Role of heat shock protein 70 in hepatic ischemia-reperfusion injury in mice

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Kuboki S, Schuster R, Blanchard J, Pritts TA, Wong HR, Lentsch AB. Role of heat shock protein 70 in hepatic ischemia-reperfusion injury in mice. Am J Physiol Gastrointest Liver Physiol 292: G1141–G1149, 2007. First published December 21, 2007; doi:10.1152/ajpgi.00491.2006.—It is well established that liver ischemia-reperfusion induces the expression of heat shock protein (HSP) 70. However, the biological function of HSP70 in this injury is unclear. In this study, we sought to determine the role of HSP70 in hepatic ischemia-reperfusion injury in mice. Male mice were subjected to 90 min of partial hepatic ischemia followed by up to 8 h of reperfusion. HSP70 was rapidly upregulated after reperfusion. To explore the function of HSP70, sodium arsenite (8 mg/kg iv) was injected before surgery. We found that this dose induced HSP70 expression within 6 h of treatment. Induction of HSP70 with arsenite resulted in a >50% reduction in liver injury as determined by serum transaminases and histology. In addition, arsenite similarly reduced liver neutrophil recruitment and liver nuclear factor-κB activation, and attenuated serum levels of tumor necrosis factor-α and macrophage inflammatory protein-2, but increased levels of interleukin (IL)-6. In HSP70 knockout mice, arsenite did not protect against liver injury but did reduce liver neutrophil accumulation. Arsenite-induced reductions in neutrophil accumulation in HSP70 knockout mice were found to be mediated by IL-6. To determine whether extracellular HSP70 contributed to the injury, recombinant HSP70 was injected before surgery. Intravenous injection of 10 μg of recombinant HSP70 had no effect on liver injury after ischemia-reperfusion. The data suggest that intracellular HSP70 is directly hepatoprotective during ischemia-reperfusion injury and that extracellular HSP70 is not a significant contributor to the injury response in this model. Targeted induction of HSP70 may represent a potential therapeutic option for postischemic liver injury.

Liver; inflammation; neutrophils; cytokines

ISCHEMIA AND REPERFUSION of the liver often occurs as a result of trauma, sepsis, and liver surgery. The hepatocellular dysfunction/cell death caused by this insult results in severe intracellular oxidative stress and oxidant-mediated killing by recruited neutrophils (16, 17). Hepatocytes possess a number of intracellular cytoprotectants that help protect the cell from oxidative injuries. Notable among these are the antioxidants glutathione, superoxide dismutase, and heme oxygenase (10, 12, 35). These systems have been well studied and are known to counter oxidative damage to hepatocytes during ischemia-reperfusion injury. Another cytoprotectant protein, heat shock protein 70 (HSP70), is known to be expressed during hepatic ischemia-reperfusion (4), but whether it plays a significant role in protecting hepatocytes from injury during ischemia-reperfusion has not been elucidated.

HSP70 is a 70-kDa protein originally described as a molecular chaperone and is intimately involved in trans-mitochondrial protein transport (18). In addition to its properties as a chaperone, HSP70 also functions as an important cytoprotectant. A number of studies have examined the protective nature of HSP70 to a variety of insults and have found that it is highly protective against oxidative stress and apoptosis. Overexpression of HSP70 in WEHI-S cells protected them from tumor necrosis factor (TNF)-dependent and free radical-dependent killing by monocyes (15). In parallel, overexpression of HSP70 in the hepatocyte cell line HepG2 protected against H2O2-induced killing in association with stabilization of the mitochondrial membrane potential (13). Likewise, induction of HSP70 by geranylgeranylacetone prevented hepatocyte apoptosis induced by H2O2 (14). The mechanism by which HSP70 inhibits apoptosis has been well studied. There is abundant evidence that HSP70 inhibits events upstream of caspase-3, and multiple laboratories have reported that HSP70 binds to the apoptotic-protease-activating factor-1 and prevents the recruitment of procaspase-9 to the apoptosisosome (3, 21, 31).

