sequencing reaction was carried out in a thermocycler GenAmp PCR System 9700(AB) with one cycle of 96°C for 3 min and then 25 cycles of 96°C for 30 s, 50°C for 15 s and 60°C for 4 min or was carried out in a 9800 Fast Thermal Cycler (AB) with one cycle of 96 °C for 1 min then 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 1 min. To obtain "clean" electropherograms, the sequencing products were doubly purified, first using MontageTM SEQ 96 Sequencing Reaction cleanup Kit (Millipore Bedford, MA USA) according to manufacter protocol. Automatic sequencing was carried out in a capillary electrophoresis ABI3730 (AB). Each sample was sequenced in both forward and reverse directions; and analized by SeqScape v 2.5 (AB) software.

Statistics analysis

Statistical calculations were performed using PASW Statistics 18 (IBM SPSS Statistics) and R for Windows (SNPassoc library, Gonzalez et al, 2007). Unless otherwise indicated, $p < 0.05$ was considered to be statistically significant.

Initially, before the statistical analysis, EFV pharmacokinetic data (all dependent variables) were log-transformed to enhance the normality of their distribution and the homogeneity of their variances. Besides, genotyping data were filtered through genotype call rate (> 90% completeness), the Hardy-Weinberg equilibrium (HWE) test (*p*-value > 0.001) and a minor allele frequencies (MAF) criterion (> 1%) to ensure their adequate quality.

Relationship between patient demographic variables (age, body mass index (BMI), gender and race) and EFV PK parameters ($C_{min\ ss,.}$ $C_{max\ ss}$, $t_{1/2}$, K_e, V/F and CL/F) was analyzed by linear regression for quantitative variables (age, BMI) and t test for dichotomous variables (gender, race).

A linear regression was also carried out to evaluate association between individual SNP markers and EFV PK parameters in the context of additive and dominant genetic effect model. In the case that analyzed SNPs had less than five observations for the patients with homozygous genotype, these were always pooled with patients with heterozygous genotype. Age, gender, race and BMI were included as covariates in this regression model.

Because of the known influence of CYP2B6 516 G>T (the most significant SNP of CYP2B6 gene) on EFV PK parameters and to enhance detection of weaker effects, linear regression was also performed after adjusting for this SNP (rs3745274), using as dependent variable the residuals obtained in an univariate regression of log-EFV pharmacokinetic parameters on this SNP.

Finally, the combined effect of several SNPs and phenotypic covariates on the EFV pharmacokinetic parameters was performed by multiple linear regression. A forward stepwise procedure was used to obtain a minimal predictive model that could be useful for the determination of the individual's EFV concentration or metabolizer ability.

Results

Study population and pharmacokinetic parameters

One hundred and twenty-five HIV-infected patients treatment with EFV, which met all inclusion criteria, were finally enrolled in the study for genotype-phenotype analysis. The baseline demographic characteristic and pharmacokinetic parameters are sumarized in Table 2. Most patients were Caucasian ancestry (96.8%) and male (66.4%). Besides, most of them had good clinical evolution, because of the mean CD_{4+} lymphocytes cell count was 423 x 10^6 cells/mL and ninety-four patients (75,2%) had indetectable (< 40 copies/mL) plasma HIV RNA load. Among EFV PK parameters and according to margin therapeutic of EFV ($C_{min,ss}$ =1-4 mg/L),[41] a considerable percentage of patients (21.4%) had not therapeutic concentrations. So, eleven patients (8.7%) had concentrations above 4 mg/L (mean $C_{min\ ss}$ = 6.57 (± 3.52)) and sixteen patients (12.7%) under 1 mg/L (mean $C_{min\ ss}$ = 0.83 (± 0.11)), respectively.

Table 2. Demographic characteristics and EFV pharmacokinetic parameters of study population (n = 125).

CHARACTERISTICS	VALUES Mean ± SD (range) or n (%)
Demographic factors	
Age (years)	44,5 ± 9,52 (18-77)
Male	83 (66.4)
Race/ethnicity	
Caucasian	121 (96.8)
Others	4 (3.2)
BMI (Kg/m^2)	23,0 ± 3,36(13.90-36.90)
Pharmacokinetic parameters of EFV	
$C_{min\ ss}$ (mg/L)	2.23 ± 1,76 (0.62-12.84)
$C_{max\ ss}$ (mg/L)	4,38 ± 1,77 (2.48-14.15)
CL /F (L/h)	7,95 ± 3,19 (0.61-16.10)

Genetic polymorphisms

A total of 90 SNPs in genes coding for proteins involved in the metabolism and transport of EFV were analyzed. All patients were genotyped for 8 SNPs in CYP2B6, 6 SNPs in CYP2A6, 25 SNPs in CYP2D6, 3 SNPs in CYP2C8, 3 SNPs in CYP2C19, 4 SNPs in CYP2C9, 2 SNPs in CYP3A4, 7 SNPs in CYP3A5, 5 SNPs in MDR1, 3 SNPs in MRP1, 8 SNPs in MRP2, 11 SNPs in MRP4, 1 SNPs in UGTB7, 3 SNPs in BCRP and 1 SNPs in ABCA1.

For all genetic polymorphisms assayed, genotype data (including rs number, gene, gene position, genotype frequencies, HWE, genotyping rate and MAF) are listed in table 1. The observance frequencies of known SNPs were in according to published data. However, twenty-four were monomorphisms, ten had MAF <1% and other six were not in HWE equilibrium; so they had to be excluded from final analysis. This resulted in a total of 50 SNPs available for statistical analysis.

Statistical analysis

Only age and BMI were statistically significant results in primary analysis of association between demographic variables examined and PK parameters of EFV (see table 3).

Table 3. Primary analysis of association between demographic variables and EFV pharmacokinetics parameters.

Variable	EFV pharmacokinetic parameters					
	$C_{min\ ss}$		$C_{max\ ss}$		CL /F	
Age	r= -0.159	P= 0.0763	r= -0.148	P= 0.1005	r= 0.195	P= 0.0295
BMI	r= -0.066	P= 0.4639	r= -0.288	P= 0.0011	r= 0.174	P= 0.0526
Gender						
Female	1.841		4.008		9.751	
Male	2.432	P=0.0643	4.315	P=0.6262	9.048	P=0.2487
Race						
Caucasian	2.190		4.165		9.338	
Others	3.575	P=0.313	5.585	P=0.513	7.968	P=0.404

However, linear regression analysis of genetic polymorphisms on these PK parameters (adjusting for demographic variables) revealed that a total of 6 SNPs (2 in CYP2B6, 2 in CYP2A6, 1 SNPs of CYP2C19 and other in MRP4) were individually associated with them. These results are presented in table 4.

Table 4. Results from linear regression on EFV pharmacokinetic parameters (adjusting for gender, age, race and BMI).

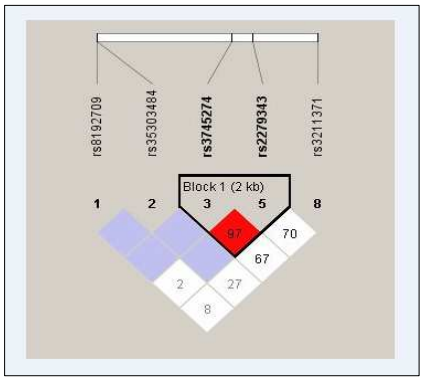
Genetic polymorphisms		**EFV pharmacokinetic parameters**					
rs number	Genotype	$C_{min\ ss}$ (mg/L)	p-value	$C_{max\ ss}$ (mg/L)	p-value	*CL/F* (L/h)	p-value
rs3745274	CYP2B6 516 G>T		1.64 e^{-19}		9.24 e^{-19}		1.33 e^{-16}
	GG (n=76)	1.58		3.54		10.77	
	GT (n= 41)	2.35		4.47		7.76	
	TT (n=8)	7.42		9.27		3.01	
rs2279343	CYP2B6 785 A>C		1.13 e^{-16}		1.70 e^{-16}		4.98 e^{-14}
	AA (n=73)	1.56		3.56		10.67	
	AG (n=45)	2.37		4.37		8.12	
	GG (n=8)	7.42		9.27		3.01	
rs4244285	CYP2C19 681 G>A		0.003		0.007		0.004
	GG (n=93)	2.43		4.42		8.85	
	AG/AA (n=27)	1.55		3.52		10.76	
rs28399435	CYP2A6 86 G>A		0.039		0.020		0.054
	GG (n=113)	2.15		4.13		9.36	
	AG/AA (n=9)	3.61		5.59		7.16	
rs8192726	CYP2A6 1836 G>T		0.044		0.039		0.058
	GG (n=112)	2.14		4.12		9.45	
	GT/TT (n=12)	3.16		5.18		7.48	
rs1751034	MRP4 3463 A>G		0.058		0.030		0.034
	AA (n=85)	2.41		4.41		8.84	
	AG/GG (n=40)	1.87		3.78		10.23	

As expected, the 2 SNPs in CYP2B6 (516 G>T (rs3745274) and 785 A>C (rs2279343)) were those had a closer association with all EFV PK parameters ($p < 0.001$). However, these SNPs are strongly linked (see figure 1) and, in fact, when both are included in the same regression model only CYP2B6 516 G>T (rs3745274) remains significant, so this SNP could be used as representative of

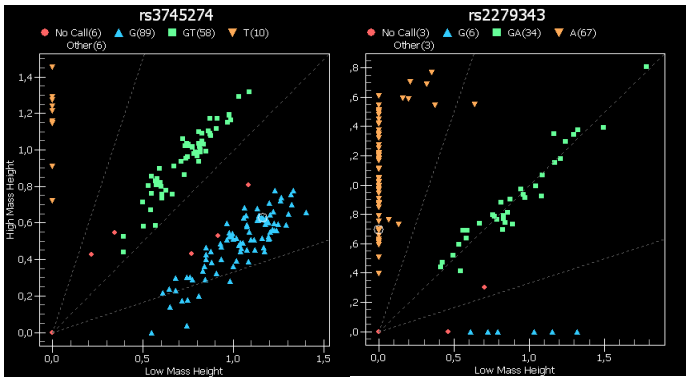
the two. Besides, because of the major effect of this SNP on EFV PK parameters and the small number of patients with homozygous genotype (T/T) some of the other observed associations could be spurious. For this reason we repeated the analysis but using as dependent variable the residuals (predicted-observed values) from a previous regression of rs3745274 on EFV PK parameters.

