Transpl Int (2001) 14: 351–360
© Springer-Verlag 2001

ORIGINAL ARTICLE

Claudio A. Redaelli
Markus Wagner
Christoph Kulli
Ying-Hua Tian
Darius Kubulus
Luca Mazzucchelli
Andreas C. C. Wagner
Martin K. Schilling

Hyperthermia-induced HSP expression correlates with improved rat renal isograft viability and survival in kidneys harvested from non-heart-beating donors

Received: 3 November 2000
Revised: 27 February 2001
Accepted: 29 May 2001

C.A. Redaelli (✉) · M. Wagner
C. Kulli · Y.-H. Tian · M. K. Schilling
Department of Visceral and Transplantation Surgery, Inselspital, University of Bern, 3010 Bern, Switzerland
e-mail: claudio.redaelli@insel.ch
Tel.: + 41-31-6324535
Fax: + 41-31-3624101

A.C.C. Wagner
Department of Clinical Research, Inselspital, University of Bern, Bern, Switzerland

L. Mazzucchelli
Department of Pathology, Inselspital, University of Bern, Bern, Switzerland

D. Kubulus
Department of Clinical and Experimental Surgery, University of Saarland, Homburg, Germany

Present address: A. C. C. Wagner, Department of Medicine II, Klinikum Grosshadern, Ludwig-Maximilians-University, Munich, Germany

Abstract Transient sublethal hyperthermia followed by recovery from heat stress, referred to as heat shock preconditioning, exerts a protective effect on ischemia/reperfusion-induced injury in many systems. This effect is considered to be correlated to heat shock proteins (HSPs) and might be a critical factor in kidney graft function and survival. This study was designed to examine the impact of heat shock preconditioning on kidney isograft function and survival in a model utilizing non-heart-beating (NHB) donors. Four groups of male Lewis rats (n = 10/group) subjected either to whole body hyperthermia (groups A and C) or to sham anesthesia (groups B and D) were allowed 24 h recovery. Thereafter, 20 min of warm ischemia (A/B), and in a separate set of experiments 40 min of warm ischemia (C/D), were induced by suprarenal aortic cross clamping before renal procurement. After 24-h preservation with University of Wisconsin solution at 4 °C, orthotopic kidney transplantations were performed to syngeneic bilaterally nephrectomized recipients. Tissue specimens were taken to determine HO-1/HSP32, 72, and 90 induction by Western blot analysis. Renal function was measured by means of serum creatinine and creatinine clearance on days 0, 3, and 7 as well as urine volume, protein content, and creatinine levels daily. HO-1/HSP32 and HSP72 were found to be expressed constitutively. Moreover, heat shock strongly induced renal HSP72 and HSP32/HO-1, and to a lesser extent HSP90, expression. For recipients of group A grafts, the graft survival rate was 10/10, whereas it was 7/10 (70%) in recipients of group B grafts (log rank $p < 0.05$). Following 40 min of warm ischemia, 6/10 (60%) recipients survived, whereas all sham treated animals died with anuria within 6 days (log rank $p = 0.01$). Heat shock preconditioning strongly improved graft viability and reduced functional impairment. Creatinine clearance (CRC) on day 3 post Tx was 0.43 ± 0.24 ml/min in preconditioned animals (group A) and 0.07 ± 0.09 ml/min ($p < 0.001$) in sham preconditioned (group B), whereas it was 0.91 ± 0.33 ml/min and 0.03 ± 0.02 ml/min ($p < 0.00001$) on day 7 post Tx. Following 40 min NHB time, CRC in survivors of preconditioned graft recipients (group C) was 0.32 ± 0.2 ml/min (day 3 post Tx) and 0.23 ± 0.08 ml/min (day 7 post Tx) and was significantly better than CRC of group B ($p < 0.01$ and $p < 0.00001$, respectively). CRCs prior to NHB procedures were comparable in all animals ranging between 1.31 and 1.72 ml/min. Serum creatinine as well as proteinuria were significantly increased after transplantation in both groups but

recovered within 5 days in recipients of preconditioned grafts, whereas kidneys from donors without HP did not recover function. Histological alterations were also diminished following HP. Hyperthermic preconditioning induces strong and long lasting HO-1/HSP32, HSP72, and HSP90 expression in rat kidneys. HP increases survival following transplantation and improves renal graft function including proteinuria, volume output, and creatinine clearance. HSP induction might be used to develop novel approaches in clinical transplantation.

Keywords Heat shock protein · Heme oxygenase-1 · Non-heart-beating-donor · NHBD · Kidney transplantation · Survival

Introduction

The demand for renal transplantation has increasingly outstripped the supply of donor organs especially over the past 10 years. Although related and unrelated living donation is being promoted as one option for increasing the donor pool, it is unlikely that this will in itself be able to bridge the gap. Non-heart beating donors (NHBD) can provide an alternative supply of organs, which could substantially increase the donor pool. The main difference between cadaveric heart-beating donors and non-heart-beating donors is the extent of warm ischemia to which the kidney is subjected before flush out. Since the first successful kidney preservation experiments with donor procurement at 32 °C [5], the warm perfusion method has been proposed to enable functional evaluation of kidneys prior to transplantation, to resuscitate kidneys following warm ischemic damage equivalent to NHB donor kidneys, and to prevent reperfusion injury [6, 7].

Transient sublethal hyperthermia and the recovery from exposure to heat are referred to as heat shock preconditioning. It provides a cytoprotective effect on ischemia/reperfusion-induced injury in many systems. There is mounting evidence that this intracellular protective response to thermal stress through heat shock proteins (HSPs) [22] is a critical factor in kidney graft function and survival. Expression of these proteins is, however, induced by almost any kind of stress, and the clinical impact of these proteins is currently a matter of intense research [12]. Cell protection by induction of HSP expression has been reported by numerous investigators for a wide variety of systems in recent years [3, 4, 10, 17, 28, 33, 34, 36, 38, 40, 42]. HSPs are ubiquitous and consist of several structurally unrelated groups of proteins. These are named according to their apparent molecular weight, such as HSP70 and HSP90 [14, 16, 41].

Furthermore, heme oxygenase (HO), a central enzyme of heme metabolism, represents another HSP with a size of 32 kDa. Three isoforms (HO-1, HO-2, and HO-3) have been identified so far [24, 25, 37]. Of these, HO-1 catalyzes the breakdown of heme into equimolar amounts of biliverdin, carbon monoxide, and iron [13] and is recognized as a major heat shock/stress response protein. In clinical transplantation, ischemia/reperfusion (I/R) injury is one of the most important causes of early graft loss and, during the reperfusion period of grafting, augmented heme transfer occurs, a potential damaging effect resulting in a direct attack and impairment of several intracellular targets such as the cytoskeleton, metabolic enzymes and DNA [20]. This raises the possibility that HSP inducing pretreatment may attenuate ischemia and reperfusion injury that occur during the warm ischemia period of NHBD grafts. Thus, improving warm ischemia tolerance will likely lead to better transplant function of NHBD kidney transplants.

