Cardiotrophin-1 Improves Kidney Preservation, Graft Function, and Survival in Transplanted Rats

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Background. Cold ischemia-reperfusion injury is unavoidable during organ transplantation, and prolonged preservation is associated with poorer function recovery. Cardiotrophin-1 (CT-1) is an IL-6 family cytokine with cytoprotective properties. This preclinical study in rats tested whether CT-1 mitigates cold renal ischemia-reperfusion injury in the context of the transplantation of long-time preserved kidneys. **Methods.** Kidneys were flushed with cold (4°C) University of Wisconsin solution containing 0.2 μg/mL CT-1 and stored for several periods of time at 4°C in the same solution. In a second approach, kidneys were first cold-preserved for 6 hours and then were perfused with University of Wisconsin solution containing CT-1 (0, 16, 32, or 64 μg/mL) and further cold-preserved. Organ damage markers were measured in the kidneys at the end of the storage period. For renal transplantation, recipient consanguineous Fischer rats underwent bilateral nephrectomy and received a previously cold-preserved (24 hours) kidney as described above. Survival and creatinine clearance were monitored over 30 days. **Results.** Cardiotrophin-1 in perfusion and preservation fluids reduced oxidative stress markers (superoxide anion and inducible nitric oxide synthase), inflammation markers (NF-κB and tumor necrosis factor-α), and vascular damage (vascular cell adhesion molecule-1) and activated leukemia inhibitory factor receptor and STAT-3 survival signaling. Transplantation of kidneys cold-preserved with CT-1 increased rat survival and renal function (ie, lower plasma creatinine and higher creatinine clearance) and improved kidney damage markers after transplantation (ie, lower superoxide anion, tumor necrosis factor-α, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 and higher NF-κB). **Conclusions.** Cardiotrophin-1 represents a novel therapeutic strategy to reduce ischemia-reperfusion and cold preservation injury to rescue suboptimal kidneys and, consequently, to improve the clinical outcomes of renal transplantation.

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For medical and economic reasons, kidney transplantation is the preferred and definitive treatment for end-stage renal disease (ESRD).[1] Kidneys from deceased donors are a major transplantation source, and they need to be preserved during the period from dissection to grafting to maintain viability. Although cold storage is the standard preservation method,[2] it nonetheless results in some degree of renal injury that negatively impacts graft function and survival,[3-7] in an inverse relationship with preservation time.[8] Renal injury also increases graft immunogenicity, rejection, and mortality.[9-14] Cold ischemia impairs osmoregulation and aerobic metabolism,[15] leading to mitochondrial swelling, activation of the mitochondrial apoptotic pathway, increased oxidative stress,[16] and, finally, acute tubular necrosis.[17] Upon transplantation, additional injury occurs as a consequence of kidney reperfusion. Oxidative stress,[18] alterations in calcium homeostasis, and deregulation of cellular pH homeostasis trigger the activation of phospholipases and proteolytic enzymes, including caspases, which contribute to further damage and inflammation.[17,19]

Despite the implementation of preservation methods, a significant number of preserved kidneys still do not function properly after grafting. Even temporarily delayed graft function increases the rate of graft failure within 1 year after grafting.[20,21] Thus, new preservation strategies are necessary to increase the

number of adequate kidneys available for transplantation and to improve graft outcomes. Cardiotrophin-1 (CT-1) is a member of the IL-6 family of cytokines that acts by binding and activating a receptor complex containing gp130 and leukemia inhibitory factor receptor (LIFR).[22] Cardiotrophin-1 exerts potent antiapoptotic effects on hepatocytes,[23] cardiomyocytes,[24] and neurons.[25] In these cells, CT-1 activates survival signaling pathways, including signal transducers and activators of transcription 3 (STAT-3), extracellular-regulated kinase (Erk1/2), and protein kinase B. Administration of CT-1 protects rats or mice from liver damage induced by ischemia/reperfusion[26] and transplantation,[27] ischemic cardiac injury,[28,29] ischemic brain damage,[30] and acute kidney injury induced by ischemia/reperfusion.[31] Endogenous CT-1 also participates in organ protection as CT-1 null mice were found to be more sensitive to ischemic liver damage compared to the wild type,[26] and the protective effect conferred by ischemic preconditioning in normal mice is abrogated in CT-1–deficient mice.[26]

In light of our previous studies that demonstrated a protective effect of CT-1 on the oxidative stress and inflammation that occur during warm renal ischemia,[31] the present study aimed to determine whether CT-1 added to preservation fluid protects the kidneys from cold preservation-induced damage and therefore improves transplantation outcomes and functionality.

MATERIALS AND METHODS

All animal procedures were conducted in accordance with the guidelines for animal use published by the Conseil de l'Europe (No. L358/1-358/6, 1986) and by the Spanish Government (BOE No. 67, pp. 8509-8512, 1988, and BOE No. 256, pp. 31349-31362, 1990). The Bioethical Committee of the University of Salamanca approved all experiments. Unless otherwise indicated, all reagents were purchased from Sigma-Aldrich (Madrid, Spain).

Male Fisher rats were used. Kidneys were extracted from donor rats and preserved in different conditions, following preservation protocols 1 and 2, described below. At the end of the preservation period, a subset of kidneys were used for biochemical analysis, while the others were transplanted into recipient rats, according to Transplantation Protocol 1 (used after Preservation Protocol 1) and Transplantation Protocol 2 (used after Preservation Protocol 2). Figure 1 shows a flow chart of all the experimental protocols.

