Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Danshen protects kidney grafts from ischemia/reperfusion injury after experimental transplantation

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Keywords
anti-oxidation, apoptosis, cold storage, Danshen, kidney transplantation, regeneration, reperfusion injury, tubular damage.

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Received: 5 May 2008
Revision requested: 6 June 2008
Accepted: 8 September 2008

doi:10.1111/j.1432-2277.2008.00770.x

Summary

Danshen (DS) is used for treatment of various ischemic events in the traditional Chinese medicine. Hence, this study was designed to investigate its effect on ischemia/reperfusion injury (IRI) after experimental kidney transplantation (eKTx). Nephrectomized Sprague–Dawley rats underwent eKTx. Some animals were infused with 1.5 ml DS 10 min before surgery. Kidney grafts were transplanted after cold storage for 20 h in Histidine–Tryptophane–Ketoglutarate solution. After reperfusion blood samples were collected for blood urinary nitrogen (BUN), creatinine, lactate dehydrogenase (LDH), and alanine transaminase. Further, tissue was assessed for morphologic and pathophysiologic changes. Donor preconditioning with DS (DS-d) significantly decreased BUN, creatinine, LDH, and aspartate aminotransferase to 65–97% of controls while preconditioning of the recipient (DS-r) decreased values to 58–82% ($P < 0.05$). Tubular damage and caspase-3 decreased significantly in both DS-d and DS-r (DS-d: 96% and 67%, DS-r: 83% and 75% of controls) while heat shock protein 72 and superoxide dismutase increased significantly (DS-d: 143% and 173%, DS-r: 166% and 194% of controls). Further, inducible nitric oxide synthase and tumor necrosis factor-α decreased (DS-d: 84% and 61%, DS-r: 79% and 67% of controls) after DS. Preconditioning of both donors and recipients with DS significantly reduces IRI and thus improves graft function after eKTx.

Introduction

Ischemia/reperfusion injury (IRI) still is one of the major problems in kidney transplantation (KTx). Thus, the importance of novel protective drugs to reduce IRI in kidney grafts after transplantation is obvious. IRI can induce apoptosis and necrosis in renal cells, initiated through either the mitochondrial pathway or through the receptor-mediated pathway. These pathways are triggered by a variety of stressors such as hypoxia or oxidative stress and the binding of tumor necrosis factor (TNF)-α amongst others to their respective receptors [1–4]. This ultimately leads to apoptosis and inflammation [5,6]. TNF-α induces neutrophil proliferation and participates in inflammatory disorders of infectious and noninfectious origin [7].

Ischemia/reperfusion (I/R) after KTx normally results in serious oxidative stress to the renal cells. Peresleni *et al.* [8] have shown that oxidative stress to epithelial green monkey kidney (BSC-1) cells increased immunodetectable inducible nitric oxide synthase (iNOS), elevated NO release and nitrite production, and decreased cell viability. Further, Yu *et al.* [9] reported that an iNOS inhibitor prevented hypoxic cellular damage in freshly prepared rat proximal tubules. Generally, renal cells exhibit defensive mechanisms including various

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anti-oxidative enzymes, i.e. superoxide dismutase (SOD) [10]. Decreased SOD-levels are present after I/R and are associated with lipid peroxidation and injury to the graft. High levels of SOD gene transduction could minimize I/R-induced acute renal failure [11]. Sublethal cellular stress caused by ischemia can induce the synthesis of cytoprotectant heat shock proteins (HSPs). Renal expression of HSP72 a few hours after I/R marks the onset of cellular regeneration metabolism in response to I/R-induced stress [12].

Over the past few years, we have investigated protective effects of amino acids and other substances in experimental transplantation models [13–16]. Recently herbal medicines are attracting increased global attention. Their usage has been widely accepted in many fields of medicine. Danshen (DS) has been used to treat heart conditions, strokes, and other ischemic conditions in traditional Chinese medicine since decades. Results from both experimental and clinical studies support the traditional uses to some degree. DS has proven to have beneficial effects in cerebral and intestinal reperfusion injury, to improve cerebral blood flow in the ischemic hemisphere and to inhibit platelet aggregation in animal models [17,18]. DS is known to reduce platelet aggregation and fibrin production. In several studies, DS has been reported to have vasodilating and anti-bacterial activities and protective effects in rats with chronic renal failure [19,20]. DS also seems to inhibit lipid peroxidation and gentamicin-catalyzed formation of superoxide and hydroxyl radicals, which play a pivotal role for the development of IRI [21,22].

Taken together, although many investigations on DS have been performed, its potential protective effects on kidney grafts against IRI are yet to be elucidated *in vivo*. In this study, a clinically relevant experimental transplantation model was used to determine whether DS could protect kidney from IRI via mechanisms including anti-oxidation, anti-apoptosis, downregulation of inflammatory mediators and regeneration. Further, a comparison was made to indicate whether it would be better to apply DS in donors or in recipients.