To address the question properly, in the present study we investigated the impact of induced HSP expression on warm ischemia tolerance, and on kidney graft survival and function using an NHBD kidney transplant model. In addition, to understand the relative contribution of these HSPs to the protective mechanism of hyperthermic preconditioning, the correlation between the time course of the HSP expression and the induced effect was also studied.

Materials and methods

Organ procurement and hyperthermia

Animal experiments were performed in accordance with the principles of laboratory animal care (NIH publication 86–23) as well as with national guidelines for the care and use of laboratory animals and were approved by the local authorities. Male Lewis rats (250–320 g) had free access to regular chow and water. Hyperthermia was performed as previously described [26, 40]. In brief, animals were anesthetized by intraperitoneal injection of pentobarbitone sodium (50 mg/kg body weight) and body temperature was monitored with a rectal thermometer. Heat shock was induced using a heat pad and lamp. The colonic temperature of the heated animals was slowly elevated to 42 °C and maintained for 20 min. Animals were also given 5 ml of isotonic saline intraperitoneally to avoid dehydration. Since the highest expression of all HSPs was found 24 h after hyperthermia, warm ischemia exposure was performed 24 h after preconditioning. In order to test for beneficial effects of hyperthermic preconditioning, we first exposed the donor animals to warm ischemia for 20 min (groups A and B). Later, in a separate set of experiments, warm ischemia exposure was extended to a lethal range of 40 min (groups C and D). NHB conditions were simulated by in situ clamping of the infradiaphragmatical aortic segment prior to kidney explantation (groups A and C).

Kidneys were then flushed for 5 min with 10 ml of 4 °C cold University of Wisconsin (UW) solution via the renal artery and removed. Kidneys were surface cooled with UW solution and stored

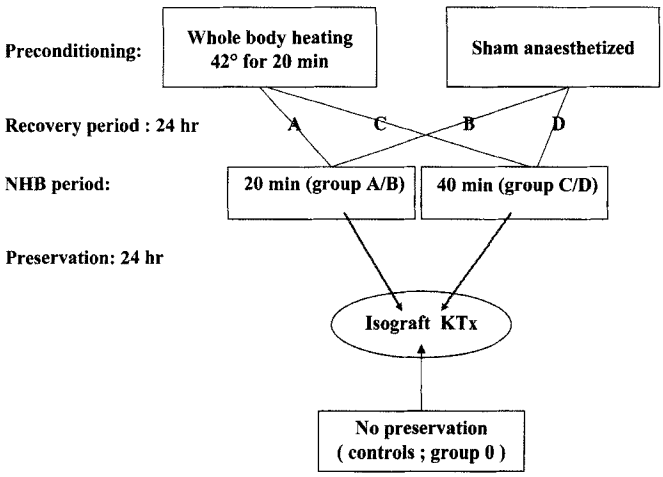

Fig. 1 Schematic diagram of the protocol of the study. Groups A/C were subjected to hyperthermic preconditioning (HP), whereas groups B/D underwent sham anesthesia 24 h prior to warm ischemia and graft harvesting. Grafts of group 0 were not subjected to any stress prior to functional assessment

at 4 °C for 24 h. For each experimental group, kidneys from ten sham treated rats not exposed to hyperthermia were identically treated and procured with UW solution. In our rat kidney transplantation model, a cold graft preservation period of up to 24 h is generally well tolerated and no recipient mortality is observed. The protocol of the study is shown in Fig. 1.

Transplant experiments

For transplantation, recipients were anesthetized with pentobarbitone sodium (50 mg/kg body weight), both recipient kidneys were removed, and single isograft kidney transplantation was performed. Removal of both native recipient kidneys was felt necessary for true assessment of graft function. Donor kidneys were rinsed with 5 ml Ringer's lactate solution and transplanted orthotopically into recipients. Renal vessels were connected with a 10/0 Prolene suture and ureters were connected via intraluminal polyethylene cuffs. Transplantation time ranged between 15 and 20 min in all groups. After transplantation, all animals were kept in metabolic units for renal function analysis. Surviving animals were sacrificed on the seventh postoperative day. Urine volume, protein content, and creatinine levels were measured daily. Serum creatinine, urea, and protein levels as well as creatinine clearance were measured on day 3 and day 7 post-transplant. Kidney grafts were also removed for histological evaluation in separate transplant experiments on the fifth post-transplant day (n = 6/group).

Determination of HSP expression by Western blotting

One-dimensional gel electrophoresis was performed using minigels (Bio-Rad, Richmond Calif.) as previously described. Five μg of protein were loaded per lane. After gel electrophoresis, proteins were transferred to nitrocellulose membranes as described earlier [40]. Protein detection was performed as described previously using the indicated concentration of antibody (1:1000 for monoclonal antibodies and 1 μg/ml for polyclonal antibodies). Labeled proteins were visualized by enhanced chemiluminescence following the manufacturers' procedures using the horseradish peroxidase-coupled secondary antibodies supplied with the kit at a 1:5000 dilution, followed by autoradiography. Densitometry of renal HSP expression was performed as described previously [40]. For densitometry, samples were loaded on one-dimensional minigels for Western blotting. To normalize for differences in quality of different antibody batches, Western transfer efficiency, and exposure times, appropriate control samples were included in each gel. The signals obtained through Western analysis and autoradiography were expressed as percentage density increase compared with those for shams not exposed to hyperthermia.

Histopathological analysis

Histological changes of the kidney graft specimens were graded on the fifth day after transplantation in a blinded fashion by an experienced pathologist (L.M.). Rat kidneys were fixed for 24 h in buffered 4% formalin, embedded in paraffin and 4-μm thick tissue sections were cut and stained with heamatoxylin and eosin. The light microscopic changes characteristic of acute tubular injuries have been previously described in detail [30, 31]. In this study we evaluated tubulointerstitial damage (tubulointerstitial score) [39] with seven histological parameters, i.e. cell swelling and vacuolization, nuclear pyknosis and karyorrhexis, cell lysis, cell sloughing, loss of proximal tubules brush border, and increase in tubular diameter, as well as the presence of protein casts and interstitial mononuclear inflammatory infiltrates. To each parameter we assigned a semiquantitative score (0–3) reflecting the severity of changes (score 1, less then 10% of the tissue section involved; score 2, 10–50%; score 3 > 50%)

Chemicals and antibodies

Mouse monoclonal isoform-specific antibodies against inducible HSP-72, HSP-32/HO-1, and HSP-90 were from StressGen (Victoria, British Columbia, Canada). Detection reagents for enhanced chemiluminescence and horseradish peroxidase coupled anti-mouse and anti-rabbit antibodies were from Amersham (Arlington Heights, Ill.). Polyacrylamide, molecular weight, and isoelectric focusing standards were from Bio-Rad (Hercules, Calif.). All other chemicals were purchased from Sigma Chemie, Buchs, Switzerland.

