Human heat shock protein 27-overexpressing mice are protected against acute kidney injury after hepatic ischemia and reperfusion

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Park SW, Chen SW, Kim M, D’Agati VD, Lee HT. Human heat shock protein 27-overexpressing mice are protected against acute kidney injury after hepatic ischemia and reperfusion. Am J Physiol Renal Physiol 297: F885–F894, 2009. First published August 5, 2009; doi:10.1152/ajprenal.00317.2009.—Liver ischemia-reperfusion injury (IRI) causes acute kidney injury (AKI) in mice characterized by renal endothelial cell apoptosis, renal tubular necrosis, inflammation, and filamentous (F)-actin disruption. Since heat shock protein 27 (HSP27) protects against apoptosis, necrosis, and stabilizes F-actin, we questioned whether overexpression of human HSP27 (huHSP27 OE) in mice would attenuate AKI after liver IRI. Twenty-four hours after hepatic IRI, HSP27 wild-type (WT) mice developed acute liver and kidney injury with elevated plasma alanine aminotransferase and creatinine, a reduced glomerular filtration rate, and histological evidence of renal endothelial cell apoptosis and tubular injury (necrosis, vacuolization, and F-actin disruption). The huHSP27 OE mice, however, were significantly protected against both liver and kidney injury after hepatic IRI. The huHSP27 OE mice also showed less induction of several proinflammatory mRNAs (TNF-α, MIP-2, and keratinocyte-derived cytokine), neutrophil infiltration, and reduction in apoptosis (terminal deoxynucleotidyl transferase biotin-DUTP nick end-labeling assay and DNA laddering) in the kidney compared with the HSP27 WT mice. Moreover, the huHSP27 OE mice showed significantly less disruption of F-actin in renal proximal tubules and better preserved vascular endothelial cell integrity compared with the huHSP27 OE mice. Finally, the kidney plays a major role in the hepatoprotective effects of huHSP27 overexpression as the hepatoprotection was reduced or abolished in mice subjected to unilateral or bilateral nephrectomy, respectively. Our results show that overexpression of huHSP27 protects against hepatic injury and AKI associated with liver IRI in vivo. Harnessing the mechanisms of cytoprotection with renal HSP27 may lead to new therapies for the perioperative AKI and liver injury associated with liver IRI.

acute renal failure; apoptosis; endothelial cell; inflammation, necrosis

ACUTE KIDNEY INJURY (AKI) is one of the most challenging but unresolved clinical problems in medicine. In particular, AKI is extremely frequent in patients with either acute and chronic liver failure, and the mortality rate in patients with combined kidney and liver dysfunction is extremely high (11). Hepatic ischemia-reperfusion injury (IRI) is a major cause of liver failure and is frequently associated with major hepatic resection, liver transplantation, or septic shock (12). In addition, AKI is a common complication before or after orthotopic liver transplantation and exponentially increases patient mortality (11). Furthermore, even mild AKI can lead to drastic increases in mortality and morbidity in the intensive care unit, implying that kidneys can modulate and/or amplify extrarenal organ dysfunction (5).

We previously demonstrated that mice subjected to severe hepatic IRI develop AKI in <24 h characterized by early renal peritubular capillary endothelial cell apoptosis and rapid disruptions of the renal proximal tubule filamentous (F)-actin cytoskeletal architecture with subsequent proximal tubular necrosis and inflammation (24). Moreover, profound cortical vacuolization, tubular simplification, peritubular/interstitial neutrophil infiltration as well as significant juxtaglomerular hyperplasia were observed in mice that developed AKI after liver IRI (24). Therefore, we hypothesized that blocking renal endothelial cell apoptosis, tubular necrosis, and preserving the F-actin cytoskeleton would provide protection against AKI incurred after liver IRI.

