Heat Preconditioning Attenuates Renal Injury in Ischemic ARF in Rats: Role of Heat-Shock Protein 70 on NF-κB–Mediated Inflammation and on Tubular Cell Injury

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Although heat preconditioning has been known to be protective in various types of injury, the precise molecular mechanism for this is unclear. Recent observations that indicate that previous heat shock has an anti-inflammatory, antiapoptotic effect led to this investigation of the in vivo effect of heat preconditioning on NF-κB activation and inflammation and also on tubular cell injury in ischemic acute renal failure (ARF). Heat preconditioning provided marked functional protection and also reduced histologic evidence of tubular necrosis. Ischemia/reperfusion–induced NF-κB activation was suppressed by heat preconditioning with a subsequent decrease in monocyte chemoattractant protein-1 expression and inflammatory cell infiltration. Heat preconditioning also suppressed the accumulation of phosphorylated inhibitory κBα (IκBα) with a resultant depletion of cytoplasmic IκBα, indicating that heat preconditioning blocked the activation of the IκB kinase complex. Tubular cell apoptosis, determined by terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling staining, also was decreased by heat preconditioning, and this was accompanied by decreased caspase 3 activation. Among several heat-shock proteins (HSP), HSP-70 was induced primarily by heat preconditioning. Inhibition of HSP-70 by quercetin almost completely reversed the functional protection that was provided by heat preconditioning. These data provide evidence that HSP-70 affords protection via inhibition of NF-κB–mediated inflammation and also inhibition of the cell death pathway in ischemic ARF. Further elucidation of the cytoprotective mechanism of stress proteins could facilitate new target or drug development in the treatment of ARF.


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apoptosis in ischemic ARF in rats. We found that the beneficial effect of heat preconditioning is mediated partially by its inhibitory effect on NF-κB pathway–mediated inflammation as well as by attenuation of tubular cell apoptosis and necrosis.

Materials and Methods

Animals and Experimental Protocol

Male Sprague-Dawley rats that weighed 150 to 200 g were purchased from Orient (Orient Bio Department, Kyungki-Do, Korea) and had free access to water and food before manipulation. Animal care was in accordance with the criteria established by the animal care committee of Korea University for the care and use of laboratory animals in research. Rats were randomly assigned to one of four groups: sham (n = 6), heat preconditioning + sham (HP; n = 6), I/R (n = 6), and heat preconditioning + I/R (HP+I/R; n = 6). For heat preconditioning, rats were anesthetized with an intraperitoneal injection of 100 mg/kg ketamine and 12.5 mg/kg xylazine and placed on a heating pad until their rectal temperature reached and was maintained at 42 ± 0.5°C for 15 min. Sixteen hours after heat preconditioning, ischemic injury was introduced by bilateral clamping of the renal pedicles for 40 min. The animals were kept at a constant temperature (37°C) during this procedure and allowed to recover after intraperitoneal instillation of 1 ml of warmed normal saline. Sham operation was performed in a similar manner, except for clamping of the renal pedicles. At the time of reperfusion and after 1, 6, and 24 h of reperfusion, rats were killed and blood was collected by intracardiac puncture. These time points were introduced by bilateral clamping of the renal pedicles for 40 min. The preconditioning ameliorates or delays renal injury. For another set of experiments, heat preconditioning was introduced as described previously. Rats were killed at 1, 6, and 14 h after heat to determine the degree of expression of various HSP. To define the role of specific HSP in heat preconditioning–induced renal protection, quercetin (20 mg/kg, intraperitoneally; Sigma Aldrich, St. Louis, MO) was administered 1 h before heat, and the rats were killed at 14 h after heat and at 24 h after I/R, respectively.