Figure 1. Haploview analysis (A) and genotyping clusters (B) of CYP2B6 gen.

A)



B)



After adjusting for rs3745274, 5 SNPs (1 in CYP2B6,1 in CYP2A6, 2 in MRP4 and 1 in MDR1) showed significant or suggestive probabilities ($p < 0.10$) (see table 5).

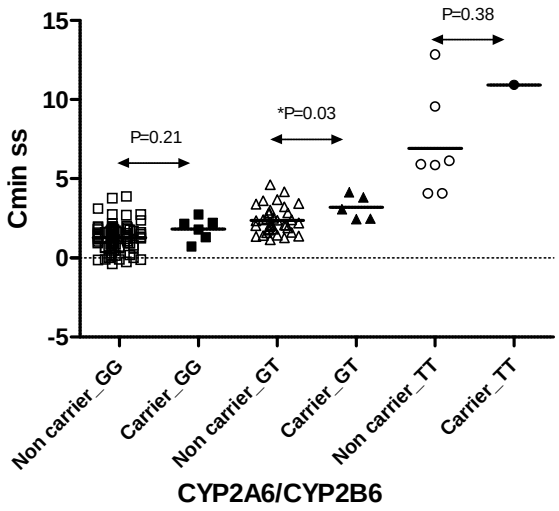
Table 5. Results of linear regression of each SNP on residual EFV after adjusting for rs3745274.

		p-value		
rs number	Genotype	$C_{min\ ss}$	$C_{max\ ss}$	*CL/F*
rs8192709	**CYP2B6 64 C>T**	**0.132**	**0.177**	**0.048**
rs1557070	**MRP4 1497 C>T**	**0.073**	**0.121**	**0.042**
rs1751034	**MRP4 3463 A>G**	**0.096**	**0.045**	**0.056**
rs8192726	**CYP2A6 1836 G>T**	**0.033**	**0.032**	**0.060**
rs9282564	**MDR1 61 A>G**	**0.056**	**0.072**	**0.103**

Respect to SNP in CYP2A6 (1836 G>T (rs8192726)), there was a significant correlation with $C_{max\ ss}$ and $C_{min\ ss}$ values. Thus, EFV $C_{min\ ss}$ of patients with heterozygous genotype for this SNP were significantly higher than those of patients with other genotype. Besides, this correlation was independent of influence of SNP CYP2B6 516 G>T (rs3745274), as shown figure 2, which is also

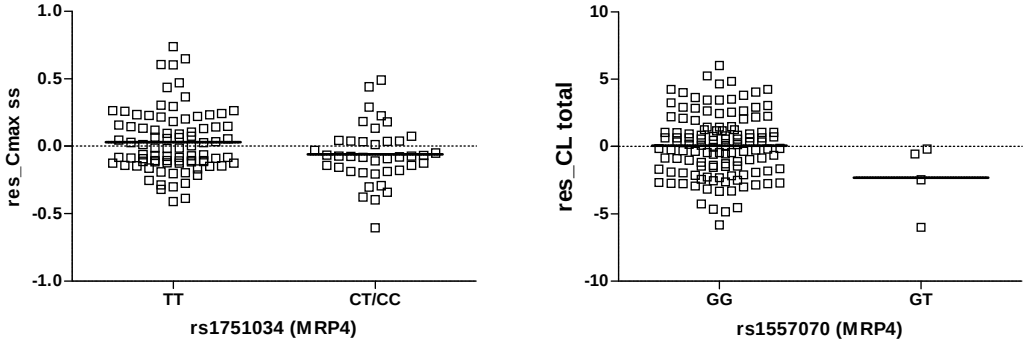
illustrated with the results of a two-way ANOVA (p< 0.0001 for rs3745274, p= 0.03 for rs8192726 and p= 0.63 for interaction).

Figure 2. Correlation $C_{min\ ss}$ and CYP2A6 genotype.



Among SNPs in MRP4 gene, their influence on EFV PK parameters also reached statistical significance (p < 0.05). Two SNPs (1497C>T (rs1557070) and 3463 A>G (rs1751034)) had good correlation with CL/F and $C_{max\ ss}$, respectively as shown figure 3.

Figure 3. Correlation PK parameters of EFV and SNPs in MRP4 gene.



To obtain a combination of genotypes showing the strongest association with EFV PK parameters and demographic characteristics, we performed a multiple linear regression analysis with forward stepwise selection of variables. A summary of results of the final models are shown in table 6.

As expected, the SNP CYP2B6 516 G>T (rs3745274) was the first variable to enter the final models of all dependent variables analyzed (PK parameters). But other SNPs were also significantly associated. Thus the effect of SNPs in MRP4 was strongly confirmed with the inclusion of various of them in the final models. Among demographic factors, gender and BMI also managed to enter in final models.

The coefficients of determination (R^2) for the regression were 0.54, 0.59 and 0.52 for final model of $C_{min\ ss}$, $C_{max\ ss}$ and CL/F , respectively; which indicated that about 50% of the total variance in differents EFV PK parameters could be explained by these models.

Table 6. Selected models in the multiple linear regressions. For each SNP is shown the Prob >F (order in which each variable is entered into the model).

	DEMOGRAPHIC FACTORS		GENETIC POLYMORPHISMS						
DEPENDENT VARIABLE	Gender	BMI	rs3745274 (CYP2B6)	rs1557070 (MRP4)	rs12875235 (MRP4)	Rs1751034 (MRP4)	Rs2274407 (MRP4)	rs28399435 (CYP2A6)	R square
$C_{min\ ss}$	0.0097 (2)		$5.29*10^{-14}$(1)	0.0232 (4)			0.0225 (3)		0.54
$C_{max\ ss}$		0.0248 (2)	$7.03*10^{-13}$ (1)	0.0180 (4)	0.0254 (3)		0.0295 (5)	0.038 (6)	0.59
CL/F			$1.95*10^{-12}$ (1)	0.0181 (4)		0.0238 (2)	0.0121 (3)		0.52

Discussion

The main objective of this study was to investigate the impact of genetic factors in the PK of EFV, due to the existence of a high interindividual variability that has not been fully explained by other factors. This variability has also been demonstrated in this study. Thus, the percentage of patients with concentrations outside the therapeutic range was approximately 20%, which is consistent with other studies conducted on this subject.[19,42,43] This implies that the response to treatment with EFV also differs from one patient to another and that is further accentuated the need to find the factors involved in it. Moreover, the understanding of the contribution of these factors on the PK variability of EFV could be very important in clinical practice to optimize treatment with this drug and to administer the appropriate dose for each patient with maximum safety and efficiency.

However, a major limitation of previous studies has been that most of them have conducted individualized analysis of certain genetic polymorphisms in certain genes,[18-21,23,28-29,34] but pharmacological response is much more complex and would need to consider all polymorphisms together. Since, due to possible interactions between the different SNPs in genes of metabolizing enzymes and transporters, the influence of these polymorphisms on PK of EFV could be totally different if they are analyzed independently.

On the other hand, it is very important to make a good selection of candidate genes, since in the ADME process of EFV is not yet defined clearly what are the metabolizing enzymes and transporters that primarily are implicated. Thus, although the main route of metabolism of EFV is through the CYP2B6 isoenzyme,[15] we must take into account also the genes encoding enzymes involved in secondary metabolism. Besides, do not forget the important role transport proteins play (located in different anatomical structures of the body) in the process of distribution of this drug, which can affect both its efficiency and its toxicity.

Therefore, we have wanted to perform a more complete analysis, getting to be one of the studies that a largest number of SNPs analyzed (total 90) in greater number of genes encoding metabolizing enzymes and transport proteins (see

table 1). The obtained results show that about 50% of variability of PK parameters may be explained by genetic factors. Among the most significant SNPs are mainly those associated with the genes encoding CYP2B6 and CYP2A6 enzymes and MRP4 transport protein.

Definitely, the CYP2B6 isoenzyme is the main involved in the PK variability of EFV, which has been reflected in the different statistical analysis performed in this study. Although several SNPs (516 G>T, 785 A>G and 64 A>G) had showed a significant relationship in preliminary analysis, the two most implicated from the beginning were the 516 G>T and 785 A>G, which have been widely studied and linked with a decrease in the activity of this isoenzyme. In our study, their influence significantly affected all PK parameters, so in patients with homozygous genotype (T/T), an increase of 1.58 to 7.42 µg/mL in $C_{min\ ss}$, 3.54 to 9.27 µg/mL in $C_{max\ ss}$ and a decrease of 10.77 to 3.01 L/h in CL/F were observed. These data are consistent with other studies.[29,33,44] Besides, in the multivariate analysis, the 516 G>T (rs3745274) was always the first variable to enter in final models of different PK parameters, explained about 45% of their total variance. Our data confirm that the impact of these polymorphisms might have on treatment with EFV is very important, especially regarding the toxicity of this drug, since several studies have shown that high EFV plasma concentrations are related to an increased risk of adverse effects.[8-11] In this sense, the possibility of knowing these genotypes before prescribing this drug would be very useful in clinical practice to optimize EFV treatment.

