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Hyperthermia Preconditioning Induces Renal Heat Shock Protein Expression, Improves Cold Ischemia Tolerance, Kidney Graft Function and Survival in Rats

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Key Words

Hyperthermia preconditioning · Kidney transplantation · Heat shock protein

Abstract

Background: Evidence indicates that hyperthermia preconditioning (HP) can be protective in kidney transplantation, possibly through increased heat shock protein (HSP) expression. A detailed study about individual HSPs and functional preservation is lacking, however. Therefore, we studied the effects of HP on kidney graft survival, function and HSP expression. ***Methods:*** Male Lewis rats were or were not subjected to whole-body hyperthermia 24 h prior to kidney procurement. Kidneys were stored in UW solution at 4°C for 32, 40 or 45 h. Recipient kidneys were both removed and single isografts transplanted orthotopically. ***Results:*** HP strongly induced HSP72 and HSP32 expression. Following 32-hour cold ischemia, most animals survived even without prior HP. However, HP strongly reduced functional impairment induced by cold ischemia. Following 40-hour cold ischemia, kidneys from donors without HP did not recover function and all animals died within 3 days. In contrast, HP-exposed kidneys tolerated 40-hour storage significantly better, with 44% of rats surviving until sacrifice on day 7. In these animals, renal function was still better compared to animals with 32-hour-stored kidneys without HP. Histological alterations were also diminished following HP. ***Conclusion:*** Our data show that HP induces renal HSP72 and, for the first time, HSP32. HP increases survival following transplantation and acts by improving several parameters of kidney function including proteinuria, volume output and creatinine clearance.

Objective

Kidney transplantation has become an effective therapy for the treatment of various end-stage kidney diseases. However, demand greatly exceeds supply and several limitations with respect to both pretransplantational preservation as well as posttransplantational graft function exist. Since organs are scarce, efforts to improve donor kidney preservation prior to transplantation as well as function following transplantation are necessary. Preservation/cold ischemia time following explantation, required

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for determination of appropriate recipients, organ transport and preparation of the recipient for transplantation is a prominent limiting parameter for kidney transplant function. Cold preservation typically causes renal apical tubular and medullary damage with cytoskeletal alterations, mitochondrial injury and consecutive delayed graft function [1, 2]. Improving cold ischemia tolerance will, therefore, likely lead to better transplant availability and function.

Increased expression of protective proteins in response to thermal stress has led to the discovery of the so-called heat shock proteins (HSPs) [3]. Expression of these proteins is, however, induced by almost any kind of stress, and the clinical importance of these proteins is currently a matter of intense research. [4]. HSPs are ubiquitous and comprise several structurally unrelated groups of proteins. These are named according to their apparent molecular weight, such as HSP70, HSP90 or HSP32 [5–7]. HSPs help the cell survive an acute episode of stress, and their increased expression also seems to protect cellular function and survival during following episodes of stress. Because of the obvious pathophysiological and medical implications, it is this protective potential of HSPs that has been the focus of interest in recent years.

Among the best characterized HSPs are members of the HSP70 family [8], which include constitutively expressed isoforms such as HSC70 (heat shock cognate) as well as stress-inducible isoforms, not expressed in unstressed tissue, such as HSP72. In addition, heme oxygenase (HO), a crucial enzyme of heme metabolism, represents another HSP protein with a size of 32 kD. Three isoforms (HO-1, HO-2 and HO-3) have been identified [9–11]. Of these, HO-1 catalyzes the breakdown of heme into equimolar amounts of biliverdin, carbon monoxide and iron [12] and is recognized as a major heat shock/stress response protein.

HSP70-mediated organ protection following preconditioning with hyperthermia or other stressors to induce HSPs has been reported by numerous investigators for a wide variety of systems including kidney transplantation models in recent years [13–18]. Similarly, HSP32 has been implicated in mediating protection. However, renal HSP32 expression following preconditioning has not been investigated, and there are insufficient data on the effects of hyperthermia preconditioning (HP) on functional and histological parameters following kidney transplantation.

We now report that HP can strongly improve cold ischemia tolerance as well as kidney graft function and recipient survival after both regular as well as extended cold ischemia times in a syngeneic kidney transplantation model. HP also ameliorates histological alterations following cold ischemia. In addition to induction of renal HSP72, for the first time, we also found increased expression of renal HSP32/HO-1 following HP.

Materials and Methods

Organ Procurement and Hyperthermia

Animal experiments were performed in accordance with the national and international 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. Donor animals but not recipients underwent HP to increase HSP expression in kidney grafts. Hyperthermia was performed as previously described [16, 19]. In brief, donor animals were anesthetized by intraperitoneal injection of pentobarbitone sodium (50 mg/kg body weight) and body temperature was monitored with a rectal thermometer (groups control I, A, C and E). In addition, animals were subjected to anesthesia without heat stress (groups 0, control II, B, D and F). During HP and sham HP, all animals were given 5 ml of isotonic saline intraperitoneally to avoid dehydration. Twenty-four hours after HP, when HSP expression peaked, kidney grafts were harvested. Ischemic preservation times varied between groups and ranged from 24 h (groups control I, II), 32 h (groups A, B) and 40 h (groups C, D) up to 45 h (groups E, F). Due to high mortality after extended cold ischemia times, 45 h rather than 48 h was chosen as the longest cold ischemia interval studied.