HSP70 gene expression is regulated by the transcription factor, heat shock factor 1 (HSF1; see Ref. 18). HSF1 exists in the cytoplasm of resting cells in monomeric form. With cellular stress, such as trauma/shock or ischemia/hypoxia, intracellular Ca2+ concentration increases and protein kinase C phosphorylates HSF1 monomers, leading to their trimerization (8, 33). HSF1 trimers then translocate in the nucleus, bind DNA, and induce transcription of HSP70. Intracellular HSP70 has been shown to directly interact with the transcription factor nuclear factor (NF)-κB, and prevent its activation (11). NF-κB is a transcription factor that is thought to have both proinflammatory and hepatoprotective roles in the hepatic response to ischemia-reperfusion (9, 20, 23, 28). Thus interference by intracellular HSP70 of NF-κB activation following hepatic ischemia-reperfusion may represent another protective mechanism.

In contrast, release of HSP70 in the extracellular compartment (i.e., serum) has also been documented. Extracellular HSP70 has been proposed to function as a proinflammatory cytokine, signaling through toll-like receptors 2 and 4 (2). Serum levels of HSP70 in trauma patients correlates with survival, whereas in patients undergoing liver resection serum HSP70 was associated with liver dysfunction (19, 29). We have previously shown that serum levels of HSP70 are elevated...
following hepatic ischemia-reperfusion (27); however, the significance of this finding has not been established. In the current studies, we sought to examine the function of HSP70 during hepatic ischemia-reperfusion injury. To do this, we used HSP70 knockout mice and sodium arsenite (as an inducer of HSP70 expression).

MATERIALS AND METHODS

Hepatic ischemia-reperfusion injury model. Male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) and wild-type (on a B6129F1/Tac background) or HSP70 knockout mice (Taconic, Germantown, NY) were used in these experiments. All mice weighed 25–30 g. This project was approved by the University of Cincinnati Animal Care and Use Committee and was in compliance with the National Institutes of Health guidelines. The animals underwent either sham surgery or nonlethal ischemia-reperfusion. Partial hepatic ischemia was induced as described previously (21). Briefly, mice were anesthetized with pentobarbital sodium (60 mg/kg ip). A midline laparotomy was performed, and an atraumatic clip was used to interrupt blood supply to the left lateral and median lobes of the liver. After 90 min of partial hepatic ischemia, the clip was removed to initiate hepatic reperfusion. Sham control mice underwent the same protocol without vascular occlusion. Some mice were pretreated with normal saline or 8 mg/kg sodium arsenite intravenously before induction of ischemia. Additionally, some mice were injected intravenously with 3 mg/kg nonspecific IgG or rabbit polyclonal antibodies to interleukin-6 (IL-6) 30 min before ischemia. Other mice were injected intravenously with 10 µg recombinant human HSP70 (rHSP70) 2 h before ischemia. rHSP70 was generated as previously described (37). There was a low level of contamination by lipopolysaccharide (9.8 ng/µg) in these preparations; therefore, 98 ng of lipopolysaccharide (Escherichia coli, serotype 0111:B4; Sigma Chemical, St. Louis, MO) were added to the vehicle (phosphate buffered saline). Mice were killed after the indicated periods of reperfusion, and blood and samples of the left lateral lobe were taken for analysis. Liver samples were not pooled for analysis.