Materials and methods

Animals and drugs

Female Sprague–Dawley (SD) rats weighting 220–250 g (Animal Center of Heidelberg University, Germany) were used for both organ donors and recipients of the kidney graft. According to the institutional guidelines, animals were accommodated in the university's veterinary care facility and submitted to a 12 h day/night cycle. The animals had access to water and standard laboratory chow (ssniff R/M-H; ssniff Spezialdiäten, Soest, Germany) *ad libitum*. DS used for this study was obtained from Shanghai Tongyong Medical Company Limited (Z20027936, Nr. 030214, China).

Experimental design

Inferior vena cava was used for i.v. injection of Ringer's and DS as described previously [15,16]. Donor rats were randomly divided into controls, DS-donor (DS-d) and DS-recipient (DS-r) groups. Control donors were given 1.5 ml Ringer's i.v. 10 min prior to organ harvest (nephrectomy, left). DS-d were infused with the same volume of DS at the same time. Subsequently, kidney perfusion with cold Histidine–Tryptophane–Ketoglutarate (HTK) solution and subsequent storage in HTK solution was performed before transplantation. Further, kidney grafts from naïve rats were transplanted to DS-r. The latter animals were infused with 1.5 ml DS 10 min before transplantation.

Blood was drawn at 6 h after KTx for analysis of serum blood urinary nitrogen (BUN), creatinine, lactate dehydrogenase (LDH) and aspartate aminotransferase (AST). Tissue samples taken 6 h after transplantation were used to analyze indices of tubular damage (TDI), caspase-3 expression (CI), HSP72 (HI), SOD, iNOS, and TNF-α.

Operation procedure

Donor rats were anesthetized with i.p. Narcoren® (20 mg/kg body weight; Pentobarbital-Natrium 16.0 g, Benzylalkohol 3.0 g in 100 ml Narcoren; Merial GmbH, Hallbergmoos, Germany), and i.m. Ketanest® (100 mg/kg body weight, Esketaminhydrochloride; Parke-Davis GmbH, Berlin, Germany). Donor's blood vessels and ureter were fully separated. Subsequently, the kidney was flushed via the aorta with 5 ml of 4 °C cold normal ringer solution followed by 5 ml of 4 °C HTK solution [16,23]. Left donor kidneys were cold stored in HTK solution at 4 °C for 20 h. Recipients were bilaterally nephrectomized and underwent transplantation as described elsewhere [23]. Briefly, after flushing grafts with 5 ml normal ringer solution, arterial and venous anastomoses were performed as end-to-side anastomoses to the aorta and inferior vena cava, respectively. Finally, the anastomosis of the ureter with the urinary bladder was constructed. The rat's temperature was monitored with a rectal thermometer and was kept constant between 35 and 37 °C in all cases.

Enzyme assays

Blood samples were obtained at 6 h after transplantation. Serum samples were deep-frozen at −80 °C until analysis.

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BUN, creatinine, LDH and AST activity were determined by standard methods [24].

Histology

Kidney graft samples were stored in 4% buffered formalin. For staining, samples were dehydrated and embedded in paraffin. For evaluation of tubular damage, Periodic Acid-Schiff reagent (PAS) staining was used. For each kidney, one representative cross section containing the cortex, outer medulla and inner medulla was assessed objectively for the tubular severity of IRI [16,25]. The score of tubular damage was graded according to particular histological findings from 0 to 4. Objective microscopic criteria used for estimation of tubular damage were: loss of tubular brush border, nuclear condensation, cytoplasmic swelling, loss of nuclei, presence of tubular cellular debris and casts, protein droplets and casts, protein filter in lumen and presence of atrophic tubular epithelia [26,27]. Thirty microscopic fields (×250 magnification) per kidney section were assessed and tubular damage was graded as follows: grade 0, 1, 2, 3, and 4 corresponded to a tubular damage of 0%, <25%, 25–50%, >50–75%, and >75%. In each section, the cortex (proximal convoluted tubules), outer medulla (mainly the straight portion of the proximal tubules and the thick ascending loops of Henle), and inner medulla (collecting ducts) were examined for morphologic changes as described elsewhere [12,16].

Immunohistochemistry

Kidney samples were stored in 4% buffered formalin. For staining, they were dehydrated, embedded in paraffin and sectioned at 4 μm. Immunohistochemistry for caspase-3, HSP72, SOD, iNOS, and TNF-α was performed. Sections were mounted on slides and deparaffinized in xylene and ethanol. Paraffin sections were incubated in rabbit polyclonal anti-caspase-3 antibody (DCS, Hamburg, Germany) at a 1:200 dilution and in rabbit polyclonal anti-HSP72 antibody (StressGen, Victoria, BC, Canada) at a 1:100 dilution for 1 h. Detection of SOD, iNOS, and TNF-α was performed using rabbit anti-Mn-SOD antibody (Sigma-Aldrich, St. Louis, MO, USA) at a 1:500 dilution, mouse monoclonal anti-iNOS antibody (Sigma-Aldrich) at a 1:50 dilution and rabbit anti-TNF-α (Biosource Europe, Nivelles, Belgium) at a dilution of 1:50 for 1 h, respectively. Hematoxylin–eosin was used for counterstaining and further processing for analysis. Sections from controls and study groups were blinded and examined with light microscopy at a magnification of ×250. In each group, 30 microscopic fields per section were assessed. The median value for each animal was taken to obtain mean values for the different groups. To assess tubular staining for expression of caspase-3, HSP72, SOD, iNOS, and TNF-α, the scoring system ranging from grade 0 to 4 was used, corresponding to no expression, <25%, 25–50%, >50–75%, >75–100% positive tubules [16,27].