Statistics

Results are expressed as mean ± standard deviation. Graft survival was calculated with the Kaplan–Meier product limit estimator. Differences in survival rates between the groups were tested with the log rank test in a statistical package program (SPSS Statistical Software, Chicago, Ill.). Differences between groups were analyzed by ANOVA with Bonferoni correction and unpaired Student t-test to compare between groups. Differences at $p < 0.05$ were considered statistically significant.

Results

Effect of hyperthermia preconditioning on renal protein expression of HSP32, HSP72, and HSP90

Figure 2 shows the constitutive expression of HSP90, HSP32 but not HSP72 in renal tissue before precondi-

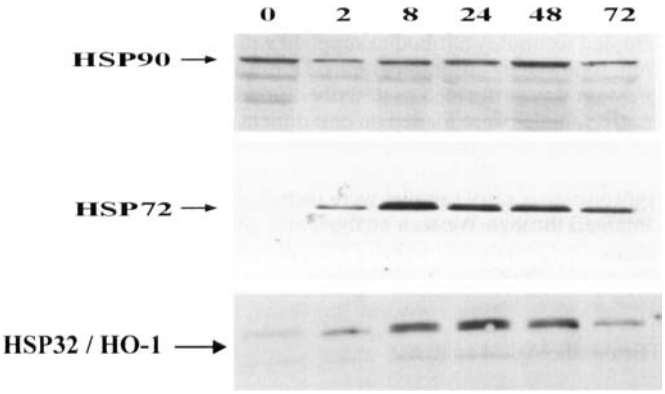

Fig. 2 Western blot analysis of HSPs HSP90, HSP72, and HSP32/HO-1 in donor kidneys prior to transplantation. The pattern of HSPs induced by whole body hyperthermia is shown in relation to the recovery period. The constitutive protein expression (0 h) is given for HSP90 and HO-1, but not for HSP72. Following hyperthermia preconditioning, HSP72 and HO-1 expression are strongly induced after 8 h, whereas HSP90 did not show a significant induction. HSP32 expression peaked at 8 h, whereas HSP72 and HSP90 expression peaked later and remained for at least 72 h after thermal stress

tioning. Heat shock in the presence of an increase in body temperature strongly induced all of the tested HSPs. HSP72 and HSP 90 expression was detected 2 h after treatment, peaked between 8 and 24 h, and returned to baseline by 72 h after thermal stress. In contrast, HSP 32 (HO-1) induction peaked at 8 h following hyperthermia preconditioning, remained elevated for up to 48 h, and decreased markedly thereafter. Normothermic sham rats receiving the same dose of pentobarbital sodium as well as the surgical stress of transplantation did not express any HSPs in the renal tissue. Warm ischemia prior to kidney grafting did not alter the expression of induced renal HSP 32, 72, and 90 levels regardless of the duration of warm ischemia exposure (data not shown).

Effect of hyperthermia preconditioning (HP) on survival of NHBD graft recipients

Kidney graft exposure to warm ischemia for up to 20 min did not lead to recipient death or major graft dysfunction regardless of preconditioning (data not shown). Following NHB exposure for 20 min to the transplanted renal graft, 30% of the recipients (3/10) without graft HP (Fig. 3, dashed black line) died within the first 6 days after transplantation, whereas all recipients of preconditioned grafts survived until study termination (solid black line, $p < 0.05$ group B versus group A).

Following extension of the NHB period up to 40 min, all recipients of normothermic sham kidneys (group D) died prior to sacrifice on day 7 post-transplant (dashed-dot gray line). In the preconditioned transplant model (group C) a 60% survival rate (6/10) was observed (dotted gray line, $p = 0.01$ group D versus group C).

Effect of hyperthermia preconditioning on renal graft function

Kidney grafts from donors not exposed to hyperthermia preconditioning showed severely reduced graft function after 20 min of warm ischemia. Prior to the NHB period, there was no difference in serum and urine creatinine concentrations, as well as protein and volume output in

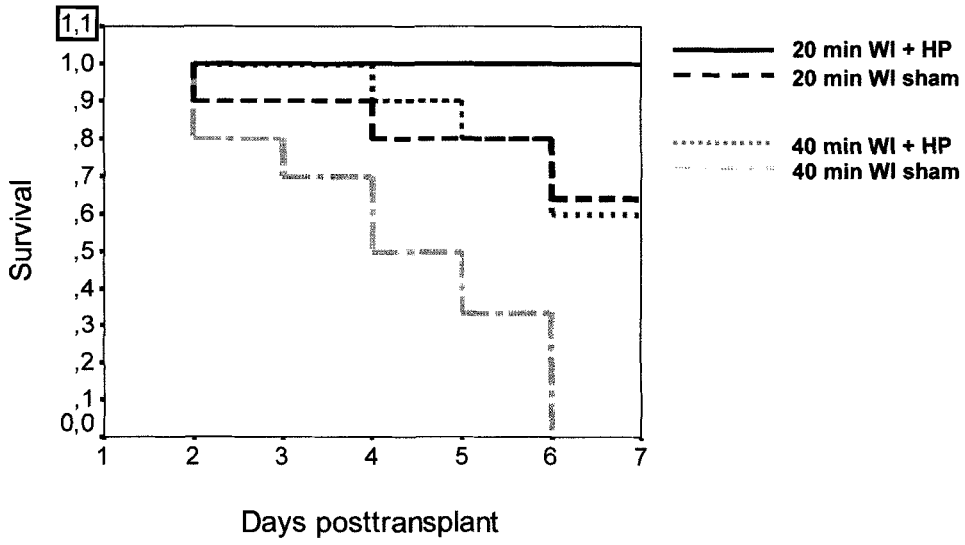

Fig. 3 Kaplan–Meier survival curves of rats recipient of an orthotopic kidney transplant. Survival rates of bilaterally nephrectomied and single renal isografted recipients with HP (groups A/C) or without HP (groups B/D) in regard to warm ischemia (WI) exposure time. 20 min WI with HP (*solid black line*) and without HP (*dashed black line*); (Log rank test: $p = 0.048$; group A versus B). 40 min WI with HP (*gray dotted line*) and without HP (*gray dash-dotted line*); (Log rank test: $p < 0.0007$; group C versus D)