Heat shock protein 27 (HSP27) is a member of a family of chaperone proteins that are upregulated in response to increases in temperature, as well as a wide range of cellular stresses including hypoxia, ischemia, and exposure to toxic drugs (2, 3, 6, 14). Furthermore, HSP27 is a potent antiapoptotic protein, reduces necrosis in many cell types, and is a key stabilizer of the F-actin cytoskeleton (18, 23, 34); all of these cellular effects could potentially lead to protection against AKI induced with liver IRI (29, 32, 36). Therefore, we utilized a murine model of liver IRI-induced AKI and tested the hypothesis that mice with global overexpression of human HSP27 (huHSP27) would show an increased resistance to AKI induced by liver IRI. We also tested whether huHSP27 overexpression in the kidney with subsequent reduction in AKI is at least partially responsible for reduced hepatic injury after liver IRI in huHSP27-overexpressing (OE) mice. We show that overexpression of huHSP27 in mice reduces renal injury, decreases renal inflammation and apoptosis, and preserves F-actin and vascular permeability compared with HSP27 wild-type (WT) mice after liver IRI. Moreover, removing kidneys from huHSP27 OE mice abolishes the hepatoprotective effects of huHSP27 overexpression.

MATERIALS AND METHODS

Mice. Heterozygous breeder pairs of mice overexpressing human HSP27 were generously provided by Dr. Jacqueline de Belleroche (Dept. of Neuromuscular Diseases, Faculty of Medicine, Imperial College, London, UK). The generation and initial characterization of the huHSP27 OE and WT mice with a C57BL/10 and CBA/Ca background have been described previously (1). The huHSP27 OE transgenic mice overexpress human HSP27 tagged with hemagglutinin to track the expression of the transgene. The huHSP27 OE transgenic mice show widespread and robust expression of huHSP27 protein in the brain, kidney, liver, heart, spinal cord, muscle, lung, and pancreas (1). Because of the concerns for the potential genetic variability associated with a noncongenic strain of mice, we bred our heterozy-
gous huHSP27 OE mice with C57BL/6 (Harlan Laboratories, Indianapolis, IN) mice for four generations. We performed PCR on genomic DNA extracted from tails and performed RT-PCR for human HSP27 from total RNA extracted from every mouse studied using primers that distinguish human from mouse HSP27. In preliminary studies, we also confirmed human HSP27 protein overexpression by immunoblotting for the mouse and human forms of HSP27 (Santa Cruz Biotechnology, Santa Cruz, CA) as described previously (7).

Marine model of hepatic IRI. After Columbia University Institutional Animal Care and Use Committee approval, male huHSP27 OE or HSP27 WT mice (25–30 g) were subjected to partial liver IRI as described previously (24). In brief, left lateral and median lobes of the liver in anesthetized mice were subjected to varying period of ischemia (45, 60, or 75 min to induce mild, moderate, or severe hepatic IRI) with a microaneurysm clip occluding the hepatic triad above the bifurcation at 37°C. This method of partial, graded hepatic ischemia results in a segmental (~70%) hepatic ischemia but spares the right lobe of the liver and prevents mesenteric venous congestion by allowing portal decompression throughout the right and caudate lobes of the liver. After hepatic ischemia, the liver was reperfused, and the wound was closed. Sham-operated mice were subjected to a laparotomy and identical liver manipulations without vascular occlusion. In some mice, we performed a unilateral or bilateral nephrectomy during wound closure (F886).

Plasma alanine aminotransferase (ALT) activity 4 and 24 h after liver IRI was measured using the Infinity ALT assay kit according to the manufacturer’s instructions (Thermo Fisher Scientific, Waltham, MA). Renal function was assessed by measuring plasma creatinine 4 and 24 h after liver IRI by a colorimetric method based on the Jaffe reaction (33) and by estimating the glomerular filtration rate 24 h after liver IRI with a microanastomys clip occluding the hepatic triad above the bifurcation at 37°C. This method of partial, graded hepatic ischemia results in a segmental (~70%) hepatic ischemia but spares the right lobe of the liver and prevents mesenteric venous congestion by allowing portal decompression throughout the right and caudate lobes of the liver. After hepatic ischemia, the liver was reperfused, and the wound was closed. Sham-operated mice were subjected to a laparotomy and identical liver manipulations without vascular occlusion. In some mice, we performed a unilateral or bilateral nephrectomy during wound closure (F886).