Biochemical and Histologic Examination

Plasma creatinine levels were measured using a Hitachi 747 automatic analyzer (Tokyo, Japan). For histologic examination, paraformaldehyde–fixed (4%) and paraffin-embedded kidney tissues were stained with periodic acid-Schiff. Histologic changes in the outer medulla were evaluated semiquantitatively. Briefly, tubular damage was estimated in 8 to 10 high-power fields (HPF; ×200) per section by using a scoring system based on the percentage of damaged tubules per field (1 = <25%; 2 = 25 to 50%; 3 = 50 to 75%; and 4 = >75%), and the mean scores of each rat were compared. Detection of apoptotic cells in the kidney also was performed on paraffin-embedded kidney tissue sections using ApopTag Plus (Intergen, Purchase, NY), following the manufacturer’s protocol. The number of apoptotic cells in the outer medulla and cortex was measured semiquantitatively by counting 8 to 10 HPF (×200) in the outer medulla, and the mean number of positive cells was compared between groups.

Preparation of Cytoplasmic and Nuclear Protein Extracts

Cytoplasmic and nuclear protein extracts were prepared using NE-PER Nuclear and Cytoplasmic Extraction Kit (Pierce Biotechnology, Rockford, IL), following the manufacturer’s protocol, and the protein concentration was measured (BCA protein assay reagent; Pierce Biotechnology).

Electrophoretic Mobility Shift Assay

NF-κB consensus oligonucleotide sequence (5'-AGTGAGGGACTTCCTCCAGGC-3'; Promega, Madison, WI) was end-labeled with [γ-32P]ATP (Bio-Rad, Hercules, CA) using T4 polynucleotide kinase (Promega), and unlabeled oligonucleotide was removed by MicroSpin G-25 columns (Amersham, Piscataway, NJ). Nuclear extracts (30 μg) were incubated with 2 μl of 5× binding buffer (Promega). One micro-liter of labeled probe was added to the reaction mixture and incubated for 20 min at room temperature. HeLa cell nuclear extracts were used as a positive control; the negative control contained no nuclear extract. The specificity of this reaction was confirmed by a competition assay in which 100-molar excess of unlabeled cold NF-κB (specific inhibition) or activating protein 1 (AP-1) oligonucleotide (nonspecific inhibition) was added 10 min before the addition of the labeled probe. The protein-DNA complexes were separated on 4% nondenaturing polyacrylamide gel and visualized by autoradiography.

Western Blot

Samples of cytoplasmic, nuclear (30 μg), or total (30 μg) protein were separated by 10% SDS-PAGE and then transferred onto a polyvinylidene difluoride membrane. Western blot analysis was performed using primary antibodies for phosphorylated IκB (phospho-IκB), HSP-27, HSP-32 (Santa Cruz Biotechnology, Santa Cruz, CA), p65, IκB-α, HSP-90 (Cell Signaling Technology, Beverly, MA), or HSP-70 (BD Transduction Laboratories, Greeneland, NH). Membranes were washed three times and incubated with appropriate peroxidase-conjugated secondary antibodies. The membranes were stained with Ponceau S.
buffer, 1 mmol/L dithiothreitol, and 50 mg of protein were incubated for 1 hour at 37°C in the presence of reaction random hexamer, 0.4 U/μl RNase inhibitor, and 3,125 U/μl Multi-Scribe Reverse Transcriptase at 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min (Taqman Reverse Transcriptase Reagents, Applied Biosystem, Foster City, CA). Subsequently, real-time PCR was run in triplicate on the iCycler system (Bio-Rad) under the amplification condition of 40 cycles of 95°C for 15 s and 60°C for 60 s, with gene-specific primer and probe set designed by Beacon Designer Software (version 2; Bio-Rad) based on sequences from GenBank, which were as follows: sense 5'-GATCTCTCTCCACCAGCATG-3', antisense 5'-AGGTCTCTGTCAC-GCTTCTGGGC-3'. Taqman probes were labeled with 6-carboxy-fluorescein as a reporter dye and 6-carboxy-tetramethyl-rhodamine as a quencher dye. 18S ribosomal RNA (Taqman ribosomal control reagent; Applied Biosystem) was used as an internal control to normalize the data. The dynamic range of each primer/probe was verified by a serial dilution of cDNA template. The abundance of monocyte chemoattractant protein-1 (MCP-1) gene expression was normalized to that of 18S and was expressed as fold differences relative to sham-operated control rats.