Western blot analyses. Liver samples were homogenized in lysis buffer (10 mM HEPES, pH 7.9, 150 mM NaCl, 1 mM EDTA, 0.6% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin, 1 µg/ml aprotinin, 10 µg/ml soybean trypsin inhibitor, and 1 µg/ml pepstatin) on ice. Homogenates were sonicated and centrifuged at 5,000 rpm to remove cellular debris. Protein concentrations were determined as described for nuclear extracts. Samples containing equal amounts of protein in equal volumes of sample buffer were separated in a denaturing 10% polyacrylamide gel and transferred to a 0.1-µm pore nitrocellulose membrane. Nonspecific binding sites were blocked with TBS (40 mM Tris, pH 7.6, 300 mM NaCl) containing 5% nonfat dry milk for 12 h at 4°C. Membranes were then incubated with antibodies to HSP70 or inhibitory factor B (IkBα; Santa Cruz Biotechnology, Santa Cruz, CA) in TBS with 0.1% Tween 20 (TBST). Membranes were washed and incubated with secondary antibodies conjugated to horseradish peroxidase. Immunoreactive proteins were detected by enhanced chemiluminescence.

Electrophoretic mobility shift assay. Nuclear extracts of liver tissue were prepared by the method of Deryckere and Gannon (7) and analyzed by electrophoretic mobility shift assay. Briefly, double-stranded consensus oligonucleotides to NF-κB (Promega, Madison, WI) or HSFI1 (University of Cincinnati DNA Core) were end-labeled with [γ-32P]ATP (3,000 Ci/mmole at 10 mCi/ml; Amersham, Arlington Heights, IL). Binding reactions (total volume 15 µl) containing equal amounts of nuclear protein extract (20 µg) and 35 fmol (~50,000 cpm, Cherenkov counting) of oligonucleotide were incubated at room temperature for 30 min. Binding reaction products were separated in a 4% polyacrylamide gel and analyzed by autoradiography.

Liver neutrophil accumulation. Liver myeloperoxidase (MPO) content was assessed by methods described elsewhere (33). Briefly, liver tissue (100 mg) was homogenized in 2 ml of buffer A (3.4 mM KH2HPO4 and 16 mM Na2HPO4, pH 7.4). After being centrifuged for 20 min at 10,000 g, the pellet was resuspended in 10 vol of buffer B (43.2 mM KH2HPO4, 6.5 mM Na2HPO4, 10 mM EDTA, and 0.5% hexadecyltrimethylammonium, pH 6.0) and sonicated for 10 s. After being heated for 2 h at 60°C, the supernatant was reacted with 3,3′,5′-tetramethylbenzidine, and the optical density was read at 655 nm.

Blood and tissue analysis. Blood was obtained by cardiac puncture for analysis of serum alanine aminotransferase (ALT) as an index of hepatocellular injury. Measurements of serum ALT were made using a diagnostic kit (Wiener Laboratories, Rosario, Argentina). Serum levels of TNF-α, macrophage inflammatory protein-2 (MIP-2), and IL-6 were measured by sandwich ELISA according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN). Liver tissues were fixed in 10% formalin and then embedded in paraffin for light microscopy. Sections were stained with hematoxylin and eosin for histological examination.

Statistical analysis. All data are expressed as means ± SE. Data were analyzed with a one-way ANOVA with subsequent Student-Newman-Keul’s test. Differences were considered significant at *P < 0.05.

RESULTS

Induction of HSP70 during ischemia-reperfusion and after treatment with sodium arsenite. HSP70 has been shown to be expressed in other models of liver ischemia-reperfusion (4). We have previously documented that HSP70 is induced after ischemia and 8 h of reperfusion (27). To determine the kinetics of HSP70 protein expression in the liver after ischemia-reperfusion, we subjected liver protein extracts to Western blot analyses. Liver HSP70 was not induced by ischemia alone (Fig. 1). However, within 1 h of reperfusion, HSP70 expression was maximal and remained elevated for up to 8 h after reperfusion. To determine if HSP70 could be induced in the liver, we injected 8 mg/kg sodium arsenite intravenously and examined HSP70 expression by Western blot. Hepatic HSP70 expression peaked 6 h after injection in otherwise unmanipulated mice (Fig. 2A). In all subsequent experiments with sodium arsenite, mice were injected 6 h before ischemia or sham surgery. We next assessed whether hepatic ischemia-reperfusion induced DNA binding of HSFI1 with or without arsenite treatment. In mice undergoing sham surgery, there was no HSFI1 activation detected in mice pretreated with saline (Fig. 2B). However, pretreatment with arsenite caused activation of HSFI1. Hepatic ischemia and 1 h of reperfusion resulted in a similar increase in HSFI1 activation. When mice were pretreated with arsenite and then subjected to hepatic ischemia-reperfusion, HSFI1 activation was much greater than that in mice pretreated with saline (Fig. 2B). The degree of HSFI1 activation was directly related to the amount of HSP70 protein expressed after ischemia and 1 h of reperfusion; mice pretreated with arsenite had higher HSP70 expression than mice pretreated with saline (Fig. 2C).