Statistical analysis

Analysis of variance (ANOVA) has been used for comparison of parametric data. For discrete data, e.g. histology indices, Fisher's exact test was employed. $P < 0.05$ was considered statistically significant.

Results

Laboratory findings

Serum levels of BUN, creatinine, LDH, and AST dramatically increased in all groups after transplantation. This effect significantly decreased after preconditioning with DS. However, while donor preconditioning with DS (DS-d) significantly decreased only LDH serum levels from 3242 ± 917 U/l in controls to 2107 ± 658 U/l, preconditioning of recipients significantly decreased all serum parameters measured. In DS-r, the parameters BUN, creatinine, AST, and LDH were decreased from 105 ± 12, 1.41 ± 0.15 mg/dl, 736 ± 333, and 3242 ± 917 U/l in controls to 84 ± 11, 1.16 ± 0.15 mg/dl, 424 ± 97, and 2236 ± 353 U/l, respectively (Figs 1 and 2). Thus, both kidney graft function and injury after transplantation were significantly improved after DS especially when given to the recipient (DS-r).

Tubular damage

In controls, the macroscopic appearance of kidney grafts was pale cortex, dark-colored renal medulla region and blood stagnation. Light microscopy revealed cell-swelling, degradation in different regions and severe tubular acute damage together with interstitial congestion, swelling and infiltration of inflammatory cells. DS applied to donors and recipients both improved the macroscopic and microscopic appearance of kidney grafts. The tubular damage index (TDI) was 3.12 ± 0.14 in controls (Figs 3a and 4a). DS applied to donors decreased the TDI to 2.99 ± 0.33 ($P > 0.05$), while TDI significantly decreased to 2.59 ± 0.54 after preconditioning of the recipient (Figs 3b and 4a).

Caspase-3

Six hours after transplantation, kidney sections were caspase-3 positive, especially in renal tubules (Fig. 3c and d).

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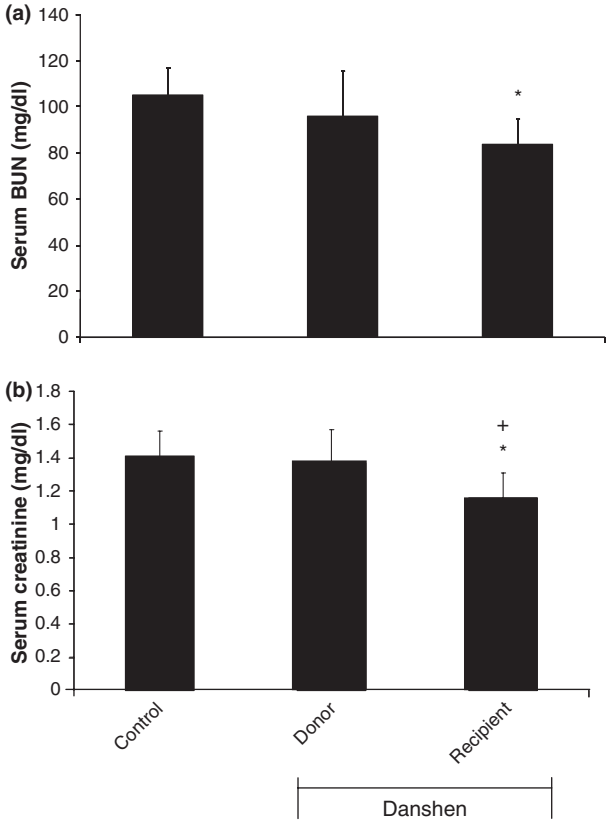

Figure 1 BUN and creatinine after transplantation. Six hours after transplantation blood was collected and serum parameters (a, BUN; b, creatinine) were determined. Values are mean ± SD ($P < 0.05$ by ANOVA, $n = 8$). *$P < 0.05$ for comparison to controls. +$P < 0.05$ for comparison between DS-donor and -recipient groups.