**Quantification of MCP-1 Protein by ELISA**

Concentration of MCP-1 protein in whole-kidney tissue was measured by ELISA (OptEIA Set Rat MCP-1; BD Biosciences, Palo Alto, CA) according to the manufacturer’s protocol. Briefly, whole-kidney tissues were homogenized in 500 μl of assay diluent (PBS with 10% FBS [pH 7.0]) and centrifuged at 12,000 rpm for 15 min at 4°C. After coating and blocking plates, MCP-1 standards and triplicates of samples were added, followed by incubation with biotinylated anti-rat MCP-1 with avidin–horseradish peroxidase. Absorbance was read at 450 nm, and the samples were run in triplicate. The samples that were incubated with specific caspase inhibitor served as a negative control.

**Statistical Analyses**

All data are presented as means ± SEM and were analyzed by an unpaired t test. P < 0.05 was considered statistically significant.

**Results**

**Heat Preconditioning Afforded Functional and Histologic Renal Protection in Ischemic ARF in Rats**

To determine the effect of heat preconditioning in I/R injury, we first evaluated biochemical and histologic renal damage. Plasma creatinine levels at 24, 48, and 96 h after I/R injury were decreased significantly in rats with heat preconditioning compared with those without (2.35 ± 0.39/3.35 ± 0.54/2.65 ± 0.41 versus 0.63 ± 0.13/1.36 ± 0.26/0.99 ± 0.13 mg/dl; P = 0.02, P < 0.01, P < 0.01; Figure 1). Histologic examination showed extensive tubular injury characterized by tubular cell necrosis, dilation of tubules, and cast formation in the outer medulla. However, kidneys from previously heat-treated rats showed less tubular injury than did kidneys from those without heat preconditioning. A semiquantitative assessment of histologic damage, demonstrating a significant beneficial effect of heat preconditioning in I/R-induced renal injury, is shown in Figure 2.

**Measurement of Caspase Activity**

The activity of caspase 3 in kidney tissue was determined by fluorometric detection of free 7-amino-4-trifluoromethylcoumarine according to the manufacturer’s protocol (ApoAlert Caspase Fluorescence Assay Kit; BD Biosciences) by using Synergy HT Multi-Detection Microplate Reader (Biotek, Woburn, MA). Briefly, kidney tissues were homogenized in 1 ml of lysis buffer, incubated on ice for 10 min, and centrifuged at 15,000 × g for 10 min at 4°C. Supernatants that contained 50 μg of protein were incubated for 1 h at 37°C in the presence of reaction buffer, 1 mMol/L dithiothreitol, and 50 μmol/L 7-amino-4-trifluoromethylcoumarine substrate conjugates. Fluorescence was read at 400/505 nm (excitation/emission), and the samples were run in triplicate. The activity of caspase 3 was expressed as a percentage increase compared with the sham-operated control group, and samples that were incubated with specific caspase inhibitor served as a negative control.

**Figure 2.** Effect of HP on renal histology in ischemic ARF in rats. Rats were placed on a heating pad for brief HP (42 ± 0.5°C, 15 min) or at room temperature and subjected to 40 min of bilateral renal pedicle clamping at 14 h after heat treatment. Twenty-four hours after reperfusion, rats were killed and histologic renal damage was determined. (A) I/R cortex. (B) HP+I/R cortex. (C) I/R medulla. (D) HP+I/R medulla. (E) Semiquantitative assessment of renal damage in medulla (n = 6 per group). *P < 0.05 versus I/R. Magnification, ×100 (periodic acid–Schiff [PAS]).
Heat Preconditioning Inhibited NF-κB Activation in Ischemic ARF in Rats