Study Groups

Eighteen rats were dedicated to study the expression of renal HSPs after HP. These separate animals were handled in an identical fashion as the HP donor animals and 1 kidney from 1 animal was sampled for each time point documented.

Survival studies were further performed on 76 animals, which were monitored until death or 1 week after transplantation. To determine control values of renal functional parameters in our setting, 10 sham control donor grafts (group 0) were isogeneically transplanted and the rats kept in metabolic cages for 7 days. Additionally, 66 donor grafts were preconditioned either with HP or sham HP and preserved 24 h (control I, n = 6/control II, n = 6), 32 h (group A, n = 10/group B, n = 9), 40 h (group C, n = 9/group D, n = 9) or 45 h (group E, n = 8/group F, n = 9). Surviving animals were sacrificed on the 7th postoperative day. Jugular venous blood for the determination of serum creatinine, urea and protein levels was collected only on days 3 and 7 to reduce stressful animal manipulations. Urine volume, protein content and creatinine levels were determined daily, however. Creatinine clearance (CRC) was accordingly calculated for days 3 and 7 only. After transplantation, all animals were kept in metabolic units for renal function analysis. In a separate set of experiments (n = 6/group), animals were sacrificed on day 3 after transplantation and kidney grafts were removed for histological evaluation. The experimental protocol, including HP, is schematically depicted in figure 1.

Arterialized Rat Renal Transplantation

For arterialized kidney transplantation, recipients were anesthetized with pentobarbitone sodium (50 mg/kg body weight), both recipient kidneys were removed and single isograft kidney transplan-

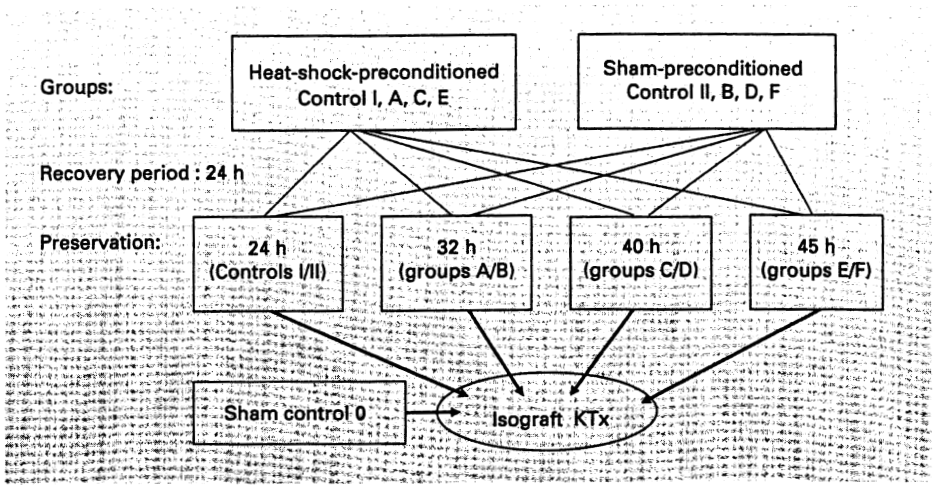

Fig. 1. Experimental flow chart. Control group 0 consisted of animals subjected to sham stress (general anesthetic 24 h prior to transplantation) prior to functional assessment. Controls I and II were subjected to a standard cold ischemia period (24 h). Furthermore, groups control I, A, C and E were subjected to HP, whereas groups control II, B, D and F underwent sham anesthesia and cold ischemic organ procurement prior to graft transplantation (KTx).

tation was performed. Removal of both native recipient kidneys was felt necessary for the true assessment of graft function. Donor kidneys from the different experimental groups (see above) 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. To eliminate operator-related discrepancy, all transplant procedures were performed by a single investigator (Y.-H.T.).

Determination of HSP Expression by Western Blotting

One-dimensional gel electrophoresis was performed using minigels (Bio-Rad, Richmond, Calif., USA) as previously described [16]. Five micrograms of protein were loaded per lane. After gel electrophoresis, proteins were transferred to nitrocellulose membranes as described earlier [16]. Protein detection was performed as described previously using the indicated concentration of antibody (1:1,000 for monoclonal antibodies and 1 μg/ml for polyclonal antibodies). Labeled proteins were visualized by enhanced chemiluminescence following the manufacturer's procedures using the horseradish-peroxidase-coupled secondary antibodies supplied with the kit at a 1:5,000 dilution, followed by autoradiography. Densitometry of renal HSP expression was performed as described previously [16]. 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. In order to equalize differences in protein loading, β-tubulin (H235) expression levels (rabbit polyclonal IgG, 200 μg/ml; Santa Cruz Biotechnology, Heidelberg, Germany) were also analyzed in all experiments.

Histopathological Analysis

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 hematoxylin and eosin. The light-microscopic changes characteristic of acute tubular injuries have previously been described in detail [20–22]. In this study, we evaluated tubulointerstitial damage (tubulointerstitial score [23]) with 7 histological parameters, i.e. cell swelling, nuclear pyknosis, cell lysis, cell sloughing, loss of proximal tubule brush border, the presence of protein casts and neutrophilic tubulitis. To each parameter, we assigned a semiquantitative score (from 0 to 3) reflecting the severity of each alteration.