Despite the undoubted importance of CYP2B6 isoenzyme, other metabolizing enzymes had also been identified as possibly responsible for this PK variability, although to a lesser extent (3%). Thus, 2 SNPs in CYP2A6 (86 A>G and 1836 G<A) showed statistical significance in different statistical analysis. Recently, some studies have examined the influence of this enzyme,[18-21] but the results are contradictory. While Kwara et al[20] argues that the influence of these genetic polymorphisms is independent of the activity of CYP2B6, Di Julio et al[21] observe only this effect when CYP2B6 activity was decreased. Our data, in accordance with the study of Kwara et al[20], support that the impact of these SNPs in the PK of EFV is largely independent of activity of CYP2B6, which had been demonstrated in several statistical analysis performed for this (see figure

2). Therefore, these results could confirm that the CYP2A6 genotyping also might be useful to optimize EFV therapy.
Regarding the rest of CYPs (CYP3A4, CYP3A5, CYP2C9, CYP2C19…) has not found any association in this study. These results are according to others previous studies.[22,23,25,26] The reasons why have not observed this relationship may be several, among them is the low frequency found for the selected SNPs in Caucasian race and, moreover, the possible little influence of these SNPs in the metabolism of EFV. In both cases, more studies would be needed to confirm these hypotheses.

Certainly the most remarkable of this work is the influence of transporters on the kinetics of EFV. So, in most statistical analysis, the SNPs (1497 C>T (rs1557070), 3463 A>G (rs1751034), 3725 G>A (rs12875235) and 912 G>T (rs2274407)) in MRP4 were significantly correlated with the PK parameters of EFV, mainly with $C_{max\ ss}$ and CL/F (see figure 3). For this last one, their values diminish in a notable percentage in patients with heterozygous or homozygous genotype for SNP 1497 C>T (rs1557070). The impact of these polymorphisms might have on efavirenz therapy are not known, since no previous studies have examined this transport protein. So it would be very necessary to clarify its role in the kinetics of EFV, due to it could also be involved in their toxicity and efficacy. In these sense, further studies in different populations and in larger numbers of patients are carried out essential.

Moreover, the influence of genetic polymorphisms in MDR1 protein transport (see table 2) remains unclear.[22-24,27,31] In our study, only 61 A>G showed suggestive significance in the preliminary statistical analysis, but none of them to enter in the final models of multivariate analysis. This could also be due to low frecuency of these SNPs in our population.

As expected, genetic factors have a significant impact on pharmacokinetic variability of EFV, much higher than other non-genetic factors such as demographic (age, BMI, gender or race). So, in our study, only gender and BMI were found to be significant only in the multivariate analysis. In the case of gender, there is considerable literature on the subject, but the results are contradictory.[45-47] The reasons why this association was found in our population might be explained because the most patients with the genotype

homozygous for the CYP2B6 516 G>T were men and this could have masked the final result. Thus further studies are needed where there is a similar proportion of both genders.

Another aspect to consider is the race, the population that we had studied was primarily Caucasian (96%) and therefore have not been able to establish differences with others. Furthermore, another limitation found in this study is that some of the selected genetic polymorphisms showed a low frequency in Caucasians, even some of them were monomorphic (see table 1); therefore has not been able to adequately establish their influence on PK EFV. For this reason, further studies should be conducted in patients of different races and with a larger sample size.

In conclusion, this work has performed a comprehensive pharmacogenetic analysis of genes encoding the major metabolizing enzymes and transporters of EFV and it has been able to establish a clear relationship between the genetic factors and the PK parameters of this drug. The SNPs in CYP2B6 (516 G>T and 785 A>G), in CYP2A6 (86 A>G and 1836 G<A) and in MRP4 (1497 C>T (rs1557070), 3463 A>G (rs1751034), 3725 G>A (rs12875235) and 912 G>T(rs2274407)) have been the most implicated. The integration of pharmacogenetic data in clinical practice can be a useful tool in the individualization of treatment with this drug.

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Capítulo IV

Population Pharmacokinetic Pharmacogenetic Model in Caucasian HIV-Infected Patients for Optimization of Efavirenz Therapy.

Original Article

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Antimicrobial Agents and Chemoterapy

Abstract

Despite extensive clinical experience with efavirenz (EFV), unpredictable inter-individual variability in efficacy and toxicity remain important limitations associated with the use of this antiretroviral. The purpose of this study was to determine the factors affecting EFV pharmacokinetics and to develop a pharmacokinetic/pharmacogenetic (PK/PG) model in a Caucasian population of HIV-infected patients.

In total, 869 EFV plasma concentrations from 128 HIV-infected patients treated with EFV were quantitatively assessed using a validated high-performance liquid chromatography technique. All patients were genotyped for 90 single nucleotide polymorphisms (SNPs) in genes coding for proteins involved in the metabolism and transport of EFV, using a MassArray platform provided by Sequenom. The influence of these polymorphisms on EFV pharmacokinetics, and the effects of demographic, clinical, biochemical, lifestyle and concurrent drug use covariates were evaluated. Plasma concentrations were fit and analyzed using a one-compartment model, with first-order absorption and elimination using nonlinear mixed-effect modeling (NONMEM program).

The CYP2B6*6 allele (a major predictor of plasma efavirenz exposure), MPR4 1497C>T (a new SNP, not previously studied) and g-glutamyltranspeptidase (GGT), were identified as major factors influencing apparent EFV oral clearance (CL/F) according to the following final model: CL/F (L/h) = (12.3 – 0.00213*GGT)* $0.640^{CYP2B6*6}$ *$0.799^{MRP4\ 1497C>T}$. The detailed genetic analysis results presented in this study might indicate that other genetic polymorphisms have no influence on the CL/F of EFV. Furthermore, our proposed model is simple and easily applicable as a tool in the optimization of EFV dosage in clinical practice.

Introduction

Efavirenz (EFV) is one of the most widely used and accepted non-nucleoside reverse transcriptase inhibitors (NNRTIs) worldwide. EFV is used in combination with two nucleoside analogue reverse transcriptase inhibitors (NRTIs), because of the efficacy and safety shown for this combined therapy in many clinical trials (15, 18, 58, 64).

Although there can be wide inter-patient differences, due to genetic and environmental factors, when comparing optimal drug concentrations to standard doses, all current treatment guidelines recommend fixed doses of antiretrovirals (ARVs). More importantly, several studies have demonstrated that drug concentrations are an important factor in patient response to ARV therapy, and that there is a significant correlation between drug exposure and efficacy or toxicity. Thus, when EFV is administered at a fixed dosage of 600 mg once daily, some patients suffer from central nervous system toxicity ($C^{ss}_{min} > 4$ µg/mL) (6, 37, 46) or fail to achieve durable viral load suppression ($C^{ss}_{min} < 1$ µg/mL) (5, 19, 20, 23, 37, 41, 47). These differential patient responses can, at least in part, be attributed to high inter-patient variability in the disposition kinetics of EFV (45).

In fact, there are multiple factors affecting the pharmacokinetic (PK) variability of EFV, including: ethnicity, gender, age, body weight, drug-drug and drug-food interactions, binding to plasma proteins, hepatic impairment, disease status, pregnancy and host genetic factors (7, 52, 59). Because of the identification of genetic polyphormisms in genes coding for proteins involved in the metabolism or transport of ARVs (which may alter these proteins activity and may explain, in part, the high inter-patient PK variability of these drugs), analysis of the influence of genetic factors on the PK of ARVs is becoming increasingly important (13, 28, 44, 50).

CYP2B6 polymorphisms are the most studied genetic polymorphisms to date. In particular, the single nucleotide polymorphism (SNP) CYP2B6 516G>T has been reported to be significantly associated with a pronounced reduction in enzyme activity and elevated EFV plasma concentrations in studies conducted on different populations (1, 9, 11, 16, 21, 25, 34, 35, 36, 39, 40, 42, 48, 51, 53, 54, 61). More recently, the SNPs CYP2B6 983T>G and 785A>G have also been reported to affect EFV plasma concentrations (21, 22, 36, 48, 53, 54, 63). These

data demonstrate that CYP2B6 poor EFV metabolizer genotypes can be used to identify individuals at risk for high EFV plasma concentrations. Genetic polymorphisms in genes coding for others CYPs (CYP3A4/5, CYP2A6, CYP2D6, CYP2C9, CYP2C19, CYP2C8), have also been analyzed in several studies, but their influence on the PK of EFV has not yet been well characterized (1, 16, 23, 34, 35, 39, 54, 55, 61). Furthermore, there is relatively limited information in the literature on polymorphisms in protein transporter genes. For example, although genetic variations in MDR1 have been the most widely analyzed (17, 23, 37, 61, 55), their influence on PKs is not clearly defined, and further studies are required to assess their clinical relevance. Similarly, genetic polymorphisms in genes coding for other protein transporters (e.g. MRP1, MRP2, MRP4...) also require further investigation.

Because only a modest part of EFV PK variability can be explained by demographic variables or the concomitant administration of other drugs, increased understanding of the influence of genetic factors on the PK of EFV could enable optimization of EFV based therapy.