To assess nuclear translocation and binding of NF-κB, we performed electrophoretic mobility shift assay. NF-κB activation was already evident at 1 h after reperfusion. Heat preconditioning suppressed the I/R-induced activation of NF-κB, whereas heat preconditioning itself had no effect on NF-κB activity. A competition assay, using a 100-molar excess of unlabeled NF-κB probe or AP-1 probe to show the specificity of reaction, was performed (Figure 3A). Addition of unlabeled NF-κB probe before the addition of labeled probe (specific inhibitor) reduced gel band intensity, but addition of unlabeled AP-1 probe (nonspecific inhibitor) had no effect. This effect of heat preconditioning on decreasing NF-κB activation also was confirmed by Western blot of p65 subunit (Figure 3B). Because activation of NF-κB is known to depend largely on phosphorylation of the serine residue of IκB and subsequent degradation, cytoplasmic protein levels of IκB and phosphorylated IκB were examined. I/R decreased the abundance of IκB, whereas the abundance of phospho-IκB was increased. Previous heat preconditioning suppressed the depletion of cytoplasmic IκB and the accumulation of phospho-IκB (Figure 3, C and D), indicating that the effect of heat preconditioning is mediated through inhibition of IκB kinase (IKK), an upstream kinase that determines the phosphorylation of IκB.

Heat Preconditioning Suppressed Expression of MCP-1 in Ischemic ARF in Rats

Because several chemokines and cytokines are known to be under the transcriptional control of NF-κB, we examined the mRNA expression of MCP-1 in bluntly dissected cortex and medulla using real-time quantitative reverse transcriptase–PCR. At 6 h after reperfusion, MCP-1 mRNA expression increased in medulla compared with cortex, and heat preconditioning significantly reduced their expressions (Figure 4A). MCP-1 protein from whole-kidney tissue as measured by ELISA also showed marked reduction by heat preconditioning (Figure 4B).

Heat Preconditioning Reduced Inflammation in Ischemic ARF in Rats

Inflammatory cell recruitment after I/R is facilitated by various mediators such as chemokines, cytokines, and adhesion molecules and is thought to contribute to renal injury. At 24 h after reperfusion, numerous ED-1– and esterase-positive leukocytes were found in the medullary interstitium, but in cortex, very few inflammatory cells were found in both groups. Heat preconditioning significantly reduced the infiltration of inflammatory cells in medulla (Figures 5 and 6).

Heat Preconditioning Reduced Caspase Activation and Apoptosis

Caspases are cysteine proteases that are responsible for apoptosis. Because the in vivo effect of heat preconditioning on apoptosis in ischemic ARF has never been examined, we measured the activation of caspase 3, an executioner caspase, and

Figure 3. Effect of HP on NF-κB activation in ischemic ARF in rats. Rats were placed on a heating pad for brief HP (42 ± 0.5°C, 15 min) or at room temperature and subjected to 40 min of bilateral renal pedicle clamping at 14 h after heat treatment. Rats were killed at 1 or 6 h after reperfusion. (A) Electrophoretic mobility shift assay (EMSA) for NF-κB. (B) Western blot of nuclear p65 protein. (C) Western blot of cytoplasmic IκB-α. (D) Western blot of cytoplasmic phosphorylated inhibitory κB (p-IκB). For EMSA, HeLa cell nuclear extracts were used as positive control and a free probe was used as negative control. One hundred-molar excess of unlabeled NF-κB probe and unlabeled activating protein 1 (AP-1) probe served as a specific inhibitor or nonspecific inhibitor, respectively.

Figure 4. Effect of HP on monocyte chemoattractant protein-1 (MCP-1) expression in ischemic ARF in rats. MCP-1 mRNA and protein expression was measured at 6 h after reperfusion by real-time quantitative reverse transcriptase–PCR (RT-PCR) and ELISA. In real-time RT-PCR, the gene expression level was normalized to that of 18S and expressed as fold differences relative to sham-operated rats. (A) MCP-1 mRNA. (B) MCP-1 protein. Data are means ± SEM (n = 6 rats per group). *p < 0.05 versus sham; †p < 0.05 versus I/R.
Figure 5. Effect of HP on anti-rat macrophage antibody (ED-1) leukocyte infiltration in ischemic ARF in rats. Eight to 10 high-power fields (HPF; \( \times 200 \)) per section were counted, and mean numbers of ED-1–positive leukocytes were compared. (A) Sham cortex. (B) I/R cortex. (C) HP + I/R cortex. (D) Sham medulla. (E) I/R medulla. (F) HP + I/R medulla. (G) Mean numbers of ED-1–positive leukocytes in medulla. Data are means ± SEM (\( n = 6 \) rats per group). \# \( p < 0.05 \) versus sham; \* \( p < 0.05 \) versus I/R. Magnification, \( \times 400 \) (ED-1 staining, 24 h).
Figure 6. Effect of HP on esterase-positive leukocyte infiltration in ischemic ARF in rats. Eight to 10 HPF (×200) were counted, and mean numbers of esterase-positive leukocytes were compared. (A) Sham cortex. (B) I/R cortex. (C) HP + I/R cortex. (D) Sham medulla. (E) I/R medulla. (F) HP + I/R medulla. (G) Mean numbers of esterase-positive leukocytes in medulla. Data are means ± SEM (n = 6 rats per group). *P < 0.05 versus sham; *P < 0.05 versus I/R. Magnification, ×400 (naphthol AS-D chloracetate esterase staining, 24 h).
also apoptosis. Heat preconditioning reduced the activation of caspase 3 at 6 h after I/R injury predominantly in cortex (Figure 7), and this was accompanied by reduced TUNEL-positive cells that also were found mainly in cortical distal tubules (Figure 8). These findings suggest that heat preconditioning decreases caspase 3-mediated apoptosis in ischemic ARF.