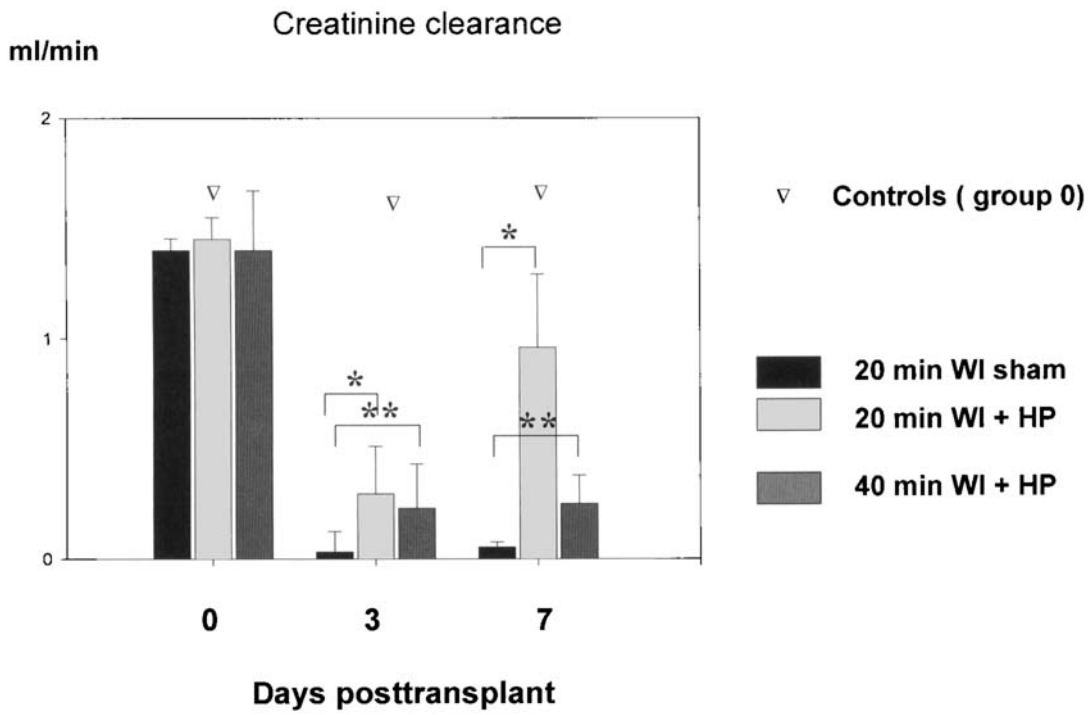

Fig. 4 Hyperthermia preconditioning improved graft function of NHBD kidney isografts. Following 20 min of warm ischemia, creatinine clearance values were poor on the third and seventh postoperative day in survivors of group B (*black bars*), and significantly decreased in comparison with function values of preconditioned grafts of group A (*gray bars*; $*p = 0.001$ and $p < 0.00001$). Creatinine clearance in survivors of group C (*dark-gray bars*) showed still significantly better values to graft function from non-preconditioned animals following 20 min warm ischemia only ($**p = 0.01$ and $p < 0.00001$). Interestingly following 20 min of anoxic period, creatinine clearance of preconditioned grafts (group A) thus did not differ significantly from creatinine clearance in healthy sham-grafted rats (group 0; *white triangles*) at the seventh day after grafting

urine of animals whose kidneys were harvested and transplanted. The corresponding creatinine clearance was also comparable and ranged between 1.31 and 1.72 ml/min per day. (Fig. 4). Three days after transplantation, creatinine clearance was 0.07 ± 0.09 ml/min in the normothermic sham group, whereas it was 0.43 ± 0.24 ml/min in the hyperthermic preconditioned group ($p < 0.001$) and, on the seventh post-transplant day, 0.03 ± 0.2 ml/min in comparison with 0.91 ± 0.33 ml/min ($p < 0.00001$). Following 40 min of NHB procedure, warm ischemic renal damage resulted in total loss of kidney function and death in all non-preconditioned animals (data not shown). Creatinine clearance in survivors of preconditioned graft recipients thus was superior on day 3 (0.32 ± 0.2 ml/min) and day 7 (0.23 ± 0.08 ml/min) post-transplant compared with graft function from control animals (group 0) following 20 min warm ischemia only ($p < 0.01$ and $p < 0.00001$, respectively).

Serum creatinine concentrations were markedly higher in sham animals than in the preconditioned group: 136.1 ± 39.8 mmol/l versus 329.6 ± 210.1 mmol/l 3 days after transplantation and 116.3 ± 51.9 mmol/l versus 460.7 ± 201.6 mmol/l 7 days after transplantation ($p = 0.03$ and $p > 0.001$, respectively; Fig. 5A). In normothermic graft recipients, urine creatinine concentration decreased rapidly after transplantation to 1851 ± 1267 mmol/l on day 3 post-transplant, whereas it was 3691 ± 1170 mmol/l in the heat shocked group ($p = 0.02$) and it remained significantly decreased until the end of the study on day 7 post-transplant (1255 ± 578 mmol/l) In contrast, grafts undergoing hyperthermia preconditioning showed a recovery and on day 7 after transplantation the corresponding urine creatinine concentration was 5291 ± 1279 mmol/l ($p < 0.0001$, Fig. 5B).

Urine output showed a similar trend to that seen with urine creatinine filtration (Fig. 5C). Urine volume quantity decreased markedly after transplantation in both study groups, but the urine output in preconditioned graft recipients reached normal values again after 6 days. Seven days after transplantation, urine output went up to 27 ± 8 ml/24 h, and was significantly higher than in the normothermic group (12 ± 4 ml/24 h; $p = 0.002$).