Kidney Cold Preservation

Preservation Protocol 1

Kidneys were obtained from 7- to 8-week-old male Fischer rats. Detailed procedures of kidney procurement and perfusion are described in (**SDC, Materials and Methods,** http://links.lww.com/TP/B588). Kidneys were perfused in situ with University of Wisconsin (UW) solution with or without 0.2 μg/mL rat CT-1 (DRO Biosystems, San Sebastian, Spain) and cold-preserved for 0.5, 6, 24, and 48 hours. Then, a piece of each kidney was snap-frozen in liquid nitrogen and preserved at −80°C. The remaining renal tissue was immediately used for superoxide anion (SOA) measurement. In a simulated

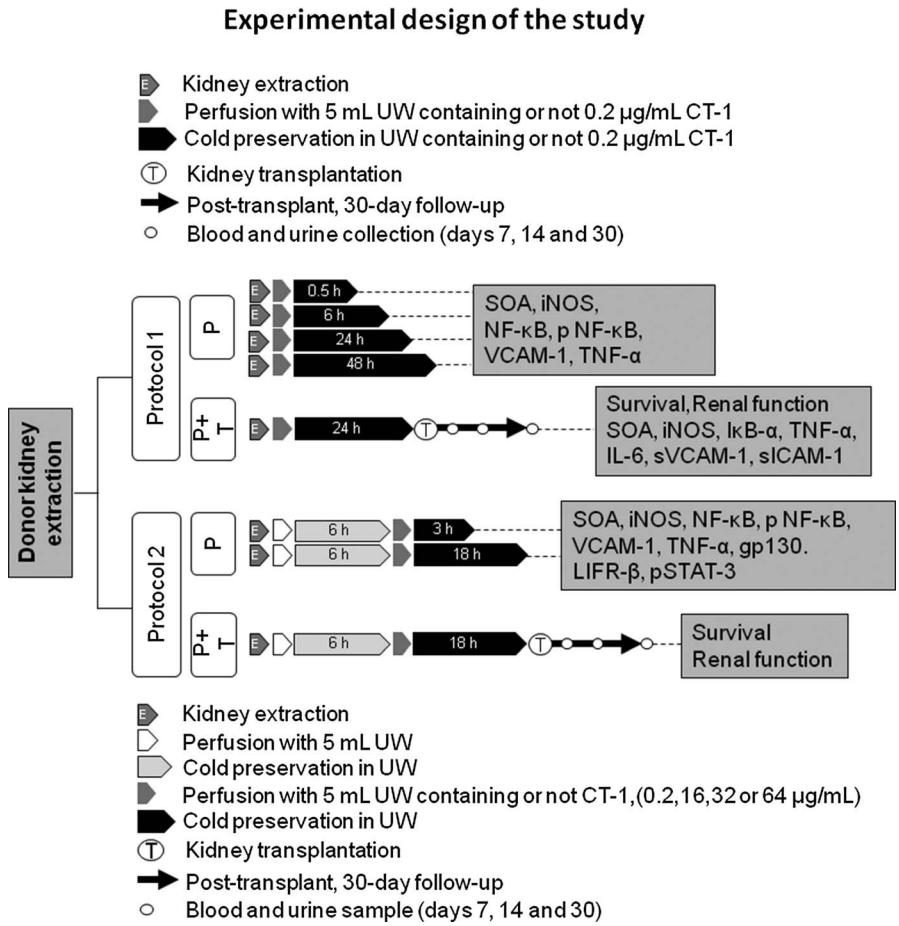

FIGURE 1. Flowchart scheme showing the experimental design of the study. P, preservation; T, transplantation.

group, kidneys were perfused with 5 mL of UW solution and immediately harvested.

Preservation Protocol 2

In a different group of animals, the kidneys were perfused in situ with UW, cold-preserved for 6 hours, perfused again with UW containing CT-1 (0, 16, 32, or 64 μg/mL), and then further preserved for 18 hours, as detailed in (**SDC, Materials and Methods,** http://links.lww.com/TP/B588). The UW solution was chosen for preservation as it has been shown to have a superior protective effect in transplanted renal grafts in mice.[15]

Kidney Transplantation

Renal transplantation was performed into male Fisher rats using cold-preserved (24 hours) kidneys as described above and in (**SDC, Materials and Methods,** http://links.lww.com/TP/B588). Transplanted rats were followed for 30 days. At the end of the experimental time, the transplanted kidney was removed, washed with cold saline solution, and immediately divided into 2 halves. One half was snap-frozen in liquid nitrogen and stored at −80°C for further analysis and the other was used immediately for SOA measurement.

Sample Analysis

For renal function studies, rats were placed in individual metabolic cages. Blood and urine samples were taken 3, 7, and 14 days after renal transplantation. Plasma and urine creatinine concentrations, SOA in renal tissues, tumor necrosis factor (TNF)-α in the preservation fluid from the reperfusion process, plasma levels of IL-6, soluble intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and the renal tissue content of phospho-STAT-3, STAT-3, phosphor-NF-κB, NF-κB, inducible nitric oxide synthase (iNOS)/NOS-2, IκB, gp130, LIFR, and CT-1 were measured as described in (**SDC, Materials and Methods,** http://links.lww.com/TP/B588).

Statistical Analysis

Statistical analysis was performed using the NCSS (2007) (Dr. Jerry L. Hintze, Kaysville, Utah, USA). Data with a normal distribution were expressed as mean ± standard deviation (SD). Scheffe test was used for multiple comparisons. For data without normal distribution, the Kruskal-Wallis test was used for multiple comparisons. Statistical significance was achieved at $P < 0.05$ or $Z > 1.96$. Progression-free survival probabilities were evaluated using the Kaplan-Meier method. Statistical differences were assessed by the log-rank test.

RESULTS

Addition of CT-1 to the Perfusion and Preservation Fluids Reduces Cold Preservation-induced Kidney Damage

Cold preservation induces an increase in SOA production and iNOS expression,[32,33] and decreased oxidative stress improves renal function and graft survival after kidney transplantation.[33] Our data revealed that after cold preservation, SOA levels at 24 and 48 hours were higher in the control group (Ctrl) than in the sham group, and the addition of CT-1 to the perfusion and preservation fluid maintained SOA at low levels, similar to those in sham animals (Figure 2A). Cold-preserved kidneys (Ctrl) had higher renal iNOS levels compared to those in the sham group, and CT-1 significantly attenuated this increase (Figure 2B).