HSP-70–Mediated Protective Effect of Heat Preconditioning in Ischemic ARF in Rats

Although heat preconditioning afforded functional and histologic renal protection in this study, the role of individual HSP was not clear. In addition, it is possible that this previous thermal stress might have other, non–HSP-related, nonspecific effects. Therefore, we examined the expression of various HSP. After heat treatment, HSP-70 expression markedly increased, compared with HSP-27, HSP-32, or HSP-90 (Figure 9A). Previous administration of quercetin, a known inhibitor of HSP-70, completely reversed the functional protection that was induced by heat preconditioning (Figure 9B), and this also was accompanied by reversal of p65 subunit downregulation by heat preconditioning (Figure 9C). These data suggest that HSP-70 is responsible for the protective effect of heat preconditioning in this study.

HSP-70 Induction by I/R Was Blunted in Heat-Preconditioned Animals

To identify the effect of induced HSP by heat preconditioning on subsequent HSP-70 induction after I/R, we performed Western blot analysis of HSP-70 at 0, 1, and 6 h after I/R. HSP-70 induction was markedly blunted in heat-preconditioned rats at 1 and 6 h compared with normothermic rats (Figure 10).

Discussion

In this study, we have demonstrated that heat preconditioning afforded functional and histologic renal protection in ischemic ARF in rats, and this was accompanied by suppression of NF-κB activation and subsequent inflammation, as well as tubular cell necrosis and apoptosis. We also found that the beneficial effect of heat preconditioning seems to be mediated primarily through HSP-70 induction.

In the kidney, I/R induces inflammatory mediators such as chemokines, cytokines, and adhesion molecules (17). These mediators are thought to initiate the inflammatory cascade that leads to leukocyte recruitment and microcirculatory compromise with resultant renal dysfunction. Because these mediators, such as TNF-α and MCP-1, are known to have κB-binding motifs in their promoter regions, their transcriptions are thought to be under the control of NF-κB (9,18). Therefore, NF-κB may be a potential therapeutic target in ischemic ARF.