In addition, warm ischemic damage resulted in significant proteinuria after transplantation in both groups (Fig. 5D). In shams, the urinary protein levels increased

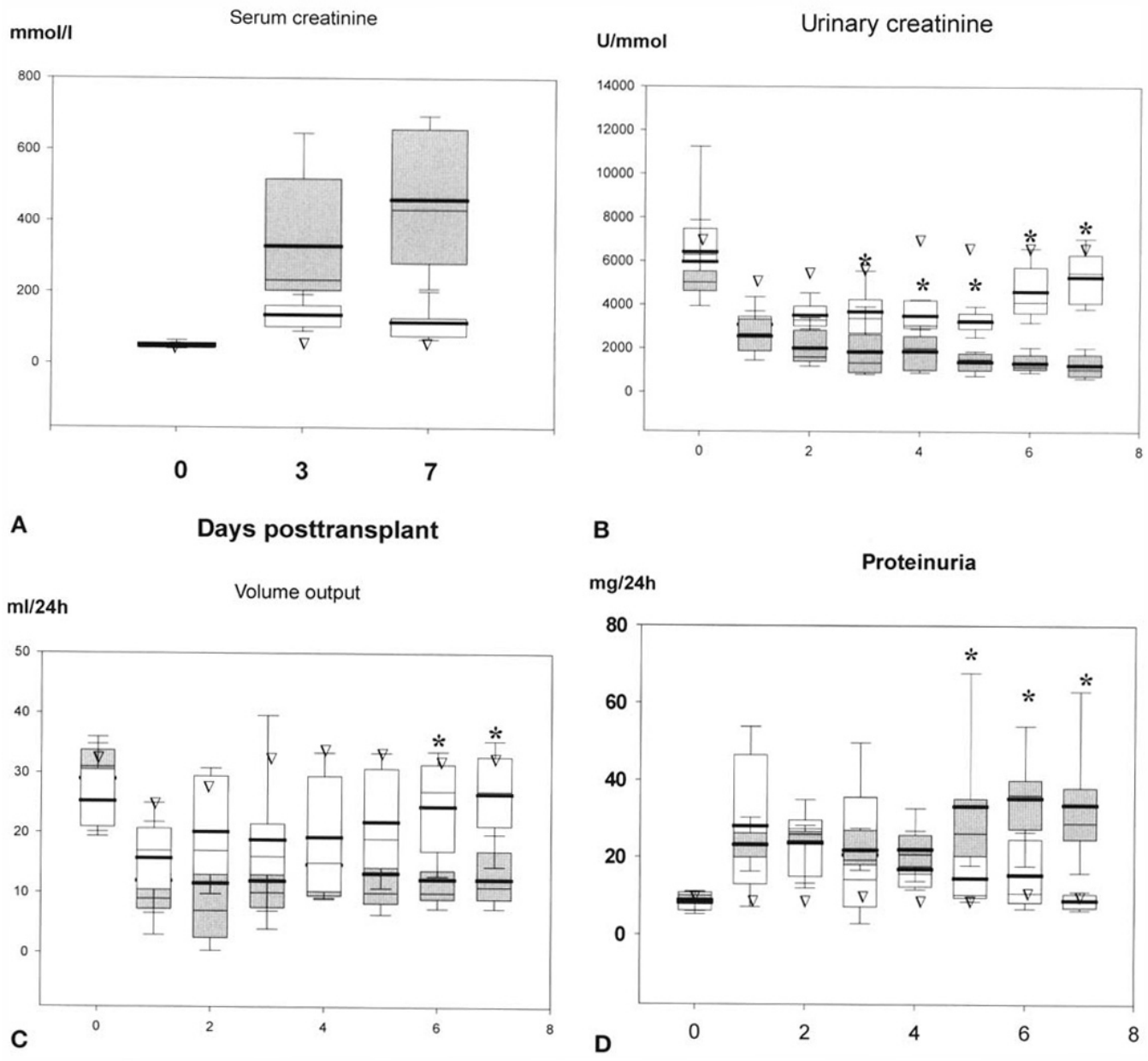

Fig. 5 A–D Serum concentrations of creatinine (**A**), urinary creatinine (**B**), urine output (**C**), and urinary protein excretion rate (**D**). Concentrations were determined in donors at time of kidney harvesting and in recipients after liver transplantation. Grafts preconditioned with heat shock prior to warm ischemia (*white box plots*) have significantly better tests after transplantation receiving statistically significant differences after 5 days ($*p < 0.05$) than renal graft receiving sham preconditioning only (*gray box plots*). Values obtained in animal recipients of a control isograft are shown as *white triangles*

gradually with a peak fivefold increase on day 7 after transplantation (basal level: 6.2 ± 0.6 mg/24 h, on day 7 post Tx: 33.6 ± 6.6 mg/24 h) Three recipients (30%) within this group died due to anuria on day 5 after showing the highest levels of proteinuria post-transplant (85.6, 68.8, and 62.2 mg/24 h). A threefold increase in urinary protein was observed in the HP group on the first day (basal level, 7.2 ± 1.2 mg/24 h; on day 1 post Tx, 20.4 ± 3.1 mg/24 h) but returned to the basal level on day 7 post Tx (8.8 ± 1.3 mg/24 h).

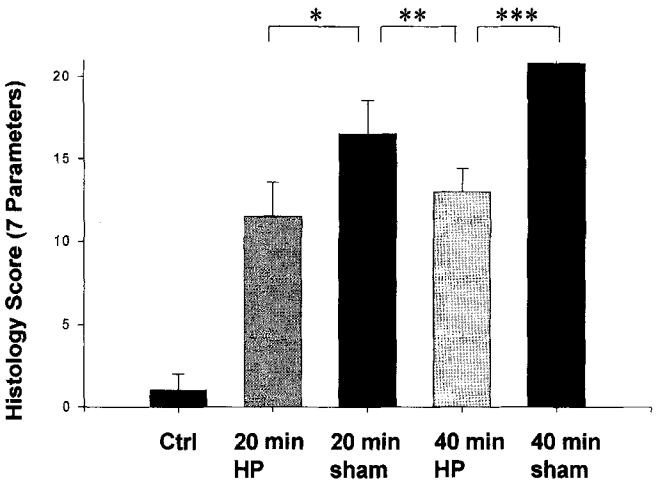

Fig. 6 Hyperthermia-induced renal organ protection gradually wears off within 96 h. Kidneys were cut and stained with H&E for histological evaluation as in Fig. 7. Warm ischemia/reperfusion-induced renal organ damage was assessed by an independent pathologist blinded to the experimental protocol. For quantification and comparison, a scoring system was used evaluating seven parameters, including cell swelling, nuclear pyknosis, cell lysis, cell sloughing, loss of proximal tubules brush border, as well as the presence of protein casts, and neutrophilic tubulitis. To each parameter we assigned a semiquantitative score (0 to 3) reflecting the severity of each alteration. Values of p are shown for differences between the study groups: $*p = 0.003$; $**p = 0.01$ and $***p = 0.000004$, respectively (ANOVA and unpaired Student's t-test)

Effects of HP on warm ischemia/reperfusion-induced organ damage

Control kidneys subjected to heat stress but not transplanted did not show any histopathological abnormalities (data not shown) while some histopathology was seen in all grafts. In all transplanted kidneys changes of acute tubular injury were more prominent in the outer medulla, corresponding to the kidney region most sensitive to hypoxemic injury. A semiquantitative tubulointerstitial score of the study groups is depicted in Fig. 6.