Chemicals and Antibodies

All antibodies were from StressGen (Victoria, British Columbia, Canada). Detection reagents for enhanced chemiluminescence and horseradish-peroxidase-coupled antimouse and antirabbit antibodies were from Amersham (Arlington Heights, Ill., USA). Polyacrylamide, molecular weight and isoelectric focusing standards were from Bio-Rad (Hercules, Calif., USA). All other chemicals were purchased from Sigma Chemie (Buchs, Switzerland).

Statistics

Results are expressed as means ± standard deviations. 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., USA). With the small number of animals used, statistical differences between groups were analyzed by the Mann-Whitney U test and Wilcoxon rank sum test to compare between groups. Differences at $p < 0.05$ were considered statistically significant.

Results

Effect of HP on Renal HSP Protein Expression

As a first step in order to characterize the effects of HP, we analyzed the pattern of HSPs induced by whole-body hyperthermia. Among several HSPs tested, we observed constitutive expression of HSP90, HSP32/HO-1, HSC70 as well as HSP60 (fig. 2 and data not shown). In contrast, HSP72 was not expressed without preconditioning. Following HP, HSP72 and HSP32/HO-1 expression were strongly induced (fig. 2), while expression of HSP90, HSP60 and HSC70 was little altered (fig. 2A and data not shown). HSP72 expression peaked at 24 h and remained