HSP are known to confer protection against diverse forms of cellular injury by having a capacity to bind to misfolded or denatured proteins and thereby prevent their irreversible denaturation (10,11). Although the beneficial effect of heat preconditioning in ischemic injury has been reported in several organs, including the kidney, the precise mechanism of protection remains unclear. Recently, several in vitro and in vivo studies suggested that one of the protective mechanisms of heat preconditioning, or HSP, is their inhibitory effect on NF-κB-mediated inflammation (19,20). However, the in vivo effect of heat preconditioning on inflammation in the kidney has never been demonstrated. Therefore, we first examined the effect of heat preconditioning on NF-κB activation and inflammation. Heat preconditioning suppressed I/R-induced NF-κB activation in the kidney at 1 h after reperfusion. These findings again were confirmed by Western blot analysis of p65 subunit of NF-κB complexes. Suppression of NF-κB activation by heat preconditioning at 1 h was accompanied by a subsequent decrease in MCP-1 mRNA and protein expression at 6 h and also by decreased leukocyte recruitment at 24 h. We divided kidney tissues into cortex and medulla by blunt dissection and found that MCP-1 expression increased significantly in medulla, where subsequent inflammation and tubular necrosis were predominant. These results are consistent with other studies (21,22) and support the possibility that heat preconditioning reduces the transcriptional activation of proinflammatory mediators and subsequent inflammation by blocking the activation of the NF-κB pathway in ischemic ARF. However, there is a limitation of using an activation assay rather than nuclear transcription reporter assay because activation of NF-κB does not necessarily lead to increased transcription. Although we cannot provide direct evidence in this study, NF-κB–dependent production of MCP-1 or other cytokines has been demonstrated in several in vitro and in vivo studies (23,24). Cao et al. (23) demonstrated that inhibition of NF-κB by in vivo transfection of decoy oligodeoxynucleotides not only prevented NF-κB activation but also decreased MCP-1 gene expression with decreased monocyte/macrophage infiltration. The role of MCP-1 in I/R injury also has been studied extensively, and it is thought to play an important role in leukocyte recruitment with further compromise of the outer medullary reflow and facilitation of tubular cell damage (17,25,26). However, the effect of NF-κB on facilitating cell survival or inhibiting apoptosis in other cell

Figure 7. Effect of HP on caspase activation in ischemic ARF in rats. Caspase 3 activities were measured in cortex and medulla at 6 h after reperfusion and were expressed as percentage increase compared with sham group rats. Samples in which specific caspase inhibitors were added served as negative control for the reactions. Data are means ± SEM (n = 6 rats per group). *P < 0.05 versus sham; **P < 0.05 versus I/R.
types also has been reported (27,28). The major pathway for NF-κB activation depends on the activation of IKK complexes, which leads to the phosphorylation of the serine residue of cytoplasmic IkB and degradation by the ubiquitin-proteasome system. However, other mechanisms that are not dependent on phosphorylation and degradation of IkB also have been reported (29). To evaluate further the inhibitory effect of heat preconditioning on NF-κB pathway, we examined IkB and phospho-IkB protein levels that recognize the phosphorylation of serine residue of IkB, using cytoplasmic protein extracts. We found that the suppression of NF-κB activation by previous heat preconditioning was secondary to decreased phosphorylation and resultant stabilization of cytoplasmic IkB. This finding might suggest that the beneficial effect of previous heat preconditioning is mediated by inhibition of IKK activation, an upstream kinase of IkB that results in inhibition of NF-κB nuclear translocation. This is in agreement with several other studies (20). However, according to several recent reports, inhibition of IkB degradation by increased phosphatase activity after heat shock also could be a possible mechanism (30).

Figure 8. Effect of HP on apoptosis in ischemic ARF in rats. Rats were placed on a heating pad for brief HP (42 ± 0.5°C, 15 min) or at room temperature and subjected to bilateral renal pedicle clamping for 40 min. Detection of apoptosis was done at 24 h. (A) Sham. (B) HP 14 h. (C) I/R. (D) HP + I/R. (E) Mean numbers of terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL)-positive apoptotic cells. Data are means ± SEM (n = 6 rats per group). *P < 0.05 versus sham; *P < 0.05 versus I/R. Magnification, ×200 (TUNEL).
proteases called caspase. HSP-70 was reported previously to decrease apoptosis through inhibition of the mitochondrial apoptotic pathway (32–34). The *in vivo* effect of HSP-70 on the mitochondrial apoptotic pathway also was demonstrated by Lee et al. (35), who observed more cytochrome c release into the cytosol with a larger infarct volume in HSP-70 knockout mice in the cerebral ischemia model. Although the inhibitory effect of previous heat stress on caspase 3 activation and apoptosis in ATP-depleted tubular cells has been reported (36), the *in vivo* effect of heat preconditioning on various caspase activation has never been assessed. Therefore, we measured caspase activation after heat preconditioning or I/R. Caspase 3 activity increased in cortex at 6 h after I/R, and heat preconditioning decreased the activations significantly. Tubular cell apoptosis, determined by TUNEL staining, was observed predominantly in cortical distal tubules and also decreased markedly by heat preconditioning. However, caspase 8 or 9 activity, which represents the activation of membrane death receptor pathway or mitochondrial pathway, did not match with those of caspase 3 (data not shown), indicating that the effect of heat preconditioning is independent of activation of caspase 8 or 9 in I/R-induced ARF. The precise biochemical pathways by which heat preconditioning decreases apoptosis is not clear in this study. Recent studies suggested that HSP-70 precipitates Bcl2, a widely known antiapoptotic protein, and this interaction between HSP-70 and Bcl2 protein may afford cytoprotection by restoring Bcl2 function, a role that is compatible with the chaperone function of this stress protein (37,38).