It has been reported that increased oxidative stress activates inflammation, SOA activates NF-κB, and in turn, that NF-κB induces TNF-α release.[34] In accordance with this, we observed an increase in renal tissue NF-κB activation and TNF-α production after cold ischemia. CT-1 administration in the preservation fluid markedly lowered NF-κB activation and TNF-α production after 24 and 48 hours of preservation. This is supported by a lower phospho-NF-κB (p65)-to-total NF-κB (p65) ratio in tissue homogenates (Figure 3A) and lower levels of TNF-α in the preservation fluid (Figure 3B), as compared with control preservation conditions.

Prolonged cold ischemia is also associated with vascular damage after grafting, in which overexpression of endothelial adhesion molecules plays a major role in monocyte infiltration during organ reperfusion.[10] Likewise, we observed a pronounced increase in renal VCAM-1 levels under cold preservation conditions. We also observed significantly lower VCAM-1 levels when the kidneys were cold-preserved in the presence of CT-1 compared to the Ctrl (Figure 3C).

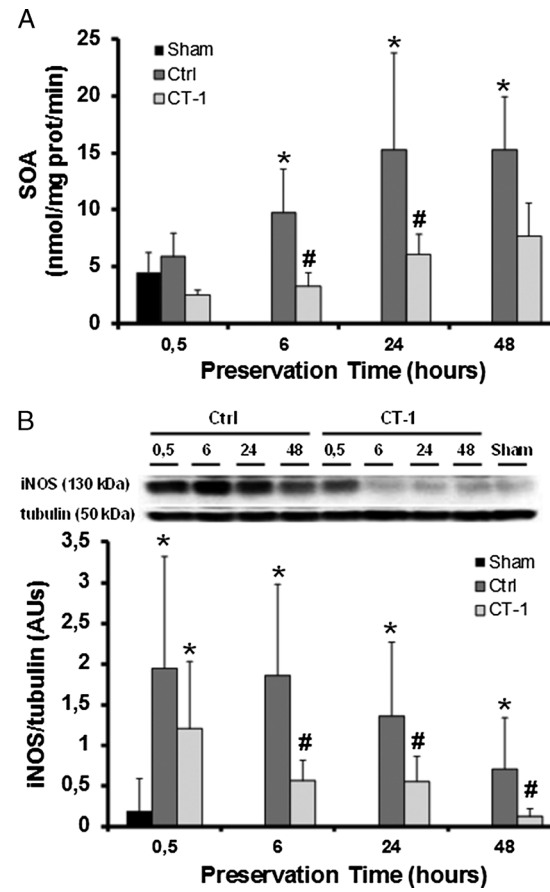

FIGURE 2. Effect of the addition of CT-1 to the perfusion and preservation fluid on kidney levels of SOA (panel A) and iNOS (panel B) after 0.5, 6, 9, and 24 hours of cold preservation. Values are expressed as AU. Values are represented as mean ± SD (sham, n = 5; Ctrl, n = 5; CT-1, n = 5). Statistically significant differences: * Z > 1.96 all groups versus sham; # Z > 1.96 CT-1 versus Ctrl same day (Kruskal-Wallis multiple-comparison test and Dunn test). AU, arbitrary units.

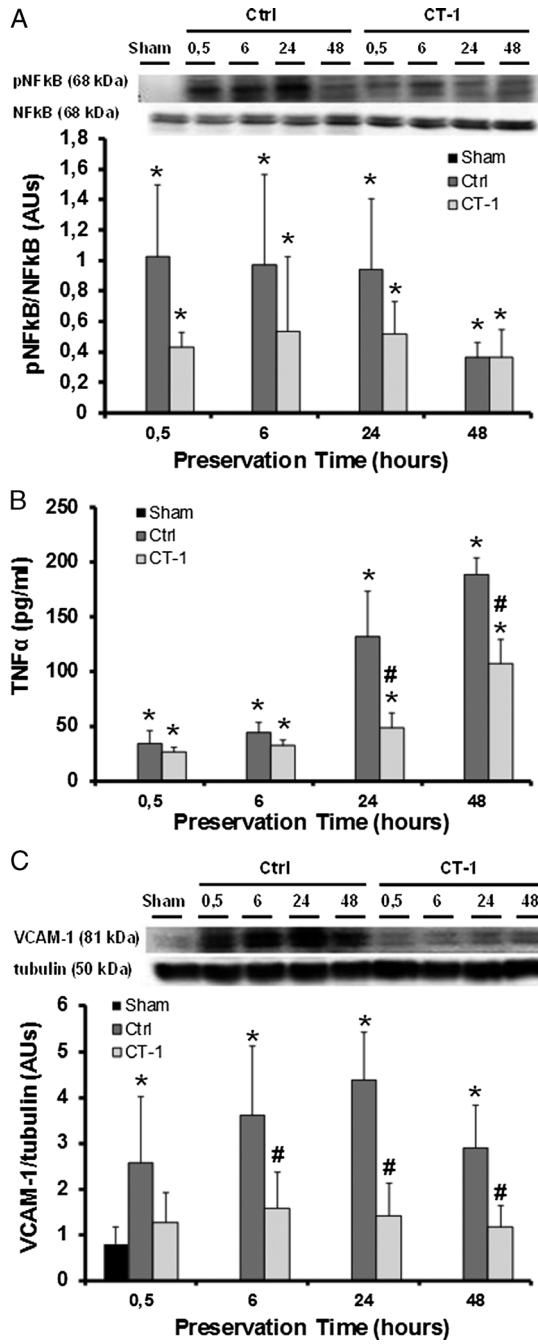

FIGURE 3. Effect of the addition of CT-1 to the perfusion and preservation fluid on the activation of Nuclear Factor-*kappa* B (NFkB, panel A), the preservation fluid concentration of Tumor Necrosis Factor-α (TNF-α, panel B), and the renal content of VCAM-1 (panel C) after 0.5, 6, 9, and 24 hours of cold preservation. Values of pNFκB/NFκB ratio and VCAM-1 content in homogenates of kidney tissue are expressed as AU. Data are represented as mean ± SD. 5 animals per group. Statistically significant differences: * Z > 1.96 all groups versus sham; # Z > 1.96 CT-1 versus Ctrl same day (Kruskal-Wallis multiple-comparison test and Dunn test).