A population-based approach, which integrates pharmacogenetic (PG) data with PK studies, is very suitable for capturing the contribution of multiple genetic factors on the PK of EFV, and, in particular, would permit the adequate characterization of PK phenotypes. However, although population PK analyses on EFV have been reported in the literature (1, 9, 14, 40, 46), only some of these have integrated PG information (1, 9, 40).

Therefore, the overall objective of this study was to develop a population-based PK/PG model in 128 HIV-infected patients, by analyzing the potential influence of a large number of SNPs (90) in genes coding for proteins involved in metabolism and transport of EFV. The results of this study could be used to improve the prediction of EFV plasma concentrations and optimize EFV ARV therapy. This study complements our previous study (9), in which we investigated the PGs of EFV, including the effects of CYP2B6, CYP3A4, and MDR1 genotypes, in 32 patients.

Material y methods

Study Population and Design

The population-based PK/PG analysis was conducted on 128 HIV-positive patients treated with EFV, from the outpatient unit of the Pharmacy Service of the University Hospital of Salamanca (Spain). Patient inclusion criteria were as follows: confirmed HIV infection; treatment with EFV for at least 3 months (at an unchanged EFV dosage for at least 1 month); adherence to the treatment regimen > 90%; age ≥ 18 years; and no co-medication with known EFV inducer or inhibitor drugs. All patients included in this study provided written, informed consent for genetic testing, and the study was subjected to approval by the ethics committee of the University Hospital of Salamanca.

All patients were initially administered 600 mg oral EFV once a day in combination with two NRTIs as part of their ARV regimen. Approximately 20% of patients required dose adjustments (range 200-1000 mg/day) to achieve therapeutic concentrations of EFV, with one patient even requiring 1600 mg/day due to the absence of a clinical reason to lower the dosage (10).

All patients were included in a therapeutic drug monitoring (TDM) program, and plasma samples for EFV assays and viral and biochemical tests were drawn periodically at 3 to 6 month intervals during follow-up visits to the hospital. Individual patient information was carefully recorded at the same time, including: dose history, sampling time, time of last dose, sex, age, weight, height, concomitant pathologies (e.g. hepatitis C virus (HCV)), concomitant treatment (e.g. ARVs and other drugs), lifestyle factors (e.g. tobacco use, alcohol consumption) and treatment adherence. Treatment adherence was measured according to dispensing records and a Simplified Medication Adherence Questionnaire (SMAQ) (33); an adherence > 90% was used as additional criteria that the coefficient of variation (CV) of the mean EFV plasma concentration/dose ratio in each patient was < 30%, according to previously observed intrapatient variability (14). Data concerning clinical evolution (CD_{4+}, plasma viral load), biochemical parameters related to liver function (alanine aminotransferase (ALT), aspartate aminotransferase (AST), g-

glutamyltranspeptidase (GGT), total bilirubin (TB), platelets (PLT) and indexes to predict liver fibrosis (APRI, FIB4, Forns)), renal function (serum creatinine (SCR)) and lipid profile (total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL)), were also collected.

The demographic and clinical characteristics of patients included in this study are shown in Table 1.

Table 1. Patient Population Demographics and Clinical Characteristics.

CHARACTERISTICS	VALUE Mean ± SD (range) or N (%)
No. of patients	128
Race [Caucasian]	124 (96,87)
Sex [Male]	86 (67,18)
Age [years]	45.06 ± 9.16 (18-77)
Body weight [Kg]	64.98 ± 12.20 (39-113)
Height [cm]	167.89 ± 8,73 (150-191)
Body mass index [kg^2/cm]	22.98 ± 3.42 (13.90-36.90)
No. of EFV concentrations analyzed	869
No. of plasma concentrations per patient	4.59 ± 2.84 (1-16)
Daily dose [mg/day]	608.75 ± 104.36 (200-1600)
EFV plasma concentration [µg/mL]	3.18 ± 1.61 (0.84-15.16)
ALT [U/L]*	47.59 ± 43.48 (4-496)
AST [U/L]*	40.71 ± 33.46 (8-380)
GGT [U/L]*	121.21 ± 156.79 (8-1612)
PLT [$x\ 10^3/\mu L$]*	212.10 ± 78.26 (27-512)
TB[mg/dL]*	0.49 ± 0.34 (0.10-4.40)
TC [mg/dL]*	191.44 ± 49.73 (55-367)
TG[mg/dL]*	156.17 ± 106.53 (36-921)
LDL[mg/dL]*	109.76 ± 42.96 (10-244)
SCR [mg/dL]*	0.82 ± 0.18 (0.30-1.60)
APRI*	0.67 ± 0.77 (0.06-6.31)
FIB4*	1.56 ± 1.58 (0.16-26.01)
FORNS*	5.04 ± 2.04 (0.20-11.48)
CD_{4+} [$x\ 10^6/\mu L$]*	416.05 ± 215.55 (9.80-1230)
PLASMA VIRAL LOAD DETECTABLE*	171 (19.67)
HCV*	336 (38.66)
SMOKER*	558 (64.21)
ALCOHOL*	324 (37.28)
ABACAVIR *	159 (18.29)
LAMIVUDINA *	600 (69.04)
EMTRICITABINA*	179 (20.59)
TENOFOVIR*	385 (44.30)
ESTAVUDINA*	95 (10.93)
DIDANOSINA*	169 (19.44)
ZIDOVUDINA*	163 (18.75)

*Data referenced to the number of EFV concentrations analyzed.

Sampling and drug assays

Most blood samples were collected at midpoint of the dosage interval, between 8 and 20 hours after EFV administration, under steady-state dosage conditions (unchanged dosage ≥1 month). The mean number of EFV plasma concentrations per patient was 4.59 ± 2.84, resulting in a final database of 869 concentrations, which were used to develop the population PK/PG model.

Blood samples (5 mL) were collected and plasma was isolated by centrifugation at 3000 g. Samples were stored at −20 °C (following virus inactivation in a water bath at 60 °C for 60 min) until analysis.

EFV concentrations were measured by HPLC (Waters, Milford, USA) with UV detection at 215 nm, following solid phase extraction using a GX-271 ASPEC (Gilson, Villiers le Bel, France). This method was validated over a concentration range of 0.5 to 10 mg/L, using 600 µL of plasma. Recovery of EFV from human plasma was 107.4%. Intra- and inter-day CV precisions were consistently < 5.7% for all internal quality controls (0.5, 2.0 and 10.0 mg/L). The quantification limit was 0.25 mg/L and absence of interference from the 21 drugs most often used in HIV patients was confirmed. Our analysis laboratory successfully participates in the International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring in HIV infection (a Dutch association for Quality assessment in Therapeutic Drug Monitoring and Clinical Toxicology [KKGT]).

SNP selection

Characterized SNPs were selected on the basis of their potential or identified influence on the functionality of enzymes and transporters of EFV, obtained from public databases (CYP alleles: www.cypalleles.ki.se; dbSNP: www.ncbi.nlm.nih.gov/SNP/ and Centre d'Etude du Polymorphisme Humain (CEPH) panel from the HapMap database: www.hapmap.org). A total of 90 SNPs (8 SNPs in CYP2B6, 6 SNPs in CYP2A6, 25 SNPs in CYP2D6, 3 SNPs in CYP2C8, 3 SNPs in CYP2C19, 4 SNPs in CYP2C9, 2 SNPs in CYP3A4, 7 SNPs in CYP3A5, 5 SNPs in MDR1, 3 SNPs in MRP1, 8 SNPs in MRP2, 11 SNPs in MRP4, 1 SNPs in UGTB7, 1 SNPs in ABCA1 and 3 SNPs in BCRP) were chosen using the above criteria and included in the analysis. SNPs investigated and their frequencies in the patients included in this study are shown in Table 2.

Table 2. Genes and SNPs investigated.

		SUBJECTS (n, %)						
GENE	**SNP**	**Wild-type**		**Heterozygous**		**Homozygous**		**N**
CYP2A6	479 T>A	118	94.40	7	5.60	0	0.00	125
	86 G>A	113	92.62	9	7.38	0	0.00	122
	459 G>A	123	98.40	2	1.60	0	0.00	125
	1093 G>A	124	99.20	1	0.80	0	0.00	125
	580 A>G	123	99.19	0	0.00	1	0.81	124
	1836 G>T	112	90.32	12	9.68	0	0.00	124
CYP2B6	785 A>G	73	58.40	44	35.20	8	6.40	125
	983 T>C	125	100.0	0	0.00	0	0.00	125
	1459 C>T	104	83.87	18	14.52	2	1.61	124
	1132 C>T	125	100.0	0	0.00	0	0.00	125
	136 A>G	118	95.93	5	4.07	0	0.00	123
	593 T>C	125	100.0	0	0.00	0	0.00	125
	516 G>T	76	60.80	41	32.80	8	6.40	125
	64 C>T	115	96.64	4	3.36	0	0.00	119
CYP2C19	- 806 C>T	82	65.60	37	29.60	6	4.80	125
	358 T>C	125	100.0	0	0.00	0	0.00	125
	681 G>A	93	77.50	23	19.17	4	3.33	120
CYP2C8	1196 A>G	91	72.80	31	24.80	3	2.40	125
	416 G>A	91	72.80	31	24.80	3	2.40	125
	805 A>T	120	96.00	5	4.00	0	0.00	125
CYP2C9	1075 A>C	125	100.0	0	0.00	0	0.00	125
	1425 A>T	103	83.74	20	16.26	0	0.00	123
	430 C>T	121	100.0	0	0.00	0	0.00	121
	1003 C>T	125	100.0	0	0.00	0	0.00	125
CYP2D6	1039 C>T	119	95.97	4	3.23	1	0.81	124
	124 G>A	125	100.0	0	0.00	0	0.00	125
	1584 C>G	73	59.84	32	26.23	17	13.93	122
	1659 G>A	124	100.0	0	0.00	0	0.00	124
	1661 G>C	42	34.43	46	37.70	34	27.87	122
	1758 G>A	124	100.0	0	0.00	0	0.00	124
	1869 T>C	125	100.0	0	0.00	0	0.00	125
	1973-1974insG	125	100.0	0	0.00	0	0.00	125
	2097 A>G	87	76.32	22	19.30	5	4.39	114
	2466 T>C	125	100.0	0	0.00	0	0.00	125
	2539-2542del AACT	124	99.20	0	0.00	1	0.80	125
	2613-2615 delAGA	109	91.60	0	0.00	10	8.40	119
	2935 A>C	125	100.0	0	0.00	0	0.00	125
	31 G>A	104	83.20	20	16.00	1	0.80	125
	3183 G>A	122	99.19	1	0.81	0	0.00	123
	3198 C>G	125	100.0	0	0.00	0	0.00	125
	4042 G>A	109	99.09	1	0.91	0	0.00	110
	CYP2D6_845_8831	125	100.0	0	0.00	0	0.00	125
	883 G>C	125	100.0	0	0.00	0	0.00	125
	100 C>T	86	72.27	27	22.69	6	5.04	119
	2850 C>T	58	47.93	33	27.27	30	24.79	121
	1023 C>T	123	100.0	0	0.00	0	0.00	123
	1846 G>A	88	72.73	27	22.31	6	4.96	121
	2549 A>del	120	96.77	0	0.00	4	3.23	124
	1707 T>del	125	100.0	0	0.00	0	0.00	125