Effects of arsenite pretreatment and associated HSP70 induction on liver ischemia-reperfusion injury. We next assessed if pretreatment with sodium arsenite had any effect on ischemia-reperfusion injury. Mice pretreated with saline and subjected to ischemia and 8 h of reperfusion had the expected level of liver injury and inflammation (Fig. 3). In these mice, serum ALT was significantly elevated above sham-operated mice (Fig. 3A), as was the amount of neutrophil accumulation, as
Histopathology showed that mice pretreated with saline followed by sham surgery had normal hepatic architecture (Fig. 3C). However, mice pretreated with saline followed by ischemia-reperfusion had substantial hepatocellular necrosis and neutrophilic infiltrates (Fig. 3D). Pretreatment with sodium arsenite reduced liver injury and inflammation as evidenced by reduced serum levels of ALT and liver MPO content (Fig. 3, A and B, respectively). Furthermore, mice pretreated with arsenite undergoing sham surgery had normal hepatic architecture (Fig. 3E), whereas those undergoing ischemia-reperfusion (Fig. 3F) displayed far less hepatocellular necrosis and neutrophil recruitment than those pretreated with saline (Fig. 3D).

We next determined whether pretreatment with arsenite had any effects on NF-κB, an inflammation-associated transcription factor known to be involved in this injury. Pretreatment with arsenite in mice undergoing sham surgery resulted in modest activation of liver NF-κB compared with mice pretreated with saline (Fig. 4A). Conversely, mice pretreated with saline and undergoing hepatic ischemia-reperfusion had very high levels of NF-κB activation. Pretreatment with arsenite markedly reduced NF-κB activation induced by ischemia-

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**Fig. 1.** Hepatic expression of heat shock protein (HSP) 70 during ischemia-reperfusion injury. HSP70 protein was assessed by Western blot. std, molecular weight standards; PC, positive control (activated macrophage cell lysates, sc-2476 Santa Cruz Biotechnology). Chemiluminescence films were quantitated by image analysis. Results are representative of duplicate experiments.

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**Fig. 2.** Effects of sodium arsenite on HSP70 expression and activation of heat shock factor 1 (HSF1). A: unmanipulated mice were injected iv with 8 mg/kg sodium arsenite, and HSP70 protein expression in the liver was monitored for up to 48 h thereafter by Western blot. Chemiluminescence films were quantitated by image analysis. Data are means ± SE with n = 3–4 mice/group; *P < 0.05 compared with 0, 3, 24, and 48 h groups (*) and 0 and 3 h groups (†). B: mice were injected iv with saline or 8 mg/kg sodium arsenite 6 h before surgery. After ischemia and 1 h of reperfusion, liver nuclear extracts were assessed by electrophoretic mobility shift assay for HSF1 activation. Results were quantitated by image analysis of autoradiograms. Data are means ± SE with n = 3–4 mice/group; *P < 0.05 compared with saline-treated groups. C: hepatic HSP70 protein expression in mice pretreated with saline or sodium arsenite before ischemia and 1 h of reperfusion. Chemiluminescence films were quantitated by image analysis. Data are means ± SE with n = 5 mice/group; *P < 0.05 compared with the saline-treated group.
reperfusion (Fig. 4A). This was associated with decreased degradation of IκBα (Fig. 4B). In mice undergoing ischemia-reperfusion, IκBα was almost fully degraded in mice receiving saline pretreatment, whereas mice receiving arsenite had far less degradation of IκBα (Fig. 4B).