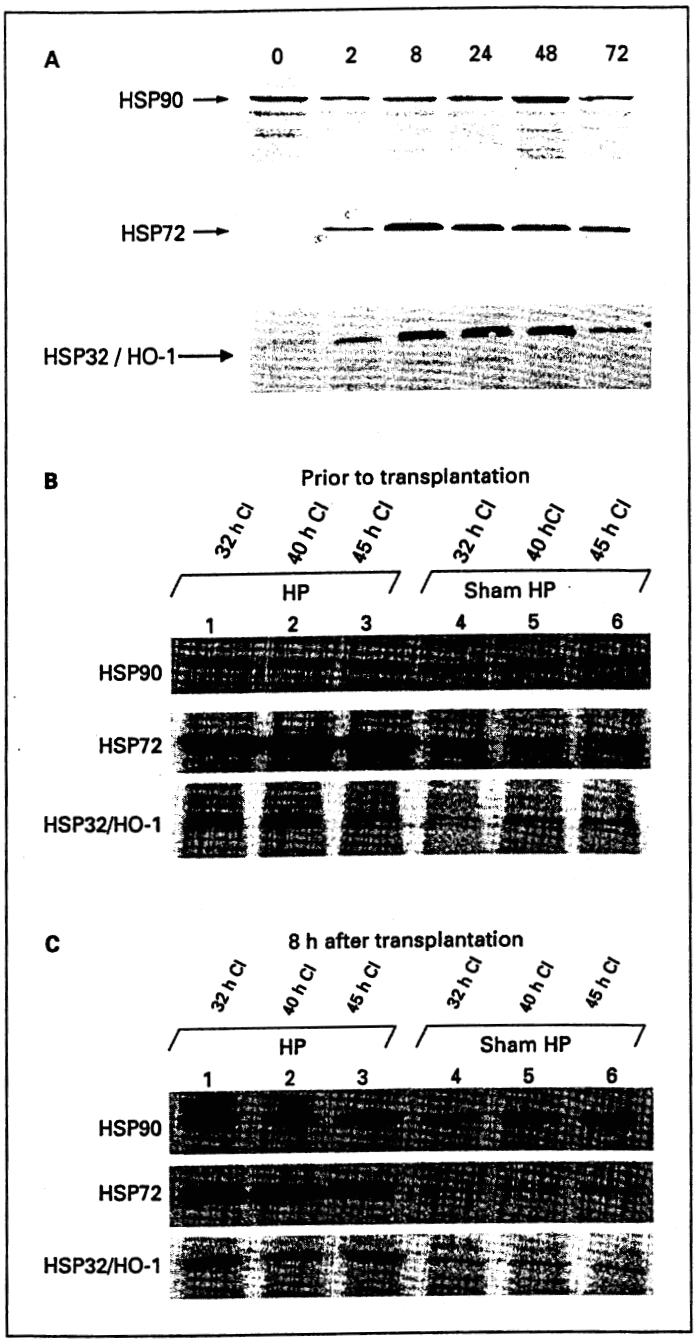

Fig. 2. A Western blot analysis of HSP90, HSP72 and HSP32/HO-1 in donor kidneys prior to transplantation in relation to the recovery period. The constitutive protein expression (0 h) is given for HSP90 and HO-1, but not for HSP72. The relative HSP expression levels analyzed by densitometry and given as mean HSP/β-tubulin ratio were: 0.01 ± 0.01 (0 h), 0.32 ± 0.02 (2 h), 1.42 ± 0.11 (8 h), 1.48 ± 0.12 (24 h), 1.04 ± 0.09 (48 h) and 0.86 ± 0.04 (72 h) for HSP72. HO-1 expression levels were 0.11 ± 0.02 (0 h), 0.21 ± 0.08 (2 h), 1.33 ± 0.13 (8 h), 1.30 ± 0.12 (24 h), 1.11 ± 0.07 (48 h) and 0.16 ± 0.1 (72 h), and those of HSP90 were 1.23 ± 0.11 (0 h), 1.01 ± 0.12 (2 h), 1.41 ± 0.10 (8 h), 1.38 ± 0.07 (24 h), 1.61 ± 0.22 (48 h) and 1.29 ± 0.24 (72 h). Data shown are representative of 3 independent experiments. **B** Western blot analysis of HSP expression in donor kidneys stored for 32, 40 or 45 h at 4 °C prior to transplantation. CI = Cold ischemia. Lanes 1–3: heat-shock-preconditioned; lanes 4–6: sham-preconditioned. The relative HSP expression levels analyzed by densitometry and given as mean HSP/β-tubulin ratio were: for HSP90, 1.20 ± 0.22, 1.27 ± 0.15, 1.31 ± 0.26 for lanes 1–3 and 1.13 ± 0.09, 1.11 ± 0.12, 1.18 ± 0.20 for lanes 4–6; for HSP72, 1.34 ± 0.14, 1.41 ± 0.20, 1.32 ± 0.09 for lanes 1–3 and 0.23 ± 0.02, 0.20 ± 0.06, 0.31 ± 0.09 for lanes 4–6; for HO-1/HSP32, 1.25 ± 0.31, 1.42 ± 0.12, 1.36 ± 0.08 for lanes 1–3 and 0.20 ± 0.02, 0.18 ± 0.04, 0.23 ± 0.07 for lanes 4–6, respectively. Data shown are representative of 6–9 independent experiments. **C** Western blot analysis of HSP expression in donor kidneys stored for 32, 40 or 45 h at 4 °C 8 h after reperfusion. CI = Cold ischemia. Lanes 1–3: heat-shock-preconditioned; lanes 4–6: sham-preconditioned. The relative HSP expression levels analyzed by densitometry and given as mean HSP/β-tubulin ratio were: for HSP90, 1.61 ± 0.32, 1.31 ± 0.25, 1.41 ± 0.18 for lanes 1–3 and 1.15 ± 0.21, 1.30 ± 0.15, 1.38 ± 0.10 for lanes 4–6; for HSP72, 1.44 ± 0.34, 1.39 ± 0.30, 1.46 ± 0.29 for lanes 1–3 and 0.17 ± 0.07, 0.11 ± 0.04, 0.28 ± 0.11 for lanes 4–6; for HO-1/HSP32, 1.30 ± 0.41, 1.23 ± 0.15, 1.33 ± 0.18 for lanes 1–3 and 0.11 ± 0.07, 0.14 ± 0.04, 0.17 ± 0.10 for lanes 4–6, respectively. Data shown are representative of 6–9 independent experiments.

at high levels for at least 72 h after thermal stress. In contrast, HSP32/HO-1 induction peaked at 8 h following HP, remaining elevated for up to 48 h and decreased markedly thereafter (fig. 2A). For practical reasons, harvesting of donor kidneys was, therefore, performed 24 h after hyperthermia. Cold ischemia prior to reimplantation (fig. 2B) as well as surgical stress of transplantation (fig. 2C) did not alter the expression levels of renal HSP32 and HSP72 levels regardless of the duration of cold ischemia storage.

Effect of HP on Survival

Kidney graft storage for up to 24 h did not lead to recipient death in sham HP (0/6) as well as in HP-preconditioned (0/6) donor recipients. Following 32-hour cold ischemia preservation, 3/9 (33%) animals without HP (group B) died prior to sacrifice on day 7 after transplantation. In contrast, 0/10 recipients of preconditioned grafts (group A) died until study termination (fig. 3, black thin line, $p < 0.05$).

Following 40-hour cold ischemia preservation, 9/9 (100%) recipients of grafts from sham-preconditioned animals (group D) died within 2–3 days postoperatively. In contrast, 4/9 (45%) recipients with grafts from preconditioned donors (group C) survived until sacrifice on day 7 ($p < 0.001$, gray bold dotted line). Even following 45-hour cold ischemia storage, 37% (3/8) of preconditioned graft recipients (group E) survived compared to 0/9 survivors in group F ($p < 0.0001$; black bold line).

Effect of HP on Renal Graft Function

Cold ischemia up to 24 h showed no major graft dysfunction regardless of preconditioning (fig. 4, dashed lines). Following 32-hour cold ischemia preservation,