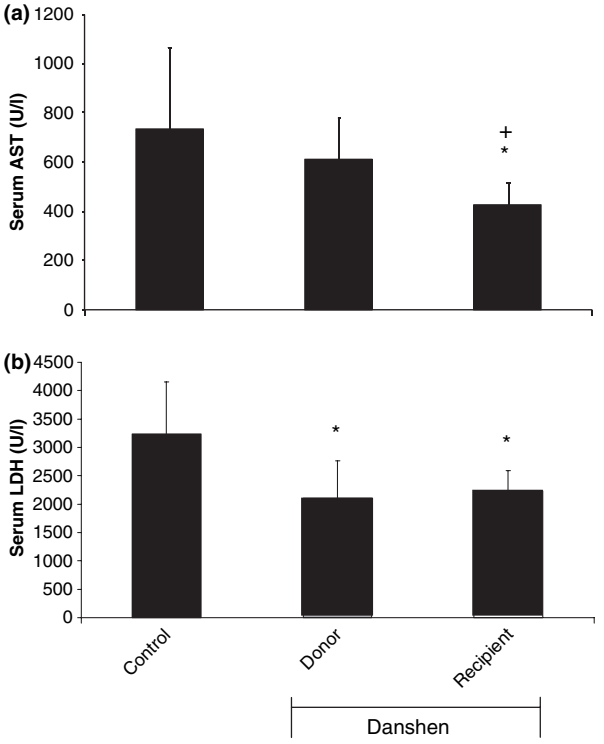

Figure 2 LDH and AST after transplantation. Six hours after transplantation blood was collected and serum parameters (LDH, AST) were determined. Values are mean ± SD ($P < 0.05$ by ANOVA, $n = 8$). *$P < 0.05$ for comparison to controls. +$P < 0.05$ for comparison between DS-donor and -recipient groups.

The number of caspase-3 positive tubules was markedly higher in controls and the caspase index was 2.82 ± 0.25 (Figs 3c and 4b). DS given to recipients decreased caspase-3 expression to 1.88 ± 0.75 ($P < 0.05$) (Fig. 3d), while its effect was not significant in preconditioned donors (DS-d) with 2.11 ± 0.77 (Fig. 4b).

Heat shock protein 72

Heat shock protein 72 was used to detect regeneration, as it corresponds to renal cell viability and anti-apoptotic tendency. The highest expression of HSP72 was found in the inner medulla, followed by the outer medulla and cortex regions of the transplanted kidneys. The highest expression of HSP72 occurred after DS-treatment (Figs 3f and 4c). The lowest HSP72 expression was found in controls (Fig. 3e). HSP72 staining was remarkable high in renal tubular cell nuclear and cytoplasm (Fig. 3f). DS given to donors and recipients increased HSP72 expression from 1.35 ± 0.08 in controls (Figs 3e and 4c) to 1.93 ± 0.35 (DS-d) ($P < 0.05$) and 2.24 ± 0.28 (DS-r) ($P < 0.05$), respectively (Fig. 4c). Comparison between the DS-treated groups showed a much higher HI value for DS-r than for DS-d ($P < 0.05$) (Fig. 4c).

Mn-SOD

The index for Mn-SOD expression in kidney tissue increased significantly after DS treatment. In DS-d and DS-r, the Mn-SOD index increased from 1.42 ± 0.25 in controls (Figs 5a and 6a) to 2.46 ± 0.43 ($P < 0.05$) and 2.75 ± 0.49 ($P < 0.05$) respectively (Figs 5b and 6a); however, there was no significant difference between DS-d and DS-r.

Inducible nitric oxide synthase

Renal I/R produced a significantly increased expression of iNOS (Fig. 5c). Application of DS in recipients reduced iNOS expression. In controls, the value was 2.52 ± 0.37 (Fig. 6b), while DS administered to donors and recipients

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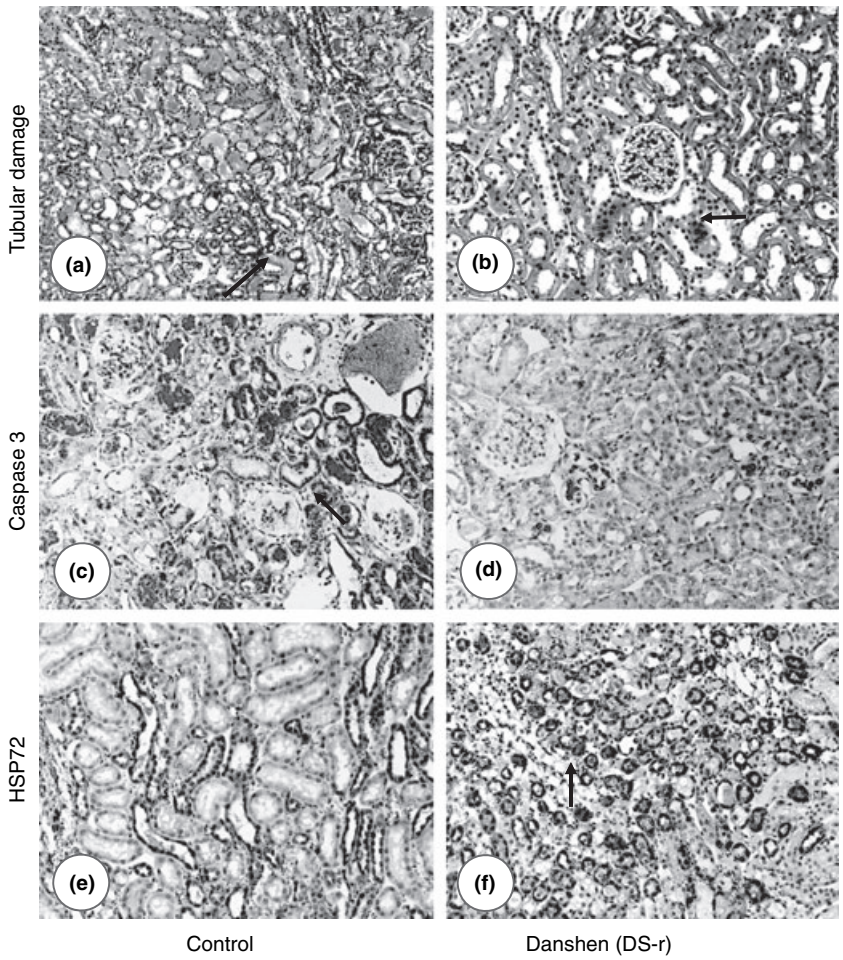