NF-κB is known to regulate the expression of a number of inflammatory cytokines, including TNF-α and MIP-2, and HSF1 is known to regulate IL-6 (30). Because each of these cytokines is known to contribute to (TNF-α and MIP-2) or protect against (IL-6) hepatic ischemia-reperfusion injury (5, 6, 21), we assessed the levels of these mediators. In saline-pretreated mice, all three cytokines were elevated after ischemia and 1 or 8 h of reperfusion (Fig. 5). Pretreatment with arsenite resulted in significantly decreased TNF-α and MIP-2 expression. Interestingly, arsenite pretreatment itself resulted in elevated IL-6 expression in sham-operated mice (Fig. 5). After 1 h of reperfusion, arsenite-treated mice had much higher levels of serum IL-6 than saline-treated
mice. However, after 8 h of reperfusion, IL-6 levels in arsenite-treated mice were lower than those in saline-treated mice (Fig. 5).

**Extracellular HSP70 does not affect hepatic ischemia-reperfusion injury.** Because extracellular HSP70 has been implicated in recovery from traumatic injury (28), as well as organ dysfunction after liver resection (19), it is unclear whether circulating HSP70 plays a positive or negative role in organ injury. To determine if hepatic ischemia-reperfusion injury could be modulated by circulating HSP70, we evaluated the effects of rHSP70 in our model of injury. Mice underwent

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**Fig. 4.** Effects of sodium arsenite on hepatic nuclear factor (NF)-κB activation and inhibitory factor κBα (IκBα) protein degradation during ischemia-reperfusion injury. Mice were injected iv with saline or 8 mg/kg sodium arsenite 6 h before surgery. Samples were analyzed after ischemia and 1 h of reperfusion. A: liver nuclear extracts were analyzed by electrophoretic mobility shift assay for NF-κB activation. Results were quantitated by image analysis of autoradiograms. Data are means ± SE with n = 3–4 mice/group; *P < 0.05 compared with saline-treated groups. B: liver lysates were assessed by Western blot for protein expression of IκBα. Chemiluminescence films were quantitated by image analysis. Data are means ± SE with n = 3–4 mice/group; *P < 0.05 compared with the saline-treated group.

**Fig. 5.** Effects of sodium arsenite on serum levels of tumor necrosis factor (TNF)-α, macrophage inflammatory protein (MIP)-2 and interleukin (IL)-6 during hepatic ischemia-reperfusion injury. Mice were injected iv with saline or 8 mg/kg sodium arsenite 6 h before surgery. Serum samples were analyzed by ELISA for TNF-α, MIP-2, and IL-6 after ischemia and 1 or 8 h of reperfusion. Data are means ± SE with n = 5–9 mice/group. *P < 0.05 compared with saline-treated mice.
hepatic ischemia and were injected intravenously with either vehicle or 10 µg of rHSP70 2 h before ischemia. The 10-µg dose has been shown to result in substantial hepatic uptake of rHSP70 (34). Injection of rHSP70 had no effects on liver injury (serum ALT) or neutrophil accumulation (MPO content) after 8 h of reperfusion (Fig. 6).