Perfusion With Higher Concentrations of CT-1 After 6 Hours of Cold Preservation Also Reduces Kidney Damage

The previous data indicate that supplementation of the perfusion and storage fluid with CT-1 reduces cold ischemia-induced kidney damage. However, adding CT-1 from the beginning of the preservation process will be difficult in many cases in real clinical practice. After kidney dissection from the donor, the recipient's identity is not immediately known, and the addition of CT-1 will require written authorization from recipient, at least in the first clinical trials. Therefore, we also assessed whether adding CT-1 to the fluid after 6 hours of preservation shows a beneficial effect on renal preservation. We observed that CT-1 does protect the kidneys in these conditions, although much higher concentrations (ie, over 2 orders of magnitude) are necessary to achieve a similar effect. According to all parameters analyzed, including SOA (Figure 4A), iNOS (Figure 4B), phospho-NF-κB (p65)-to-total NF-κB (p65) ratio in tissue homogenates (Figure 5A) and TNF-α in the preservation fluid (Figure 5B), 32 to 64 μg/mL CT-1 is necessary when added 6 hours later, whereas only 0.2 μg/mL CT-1 (Ctrl+) is needed if added from the beginning of the preservation process.

CT-1 Activates Survival Signaling Under Cold Conditions

CT-1 signals through the gp130/LIFR complex, which activates intracellular pathways including the STAT-3 pathway.[22,35]

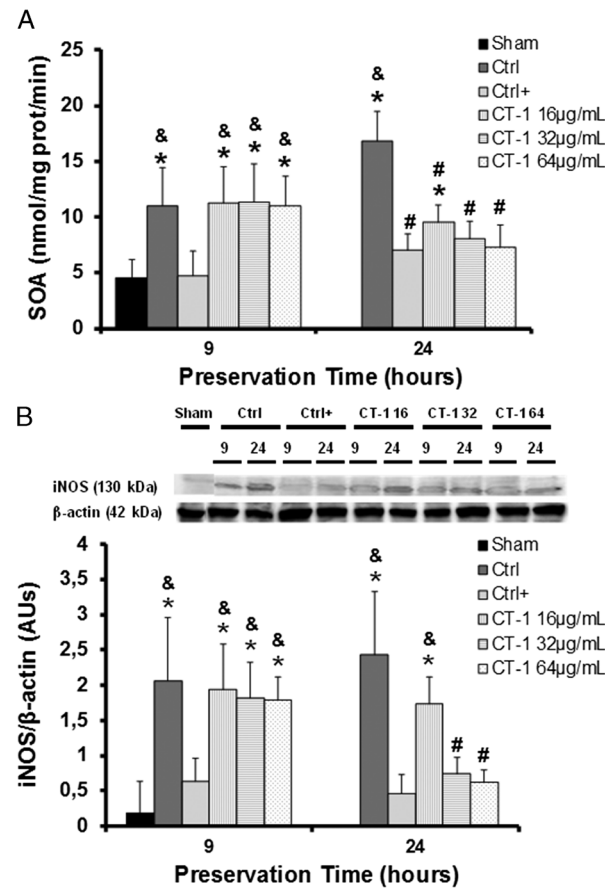

FIGURE 4. Effect of kidney perfusion with CT-1-containing perfusion fluid after 6 hours of cold storage on the kidney levels of SOA (panel A) and iNOS. Renal iNOS content values are expressed as arbitrary units (AU). Values are represented as mean ± SD. 6 animals per group, except sham (n = 5). Panel A: * $P < 0.05$ all groups versus sham; # $P < 0.05$ CT-1 groups versus Ctrl same day; & $P < 0.05$ CT-1 groups versus Ctrl+ same day (2-way ANOVA and Scheffe test was used for multiple comparisons). Panel B: * Z > 1.96 all groups versus sham; # Z > 1.96 CT-1 groups versus Ctrl same day; & Z > 1.96 CT-1 groups versus Ctrl+ same day (Kruskal-Wallis multiple-comparison test and Dunn test).

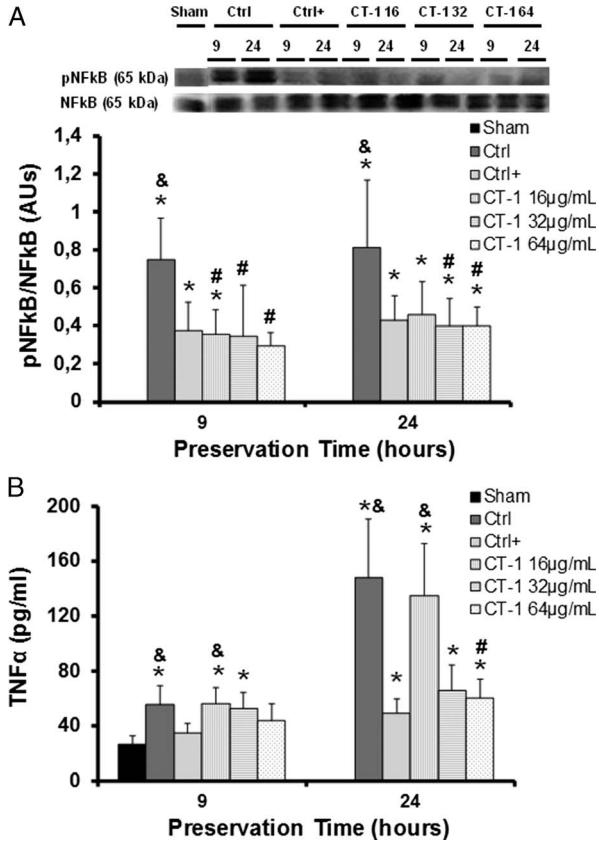

FIGURE 5. Effect of kidney perfusion with CT-1-containing perfusion fluid after 6 hours of cold storage on kidney activation of Nuclear Factor-*kappa* B (NFkB, panel A) and Tumor Necrosis Factor-α (TNF-α, panel B) levels in the preservation fluid after 9 and 24 hours of cold preservation. NFkB activation, expressed as the pNFκB/NFκB ratio, is expressed as AU. Data are represented as mean ± SD (sham, n = 5; Ctrl, n = 6; Ctrl+, n = 6; CT-1 16 µg, n = 6; CT-1 32 µg, n = 6; CT-1 64 µg, n = 6). Statistically significant differences: panel A: * Z > 1.96 all groups versus sham; # Z > 1.96 CT-1 groups versus Ctrl same day; & Z > 1.96 CT-1 groups versus Ctrl+ same day (Kruskal-Wallis multiple-comparison test and Dunn test). Panel B: * $P < 0.05$ all groups versus sham; # $P < 0.05$ CT-1 groups versus Ctrl same day; $P < 0.05$ CT-1 groups versus Ctrl+ same day (2-way ANOVA and Scheffe test for multiple comparisons).