Figure 3 Histology for tubular damage and immunohistochemistry for the expression of caspase-3, HSP72 in kidney tubules after transplantation. Kidney tissue was taken 6 h after transplantation and was processed for light microscopy by PAS staining and immunohistochemistry. Control (a), Danshen-recipient (b) for tubular damage index; control (c) and Danshen-recipient (d) for caspase-3 expression; control (e) and Danshen-recipient (f) for HSP72 expression. Tubular damage was shown in the medulla regions. Control (a) showed severely damaged tubules with complete loss of brush border, focal denudation, necrotic material in lumen and denuded basement membranes while Danshen (DS-r) (b) showed only mild tubular injury. Caspase-3 expression was markedly increased in controls (c) and reduced by Danshen treatment (d). There was an increased number of HSP72-positive cells after Danshen (DS-r, DS-d) (f) compared with controls (e). Pictures depicting typical pattern of histology. Magnification: ×200.

reduced these values to 2.12 ± 0.26 and 2.01 ± 0.2 ($P < 0.05$) respectively (Figs 5d and 6b).

Tumor necrosis factor-α

As a key mediator in the local inflammatory immune response, high levels of TNF-α correlate to increased risk of cytotoxicity. In this study, kidney sections have shown expression of TNF-α in renal tubules (Fig. 5e and f). The number of TNF-α-positive tubules was markedly higher in controls: 2.91 ± 0.55 (Figs 5e and 6c). DS applied to both donors and recipients significantly decreased these values to 1.78 ± 0.47 ($P < 0.05$) and 1.95 ± 0.31 ($P < 0.05$), respectively (Figs 5f and 6c).

Discussion

This study clearly demonstrates for the first time that I/R-induced impairment of kidney grafts, resulting in decreased renal function and extensive tubular damage, can be diminished by application of DS to donors or recipients. Even though DS protects kidney grafts from IRI once applied to both donors and recipients, it seems that recipient preconditioning with DS protects kidney grafts better.

The observed effects are significant but may only have marginal effects on kidney function. Because of its decade-long traditional usage and its proven safety, DS remains an interesting substance for further investigations.

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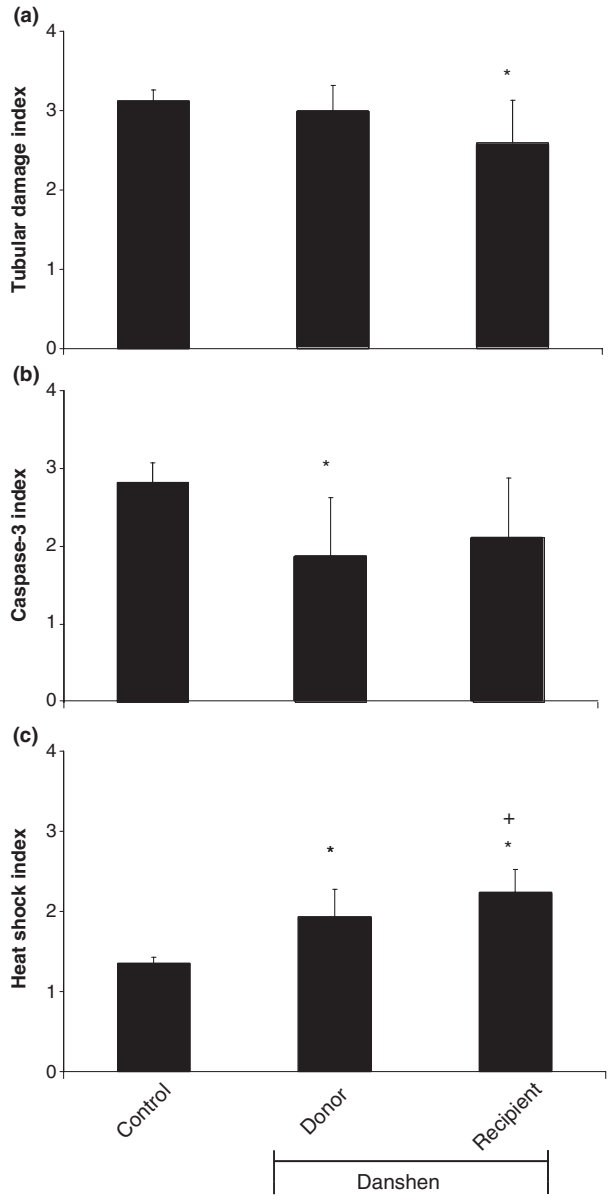