Table 2. Continued.

		SUBJECTS (n, %)						
GENE	**SNP**	**Wild-type**		**Heterozygous**		**Homozygous**		**N**
CYP3A4	-392 A>G	115	92.00	8	6.40	2	1.60	125
	878 T>G	124	100.0	0	0.00	0	0.00	124
CYP3A5	14690 G>A	121	96.80	4	3.20	0	0.00	125
	31551 T>C	125	100.0	0	0.00	0	0.00	125
	-86 G>A	124	99.20	1	0.80	0	0.00	125
	-74 C>T	117	93.60	8	6.40	0	0.00	125
	29753 T>C	124	100.0	0	0.00	0	0.00	124
	17163 G>T	119	95.20	6	4.80	0	0.00	125
	6986 A>G	104	83.20	19	15.20	2	1.60	125
MDR1	3435 C>T	39	31.45	55	44.35	30	24.19	124
	1236 C>T	46	37.10	56	45.16	22	17.74	124
	1199 G>A	114	91.94	10	8.06	0	0.00	124
	TAG6	48	38.40	55	44.00	22	17.60	125
	61 A>G	112	89.60	13	10.40	0	0.00	125
MRP1	825 T>C	50	40.65	64	52.03	9	7.32	123
	1062 T>C	54	43.20	62	49.60	9	7.20	125
	816 G>A	114	91.20	11	8.80	0	0.00	125
MRP2	259 G>T	123	98.40	2	1.60	0	0.00	125
	3563 T>A	110	88.00	15	12.00	0	0.00	125
	1249 G>A	81	65.32	40	32.26	3	2.42	124
	3972 C>T	46	36.80	55	44.00	24	19.20	125
	1058 G>A	124	100.0	0	0.00	0	0.00	124
	-24 C>T	75	60.00	41	32.80	9	7.20	125
	4488 C>T	109	87.90	15	12.10	0	0.00	124
	4544 G>A	109	87.90	15	12.10	0	0.00	124
MRP4	559 G>T	114	91.20	11	8.80	0	0.00	125
	3724 G>A	124	99.20	1	0.80	0	0.00	125
	3725 G>T	96	76.80	27	21.60	2	1.60	125
	1497 C>T	121	96.80	4	3.20	0	0.00	125
	3463 A>G	85	68.00	34	27.20	6	4.80	125
	969 G>A	56	44.80	53	42.40	16	12.80	125
	951 G>A	53	43.09	53	43.09	17	13.82	123
	912 G>T	100	80.00	24	19.20	1	0.80	125
	4131 T>G	44	35.48	62	50.00	18	14.52	124
	3310 T>C	124	99.20	1	0.80	0	0.00	125
	669 C>T	87	69.60	35	28.00	3	2.40	125
UGTB7	*1C	40	32.00	66	52.80	19	15.20	125
ABCA1	2649A>G	75	60.48	43	34.68	6	4.84	124
BCRP	376 C>T	125	100.0	0	0.00	0	0.00	125
	34 G>A	107	85.60	17	13.60	1	0.80	125
	421 C>A	107	85.60	16	12.80	2	1.60	125

Genotyping analysis

All genotyping experiments were conducted at the Spanish National Genotyping Center (CeGen).

Genomic DNA was isolated automatically from 5 mL whole human blood with magnetic bead technology using the Chemagic Magnetic Separation Module I and the Chemagic DNA kit, according to the manufacturer's instructions (Chemagen AG, Baesweiler, Germany).

Genotyping was mainly performed using a MassArray platform provided by Sequenom. This method involves multiplex PCR amplification of up to 7 SNPs. Excess nucleotides are removed by treatment with shrimp alkaline phosphatase, and multiplex primer extension is performed using a mix of deoxy- and dideoxynucleotides, so that products of different masses are obtained for each allele of each SNP. Resulting products are resolved by mass spectrometry (MALDI-TOF).

Prior to genotyping by Sequenom, two genes (CYP2B6 and CYP2D6) were amplified and separated from their respective pseudogenes, using different methodologies as previously described (56). In addition, some SNPs of CYP2A6 (rs28399454, rs34816076) and CYP3A5 (rs28365095) could not be analyzed by Sequenom, and were instead analyzed using Real Time PCR Taqman drug Metabolism Genotyping assays.

Population Pharmacokinetic/Pharmacogenetic Model Development

A population-based PK/PG model of EFV was built using NONMEM (version VI; double precision, level 2.0) (4). The first-order conditional estimation method (FOCE) in conjunction with a Laplace approximation was used for all models tested during model development.

A one-compartment, open kinetic model with first-order absorption and elimination (specified in NONMEM using the ADVAN2 and TRANS2 routines), was assumed. Because of the nature of the data, the absorption rate constant (ka) could not be estimated and was fixed at 0.3 h^{-1}, a ka value

previously reported (14). Therefore, estimated fixed-effect PK parameters included the apparent CL/F and the apparent distribution volume (V/F). Both additive and exponential error models were tested to explain inter-individual and residual variability, as described in our previous study (9).

To elucidate preliminary relationships between individual PK parameters obtained using a Bayesian maximum *a posteriori* estimation (the POSTHOC option in NONMEM) and covariates, a graphical approach to exploratory data analysis and the stepwise generalized additive model (GAM) implemented in Xpose were used (29). Resulting, potentially important covariates were then selected and incorporated stepwise into the basic model to develop intermediate and full models.

The inclusion of a fixed-effect parameter in the basic model quantifies the relationship between a particular PK parameter and covariate, allowing determination of whether the covariate significantly improves the ability of the model to predict the observed concentration-time profile. Quantitative covariates (age, total body weight (TBW), body mass index (BMI), ALT, AST, GGT, TB, PLT, TC, TG, LDL and SCR) were included using linear and nonlinear methods, including log-transformation. Discrete covariates (gender and concomitant drugs) were tested in the model as binary variables (0 or 1 for female or male, and 0 or 1 to indicate the absence or presence of a concomitant drug during the treatment, respectively). With respect to PG covariates, these variables took values of 0, 1 or 2 to indicate wild-type, heterozygous or homozygous genotypes, respectively. All discrete covariates were examined using a multiplicative model, in order to obtain the fractional increase or decrease in the associated PK parameter.

The criteria for retention of a particular covariate in the model were as follows:

- The objective function value difference (OFVD) between two hierarchical models must be at least 3.84 (degrees of freedom = 1) in order to achieve the desired level of statistical significance ($p < 0.05$).
- Reduction in unexplained inter-individual variability for the associated PK parameter.
- Randomly distributed weighted residuals.
- Closer relationship between predicted and observed concentrations.

- The 95% confidence interval (CI) of the covariate effect must exclude zero.
- Standard errors (SE) for estimated fixed and random parameters cannot be greater than 25 and 50%, respectively (2).

In addition, for a covariate to be selected as clinically relevant, a change in typical PK parameter estimates of ± 20% was required.

The generated full model was then subjected to backwards elimination, where each model parameter was fixed to zero, using more stringent criteria for statistical significance ($p < 0.01$).

Final Model Validation

Final model suitability was evaluated using pseudoresiduals, a validation approach proposed by Mesnil *et al.* (38). Monte Carlo simulation was applied to mimic the mean EFV concentrations in adult patients receiving standard doses of EFV (600 mg/day), to assign individual characteristics (those included in the final model) to the simulated population. 1000 random concentrations were generated for each simulated patient, and pseudoresiduals were computed as described in Comets *et al.* (12).

Complementary statistical analyses were performed using SPSS (version 15, SPSS, Inc., Chicago, IL) (57).

Results

The mean EFV plasma concentration was measured to be 3.18 (± 1.61) μg/mL, with a range between 0.84 and 15.16 μg/mL; suggesting high inter-individual EFV drug disposition variability.