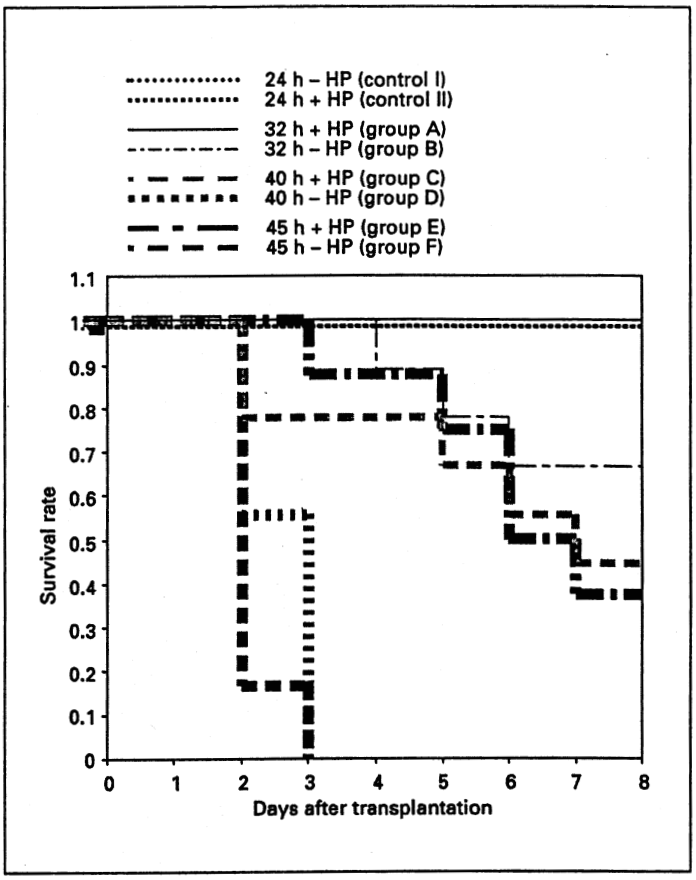

Fig. 3. Kaplan-Meier survival curves of rats recipient of an orthotopic kidney transplant. Survival rates of bilaterally nephrectomized and single renal isografted recipients with HP (groups control I, A, C, E) or without HP (groups control II, B, D, F) in relation to the cold ischemia preservation period. After 32 h of cold ischemia with HP (thin black line) and without HP (thin dash-dotted line): log rank test, group A versus B, $p = 0.038$; 40 h of cold ischemia with HP (gray bold dashed line) and without HP (gray bold dotted line): log rank test, group C versus D, $p < 0.001$; 45 h of cold ischemia with HP (black bold dash-dotted line) and without HP (black bold dotted line): log rank test, group E versus F, $p < 0.0001$.

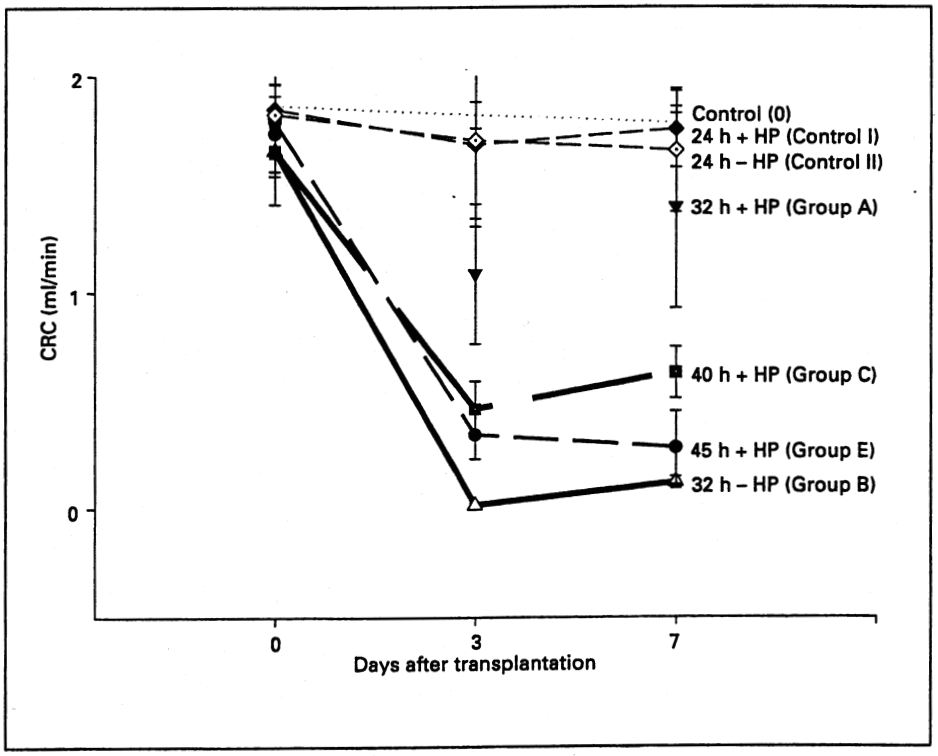

Fig. 4. HP improved graft function of kidney isografts exposed to prolonged cold ischemic organ storage. CRC of survivors of a single kidney isograft undergoing either 0, 24, 32 ,40 or 45 h of cold organ preservation. Cold ischemia up to 24 h showed no major graft dysfunction regardless of preconditioning (thin dashed lines). Following 32 h of cold ischemia preservation, grafts from donors not exposed to HP (group B) showed severely impaired graft function (solid black line). CRC was significantly decreased in comparison with function values of preconditioned grafts of group A (solid gray line: $p = 0.034$ and 0.025). CRC in survivors of group C (long dashed line) showed still significantly better values compared to graft function from nonpreconditioned animals following 32 h of cold ischemia only ($p = 0.001$). Interestingly, following 32 h of cold ischemia, CRC of preconditioned grafts (group A) thus did not differ significantly from that of healthy sham-grafted rats (group 0) on the 7th day after grafting.