**HSP70 does not influence hepatic inflammation.** To more directly examine the function of HSP70 during hepatic ischemia-reperfusion injury, we employed HSP70 knockout mice. Unexpectedly, HSP70 knockout mice did not differ from wild-type mice in their response to hepatic ischemia-reperfusion (Fig. 7). HSP70 knockout mice had similar serum ALT and liver MPO content as wild-type mice (Fig. 7, A and B, respectively). In addition, no differences were observed in liver histopathology. Both wild-type and HSP70 knockout mice undergoing sham surgery had normal liver architecture (Fig. 7, C and F, respectively). After ischemia and 8 h of reperfusion, hepatocellular necrosis and neutrophilic infiltration were similar in wild-type and HSP70 knockout mice (Fig. 7, D and G, respectively). We next evaluated whether the effects observed with arsenite treatment were the result of HSP70. In these experiments, wild-type and HSP70 knockout mice were pretreated with arsenite and evaluated for liver injury after ischemia-reperfusion. Unlike its effects in wild-type mice, arsenite showed no benefit on liver injury in HSP70 knockout mice (Fig. 7A). However, arsenite treatment reduced liver MPO content in both wild-type and HSP70 knockout mice (Fig. 7B). These results were supported by histology, with wild-type mice that were pretreated with arsenite showing reduced amounts of necrosis and neutrophil accumulation (Fig. 7E) and HSP70 knockout mice treated with arsenite showing high levels of necrosis but reduced neutrophil infiltration (Fig. 7H).

Finally, because our earlier results showed that arsenite pretreatment induced IL-6 production (Fig. 5), and that knockout of HSP70 had no effect on neutrophil recruitment, we next examined whether increased IL-6 production may be responsible for the reduced neutrophil accumulation. HSP70 knockout mice were pretreated with saline or arsenite and subsequently treated with anti-IL-6 antibodies 30 min before induction of ischemia. HSP70 knockout mice pretreated with saline and receiving anti-IL-6 had similar liver MPO content after ischemia and 8 h of reperfusion as mice receiving only saline pretreatment (Fig. 8). However, blockade of IL-6 partially reversed the arsenite-induced reduction in liver MPO content (Fig. 8), suggesting that arsenite-induced IL-6 was at least partially responsible for the reduction in neutrophil accumulation.

**DISCUSSION**

The present study suggests that HSP70 may be hepatoprotective during ischemia-reperfusion injury. Although previous studies, including our own work, have shown that HSP70 is upregulated after liver reperfusion (4, 27), the manner in which HSP70 protects against liver injury has not been previously studied. Here we show that HSP70 is induced early after reperfusion and its expression remains elevated for at least 8 h after reperfusion. This increased expression coincided with increased activation of the transcription factor HSF1. Interestingly, direct application of HSP70 knockout mice in these studies revealed no phenotype, and analysis of these studies alone would suggest that HSP70 is not an important protein in this response. However, the lack of phenotypic differences in knockout vs. wild-type mice may be a result of genetic compensation. Although our data do not confirm this possibility, we show that treatment with sodium arsenite induces HSP70 expression in the liver and that this is accompanied by marked decreases in liver injury after ischemia-reperfusion. In HSP70 knockout mice, treatment with arsenite did not reduce liver injury, suggesting that HSP70 is required for the hepatoprotective effects of arsenite. Supporting this concept are previous in vitro studies that demonstrated that...
Fig. 7. Response of HSP70 knockout mice to hepatic ischemia-reperfusion injury. Wild-type or HSP70 knockout mice were injected iv with saline or 8 mg/kg sodium arsenite 6 h before surgery. Samples were analyzed after ischemia and 8 h of reperfusion. A: liver injury was measured by serum levels of ALT. Data are means ± SE with n = 4–7 mice/group. *P < 0.05 compared with saline-treated mice. B: neutrophil accumulation was determined by liver content of MPO. Data are means ± SE with n = 4–6 mice/group. *P < 0.05 compared with saline-treated mice. Liver histology in sham-operated wild-type (C) or HSP70 knockout (F) mice pretreated with saline showed normal hepatic architecture. After ischemia-reperfusion, livers from saline-pretreated wild-type (D) and HSP70 knockout (G) mice had large areas of necrosis with marked neutrophilic infiltrates. In contrast, livers from arsenite-pretreated wild-type mice had far less liver injury and neutrophil accumulation (E), whereas HSP70 knockout mice had marked necrosis with reduced neutrophilic infiltrates (H). For C–H, original magnification was ×50.
overexpression of HSP70 in HepG2 cells limited H₂O₂-induced cell death (13).