A one-compartment model with first-order absorption and elimination fit the data appropriately. In the basic model, which did not consider covariates, mean values for CL/F and V/F were 9.61 L/h (SE = 4.03%) and 291 L (SE = 15.4%), with inter-individual variability values (well described by proportional error models) of 43.7% and 100.29%, respectively. Residual variability, also according to a proportional error model, was 17.29%.

Graphical exploratory analysis of the correlation between individual Bayesian CL/F and V/F values estimated by NONMEM (using the POSTHOC option) and non-genetic covariates by GAM, revealed that age, sex, GGT and concomitant treatment with lamivudine and emtricitabine showed a specific influence on CL/F. Whereas only BMI, and to a lesser extent TBW, showed any influence on V/F. Table 3 summarizes the relevant models that take these covariates into consideration. Despite the observed decrease in OFV when included in the model, only age and GGT had a statistically significant affect on CL/F, whereas no covariates had a statistically significant affect ($p > 0.5$) on V/F (see Table 3; Model 6). Although this intermediate model resulted in a reduction in OFV of 17.92 ($p < 0.01$), the inter-individual variability in CL/F and the residual were insignificantly reduced.

With respect to PG covariates, expressed as SNPs, only 10 (CYP2B6 516G>T, CYP2B6 785 A>G, CYP2B6 1459C>T, CYP2A6 86G>A, CYP3A4 392A>G, CYP2C19 681 G>A, MDR1 61A>G, MRP4 3463A>G, MRP4 1497C>T, MRP4 912 G>T) of the 90 SNPs analyzed showed a specific influence on individual CL/F in preliminary exploratory GAM analysis. These SNPs were added stepwise to the intermediate PK/PG model (see Table 3; Model 6). However, only two CYP2B6 SNPs (516 G>T and 785 A>G) showed a change in CL/F ≥ 20 % (the value required to be selected as a clinically relevant covariate). Because they both define allele 6 of this isoenzyme (CYP2B6*6), these SNPs were included in the model as a single covariate, resulting in a reduced OFV of 153.169 units

with respect to the basic model and a >33% change in CL/F inter-individual variability (28.86% *vs.* 43.70%). When this genetic covariate was included in the model, a deficient estimation of the contribution from the parameter quantifying age was observed (SE > 50%). Thus age was excluded as a predictor of EFV CL/F. In fact, following exclusion of age, OFV decreased 14.291 units with respect to the previous model (see Table 3; Model 8 vs. Model 7).

Table 3. Representative PK/PG population models tested for CL/F.

Number	Model description	Covariate	OFV	Model used for comparison	ΔOFV	CV $_{CL/F}$ (%)	CV $_{V/F}$ (%)	σ (%)
1	CL= θ_1	Basic model	182.464			43.70	100.99	17.29
2	CL= θ_1*TBW	TBW	224.645	1	-42.181	43.59	98.89	17.83
3	CL= θ_1*BMI	BMI	286.811	1	-104.347	43.70	99.80	18.38
4	CL= θ_1+ θ_3*AGE	AGE	178.312	1	4.152	43.24	98.29	17.35
5	CL= θ_1+ θ_3*GGT	GGT	167.448	1	15.016	43.13	98.84	17.12
6	CL= θ_1+ θ_3*AGE+ θ_4*GGT	AGE, GGT	164.540	1	17.924	42.54	99.55	16.91
7	CL= (θ_1+ θ_3*AGE+ θ_4*GGT) $\theta_5^{CYP2B6*6}$	AGE, GGT CYP2B6*6	29.295	6	153.196	28.86	95.34	16.73
8	CL= (θ_1+ θ_3*GGT) $\theta_4^{CYP2B6*6}$	GGT, CYP2B6*6	15.004	7	14.291	29.75	90.11	16.49
9	CL= (θ_1+ θ_3*GGT)* $\theta_4^{CYP2B6*6}$ $\theta_5^{CYP2C19\ 681G>A}$	CYP2C19 681 G>A	14.993	8	0.011	29.75	90.11	16.49
10	CL= (θ_1+ θ_3*GGT)* $\theta_4^{CYP2B6*6}$ *$\theta_5^{MDR1\ 61A>G}$	MDR1 61 A>G	14.615	8	0.389	29.68	90.28	16.49
11	CL= (θ_1+ θ_3*GGT)* $\theta_4^{CYP2B6*6}$ *$\theta_5^{CYP3A4\ -392A>G}$	CYP3A4 -392A>G	11.354	8	3.650	29.53	95.50	16.40
12	CL= (θ_1+ θ_3*GGT)* $\theta_4^{CYP2B6*6}$ *$\theta_5^{MRP4\ 969G>A}$	MRP4 969 G>A	9.390	8	5.614	29.30	91.05	16.43
13	CL= (θ_1+ θ_3*GGT)* $\theta_4^{CYP2B6*6}$ *$\theta_5^{MRP4\ 3463A>G}$	MRP4 3463 A>G	7.083	8	7.921	29.36	90.88	16.43
14	CL= (θ_1+ θ_3*GGT)* $\theta_4^{CYP2B6*6}$ *$\theta_5^{MRP4\ 1497C>T}$	MRP4 1497 C>T	6.359	8	8.645	29.26	95.59	16.34

Although inclusion of the remaining eight SNPs initially selected in this new model (see Table 3; Model 8) demonstrated that, MRP4 969G>A, MRP4 3463A>G and MRP4 1497C>T were statistically significant, because the OFV was reduced by more than 3.84 units, only inclusion of MRP4 1497C>T changed the magnitude of CL/F >20%. Thus, only MRP4 1497C>T was included in the final model. However, inclusion of MRP4 1497C>T in the final model insignificantly influences the inter-individual variability of CL/F. Table 3 summarizes the most significant models evaluated, and includes the main parameters used in model discrimination.

In conclusion, the final model adopted for CL/F was as follows:

$$CL/F = (\theta_1 + \theta_3 * GGT) * \theta_4^{CYP2B6*6} * \theta_5^{MRP4\ 1497C>T}$$

where θ_1 through θ_5 are fixed parameters and CYP2B6*6 and MPR4 1497C>T were assigned values 0, 1, or 2 for patients with wild-type [G/G], heterozygous [G/T], or homozygous [T/T] genotypes, respectively.

Regarding V/F, none of the covariates could be included in the final model, not even TBW and BMI which were previously selected by GAM, because these covariates did not fulfill the required statistical criteria. This may explain why $CV_{V/F}$ barely reduced, from 100.29 % in the basic model to 95.59 % in the final model. In fact, the residual variability decreased less than 5% in the final model in comparison with the basic model (16.34% *vs.* 17.29%). Table 4 shows the parameters included in the final population PG/PK model proposed for EFV.

Table 4. Efavirenz Population PG/PK parameters in the final model[a].

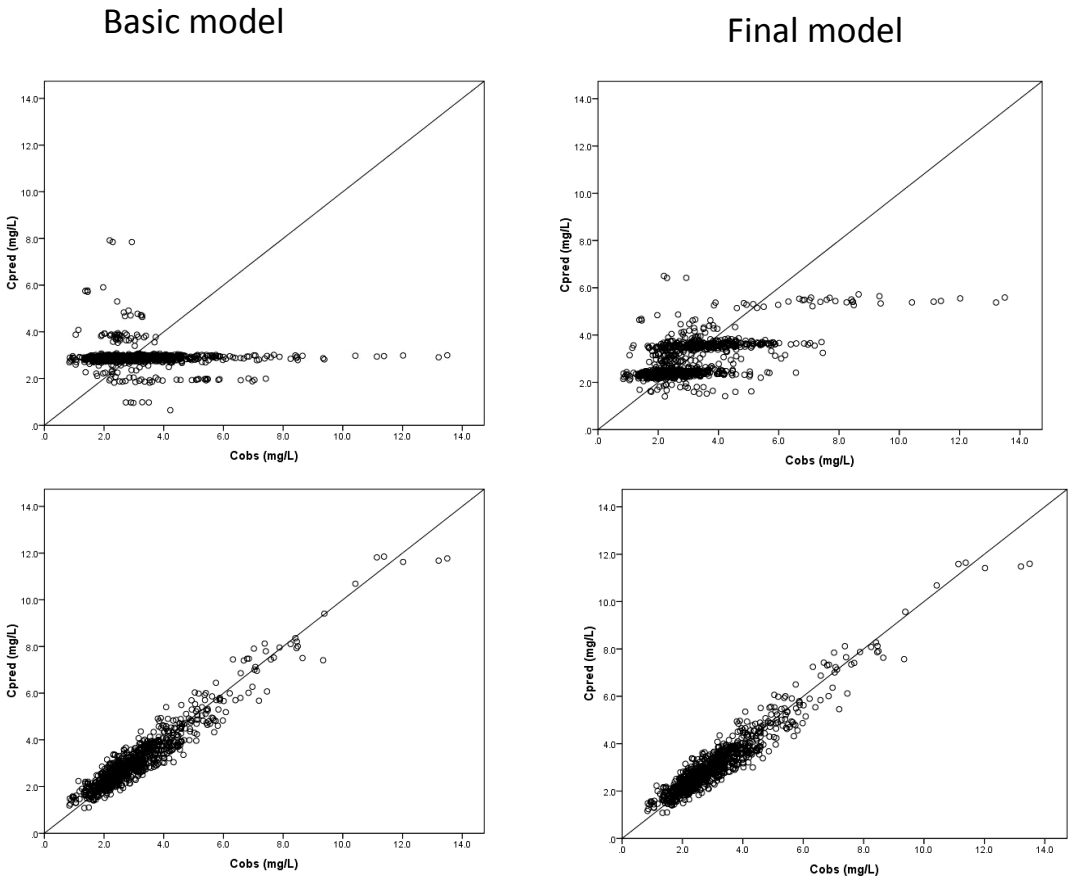
Parameter	Estimate value	SE (%)
θ_1 (liters/h)	***12.3***	***3.17***
θ_2 (liters)	***264***	***14.6***
θ_3	***-0.00213***	***36.6***
θ_4	***0.640***	***1.80***
θ_5	***0.799***	***19.1***
$CV_{CL/F}$ (%)	***29.26***	***16.0***
$CV_{V/F}$ (%)	***95.59***	***20.2***
σ (%)	***16.34***	***7.75***

[a] Final model: $CL/F = (\theta_1 + \theta_3 * GGT) * \theta_4^{CYP2B6*6} * \theta_5^{MRP4\ 1497C>T}$.