grafts from donors not exposed to HP (group B) showed severely impaired graft function with reduced urinary volume and CRC levels as well as increased serum creatinine and urinary protein in comparison to preconditioned grafts. HP preserved CRC well which averaged 1.08 ± 0.64 ml/min in group A (fig. 4, gray solid line, black triangles) compared to 0.2 ± 0.02 ml/min in group B on the 3rd postoperative day (black solid line, white triangles; $p = 0.034$). Corresponding values on the 7th postoperative day were 1.39 ± 0.93 ml/min compared to 0.11 ± 0.06 ml/min, respectively ($p = 0.025$). For comparison, CRC of untreated control grafts (group 0, dotted line) averaged 1.91 ± 0.21 ml/min on day 3 and 1.78 ± 0.32 ml/min on day 7 after grafting.

Following 40 and 45 h of preservation, cold ischemic renal damage resulted in total loss of kidney function in all sham-preconditioned grafts within 7 days (groups D, F). CRCs in survivors of preconditioned graft recipients of group C (fig. 4, black long dashed line, black squares) averaged 0.46 ± 0.25 ml/min on day 3 and 0.62 ± 0.26 ml/min on day 7 and were statistically significantly better than CRCs of group B ($p = 0.028$ and $p = 0.001$, respectively). Even after 45 h of storage (group E), CRCs of preconditioned grafts (fig. 4, dashed line, black circles) compared favorably (0.34 ± 0.27 ml/min on day 3 and 0.28 ± 0.37 ml/min on day 7) to those of group B (sham HP, 32-hour storage).

Improved CRC rates on days 3 and 7 after transplantation in recipients of preconditioned grafts were mirrored by improved urine volume, urine creatinine and serum creatinine values throughout the study period. Thus, urinary creatinine concentration decreased rapidly from 5,501 ± 643 mmol/l in untreated controls (group 0) to 2,151 ± 1,267 mmol/l on day 3 and 1,855 ± 578 mmol/l on day 7 after transplantation of sham-preconditioned and 32-hour-stored grafts in group B. In contrast; preconditioned grafts (group A) showed higher urinary creatinine levels throughout, reaching 3,891 ± 1,279 mmol/l ($p < 0.001$) on day 7 after transplantation. Urine creatinine concentrations in animals of group C and E were similar to those of group B despite the longer preservation intervals (40 and 45 h, respectively). For comparison, urinary creatinine of untreated animals (group 0) averaged 5,006 ± 978 mmol/l throughout the study period.

Similarly, urinary volume decreased markedly after transplantation in all study groups, with a continuous recovery to normal values seen only in group A. Thus, 7 days after transplantation, urine output in group A went up to 33 ± 8 ml/24 h and was significantly higher compared to group B (16 ± ml/24 h; $p < 0.001$). After 40 and 45 h of cold ischemia, preconditioned kidney grafts (groups C and E, respectively) produced similar urine amounts compared to sham-preconditioned grafts stored for only 32 h (group B). On day 7, urine output was 9.3 ± 2.1 ml in group C and 5.6 ± 0.8 ml in group E, respectively.

For comparison, the urinary volume of untreated animals (group 0) showed a reactive hypovolemia on the first postoperative day but bounced back to an average volume output of 34 ± 8 ml/24 h on the following days.

Serum creatinine levels were accordingly higher in recipients of sham-preconditioned grafts: exact values were 311 ± 210 versus 166 ± 53 mmol/l 3 days after transplantation and 261 ± 64 versus 90 ± 112 mmol/l 7 days after transplantation in group B compared to group A ($p < 0.02$ and $p < 0.03$, respectively). Serum creatinine values in groups C and E were 237 ± 111 and 299 ± 176 mmol/l for day 3, 187 ± 65 and 201 ± 110 mmol/l on day 7.

For comparison, the serum creatinine of untreated animals (group 0) averaged 76 ± 43 and 82 mmol/l on days 3 and 7, respectively.

In addition, cold ischemic damage resulted in significant proteinuria. Sham-preconditioned grafts after 32-hour storage excreted up to four times as much protein compared to untreated controls (group 0). Values on day 7 after transplantation were 70.25 ± 15.1 mg/24 h compared to 16.1 ± 3.3 mg/24 h. The 3 animals in group B dying (30%) due to anuria on day 4 showed the highest levels of proteinuria (79.6, 78.8 and 78.2 mg/24 h). In contrast, the urinary protein loss of preconditioned grafts in group A was significantly higher compared to untreated controls (group 0) on the second day only with 32.1 ± 5.2 mg/24 h compared 15.2 ± 2.2 mg/24 h. Proteinuria of preconditioned grafts then returned close to normal values on day 7 (19.1 ± 3.2 mg/24 h). While increased compared to untreated animals, urinary protein loss of preconditioned grafts stored for 40 h (group C) and 45 h (group E) averaged 56.2 ± 7.3 and 64.7 ± 2.1 mg/24 h on day 7 (fig. 4) but was still lower compared to sham-preconditioned grafts stored for only 32 h (group B).

Effects of HP on Cold Ischemia/Reperfusion-Induced Histological Alterations

Some histopathology was seen in all grafts. Generally, 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 figure 5.