With the aim of determining whether CT-1 activates its receptor and intracellular signaling under cold preservation conditions, we studied the expression of CT-1 receptors and STAT-3 activation in these conditions. As shown in Figure 6A, there were no differences in the expression of gp130 between the Ctrl and sham group. When CT-1 was included in both the perfusion and preservation fluids, gp130 levels were higher after 24 hours of preservation. The same result was observed when CT-1 was administered only in the reperfusion fluid, although higher concentrations were necessary (ie, 64 µg/mL). Cold preservation increased the expression level of LIFR. All the groups receiving CT-1 showed higher levels of LIFR compared to the sham group, but the differences did not reach statistical significance due to the great variability of this parameter (Figure 6B). STAT-3 is activated by phosphorylation, and this was assessed from the pSTAT-3/STAT-3 ratio. Under normal (sham group) and control preservation conditions (Ctrl), no STAT-3 phosphorylation was detected. However, when CT-1 was added to the preservation fluids, significant

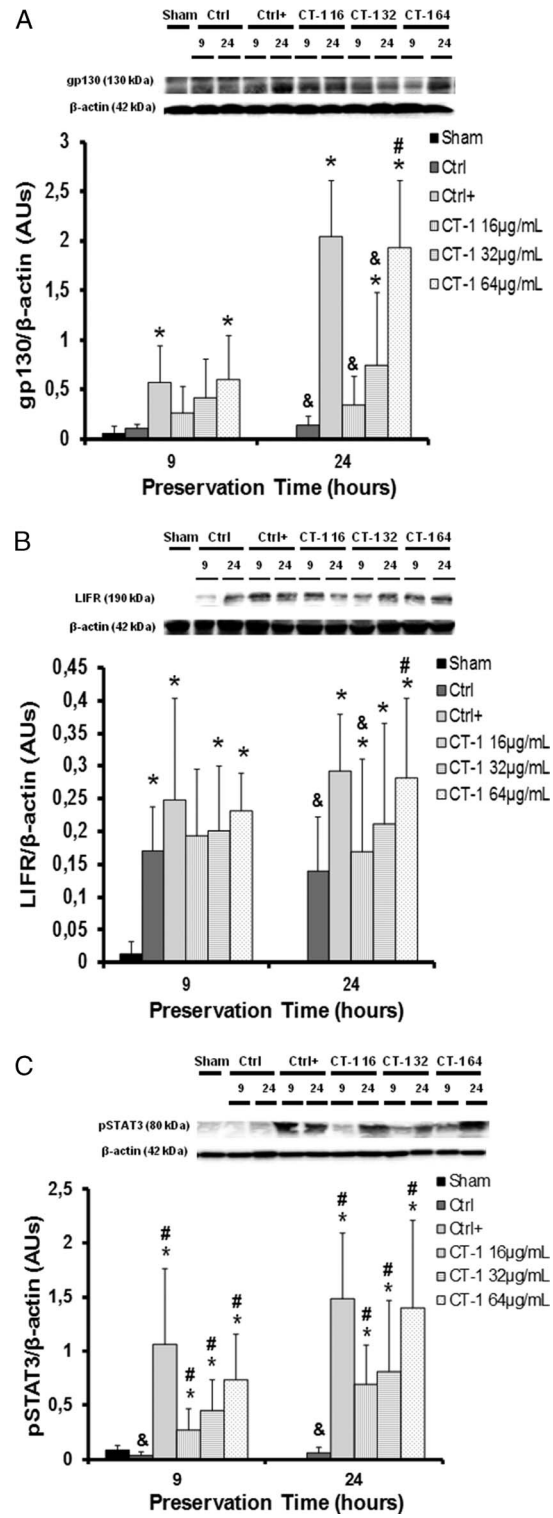

FIGURE 6. Effect of kidney perfusion with CT-1-containing perfusion fluid after 6 hours of cold storage on kidney levels of glycoprotein-130 (gp130, panel A), LIFR-*beta* (LIFR, panel B), and activation of Signal Transducers and Activator 3 (STAT3. Panel C) Values are expressed as arbitrary units (AU) and represented as mean ± SD. (sham, n = 5; Ctrl, n = 6; Ctrl+, n = 6; CT-1 16 µg, n = 6; CT-1 32 µg, n = 6; CT-1 64 µg, n = 6). Statistically significant differences: panels A and B: * Z > 1.96 all groups versus sham; # Z > 1.96 CT-1 groups versus Ctrl same day; & Z > 1.96 CT-1 groups versus Ctrl+ same day (Kruskal-Wallis multiple-comparison test and Dunn test). Panel C: * $P < 0.05$ all groups versus sham; # $P < 0.05$ CT-1 groups versus Ctrl same day; & $P < 0.05$ CT-1 groups versus Ctrl+ same day (2-way ANOVA and Scheffe test for multiple comparisons).

STAT-3 activation occurred. When CT-1 was added after 6 hours of preservation, STAT-3 activation was also attained, although much higher concentrations of the cytokine were needed to achieve a similar effect (Figure 6C). These results indicate that even in cold conditions, CT-1 activates the receptor and the survival signaling, which may account for the observed protective effect.