Histology and quantification of renal injury after hepatic IRI. Twenty-four hours after reperfusion, both kidneys were collected and fixed in xylene and rehydrated through graded ethanols to water. In situ terminal deoxynucleotidyl transferase biotin-dUTP nick end-labeling (TUNEL) assay was used for detecting DNA fragmentation in kidney sections obtained at 24 h after hepatic IRI. For the TUNEL assay, fixed kidney sections were incubated with TUNEL staining solution containing terminal deoxynucleotidyl transferase (TdT), biotin-dUTP, and substrate buffer. After incubation with TdT, sections were treated with avidin-biotin-peroxidase complex (ABC) reagent. The sections were then incubated with diaminobenzidine (DAB) substrate, washed, and counterstained with hematoxylin. For negative controls, sections were processed in the same manner, except that the TdT was omitted.

Fig. 1. A: plasma alanine aminotransferase (ALT) and creatinine (Cr) levels in heat shock protein 27 wild-type (HSP27 WT) and human HSP27-overexpressing (huHSP27 OE) mice subjected to sham operation (sham) and 45-, 60-, or 75-min liver ischemia and 24 h of reperfusion injury (IRI). *P < 0.01 vs. sham group. **P < 0.01 vs. sham group. ***P < 0.05 vs. 45-min liver ischemia group. +++P < 0.05 vs. 60-min liver ischemia group. B: plasma ALT and Cr levels in HSP27 WT and huHSP27 OE mice subjected to sham operation (sham) and 60-min liver IRI. The blood samples were collected at 4 and 24 h after reperfusion. Values are means ± SE. *P < 0.01 vs. sham group. **P < 0.01 vs. sham group. ***P < 0.05 vs. 4-h reperfusion group.
were then stained with 1:40 dilution of Alexa Fluor (Red)-conjugated Phalloidin in PBS from H11011 6.6/H9262 M (200 U/ml) stock solution (Invitrogen, Carlsbad, CA) for 30 min at 37°C in a humidified chamber in the dark. Sections were then washed twice in PBS and mounted with Vectashield (Vector Laboratories, Burlingame, CA). F-actin images were visualized with an Olympus IX81 epifluorescence microscope (Tokyo, Japan) and captured and stored using SlideBook 4.2 software (Intelligent Imaging Innovations, Denver, CO) on a personal computer. The mean fluorescent intensity after background correction was calculated from five random blinded sections with identical surface areas per slide to quantify F-actin degradation after liver IR. To minimize the variations in fluorescent intensity, slides from sham-operated animals and animals subjected to liver IRI were processed together.

Analysis of kidney vascular permeability after hepatic IRI. Changes in kidney vascular permeability were assessed by quantitating extravasations of Evans blue dye (EBD) into the tissue as described (24). Briefly, 2% EBD (Sigma Biosciences, St. Louis, MO) was administered at a dose of 20 mg/kg (iv) 24 h after liver IRI. One hour later, mice were killed and perfused through the heart with PBS and EDTA with 10 ml cold saline with heparin (100 U/ml). Both kidneys were then removed, allowed to dry overnight at 60°C, and the dry weights were determined. EBD was extracted in formamide (20 ml/g dry tissue; Sigma Biosciences), homogenized, and incubated at 60°C overnight. Homogenized samples were centrifuged at 5,000 g for 30 min, and the supernatants were measured at 620 and 740 nm in a spectrophotometer. The extravasated EBD concentration was calculated against a standard curve, and the data are expressed as micrograms of EBD per gram of dry tissue weight.

Protein determination. Protein contents were determined with a bicinchoninic acid protein assay kit (Pierce Chemical, Rockford, IL), using BSA as a standard.