In addition, examination of a scatter plot of weighted residuals *versus* predicted concentrations obtained from the final model revealed a significant improvement in pattern (random distribution) with respect to the basic model, in agreement with the OFV decrease (difference in OFV, 176.105; $p < 0.01$). With the exception of GGT (SE= 36.6%), random and fixed-effect parameters were estimated with an SE of < 20%. Figure 1 shows scatter plots of measured EFV concentrations *versus* EFV concentrations predicted by the basic and the final models. These plots show an improvement in fit for the final model, reflected as tighter scatter around the identity line. Furthermore, the linear regression correlation coefficient between observed *versus* fitted

concentrations was 0.648 for the final model; significantly better than the correlation coefficient of 0.07 obtained for the basic model.

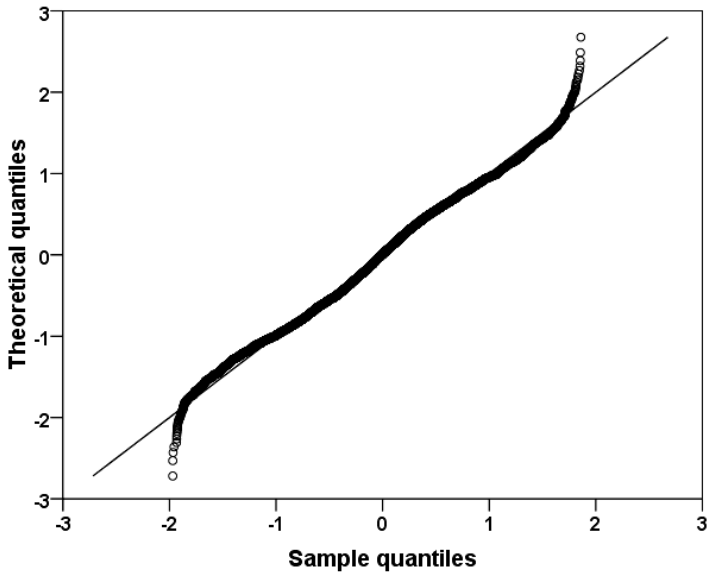
Figure 1. Scatter Plot of measured EFV plasma concentrations (Cobs) versus EFV concentrations predicted (Cpred) by the basic (left panels) and the final (right panels) models.



The results obtained during model validation support the final proposed model in a simulated population as described in the Material and Methods section. The quantiles of normalized pseudo-residuals are plotted against theoretical quantiles from a uniform distribution in Figure 2.

Model adequacy was formally tested using a Kolmogorov–Smirnov test, to compare pseudo-residuals to uniform distribution over [0, 1], resulting in a p-value of 0.066, which is higher than the empirical value of 0.010. Therefore, the model can be considered to be adequate.

Figure 2. Quantile-quantile plot of pseudoresiduals for simulated patients versus the uniform distribution: observed values are plotted against theorical quantiles for a uniform distribution over [0, 1].



Discussion

The overall aim of this study was to develop a PK/PG model for a Caucasian population of HIV-infected patients, which can be used as a tool for optimization of EFV dosage in clinical practice, to minimize the inter-individual EFV PK variability partly responsible for important differences in clinical response to EFV treatment. Although some population PK/PG models have been previously reported using similar methodology (1, 9, 40), this is the first study to extensively examine the combined effects of a large number (90) of different SNPs (some new SNPs and others previously identified as functional) present in genes coding for the main metabolizing enzymes and drug transporters in a Caucasian population of HIV-infected patients.

Although the kinetics of EFV seem to be better described by a two-compartment model (3, 30, 62), owing to the nature of the data (e.g. sparse TDM data), a one compartment linear model was used in this study. This simple model appeared to describe our data adequately, and has been widely used by others for EFV (9, 14, 42, 46, 48).

The number of covariates examined in this study, especially those related to genetic polymorphisms, was very high (>150). GAM analysis was able to significantly reduce the number of covariates to those most likely to influence CL/F and V/F, which simplified the population model building process.

Although preliminary results suggested the incorporation of gender and age for CL/F and BMI or TBW for V/F, in the end no demographic covariates could be included in the final model according to statistical criteria. Despite some controversy in the literature regarding sex and body size (TBW or BMI), these results are consistent with most EFV population studies (9, 14, 30, 31, 46). For example, the influence of sex may be race specific, because African females appear to have a metabolizing capacity of 70% compared to African males (7, 42). However, this influence has not been well demonstrated in Caucasian populations (9, 30, 46). Regarding TBW and BMI, some studies have shown an influence on CL/F (1, 43, 59, 65).

With respect to clinical covariates, only biochemical markers of liver function showed an influence on CL/F. This correlation seems logical, because EFV is eliminated primarily through hepatic metabolism. In addition, liver toxicity (as indicated by a severe increase in liver enzymes) related to EFV occurs in 1 to 8% of HIV-patients (49), and has been attributed to, among other reasons, EFV accumulation or dose-dependent mechanisms (32). However, because of the tight correlation between different biochemical markers, only GGT was selected as the best predictor with the greatest influence on this PK parameter. This result should be interpreted with caution, because the influence of GGT is only significant at the very high values attributed to impairment of liver function. Thus, a 20% decrease in CL/F was obtained for rare values > 1155 UI/L. However this result could be useful for predicting the risk of EFV toxicity in patients with these GGT values who are receiving standard EFV doses.

Although clinically important interactions can occur when EFV is used in combination with protease inhibitors (PIs), in this study, no patients received this kind of drug. Therefore the influence of PIs could not be analyzed. However, other concomitant ARVs used, such as NRTIs, did not show a significant correlation with any of the estimated EFV PK parameters. Although inclusion of lamivudine, zidovudine, and emtricitabine on CL/F significantly reduced the OFV, this parameter was insignificantly changed by factors of 1.05, 0.98 and 1.08 respectively. Thus, the 95% CI included unity, and these parameters were not included in the final model. In general, most studies confirm that co-administration of EFV with these ARVs does not significantly affect the PK of EFV (14, 40), and only Stöhr (59) has reported a 25% reduction in EFV plasma concentration when used in combination with zidovudine.

Previous studies have examined the influence of genetic covariates on the kinetic behavior of EFV (7, 9, 11, 21, 22, 36, 37, 42, 46, 51, 54, 61, 63). In general, these studies have primarily focused on genetic polymorphisms in CYP2B6, the enzyme responsible for the major metabolic pathway of EFV. More recently, some researchers have conducted extensive studies that have analyzed a larger number of genetic polymorphisms in genes coding for enzymes responsible for minor EFV metabolic pathways, as well as several transporters involved in different EFV kinetic processes (1, 9, 16, 34, 40, 48).

The CYP2B6 gene is highly polymorphic, with numerous SNPs and associated haplotypes, a higher frequency of which are observed in black populations (≈ 45%) compared to Caucasians (≈ 22%) or Asians (≈ 17%), which have been much less studied (26, 27). In our study, we individually analyzed the influence of several SNPs in CYP2B6 (983 T>C, 1459 C> T, 136 A> G, 64 C>T, 593 T> C, 1132 C>T; 516 G>T and 785A >G) previously selected for their ability to reduce the activity of this enzyme. Of these, only 516 G>T and 785 A>G, which constitute the CYP2B6*6 allele, are able to explain an important part of EFV CL/F inter-individual variability. None of the remaining SNPs analyzed show any significant influence, although these results are inconclusive because of the low frequency or even absence of these SNPs in the population studied (see Table 2). To date, SNPs 1459 C>T and 64 C>T have not been identified as responsible for significant changes in EFV metabolism, whereas SNPs 983 T>C, 136 A>G, 593 T>C and 1132 C>T are associated with high EFV plasma concentrations, especially when present with the polymorphism 516 G>T and in black populations (22, 53).

Univariate, stepwise inclusion of the SNPs analyzed from CYP3A5 and CYP3A4 (see table 2) indicate a statically insignificant influence on EFV CL/F. The selection of these SNPs was again made based on their involvement in reducing the activity of these isoenzymes. These results do not indicate a significant influence, consistent with previous studies, including those conducted on black populations (17, 39, 55, 61, 60), suggesting that these isoenzymes play a minor role in EFV metabolism. Although in a detailed analysis of these isoenzymes, Arab-Alamedine *et al.* (1) concluded that the SNP 17163 G>T of CYP3A4 does influence CL/F, again this effect was only observed in patients with impaired CYP2B6.

None of the CYP2A6 SNPs (see Table 2) were included in the final model, although 86 G>A did show some influence on EFV CL/F in the preliminary GAM analysis. To date, the CYP2A6 isoenzyme has been little studied, although its contribution to EFV metabolism is gaining in importance. Thus, some population PK studies (1, 16) have reported that some CYP2A6 polymorphisms in patients with CYP2B6 slow metabolizer genotypes were associated with a higher drug area under the curve (AUC) and lower CL/F.