Figure 4 Indices of tubular damage, caspase-3 and HSP72 after transplantation. (a) The indices of tubular damage (TDI), (b) caspase-3 (CI) and (c) HSP72 (HI) were graded on a scale from 0 to 4 by using microscopic criteria as described in Materials and methods. Values are mean ± SD ($P < 0.05$ by Fisher's exact test, $n = 8$). $*P < 0.05$ for comparison to controls. $+P < 0.05$ for comparison between Danshen-donor and -recipient groups.

Danshen is the root of salvia miltiorrhiza from Lamiacae family in the traditional Chinese herbs, which belongs to a class of substances that promote circulation and dissipate blood stasis. More than 15 kinds of chemical structures extracted from DS provide a wide spectrum of anti-oxidative activity. Salvianolic acid A, B and rosmarinic acid, three water-soluble components of DS, were proved to inhibit lipid peroxidation in microsomes and the superoxide production in a xanthine–xanthine oxidase system via the potential mechanism of scavenging superoxide anion radicals [21,28].

Danshen can also adjust the metabolism of blood lipid, improve rheology, body microcirculation and activity of fibrinolysis, reduce viscosity of blood and inhibit platelet aggregation. Lee [29] reported the major cause of death in kidney transplants was renal vascular thrombosis and subsequent renal graft dysfunction. Zhang *et al.* showed that the inhibition rate of DS on rats' platelet aggregation reached 48.12%, which markedly inhibited the formation of thrombi. One assumed mechanism is that increased contents of cAMP after application of DS can inhibit phosphor-esterase and epoxy enzyme and reduce the formation of prostaglandin peroxide. DS can also activate protein kinase, phosphorylate the protein ingredient of the membrane, and alter its tendency to induce the aggregation of platelets [30]. Further, Cao *et al.* [31] indicated that the anti-thrombotic action of DS is as powerful as aspirin, using aspirin as a control. As we know, impairment of the microcirculation is a critical feature of IRI, causing 'no reflow' phenomenon. I/R-induced morphologic changes are believed to be responsible for delayed graft function in human kidney grafts with high levels of BUN and creatinine as indices [12,32,33]. Long-time cold storage or IRI normally increase blood viscosity and lead to platelet aggregation. This finally results in impairment of the microcirculation and renal dysfunction. Data provided here shows that renal graft function was improved significantly with decreased levels of both BUN and creatinine after DS treatment (Fig. 1), which supports the rheologic effects of DS.

Both, LDH and AST are regarded as nonspecific markers of extensive cellular deterioration. During renal tubular cell cold hypoxic preservation, considerable amounts of LDH are released [34]. As a marker of cellular disruption and necrosis, AST is present within the proximal tubule and can be released following renal injury [35]. In this study, both LDH and AST markedly increased after long-time cold ischemia and I/R. However, LDH and AST were significantly reduced after DS treatment, suggesting a protective effect of DS on renal cells against IRI (Fig. 2). Renal TDI also improved significantly after DS (Fig. 4a).

Renal cell apoptosis contributes to renal cell death including necrosis and apoptosis during cold ischemia and I/R. Free radicals have been suggested to contribute to cold-ischemia injury and a series of mitochondrial events can result in the activation of caspase-3 with subsequent apoptosis [36]. In addition, the death form of renal cells occurs at different times of renal I/R in rats