RESULTS

Reduced AKI and hepatic injury after liver IRI in huHSP27 OE mice compared with HSP27 WT mice. The plasma levels of ALT and creatinine in sham-operated HSP27 WT or huHSP27 OE mice were similar (Fig. 1). Increasing the duration of hepatic ischemia (45, 60, or 75 min) resulted in increased hepatic as well as renal injury after IRI in both HSP27 WT and huHSP27 OE mice (Fig. 1A). In HSP27 WT mice subjected to 60-min liver IRI, plasma ALT significantly increased at 4 and 24 h after 60-min liver IRI (Fig. 1B). Moreover, the huHSP27 OE mice developed AKI 4 and 24 h after 60-min hepatic IRI (Fig. 1B). However, the huHSP27 OE mice showed significantly less elevation in plasma ALT and creatinine compared with the HSP27 WT mice at 4 and 24 h (Fig. 1B). The
survival rates for the HSP27 WT mice (76%, n = 21) and the huHSP27 OE mice (91%, n = 20) were not statistically different at 24 h after 60-min liver IRI (P = 0.19).

In addition to plasma creatinine, we measured the glomerular filtration rate in mice with the FITC-inulin clearance technique as described by Qi et al. (30). The glomerular filtration rate in sham-operated HSP27 WT and huHSP27 OE was similar (0.96 ± 0.11, n = 4 vs. 1.08 ± 0.18 ml·min⁻¹·100 g body wt⁻¹, n = 4). Sixty minutes of liver IRI significantly reduced the glomerular filtration rate in HSP27 WT mice within 24 h (0.11 ± 0.05 ml·min⁻¹·100 g body wt⁻¹, n = 4). The glomerular filtration rate was significantly better preserved in huHSP27 OE mice (0.59 ± 0.2 ml·min⁻¹·100 g body wt⁻¹, n = 4, P < 0.01 vs. HSP27 WT mice subjected to liver IR) 24 h after liver IRI.

HSP27 WT mice subjected to 45-min liver IR also developed liver and kidney injury. Furthermore, the huHSP27 OE mice subjected to 45-min liver IRI demonstrated reduced liver and kidney injury compared with the WT mice at 24 h. Increasing hepatic ischemia time to 75 min increased both liver and kidney injury in HSP27 WT mice, and the huHSP27 OE mice continued to demonstrate reduced hepatic injury and improved renal function after 75-min liver ischemia and 24 h of reperfusion (Fig. 1A).

Unilateral or bilateral nephrectomy abolishes hepatic protection with huHSP27 overexpression. Twenty-four hours after unilateral nephrectomy without hepatic ischemia, mouse plasma creatinine did not change significantly (0.46 ± 0.06 mg/dl, n = 5) compared with the sham-operated mice. However, unilateral nephrectomy significantly reduced the hepatic protection provided by huHSP27 overexpression in huHSP27 OE mice subjected to liver IR (P = 0.13 vs. HSP27 WT mice subjected to liver IR, Fig. 2). Bilateral nephrectomy abolished the hepatic protection provided by huHSP27 overexpression as HSP27 WT and huHSP27 OE mice developed similar degree of liver injury after 45 min of liver ischemia and 24 h of reperfusion (P = 0.29 vs. HSP27 WT mice, Fig. 2), demonstrating that kidneys are required for huHSP27 overexpression-mediated liver protection after liver IRI. The HSP27 WT mice did not show increased liver injury after unilateral or bilateral nephrectomy.

huHSP27 OE mice show reduced renal cortical vacuolization, proximal tubular simplification, hypereosinophilia, and leukocytosis after liver IRI. We demonstrated previously that our model of partial hepatic ischemia produced graded liver necrosis with increasing duration of ischemia in mice after IRI (21). Correlating with significantly improved function, reduced liver necrosis was observed in huHSP27 OE mice subjected to hepatic IR compared with the HSP27 WT mice (data not shown). When we examined the kidneys from the HSP27 WT mice subjected to liver IR, we observed multifocal acute tubular injury including S3 segment proximal tubule necrosis, cortical tubular simplification, cytoplasmic vacuolization, and dilated lumina as well as focal granular bile/heme casts. Moreover, endothelial cell apoptosis of peritubular capillaries was also visible in H&E sections as linear arrays of apoptotic bodies corresponding to interstitial capillary-lining endothelium. The number of glomeruli with detectable juxtaglomerular apparatus in H&E sections and the number of cells per juxtaglomerular apparatus were both increased after liver IR, indicating hyperplasia of the juxtaglomerular apparatus. Represen-
proximal tubule hypereosinophilia are shown in Fig. 4. These indices of renal tubular injury were significantly less in the huHSP27 OE mice subjected to liver IRI (60-min liver ischemia and 24 h of reperfusion).