Preservation With CT-1 Improves Rat Survival and Renal Function, and Reduces Kidney Damage After Transplantation

To study the consequences of preservation with CT-1 on graft function and animal survival after transplantation, we used kidneys preserved under strict conditions (ie, for 24 hours) with or without 0.2 μg/mL CT-1. Our results show that recipients of kidneys preserved with CT-1 have strikingly superior survival (Figure 7A), better renal function (ie, normal or nearly normal), and no or few signs of kidney damage. Better renal function was demonstrated by a significantly lower plasma creatinine concentration (Figure 7B) and higher creatinine clearance (CrCl) (Figure 7C) in rats with CT-1-preserved kidneys compared to rats grafted with kidneys preserved in control conditions. The renal function of the CT-1-preserved-kidney rats was indistinguishable from that of the rats who underwent simulated surgery.

Kidney SOA production was markedly lower in rats of the CT-1 group than in the Ctrl, reaching values similar to those in the sham group (Figure 8A). Rats in the CT-1 group also had lower plasma levels of the pro-inflammatory cytokine TNF-α (Figure 8B) and higher levels of the anti-inflammatory cytokine IL-6, which were comparable to the levels found in sham rats (Figure 8C). Renal IκB was significantly higher in the Ctrl than in the sham group. The addition of CT-1, both in the perfusion and reperfusion fluid, was associated with significantly lower levels of IκB (Figure 8D). These data indicate that preservation with CT-1 leads to decreased inflammation in the grafted kidney. Plasma levels of soluble ICAM-1 (Figure 8E) and VCAM-1 (Figure 8F) were undetectable in the sham and CT-1 groups and significantly higher in the Ctrl group, which suggests effective endothelial protection by CT-1 from ischemia/reperfusion damage.

Addition of CT-1 to the Reperfusion Fluid After 6 Hours of Cold Preservation Improves the Outcomes of Renal Transplantation

Kidneys with CT-1 added after 6 hours of cold preservation were transplanted into recipient, nephrectomized rats. Our results indicate that, although much higher concentrations of CT-1 are needed, preservation in those conditions also significantly improves graft function and transplantation outcomes. In fact, rats who received kidneys preserved with CT-1 showed a much better survival rate compared with rats who received kidneys preserved under control conditions. In addition, survival after kidney transplantation using both preservation methods (ie, 0.2 μg/mL CT-1 from the beginning or 16-64 μg/mL CT-1 after 6 hours) was very similar and statistically indistinguishable (Figure 9A). Reduced plasma creatinine concentration (Figure 9B) and increased CrCl (Figure 9C), which were completely normalized by 0.2 μg/mL CT-1 from the beginning and by 34 to 64 μg/mL CT-1 after 6 hours, enabled kidneys

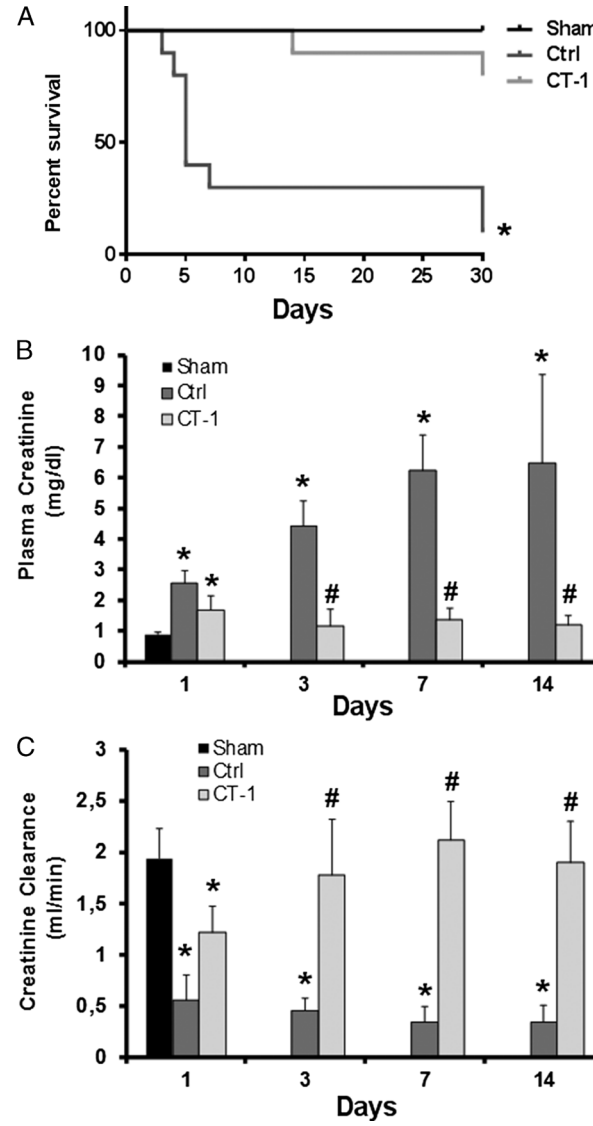

FIGURE 7. Accumulative survival in transplanted animals for 30 days (Panel A), plasma creatinine concentration (Panel B), and CrCl (Panel C) at 1, 3, 7, and 14 days after transplantation. In panel A, cumulative survival values are expressed as % of survival. 10 animals per group. In panels B and C, data are represented as mean ± SD (sham, n = 5; Ctrl, n = 5; CT-1, n = 5). Statistically significant differences: panel A: * $P < 0.05$ control versus sham; log rank (Mantel-Cox test). Panel B: * $Z > 1.96$ all groups versus sham; # $Z > 1.96$ CT-1 versus Ctrl same day (Kruskal-Wallis multiple-comparison test and Dunn test). Panel C: * $P < 0.05$ all groups versus sham; # $P < 0.05$ CT-1 versus Ctrl same day (2-way ANOVA and Scheffe test for multiple comparisons).

preserved with CT-1 in the delayed modality to perform better upon transplantation.