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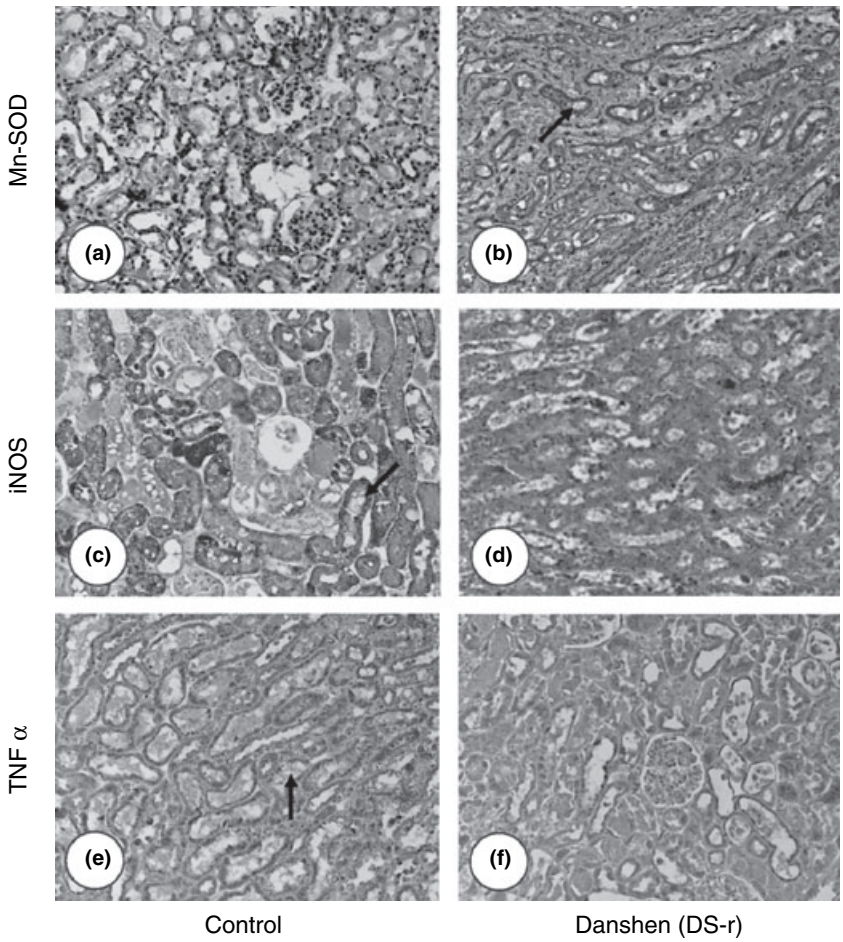

Figure 5 Expression of SOD, iNOS and TNF-α in kidney tubules after transplantation. Kidney tissue was taken 6 h after transplantation and was processed for immunohistochemistry. Control (a), Danshen-recipient (b) for SOD expression; control (c) and Danshen-recipient (d) for iNOS expression; control (e) and Danshen-recipient (f) for TNF-α expression. Control (a) showed low expression of SOD, while Danshen (b) markedly increased its expression. iNOS expression was markedly increased in controls (c) and reduced by Danshen treatment (d). Further, there was a more reduced TNF-α expression in Danshen group (f) than in controls (e). Pictures depicting typical pattern of histology. Magnification: ×200.

[37]. As an important effector, caspase-3 plays a central role in apoptosis.

In Zhao *et al.*'s [38] study, a significant inhibition of apoptosis of myocardial cells resulting from IRI was shown after DS. In similarity to our findings, the expression of caspase-3 showed an obvious increase 6 h after reperfusion in controls, suggesting renal cell apoptosis and necrosis. This tendency was greatly reduced by DS (Figs 3d and 4b). Taken together, DS might have a protective effect on renal cells through the mitochondrial-linked pathway via inhibition of caspase-3 expression.

Generation of reactive oxygen species (ROS) after reperfusion can result in oxidative damage of lipids, proteins, and nucleic acids [39,40]. Nitric oxide (NO), produced via iNOS, is implicated in the pathophysiology of renal IRI. Moreover, NO reacts with superoxide anions to form peroxynitrite, which causes injury via direct oxidant injury [41]. iNOS can be induced in the kidney by cytokines, lipopolysaccharide during I/R leading to renal cell injury. The *in vivo* and *in vitro* investigations have shown that inhibition of the expression or activity of iNOS can ameliorate or prevent renal IRI [41]. In the study by Isobe *et al.* [42], they found that the levels of iNOS expression paralleled hepatic dysfunction in rat subjected to hepatic I/R, suggesting that the expression of iNOS correlates to hepatic dysfunction induced by I/R. In our study, the expression of iNOS dramatically increased after reperfusion in controls (Figs 5c and 6b), suggesting I/R can also induce and up-regulate the expression of iNOS in kidney. However, DS can eliminate free oxygen radicals, reduce the content of lipid peroxides and improve the fluidity of mitochondrial membranes [43] (Figs 5d