huHSP27 OE mice showed reduced TNF-α, KC, and MIP-2 mRNA expression in the kidney 24 h after liver IRI. We measured the expression of proinflammatory cytokine mRNAs in the kidneys 4 and 24 h after 60-min liver IRI with RT-PCR. Hepatic IRI was associated with significantly increased proinflammatory ICAM-1, KC, MCP-1, and TNF-α mRNA expression at 4 h and ICAM-1, TNF-α, KC, MCP-1, and MIP-2 mRNA expression at 24 h in the kidneys of HSP27 WT mice (Fig. 5). However, the huHSP27 OE mice showed significantly reduced increases in TNF-α, KC, and MIP-2 mRNA 24 h after liver IRI compared with the HSP27 WT mice (Fig. 5). There were no significant differences in induction of MCP-1 or ICAM-1 mRNAs at 24 h after liver IRI. Both HSP27 WT and huHSP27 OE mice showed similar induction of proinflammatory mRNAs at 4 h after liver IRI (Fig. 5). Induction of mouse HSP27 mRNA in the kidneys was equivalent in both groups of mice after liver IRI.

huHSP27 OE mice showed reduced renal neutrophil infiltration 24 h after IRI. Sixty minutes of hepatic ischemia resulted in recruitment of neutrophils into the kidney (Fig. 6). Neutrophil infiltration 24 h after hepatic ischemia was significantly less in huHSP27 OE mice compared with the HSP27 WT mice. Figure 6 shows representative images of neutrophil immunohistochemistry of kidney sections from HSP27 WT or huHSP27 OE mice subjected to sham operation or to 60 min of liver ischemia and 24 h of reperfusion. In sham-operated HSP27 WT or huHSP27 OE mice, we were unable to detect any neutrophils. Twenty-four hours after liver IRI, we detected 27 ± 8 neutrophils/field (×200 magnification, n = 6) in the kidneys of HSP27 WT mice. The huHSP27 OE mice had significantly reduced renal neutrophil infiltration 24 h after liver IRI (6 ± 2 neutrophils/field, ×200 magnification, n = 7, *P < 0.05).

huHSP27 OE mice show decreased renal apoptosis after IRI. We utilized two techniques to detect apoptosis: TUNEL staining and DNA laddering. Twenty-four hours after 60 min of hepatic IRI, huHSP27 OE mice showed significantly reduced apoptosis of the kidney compared with the HSP27 WT mice with reduced TUNEL staining (Fig. 5A, representative of 5 experiments) and DNA laddering (Fig. 5B, representative of 4 experiments). The TUNEL staining showed that endothelial cell apoptosis was predominant in the kidney (inset).

huHSP27 OE mice show decreased degradation of renal tubule F-actin architecture after liver IRI. Sixty minutes of liver IRI resulted in a severe loss of F-actin in renal proximal tubules in 24 h. Twenty-four-hour post-hepatic IRI-induced disruptions of the F-actin cytoskeleton in renal proximal tubular epithelial cells are shown in Fig. 8A. HSP27 WT or

![Graphs](http://ajprenal.physiology.org/)

Fig. 5. Densitometric quantifications of relative band intensities from RT-PCR reactions (normalized to GAPDH). Values are means ± SE. *P < 0.05 vs. HSP27 WT sham group. #P < 0.05 vs. HSP27 WT IRI group. ++P < 0.05 vs. 4-h reperfusion group.
huHSP27 OE mice subjected to sham surgery showed intense staining in the tubular epithelial cells and in the basal plasma membrane. In contrast, kidneys from HSP27 WT mice subjected to liver IRI showed loss of F-actin staining in the tubular epithelial cells. However, the huHSP27 OE mice show significantly better preserved F-actin structure after liver IRI as the staining is quite similar to that in sham-operated mice. Mean fluorescent F-actin intensity also showed reduced renal proximal tubule F-actin degradation in huHSP27 OE mice after liver IRI (Fig. 8B).