Interestingly, the reduction in neutrophil accumulation by arsenite treatment was also observed in HSP70 knockout mice. Thus it appears that the protective effects of HSP70 are independent of the inflammatory process, or at least independent of the hepatic recruitment of neutrophils. This leaves open the manner in which sodium arsenite attenuates this response. Treatment with arsenite increased HSF1 activity, and HSF1 activation has been linked to inhibition of NF-κB activation and repression of proinflammatory cytokine expression in a number of other experimental systems (1, 25, 39). NF-κB is a well-known transcriptional regulator of proinflammatory cytokines and cytoprotective proteins (9, 28). The role of NF-κB in hepatic ischemia-reperfusion injury has not been fully elucidated. Activation of NF-κB in whole liver during ischemia-reperfusion injury is an indicator of the extent of injury. The precise function of NF-κB activation in hepatocytes during this response is controversial, and there is evidence to support both protective (20) and injurious (23) actions. Increased activation of HSF1, as observed after arsenite treatment, would be expected to reduce both NF-κB activation and proinflammatory cytokine expression, which is precisely the profile we observed (Fig. 9). Second, it is known that HSF1 regulates IL-6 expression (30) and that IL-6 reduces hepatic ischemia- and reperfusion-induced inflammation (5). In our studies, we found that neutralization of IL-6 in HSP70 knockout mice partially reversed arsenite-mediated reductions in neutrophil accumulation. These data suggest that HSF1 activated following ischemia-reperfusion and augmented by arsenite treatment results in induction of both HSP70 and IL-6. HSP70 appears to have direct protective effects on hepatocytes, and IL-6 appears to function in part by attenuating the recruitment of neutrophils to the injured liver (Fig. 9).

A limitation of our studies is the effects of arsenite other than induction of HSP70. Arsenite is a pharmacological inducer of the heat shock response, of which HSP70 induction is but one component. It is known that components of the heat shock response other than HSP70 may interfere with NF-κB signaling and resultant cytokine expression (24). It is therefore possible that some of the arsenite effects observed in our studies were unrelated to its induction of HSP70. However, our data with HSP70 knockout mice do demonstrate a requirement for HSP70 for the hepatoprotection afforded by arsenite.

We have previously reported that serum levels of HSP70 are elevated following hepatic ischemia-reperfusion (27). Elevated circulating HSP70 has also been demonstrated during trauma, septic shock, myocardial infarction, and liver resection (19, 29, 32, 38). In liver resection and myocardial infarction, circulating HSP70 was correlated with increased infection or systemic inflammation and poor outcome, whereas, in trauma, circulating levels correlate with survival (19, 29, 32, 38). Extracellular HSP70 has been shown to signal via toll-like receptors 2 and 4 (2). Interestingly, toll-like receptor 4 expressed on Kupffer cells has been shown to contribute to the development of hepatic ischemia-reperfusion injury (36). In our studies, administration of rHSP70 did not alter hepatic ischemia-reperfusion injury, suggesting that the infused HSP70 does not have any protective or injurious function in this setting.

In summary, induction of HSP70 with sodium arsenite resulted in significant protection from liver injury and inflammation. The hepatoprotective effects of arsenite were due specifically to HSP70, since HSP70 knockout mice treated with arsenite showed no protection against injury. However, the reduction in liver inflammation observed with arsenite treatment was not related to HSP70 but appeared to be, at least partly, dependent upon arsenite-induced IL-6. Intravenous injection of rHSP70 did not change the degree of inflammatory injury after ischemia-reperfusion. These data suggest that intracellular HSP70 is hepatoprotective during ischemia-reperfusion injury and that extracellular HSP70 has no impact on the injury response in this model. Targeted induction of HSP70 may be a potentially important therapeutic option for posts ischemic liver injury.

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