After 20 min of warm ischemia, sham preconditioned kidneys (group B) showed signs of a severe acute tubular injury. Cell lysis, nuclear pyknosis, and karyorrhexis as well as mononuclear infiltrates and neutrophilic tubulitis were generally detected in more then 50% of the tissue sections (Fig. 7A/B). Areas of parenchymal necrosis could be observed in each sample. Signs of tissue repair were consistently absent. In contrast, histological signs of acute tubular injury were light to moderate in the hyperthermic preconditioned group A. In particular, cell lysis, nuclear pyknosis, and karyorrhexis were totally absent. All samples of this group, however, showed light to moderate neutrophilic tubulitis or interstitial mononuclear infiltrates. Protein casts were in general rare and flattened tubular epithelial cells were not present (Fig. 7C/D).

Following 40 min of warm ischemia, HP kidneys (group C) were characterized by prominent cell sloughing, enlargement of tubular diameter, and protein casts, but relevant inflammatory infiltrates, namely neutrophilic tubulitius or mononuclear infiltrates, were absent in all grafts. Flattened epithelial cells as signs of regeneration were also generally present in preconditioned grafts of group C. (Fig. 7E/F) Histological signs of cellular ischemia, such as nuclear pyknosis and karyorrhexis were only occasionally found in these transplants, whereas it was present in over 90% of kidney grafts of group D (not shown).

Discussion

The present study demonstrated that rat kidney transplants harvested from NHB donors and their graft function are protected by heat stress against the consequences of the warm ischemic sequence prior to transplantation and that HSP expression seems to mediate this protective effect.

By using NHB donor grafts, a prior insult of warm ischemia preceding the period of cold preservation and the following revascularization result in cellular damage and the loss of cellular functions. Moreover, in first experiences with NHB donor kidneys, a dramatic increase in delayed graft function and primary nonfunction has been encountered as compared with heart beating donor grafts [11, 15, 21, 32]. This observation is not surprising considering that prior warm ischemic damage, compounded by cold ischemic organ storage, result in impaired graft function sooner than in kidneys with a single insult. Therefore, new approaches to organ preservation and viability testing have gained renewed interest with the resurgence of the NHB donor. Until now, hyperthermic graft preconditioning has played a minimal role in organ procurement and has been studied in experimental models only, despite its cytoprotective potential in reducing ischemia/reperfusion damage.

Prior hyperthermia is known to induce renal HSP synthesis, in particular HSP72 [34] which possess molecular chaperoning properties [35]. In good agreement with earlier studies, we found strongly induced expression of renal HSP72 expression following hyperthermia preconditioning [9, 11, 34, 35]. So far HSP72 has been implicated with protection of graft survival in several studies [2, 17, 34, 35]. For instance, Perdrizet and colleagues [35] similarly reported in a kidney transplant model that graft survival after prolonged warm ischemia correlated to the level of HSP72 expression. However, these studies provide no detailed analysis of hyperthermia preconditioning-induced expression of other HSPs since recipient kidneys were not removed upon trans-

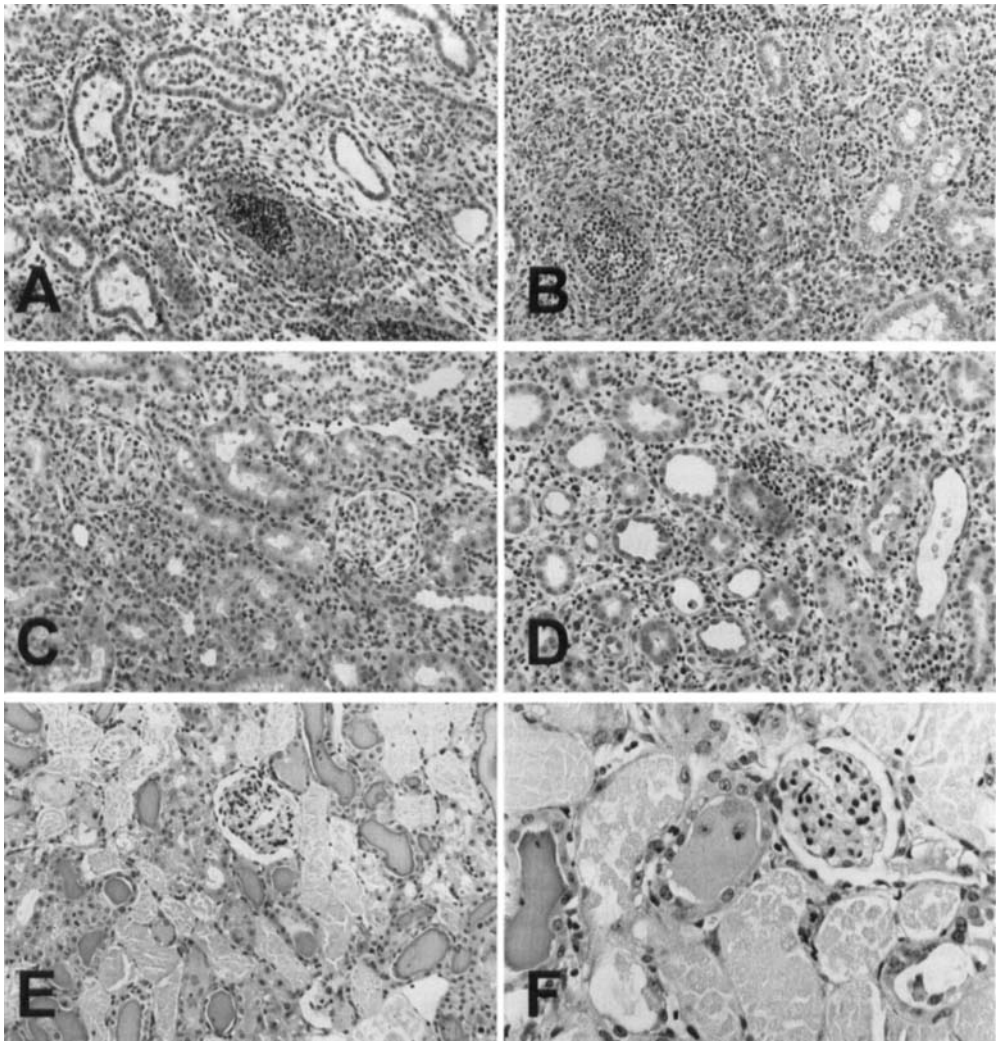

Fig. 7 **A, B** (20 min WI + sham preconditioned): Severe acute tubular injury. Note enlargement of tubular diameters, cell sloughing, neutrophilic tubulitis, and prominent interstitial mononuclear infiltrates. **C, D** (20 min WI + hyperthermic preconditioned): Mild acute tubular injury. Note mild interstitial mononuclear infiltrates by preserved nephrons. **E, F** (40 min WI + hyperthermic preconditioned): enlargement of tubular diameters and protein casts in lumen. Inflammatory infiltrates are absent. At higher magnification (**F**) flattened tubular epithelial cells in preserved nephrons are visible. Original magnification × 200 (**A–E**) and × 400