After 24 h of cold ischemia, we observed a focal slight enlargement of the tubule diameters and minimal cell sloughing in the tubules (fig. 6A). After 32 h of ischemia, histological signs of acute tubular injury were similar with or without HP. In particular, we observed appearance of protein casts, vacuolization and a mild interstitial mononuclear inflammatory infiltrate (fig. 6B, C). However, neutrophilic infiltration and casts were more prominent in sham-preconditioned grafts.

Following 40 h of cold ischemia, nuclear pyknosis and cell lysis were only observed in sham-preconditioned grafts (group D). In addition, papillary necrosis could only be found in sham-preconditioned grafts of groups D and F. In contrast, kidneys obtained from rats subjected to hyperthermia showed prominent cell sloughing while nephron integrity was well preserved (compare fig. 6D–F). Signs of regeneration characterized by a flattened epithelium were also generally present.

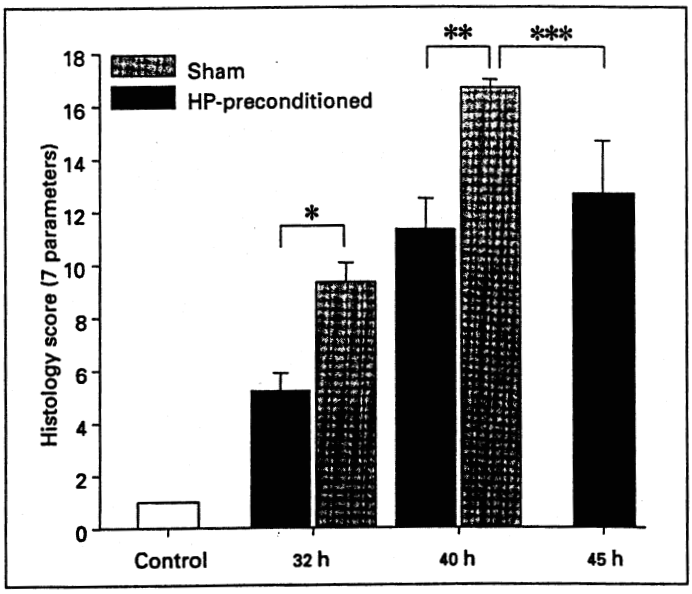

Fig. 5. Hyperthermia-induced renal organ protection gradually wears off within 96 h. Kidneys were cut and stained with HE for histological evaluation as in figure 6. Cold ischemia/reperfusion-induced renal organ damage was assessed by an independent pathologist blinded to the experimental protocol. For quantification and comparison, a scoring system was used evaluating 7 parameters, including cell swelling, nuclear pyknosis, cell lysis, cell sloughing, loss of proximal tubule brush border, the presence of protein casts and neutrophilic tubulitis. To each parameter, we assigned a semiquantitative score (from 0 to 3) reflecting the severity of each alteration. p values are shown for differences between the study groups: * $p < 0.01$, ** $p = 0.02$ and *** $p = 0.03$ (Mann-Whitney U test).

Discussion

In good agreement with earlier studies, we found a strongly induced expression of renal HSP72 following HP. So far HSP72 has been implicated with protection of graft survival in several studies [13, 24–28]. For instance, Amrani et al. [28] showed that hearts from animals subjected to heat shock before hypothermic storage demonstrated superior recovery of graft function and protection was correlated with increased inducible HSP70 protein expression. In a kidney transplant model, Perdrizet et al. [14, 17] similarly reported that graft survival after prolonged cold or warm ischemia corresponded to the level of HSP72 expression. However, these studies have mainly assessed survival in graft recipients but provide no detailed analysis of kidney function or HP-induced expression of other HSPs.

In addition to HSP72, we also report strong expression of renal HSP32/HO-1 after HP. HSP32 was even more rapidly upregulated compared to HSP72. This may indicate that hyperthermia-mediated augmentation of cold ischemia tolerance does not depend solely on HSP72. Instead, HP-mediated protection may very well also be influenced by HSP32 expression. Interestingly, HSP32, also known as HO-1, exerts a protective effect on tissue injury in many systems via its antioxidant and vasodilatative potential and may therefore also be of importance to organ transplantation [12]. Thus, it has recently been shown that the pharmacological alteration of HO-1 with protoporphyrins can influence cold ischemia tolerance in a liver graft model [29]. To our knowledge, our report implicates HO-1 for the first time as a player in HP-mediated renal protection.

We also found that HP of graft donors increased survival in graft recipients. Therefore, in addition to characterizing the pattern of HSP induction, we also wanted to investigate the effects of HP on kidney graft function in order to determine possible mechanisms of protection. Since recipient kidneys were not removed upon transplantation in most other studies [25], these studies allow no analysis of the effects of HP on renal functional parameters [14]. Therefore, we removed both native kidneys at transplantation so that functional parameters truly assessed solely graft function. In our study, HP prior to cold ischemia storage positively influenced all examined aspects of kidney graft function both morphologically as well as biochemically. In particular, functional preservation after HP was so good, compared to sham-preconditioned grafts, that CRC was not significantly reduced in single kidney graft recipients even after 32 h of cold isch-