However, other studies (35) have identified CYP2A6 genetic variations as independent predictors of EFV plasma concentrations.

Notably, of all the SNPs in isoenzymes CYP2C19, CYP2C9, CYP2C8 and CYP2D6 (see Table 2), only CYP2C19 681 G>A displayed a tendency to reduce EFV CL/F. In the end, CYP2C19 681 G>A was not included in the final model because statistical criteria (difference in OFV was < 3.84) were not met. In fact, no other studies have analyzed these polymorphisms, most likely because their contribution to EFV metabolism may be small and remains poorly understood. However, for the CYP2D6 SNPs, Fellay *et al.* (17) reported a trend of higher plasma EFV levels, but only when these SNPs were associated with polymorphisms in CYP2B6.

In contrast, studies that have analyzed the influence of polymorphisms in membrane transporters are scarce, with inconclusive results. In our study, we have examined fundamental efflux transporters, including P-glycoprotein (P-gp, encoded by the gene MDR1). MDR1 is the best studied, because several MDR1 genetic polymorphisms that affect protein expression have been shown to influence absorption and disposition of some ARVs (9, 17, 23, 24, 30, 37, 40, 55, 61). Of all the MDR1 SNPs analyzed in this study (see Table 2), only 61A>G displayed a tendency to increase EFV concentrations in carriers of the CYP2B6*6 allele (genotype T/T). However this could not be included in the final model because the resulting reduction in OFV was < 3.84; probably due to the low frequency of both polymorphisms (seen in only one patient) in our population. This observation, coupled with the fact that no previous studies have examined the influence of these SNPs, suggests that further studies on larger numbers of patients with this polymorphism are required to confirm this result. For the SNP MDR1 3435 C>T, we did not find a significant effect, despite the high frequency of occurrence (68.8%) of this polymorphism in our population. These results are in agreement with previous studies, which also did not observe any influence from this SNP (23, 24, 55, 61). However, a study by Fellay *et al.* (17) did report a decrease in EFV plasma concentrations in patients with this polymorphism. The remainder of the MDR1 SNPs analyzed in our study did not have any significant influence on CL/F or V/F. Only one of these SNPs, 2677 G>T, has been analyzed previously by other groups, who also found no influence of this SNP on the PK of EFV (23, 24).

Notably, of the efflux transporters analyzed, MRP4 appears to be the most relevant, because several SNPs from this transporter gene (1497 C>T, 3463 A>G and 969 G>A) were observed to have a specific influence on CL/F, although only 1497 C>T was included in the final model. EFV CL/F decreased by a factor of 0.79 for patients with a heterozygous genotype, possibly due to decreased protein expression, which would cause an increase in F, but not a direct effect on EFV drug metabolism. Although inclusion of this SNP in the model significantly reduced the OFV (8.645), and its coefficient implies an influence on CL/F > 20%, its contribution to reducing CL/F variability is minimal, probably due to the low frequency (3.20% heterozygous) found for this polymorphism and the fact that no patient had a double mutation (homozygous genotype).

SNPs related to MRP1 and MRP2 (see Table 2) had no influence on the PK of EFV, and no other studies have analyzed these SNPs, with the exception of Fellay *et al.* (17), who reported similar results.

It is noteworthy that in previous studies, polymorphisms in CYP2B6 have been found to significantly affect EFV pharmacokinetics. Furthermore, the influence of polymorphisms in other isoenzymes and transporters is usually observed when associated with carriers of the CYP2B6*6 allele. In our study, this allele was also observed to be the most important, however low frequency or absence of these SNPs, in combination with other polymorphisms, limited our ability to determine the influence of these combinations. Therefore, it would be interesting to conduct multicenter clinical studies encompassing larger numbers of Caucasian patients, in order to include more patients with these kinds of combinations.

According to PK parameters obtained from the final model, the average EFV CL/F in patients with normal GGT values (12-55 U/L) and without CYP2B6 isoenzyme or MRP4 transporter polymorphisms was 12.51 L/h, in agreement with previous reports (1, 9, 16, 30, 31, 46, 48). When CYP2B6 polymorphisms are included, CL/F values would be: 5.03 and 7.87 L/h for patients with T/T and G/T genotypes, respectively. The magnitude of these values is also similar to those previously reported in other studies, highlighting likely overexposure to EFV in patients with G/T or T/T genotypes who receive the standard dose of 600 mg/day (1, 9, 42). In fact, incorporation of these polymorphisms in our

population model explains over 33 % of PK inter-individual CL/F variability, and can justify its determination "*a priori*" to initiate treatment using the appropriate EFV dose for each patient. EFV CL/F when only MRP4 polymorphisms were included is reduced to 9.82 L/h. The proposed final model should be used with caution, and only when there are polymorphisms in either CYP2B6 or MRP4, but not both, because this model has been developed from data in which only one patient had both polymorphisms, and thus the ability of this model to correctly predict, "*a priori*", EFV CL/F, has not been validated. An "*a priori*" reduction to 400 and 200 mg/day for CYP2B6 G/T and T/T genotypes is recommended by our study, in agreement with others (1, 8, 9, 34, 60). However, the desirability of reducing EFV doses in patients with MRP4 polymorphisms and high GGT levels still requires further investigation of the influence of these covariates, on a larger number of patients, to confirm the results obtained in this study.

The V/F of 264 L, estimated with a higher level of uncertainty than CL/F, is within the range of values (150 to 421 L) established by other authors (1, 9, 16, 30, 31, 46, 48). The fact that it was not possible to include any covariate with V/F justifies that its variability in the final model has been insignificantly reduced and was significantly higher than that obtained for CL/F.

The proposed final model is simple, easily applicable in clinical practice and similar to that previously published by our research group (9). In addition, the added value of the model presented here is based on the larger number of patients analyzed and the detailed genetic analysis conducted on these patients, might suggest that polymorphisms other than the CYP2B6*6 haplotype (the major predictor of plasma EFV exposure) and MRP4 (not previously studied) do not influence EFV CL/F. Moreover, the model presented here considers the potential influence of high GGT values.

Finally, although model covariates explain a significant proportion of the inter-individual EFV kinetic behavior variability, this variability remains relatively high, whereas the remaining residual variability is low. Because of this, we still advise the use of TDM to appropriately adjust initial "*a priori*" doses.

Furthermore, the implementation of the population PK/PG model proposed here in a Bayesian algorithm would be useful for individualization of EFV dosages based on data obtained from TDM.

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Conclusiones generales

- Efavirenz es un componente esencial del tratamiento antirretroviral, que tiene una adecuada eficacia y seguridad según numerosos ensayos clínicos. Sin embargo, la experiencia clínica nos muestra una elevada variabilidad interindividual en la respuesta, lo cual se traduce en un importante número de cambios de tratamiento por fracasos virológicos y efectos adversos. Por tanto, son necesarias nuevas estrategias que permitan la individualización del tratamiento con este fármaco.

- Se ha demostrado que el ajuste de dosis progresivo de la dosis estandar de efavirenz de 600 mg hasta 200 mg una vez al día, dirigido por la monitorización de niveles plasmáticos y el análisis farmacogenético, consegue buenos resultados clínicos ya que permite disminuir de manera considerable los efectos adversos sin afectar la eficacia del tratamiento a largo plazo. Por tanto, la monitorización de niveles plasmáticos y el análisis farmacogenético pueden ser dos buenas estrategias en la práctica clínica para conseguir individualizar el tratamiento con efavirenz.

- Se ha realizado un exhaustivo análisis farmacogenético en un total de 125 pacientes en tratamiento con efavirenz, que ha permitido identificar de un total de 90 polimorfismos genéticos, aquellos más implicados en el metabolismo y transporte de efavirenz. Los SNPs más significativos se encuentran asociados a los genes que codifican las enzimas CYP2B6 (516 G> T y 785 A> G) y CYP2A6 (86 G> A, 1836 A>G) y la proteína de transporte MRP4 (1497 C>T, 3463 A>G, 3725 G>A y 912 G>T).

- Se ha podido establecer una clara relación entre estos polimorfismos genéticos y los parámetros farmacocinéticos ($C_{max\ ss}$, $C_{min\ ss}$ y CL/F) de efavirenz. Los resultados obtenidos muestran que alrededor del 50% de la variabilidad de los parámetros farmacocinéticos puede explicarse por factores genéticos. Por tanto, la integración de estos datos farmacogenéticos en la práctica clínica puede ser una herramienta útil en la individualización del tratamiento con este fármaco.

- Se ha desarrollado un modelo farmacocinético/farmacogenético poblacional de efavirenz en 128 pacientes con 869 concentraciones procedentes de la monitorización en pacientes VIH+ usando un modelo no lineal de efectos mixtos (NONMEN). El alelo CYP2B6*6 (el mejor predictor de la exposición a efavirenz), MPR4 1497C>T (un nuevo SNP, no previamente estudiado) and g-glutamiltranspeptidasa (GGT), fueron identificados como los factores de mayor influencia en el aclaramiento del fármaco de acuerdo al siguiente modelo: CL/F (L/h) = (12.3 – 0.00213*GGT)* $0.640^{CYP2B6*6}$ *$0.799^{MRP4\ 1497C>T}$. El modelo propuesto es simple y fácil de aplicar en la práctica clínica, por lo que podría considerarse una herramienta más para individualizar el tratamiento de efavirenz.