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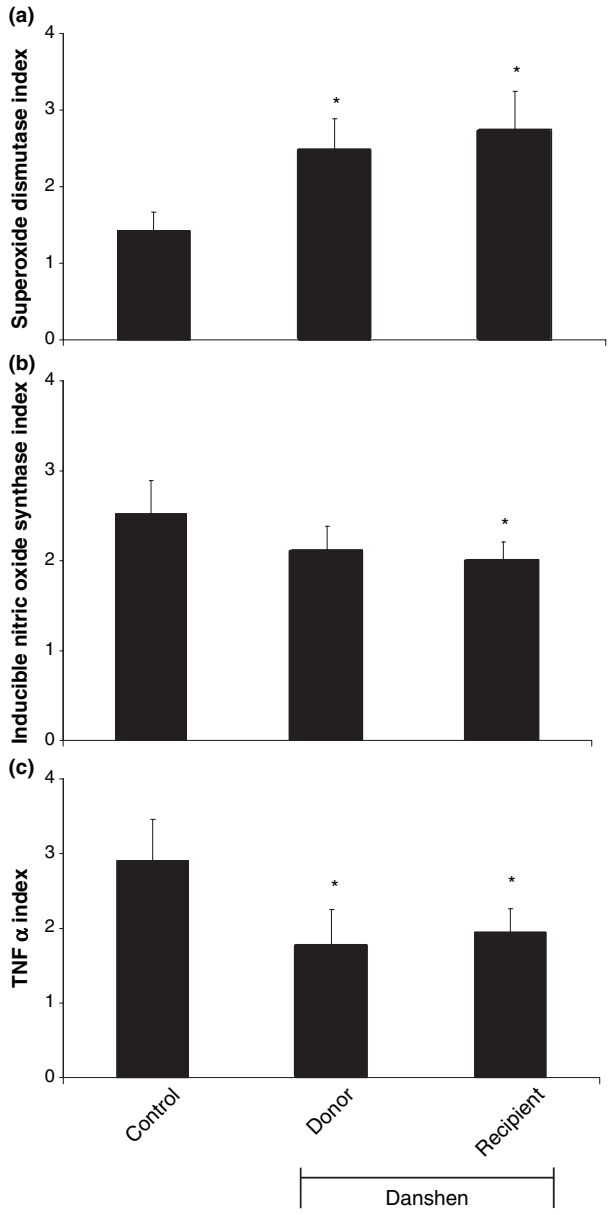

Figure 6 Indices of (a) SOD, (b) iNOS, and (c) TNF-α after transplantation. The indices of SOD, iNOS, and TNF-α were graded on a scale from 0 to 4 by using microscopic criteria as described in Materials and methods. Values are mean ± SD ($P < 0.05$ by Fisher's exact test, $n = 8$). *$P < 0.05$ for comparison to controls.

and 6b). This supports our findings of a significant reduction of iNOS expression after DS application.

Free radicals are the result of oxygen metabolism in tissues and, along with induced oxidation enzymes, they play an important role in the occurrence of IRI. In kidney, I/R results in accumulation of free oxygen radicals. Renal cells exhibit defensive mechanisms including various anti-oxidant enzymes, i.e. SOD [10], which is one of the enzymes that eliminate free radicals in cells. Zhang [44] indicated that the effect of DS on elimination of superoxide anions is greater compared with SOD. A reduced anti-oxidative capacity, which is known to occur after I/R, is associated with lipid peroxidation and injury to the graft. Indeed, in our study, the anti-oxidative capacity increased after DS (Figs 5b and 6a) and was accompanied by less cellular injury (Figs 2 and 4). Taken together, DS increased SOD expression and thus protected renal cells from IRI. DS also significantly increased HSP72 expression after reperfusion, especially in the renal medulla region (Figs 3f and 4c). The increased HSP72 accumulation after DS might have resulted from a larger number of viable cells in the medulla, which actively recovered from I/R stress. Reduced HSP72 expression in controls was probably caused by the larger amount of necrotic cells. Mao *et al.* [45] suggested that increased HSP72 could provide additional cytoprotection by inhibiting caspase-3 or by preventing caspase-3 from degrading anti-apoptotic proteins.

Chang *et al.* [46] indicated that I/R induces inflammatory cytokine gene expression such as TNF-α, interleukin (IL)-β, IL-6, IL-8, interferon-γ. These over-expressed cytokines play a critical role in the progress of organ dysfunction, including IRI and vascular wall remodeling. Locally produced TNF-α also contributes to postischemic myocardial dysfunction via direct induction of cardiomyocyte apoptosis [47]. Anti-TNF-α monoclonal antibodies have been used to reduce or inhibit TNF-α activity. One hypothetical advantage of treatment with anti-TNF-α antibodies results from its role in multiple types of inflammation [36]. In our study, the expression of TNF-α increased in controls, suggesting graft injury and renal cell apoptosis and necrosis (Fig. 5e). DS treatment led to a significant reduction in the expression of TNF-α compared with controls (Figs 5f and 6c). Taken together, DS regulated the expression of inflammatory cytokines and exhibit a relievable or beneficial effect on renal IRI.

In conclusion, applying DS to donors and recipients had significant effects on IRI in KTx. Some of our findings suggest that preconditioning recipients with DS has a greater impact on IRI than application of DS to donors. In summary, this is the first report of DS and its potential benefit to protect kidneys from experimental cold IRI after transplantation. The underlying mechanisms most likely include especially its anti-oxidative capacity.

Authorship

XG, MK, PS and JL: performed research/study. XG, GDA, HB and PS: wrote the paper. JC and RL: contributed important reagents. AN, HB and MLG: analyzed data. MWB: reviewed the manuscript and study protocol. PS: designed research/study.

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Acknowledgements

We thank Eva Eiteneuer and Heike Ziebart for helping us to perform immunohistochemistry (Department of Pathology, Ruprecht-Karls-University of Heidelberg, Germany; Director: Professor P. Schirmacher MD), and Ulf Hinz for great help with statistics.

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