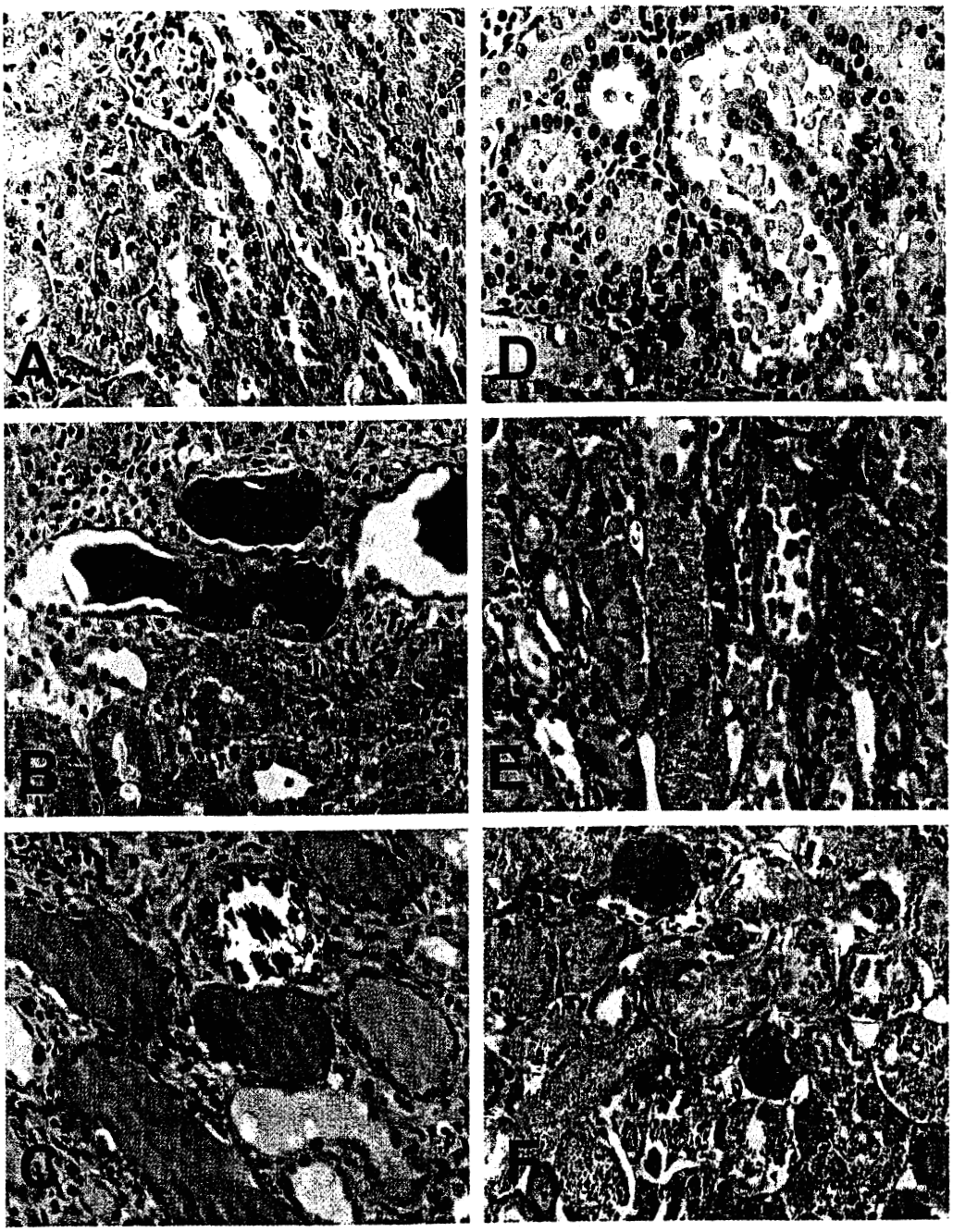

Fig. 6. A Twenty-four-hour cold ischemia. Tubular enlargement and slight cell sloughing. **B, C** Thirty-two-hour cold ischemia with and without hyperthermia, respectively. Similar histopathological findings characterized by protein casts, microcalcification and mild interstitial mononuclear infiltrates. **D** Forty-hour cold ischemia with hyperthermia. Prominent cell sloughing but preserved nephrons. **E, F** Forty-hour cold ischemia without hyperthermia. Severe cell lysis, karyorrhexis and microcalcifications. Magnification × 400.

emia. Moreover, proteinuria was clearly reduced following preconditioning, while urine volume and creatinine excretion were increased. An early high diuresis volume and low proteinuria can reportedly predict graft outcome [30, 31]. Although our study was limited to 7 days, we therefore believe that our data provide good evidence that long-term kidney graft function can also be positively influenced by HP.

In summary, the present study showed that HP is a powerful protective mechanism in the ischemic kidney. It

leads to a strong upregulation of renal HSP72, HO-1 and resulted in preservation of graft function. HP improved all aspects of kidney graft function, after conventional as well as extended cold ischemia times compared with untreated sham-preconditioned grafts. In addition, HP also enhanced survival after sublethal cold ischemia exposure.

We believe that together with renal HSP72, renal HO-1 may also be involved in HP-mediated graft protection. More studies are needed to provide us with a more detailed insight into the molecular mechanisms of these alterations. Hopefully, this will lead to the development of novel procedures in clinical transplantation.

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