Fig. 6. Representative photomicrographs of neutrophil accumulation (dark brown punctate stain, arrows) in kidney sections (magnification ×400). HSP27 WT mice subjected to 60 min of liver ischemia and 24 h of reperfusion injury exhibited neutrophil accumulation whereas the huHSP27 OE mice showed very scant neutrophil infiltration after liver IRI (representative of 6–7 experiments).

Fig. 7. A: representative fluorescence photomicrographs of kidney sections illustrating apoptotic nuclei [terminal deoxynucleotidyl transferase biotin-dUTP nick end-labeling (TUNEL) fluorescence staining, ×100] at 24 h after 60 min of liver IRI. TUNEL-positive cells were mainly peritubular capillary endothelial cells. Photographs are representative of 5 independent experiments. Inset: higher magnification of image showing TUNEL-positive cells. In the kidney, endothelial cells not proximal tubule cells underwent apoptosis. B: representative DNA ladder (of 4 experiments). Apoptotic DNA fragments were separated from intact chromatin and loaded onto the agarose gel.
huHSP27 OE mice show decreased renal vascular permeability after IRI. We measured renal vascular permeability after 60 min of liver ischemia and 24 h of reperfusion with EBD injection. EBD binds to plasma proteins, and its appearance in extravascular tissues reflects an increase in vascular permeability. Analysis of EBD extravasations in the kidneys from HSP27 WT and huHSP27 OE mice subjected to sham operation and liver IRI is shown in Fig. 9. Liver IRI increased the EBD content in the kidney for both groups; however, the increase in EBD content was significantly higher for the HSP27 WT mice compared with the huHSP27 OE mice.

DISCUSSION

The major findings of this study are that mice overexpressing human HSP27 (huHSP27 OE mice) demonstrate reduced renal and hepatic injury after liver IRI. The reduction in AKI was characterized by 1) reduced renal proximal tubule necrosis, 2) less cortical vacuolization and simplification, 3) decreased peritubular capillary endothelial cell apoptosis, and 4) reduced neutrophil infiltration coupled with less induction of several proinflammatory cytokines (TNF-α, KC, and MIP-2). Furthermore, we demonstrate that the huHSP27 OE mice showed reduced renal tubular F-actin breakdown and improved preservation of renal vascular integrity after hepatic IRI. Finally, HSP27 OE-mediated hepatic protection requires intact renal function as binephrectomized HSP27 OE mice are no longer protected against liver injury after hepatic ischemia and reperfusion.

We recently reported a murine model of liver IRI-induced AKI in which mice subjected to 60 min of warm liver ischemia developed severe and reproducible AKI in <24 h (24). We demonstrated in our previous study that plasma ALT and
The image contains text that discusses the role of HSP27 in protecting against liver injury. The text mentions the overexpression of human HSP27 in mice, which provides significant protection against liver IR, especially for the huHSP27 OE mice subjected to liver IRI. The text also notes that the overexpression of huHSP27 in the Kupffer cells may be responsible for the hepatic protection observed in huHSP27 OE mice. The text highlights the significance of the interactions between liver and kidney injury after hepatic IR, with huHSP27 overexpression producing a drastic reduction in the apoptosis of these pericapillary endothelial cells. The text concludes with a discussion on the potential mechanisms by which HSP27 may provide protective effects against liver and kidney injury.
Hepatic IR also caused significant upregulation of several proinflammatory chemokine and cytokine mRNAs in the kidney. However, unlike in the liver where the peak proinflammatory mRNA expression occurred early (~4–5 h after reperfusion), the proinflammatory mRNAs in the kidneys continued to increase and peaked ~24 h after liver IR (Fig. 5). Of many cell types in the kidney, endothelial cells are first exposed to the circulating cytokines and chemokines released from the liver. We postulate that the massive cytokine upregulation and release from the liver travels to the kidney, causing early renal endothelial dysfunction and death, with subsequent vascular impairment and neutrophil infiltration leading to proximal tubule necrosis and inflammatory changes in the kidney. Neutralization of TNF-α or IL-6 produced significant protection against both liver as well as kidney injury after liver IR (Park SW, Chen SWC, Kim M, D’Agati VD, Lee HT, unpublished observations), supporting the hypothesis that remote renal injury after liver IR is initiated by the chemokines and cytokines released from the liver.