plantation; these studies also allow no analysis of the effect of HP on renal functional parameters [35]

In addition to HSP72, we also report strong induction of renal HSP32/heme-oxygenase 1 (HO-1) and HSP90 after hyperthermia preconditioning. HSP32 was even more rapidly upregulated than HSP72. This may indicate that hyperthermia-mediated augmentation of warm ischemia tolerance does not depend solely on HSP72. Instead, HP-mediated protection may very well also be influenced by HSP32 expression.

Interestingly, HSP 32, also known as heme oxygenase-1 (HO-1), exerts a protective effect on tissue injury in many systems via its antioxidant and vasodilative potential and may therefore also be of importance to organ transplantation . The purpose of this study was to demonstrate that a principal heat-induced upregulation of HSP induces graft improvement in NHBDs. However, there are pharmacological inducers of HSPs such as metalloporphyrins, which might be used clinically after experimental evaluation. It has recently been shown that pharmacological alteration of HO-1 with protoporphyrins or nitric oxide can influence ischemia tolerance in cardiac and liver graft models [1, 19]. To characterize the pattern of HSP induction, we also wanted to investigate effects of HP on kidney graft function in order to determine possible mechanisms of protection. In good agreement with these studies, we were able to demonstrate that HP-induced HO-1 strongly correlates with improved kidney transplant viability and graft survival in NHB donor kidneys and therefore we implicate HO-1 as a player in HP-mediated renal protection. In a real clinical situation, those pharmacological inducers of HSPs could be administered at time of harvesting to mimic the effect of hyperthermia preconditioning.

We also showed that the graft survival rate was significantly improved even after an extended NHB period of 40 min followed by 24 h of cold preservation and transplantation, which was confirmed by the improvement

of graft function and histological alterations of kidney grafts, indicating better graft viability. In our study, hyperthermia preconditioning prior to warm ischemia stress positively influenced all examined aspects of kidney graft function, both morphologically as well as biochemically. In contrast to most other studies, we removed both native kidneys at transplantation so that functional parameters truly assessed solely graft function. Interestingly, functional preservations after HP was so good, compared with untreated controls, that creatinine clearance was significantly better in single kidney graft recipients even when exposed to an extended NHB period of 40 min, whereas all normothermic graft recipients stressed to 40 min of warm ischemia died within 3 days due to anuria.

Moreover, proteinuria was clearly reduced following preconditioning while urine volume and creatinine excretion were increased. Early post-transplant high diuresis volume and low proteinuria can reportedly predict graft outcome [18, 23]. Although our study was limited to 7 days, we therefore believe that our data provide good evidence that long term kidney graft function in NHB donor grafts can also be positively influenced through hyperthermia preconditioning.

These observations suggested a close relationship between the cellular protection represented by the improved graft survival rate and the induced expression of HSP72 and HO-1.

HSP90 can reportedly also contribute to organ protection and has been suggested to be involved in protection against ischemia/reperfusion injury [8, 27, 29]. The exact role of HSP 90 in our system is unclear since HP-mediated induction was relatively weak compared with HSP72 and HO-1 and also occurred rather late.

In conclusion, the present study shows strong upregulation of renal HSP72, HO-1 and, to less extent, HSP90 following hyperthermia preconditioning. Hyperthermia preconditioning improved all aspects of kidney graft function, after conventional as well as extended warm ischemia times, compared with untreated sham-preconditioned animals. We therefore believe that, together with HSP72, HO-1 may be involved in HP-mediated graft protection. More studies are needed to provide us with a more detailed insight in the molecular mechanisms of these alterations. Hopefully, this will lead to the development of novel procedures in clinical transplantation.

Acknowledgements This work was supported by grants from the Prof. Dr. Max Cloëtta Foundation Grant Program and the Swiss National Foundation SNF 31–54167.98 both awarded to C. A. Redaelli.

References

1. Amersi F, Buelow R, Kato H, Ke B, Coito AJ, Shen XD, Zhao D, Busuttil RW, Kupiec-Weglinski JW (1999) Upregulation of heme oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. J Clin Invest 104: 1631–1639
2. Amrani M, Corbett J, Boateng SY, Dunn MJ, Yacoub MH (1996) Kinetics of induction and protective effect of heat-shock proteins after cardioplegic arrest. Ann Thorac Surg 61: 1407–1411
3. Beckmann RP, Mizzen LE, Welch WJ (1990) Interaction of Hsp 70 with newly synthesized proteins: implications for protein folding and assembly. Science 248: 850–854
4. Bohen SP, Kralli A, Yamamoto KR (1995) Hold 'em and fold 'em: chaperones and signal transduction [comment]. Science 268: 1303–1304
5. Brasile L, Clarke J, Green E, Haisch C (1996) The feasibility of donor preservation at warmer temperatures. Transplant Proc 349–351
6. Brasile L, Green E, Haisch C (1997) Ex vivo resuscitation of kidneys following postmortem warm ischemia. Transplant Proc 3518–3519
7. Brasile L, Green E, Haisch C (1997) Warm ex vivo perfusion prevents reperfusion injury in warm ischemically damaged kidneys. Transplant Proc 3422–3423
8. Cumming DV, Heads RJ, Watson A, Latchman DS, Yellon DM (1996) Differential protection of primary rat cardiocytes by transfection of specific heat stress proteins. J Mol Cell Cardiol 28: 2343–2349
9. Defily DV, Chilian WM (1993) Preconditioning protects ventricular coronary arteriolar endothelium from ischemia-reperfusion injury. Am J Physiol 265:H700–H706
10. DeNagel DC, Pierce SK (1993) Heat shock proteins in immune responses. Crit Rev Immunol 13: 71–81
11. Donnelly TJ, Sievers RE, Vissern FL, Welch WJ, Wolfe CL (1992) Heat shock protein induction in rat hearts. A role for improved myocardial salvage after ischemia and reperfusion? Circulation 85: 769–778
12. Eden W, Young DB (eds) (1996) Stress proteins in medicine, Vol 1. Marcel Dekker, New York
13. Elbirt KK, Bonkovsky HL (1999) Heme oxygenase: recent advances in understanding its regulation and role. Proc Assoc Am Physicians 111: 438–447
14. Gething MJ, Sambrook J (1992) Protein folding in the cell. Nature 355: 33–45
15. Gonzalez C, Castelao AM, Torras J, Gil S, Lopez MA, Riera L, Franco E, Alsina J (1995) Long-term follow up of transplanted non-heart beating donor kidneys. Transplant Proc 27: 2948–2950
16. Hartl FU, Hlodan R, Langer T (1994) Molecular chaperones in protein folding: the art of avoiding sticky situations. Trends Biochem Sci 19: 20–25
17. Hiratsuka M, Yano M, Mora BN, Nagahiro I, Cooper JD, Patterson GA (1998) Heat shock pretreatment protects pulmonary isografts from subsequent ischemia–reperfusion injury. J Heart Lung Transplant 17: 1238–1246
18. Hohage H, Kleyer U, Bruckner D, August C, Zidek W, Spieker C (1997) Influence of proteinuria on long-term transplant survival in kidney transplant recipients. Nephron 75: 160–165