These above findings suggest that the effect of heat preconditioning might be mediated via inhibition of NF-κB–mediated inflammation and inhibition of cell death pathways, but there still are uncertainties as to the specificity of individual HSP in these circumstances or the possible nonspecific effects of other HSP molecules. We therefore performed Western blot analysis of various HSP, including HSP-27, HSP-32 (heme oxygenase-1), HSP-70, and HSP-90. In contrast to HSP-27, -32, and -90, which showed a minimal increase after heat stress, HSP-70 protein increased significantly after 1 h and reached a maximum level at 14 h. Quercetin administration, a widely known inhibitor of heat preconditioning decreases apoptosis is not clear in this study. Recent studies suggested that HSP-70 precipitates Bcl2, a widely known antiapoptotic protein, and this interaction between HSP-70 and Bcl2 protein may afford cytoprotection by restoring Bcl2 function, a role that is compatible with the chaperone function of this stress protein (37,38).

Renal tubular cells that are lethally injured after ischemic or toxic insults can die by necrosis or apoptosis (4,31). Contrary to apoptosis, which is a well-coordinated process, necrosis usually results from overwhelming cellular ATP depletion that is beyond their capability to repair and has been known to be an unregulated event. However, recent studies suggest that necrosis also is controlled by a specific program that involves many signaling cascades such as reactive oxygen species and stress kinases (14). In our study using 40-min bilateral ischemia, extensive tubular necrosis in outer medulla was observed at 24 h after I/R, and heat preconditioning significantly decreased tubular necrosis. The mechanisms of heat preconditioning or HSP-mediated cell protection from necrosis is not well understood. The direct inhibitory effect of HSP-72 on c-JUN N-terminal kinase pathways that mediate cell necrosis in addition to their function as molecular chaperones has been suggested by Yaglom et al. (14), and it is possible that induced HSP-70 in our study inhibited c-JUN N-terminal kinase–mediated tubular cell death. Apoptosis is characterized by cell shrinkage, nuclear chromatin condensation, and activation of a family of cysteine

![Figure 9](image)

**Figure 9.** Effect of quercetin (Q) on expression of heat-shock proteins (HSP), renal function, and NF-κB activation in ischemic ARF in rats. Q was administered 1 h before HP, and rats were killed at 1, 6, and 14 h after HP or 24 h after I/R. (A) Western blot of various HSP after heat-shock treatment or Q+heat-shock treatment. (B) Plasma creatinine 24 h after I/R. Data are means ± SEM (n = 6 rats per group). *P < 0.05 versus sham; **P < 0.05 versus I/R; ***P < 0.05 versus HP+I/R. (C) Western blot of nuclear p65 protein 1 h after I/R.

![Figure 10](image)

**Figure 10.** HSP-70 induction after I/R. Rats were placed on a heating pad for brief HP (42 ± 0.5°C, 15 min) or at room temperature and subjected to bilateral renal pedicle clamping for 40 min. Rats were killed at 0, 1, and 6 h after I/R.

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and further supports that induced HSP before injury is critical in providing protection.

Conclusion
We have shown that heat preconditioning resulted in attenuation of renal injury. This protective effect was accompanied by inhibition of NF-kB activation with subsequent decrease in inflammation and also decrease in tubular cell necrosis and apoptosis. HSP-70 is thought to be responsible for this beneficial effect. A better understanding of the role of stress proteins in modifying proinflammatory, proapoptotic mediators could facilitate new drug development for reducing renal injury in ischemic ARF and other diseases.

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References