DISCUSSION

The results from this study show that addition of CT-1 to the perfusion and preservation fluids, even after 6 hours of cold preservation, reduces kidney damage and improves graft function and animal survival after transplantation. These effects likely result from the ability of CT-1 to activate its gp130 and LIFR receptors and the STAT-3 survival pathway under cold conditions. An initial implication of our study is that CT-1 stands out as a good candidate additive to improve kidney preservation for transplantation, as it may increase the

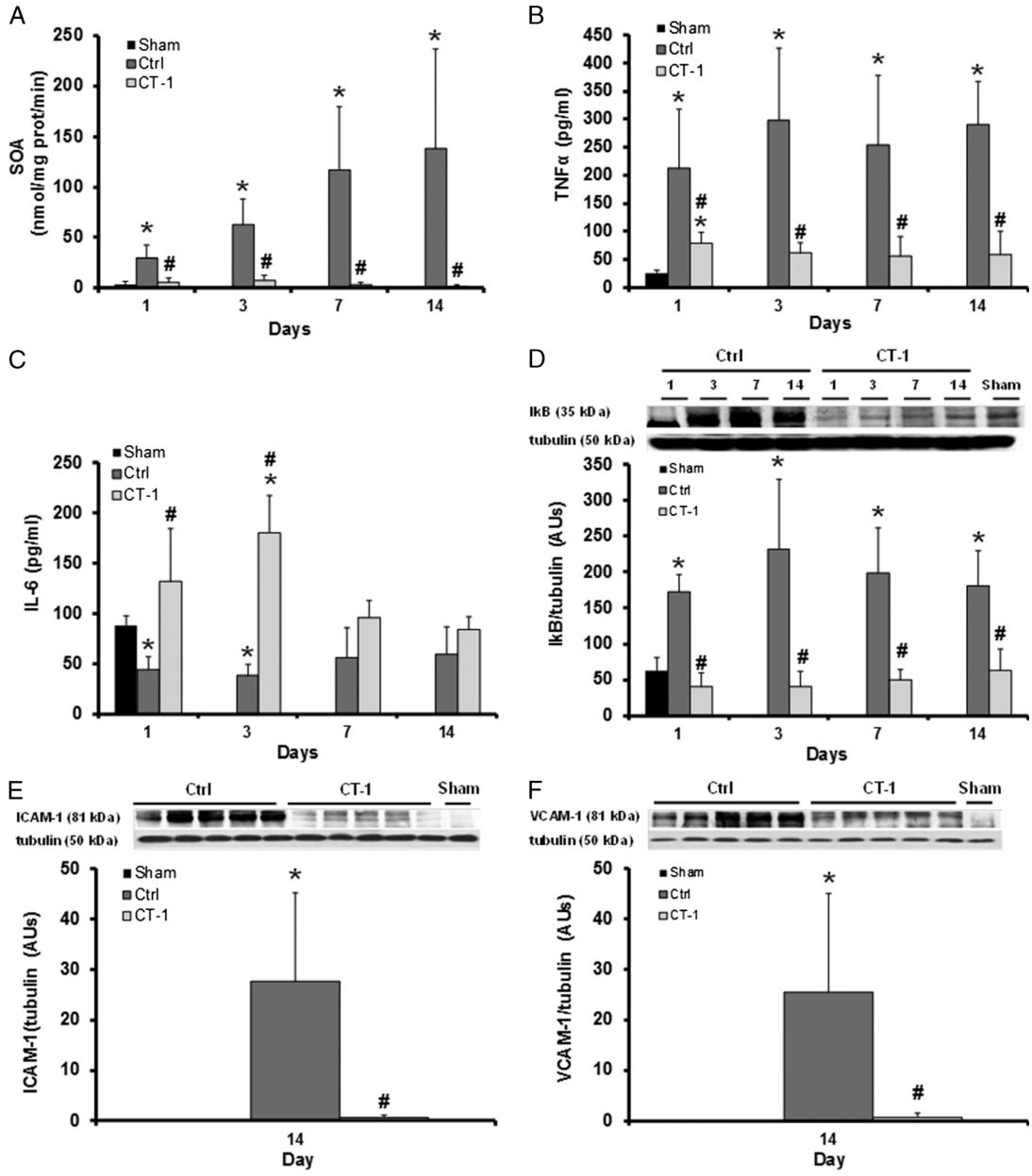

FIGURE 8. Effect of cold preservation with CT-1 on kidney levels of SOA (Panel A), concentration of Tumor Necrosis Factor-α (TNF-α, Panel B), and Interleukin-6 (IL-6, panel C), kidney levels of NFkB inhibitor (IkB, panel D), and plasma levels of ICAM-1 and VCAM-1 (panel D) at 1, 3, 7, and 14 days after transplantation. In panels D, E, and F, values are expressed as AU. Values are represented as mean ± SD (sham, n = 5; Ctrl, n = 5; CT-1, n = 5). Statistically significant differences: * Z > 1.96 all groups versus sham; # Z > 1.96 CT-1 versus Ctrl same day (Kruskal-Wallis multiple-comparison test and Dunn test).

effective storage time and the final quality of the organ to be transplanted. A second, and very interesting, implication is the potential of CT-1 to rescue suboptimal kidneys. High-quality organ supply is an extremely limiting aspect of kidney transplantation.[8] Persistent organ shortage has forced the expansion of eligibility criteria leading to the usage of marginal kidneys, which poorly tolerate cold storage resulting in increased preservation injury and higher rates of delayed graft function.[36]

Our results of strict preservation conditions (ie, cold storage for 24 hours) rendered low-quality kidneys, as demonstrated by the lack of restored renal function after transplantation. Interestingly, the addition of CT-1 to the preservation solution rescued those suboptimal kidneys, as it significantly reduced tissue damage, normalized renal function after transplantation, and greatly improved animal survival. Implementation of an additive into the preservation method encounters practical hurdles at the clinical development phase owing to unavoidable ethical routines. Specifically, adding CT-1 (or any other substance) to the preservation solution requires the recipient's consent, which in many instances is not known at the time of organ extraction. Our results indicate that CT-1 (at higher concentration) can be added later in the process of preservation and still achieve nephroprotection. This provides a window of opportunity to arrange procedural issues before adding CT-1, which will be especially attainable with the use of portable perfusion devices for organ preservation, which are currently in development.