Several proinflammatory mRNAs (TNF-α, KC, and MIP-2) examined were significantly attenuated in huHSP27 OE mice kidney after hepatic IRI. The chemokines MIP-2 and KC and cytokine TNF-α are important mediators in the pathogenesis of AKI (13). Upregulation of these proinflammatory chemokines and cytokines leads to neutrophil recruitment during AKI induced by renal IRI or nephrotoxic agent (27). Overexpression of huHSP27 may have directly but selectively attenuated the proinflammatory mRNA expression in the kidney. More likely, however, reduction in renal TNF-α expression with huHSP27 overexpression resulted in secondary reduction in MIP-2 and ICAM-1 expression in the kidney. TNF-α is known to promote the migration of inflammatory cells into the renal parenchyma via the upregulation of KC and MIP-2 in the kidney (31).

The role of neutrophils in the pathophysiology of organ injury (including the liver and the kidney) is well recognized (19). Our study demonstrated that liver IRI caused neutrophil infiltration into the kidney within 24 h. Neutrophils are activated during and after liver ischemia, and activated neutrophils attach to and then transverse the hepatic capillary endothelium into the subendothelial space, where they release enzymes and cytokines, causing direct hepatocyte injury and recruit other injurious cells, such as monocytes and macrophages (22, 28). Activated neutrophils release substances to produce further tissue injury, such as products of arachidonic acid metabolism, oxygen free radicals, and neutrophil elastase (19). huHSP27 overexpression resulted in improved kidney vascular endothelial cell integrity (indicated by reduced EBD infiltration) and most likely limited the polymorphonuclear neutrophil infiltration into the kidney.

IRI in vivo results in F-actin cytoskeleton degradation, which further compromises organ function (4, 20). For example, F-actin disruption is a well-known stimulus of apoptosis in several cell lines (35). We saw severe disruption of F-actin in renal proximal tubules after liver IRI in HSP27 WT mice. We demonstrated in this study that huHSP27 overexpression resulted in markedly better preserved F-actin of the liver as well as the kidney. An intact F-actin cytoskeleton in kidneys of mice subjected to liver IRI may have contributed to reduced AKI observed in these mice.

Global overexpression of HSP27 may not always lead to organ protection against IRI. We were surprised to discover that systemic overexpression of HSP27 increased renal injury after IRI with significantly increased KC upregulation and activation in vivo (7). Our findings after liver IRI contrast with our previous finds in the kidney where huHSL27 OE mice demonstrated increased renal inflammation and neutrophil infiltration after IRI (7). The reason for this discrepancy remains to be elucidated. It appears that renal IRI leads to heightened systemic inflammation (and enhanced plasma KC activation) that overcomes the protective effects of huHSP27 overexpression in renal proximal tubule cells. In contrast, liver IRI appears to produce a less systemic inflammatory response compared with renal IRI, leading to less KC activation and upregulation.

In summary, we demonstrate in this study that mice overexpressing huHSP27 are protected against AKI after liver IRI with reduced endothelial cell apoptosis, proximal tubule necrosis, and inflammation. The huHSP27-mediated renal protection plays a major role in hepatic protection after liver IRI in these mice. Given the protective benefit of HSP27 against hepatic IRI and that hepatic IRI is common in patients after liver surgery, liver transplantation or sepsis, our findings may have important future therapeutic implications.

GRANTS

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REFERENCES