19. Katori M, Tamaki T, Akahashi T, Anaka M, Awamura A, Akita A (2000) Prior induction of heat shock proteins by a nitric oxide donor attenuates cardiac ischemia/reperfusion injury in the rat. Transplantation 69: 2530–2537
20. Keyse SM, Tyrrell RM (1989) Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. Proc Natl Acad Sci USA 86: 99–103
21. Kievit JK, Oomen AP, de Vries B, Heineman E, Kootstra G (1997) Update on the results of non-heart-beating donor kidney transplants. Transplant Proc 29: 2989–2991
22. Lindquist S (1986) The heat-shock response. Annu Rev Biochem 55: 1151–1191
23. Matteucci E, Carmellini M, Bertoni C, Boldrini E, Mosca F, Giampietro O (1998) Urinary excretion rates of multiple renal indicators after kidney transplantation: clinical significance for early graft outcome. Ren Fail 20: 325–330
24. McCoubrey WK Jr, Huang TJ, Maines MD (1997) Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3. Eur J Biochem 247: 725–732
25. McCoubrey WK Jr, Maines MD (1994) The structure, organization and differential expression of the gene encoding rat heme oxygenase-2. Gene 139: 155–161
26. Metzler W, Hofken T, Weber H, Printz H, Goke B, Wagner AC (1999) Hyperthermia, inducing pancreatic heat-shock proteins, fails to prevent cerulein-induced stress kinase activation. Pancreas 19: 150–157
27. Morris SD, Cumming DV, Latchman DS, Yellon DM (1996) Specific induction of the 70-kD heat stress proteins by the tyrosine kinase inhibitor herbimycin-A protects rat neonatal cardiomyocytes. A new pharmacological route to stress protein expression? J Clin Invest 97: 706–712
28. Nathan DF, Lindquist S (1995) Mutational analysis of Hsp90 function: interactions with a steroid receptor and a protein kinase. Mol Cell Biol 15: 3917–3925
29. Nayeem MA, Elliott GT, Shah MR, Hastillo-Hess SL, Kukreja RC (1997) Monophosphoryl lipid A protects adult rat cardiac myocytes with induction of the 72-kD heat shock protein: a cellular model of pharmacologic preconditioning. J Mol Cell Cardiol 29: 2305–2310
30. Olsen S, Burdick LF, Koewen PA (1989) Primary acute renal failure ("acute tubular necrosis") in the transplanted kidney. Morphology and pathogenesis. Medicine 68: 173–187
31. Olsen SK (1994) Acute tubular necrosis and toxic renal injury. In: Tisher CG, Brenner BM (eds) Renal pathology, 2nd edn. Lippinicott Williams & Wilkins, Philadelphia
32. Orloff MS, Reed AI, Erturk E, Kruk RA, Paprocki SA, Cimbalo SC, Cerilli GJ (1994) Nonheartbeating cadaveric organ donation. Ann Surg 220: 578–583
33. Perdrizet GA, Heffron TG, Buckingham FC, Salciunas PJ, Gaber AO, Stuart FP, Thistlethwaite JR (1989) Stress conditioning: a novel approach to organ preservation. Curr Surg 46: 23–26
34. Perdrizet GA, Kaneko H, Buckley TM, Fishman MA, Schweizer RT (1990) Heat shock protects pig kidneys against warm ischemic injury. Transplant Proc 22: 460–461
35. Perdrizet GA, Kaneko H, Buckley TM, Fishman MS, Pleau M, Bow L, Schweizer RT (1993) Heat shock and recovery protects renal allografts from warm ischemic injury and enhances HSP72 production. Transplant Proc 25: 1670–1673
36. Pratt WB, Toft DO (1997) Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr Rev 18: 306–360
37. Shibahara S, Yoshizawa M, Suzuki H, Takeda K, Meguro K, Endo K (1993) Functional analysis of cDNAs for two types of human heme oxygenase and evidence for their separate regulation. J Biochem (Tokyo) 113: 214–218
38. Takahashi Y, Tamaki T, Tanaka M, Konoeda Y, Kawamura A, Katori M, Kakita A (1998) Efficacy of heat-shock proteins induced by severe fasting to protect rat livers preserved for 72 hours from cold ischemia/reperfusion injury. Transplant Proc 30: 3700–3702
39. Torras J, Cruzado JM, Riera M, Condom E, Duque N, Herrero I, Merlos M, Espinosa L, Lloberas N, Egido J, Grinyo JM (1999) Long-term protective effect of UR-12670 after warm renal ischemia in uninephrectomized rats. Kidney Int 56: 1798–1808
40. Wagner AC, Weber H, Jonas L, Nizze H, Strowski M, Fiedler F, Printz H, Steffen H, Goke B (1996) Hyperthermia induces heat shock protein expression and protection against cerulein-induced pancreatitis in rats. Gastroenterology 111: 1333–1342
41. Welch WJ (1992) Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. Physiol Rev 72: 1063–1081
42. Xu Y, Lindquist S (1993) Heat-shock protein hsp90 governs the activity of pp60v-src kinase. Proc Natl Acad Sci USA 90: 7074–7078