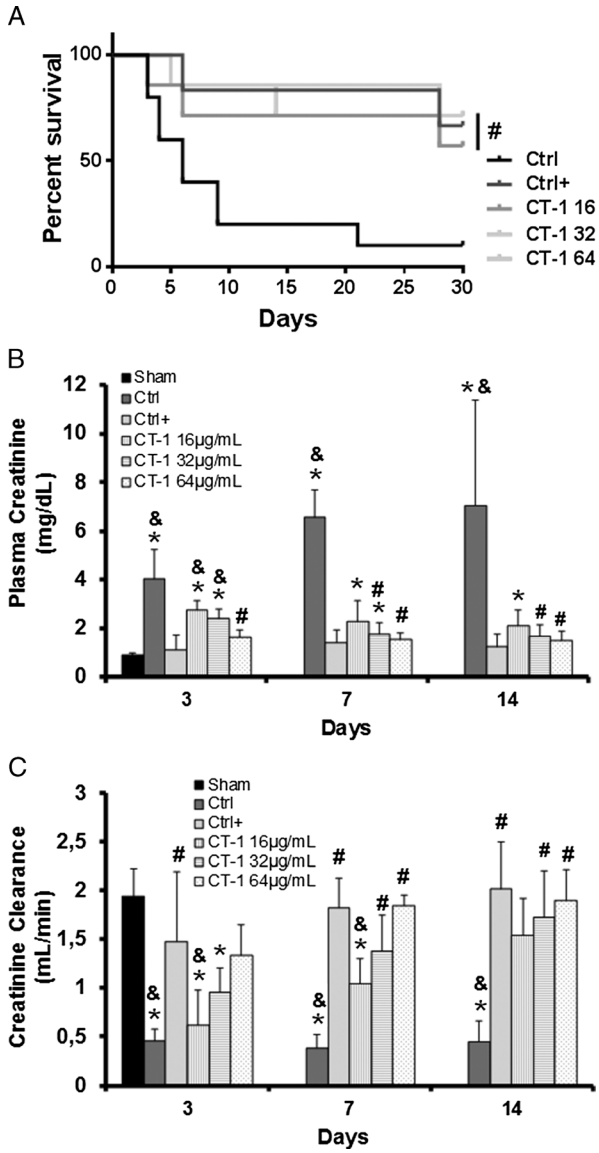

FIGURE 9. Accumulative survival in transplanted animals for 30 days (panel A), plasma creatinine concentration (panel B), and CrCl (panel C) at 3, 7, and 14 days after transplantation. Values in panel A are expressed as mean % on the posttransplantation day. Values in panels B and C are represented as mean ± SD. (sham, n = 5; Ctrl, n = 5; Ctrl+, n = 5; CT-1 16 µg, n = 5; CT-1 32 µg, n = 5; CT-1 64 µg, n = 5). Statistically significant differences: panels B and C: * $Z > 1.96$ all groups versus sham; # $Z > 1.96$ CT-1 groups versus Ctrl same day; & $Z > 1.96$ CT-1 groups versus Ctrl+ same day (Kruskal-Wallis multiple-comparison test and Dunn test).

A major characteristic of the renal protection afforded by CT-1 which makes it an interesting renoprotective candidate is that CT-1 tackles most of the key pathophysiological mechanisms responsible for kidney damage resulting from ischemia and reperfusion.[31] These mechanisms include oxidative stress and tubule-interstitial inflammation.[37] Oxidative stress significantly contributes to organ damage after ischemia/reperfusion.[38,39] Cardiotrophin-1 has been found to prevent oxidative stress in liver transplantation[27] and in other organs,[40] including the kidney after ischemia/reperfusion,[31] as well as the action of nephrotoxins, such as gentamicin[41] and iodinated contrast media.[42] Inflammation is also a major contributor to ischemia/reperfusion damage.[43] Importantly, CT-1 administration has demonstrated anti-inflammatory properties in several models of acute kidney injury.[31,35,42]

We hypothesize that IL-6 plays an important role in the anti-inflammatory effect of CT-1. IL-6 exhibits regenerative and anti-inflammatory capacities[44] by controlling the level of proinflammatory cytokines. In fact, IL-6 inhibits the LPS-induced synthesis of TNF-α in vitro[45] and in vivo.[46] In our study, in contrast to other cytokines, plasma IL-6 increased in the groups exposed to CT-1, consistent with previous findings that CT-1–induced IL-6 synthesis in endothelial cells and leukocytes.[47,48] We believe that in our model, CT-1-induced IL-6 played a net anti-inflammatory role. This is supported by the observation that the inflammatory response to several stimuli is more severe in IL-6 knock-out mice than in control mice.[49] Moreover, in our previous studies, the administration of CT-1 in the context of renal ischemia/reperfusion was associated with higher levels of IL-6 and lower levels of TNF-α, IL-1β, and interferon-γ.[31] Reversal of renal vasoconstriction and prevention of tubular apoptosis are additional mechanisms of CT-1 that may be theoretically invoked to explain its nephroprotective properties.[35]

Although experimental transplantation in rodents has facilitated research on transplant physiology and immunology and drug screening, a major limitation of these studies is that the processes involved in ischemia/reperfusion-induced renal damage differ between rodents and humans.[50] In addition, a major limitation of our study is the lack of histological analysis. Another limitation is the number of animals used to adhere to institutional, national, and international guidelines for the care and use of laboratory animals (Institute for Laboratory Animal Research. Guide for the Care and Use of Laboratory Animals, 2011. Washington, DC. The National Academies Press). Lastly, this was a descriptive study that did not assess in depth the cellular and molecular protective mechanisms of CT-1.

Finally, there is one more factor that makes CT-1 a promising additive for kidney preservation. Several molecules have demonstrated the ability to improve renal function after kidney transplantation in experimental animal models, but very few have reached the clinical setting. Interestingly, CT-1 has already been approved for human use in the context of organ transplantation. Specifically, CT-1 has been given the status of an orphan drug by the US Food and Drug Administration, used to protect the liver from ischemia/reperfusion injury inherent to the transplantation procedure (designation request 07-2449). The European Medicines Agency (EMA) has also granted CT-1 this status for the prevention of ischemia/reperfusion injury associated with solid organ transplantation (EU/3/06/396). Accordingly, the clinical development of this application of CT-1 may progress rapidly from bench to bedside.

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