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Hepatic Heat Shock and Acute-phase Gene Expression Are Induced Simultaneously after Celiotomy in the Anesthetized Pig

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Background: **The liver plays a central role in the whole organism's response to injury. Expression of hepatic acute-phase and heat-shock genes likely contributes to the restoration of homeostasis after stressful events. However, after prolonged ischemia, hepatic transcription of heat-shock genes can exclude the simultaneous transcription of acute-phase genes. The issue of whether hepatic 72-kd heat-shock protein (hsp72) gene expression is induced under perioperative conditions that do not result in prolonged liver ischemia and whether this might further affect the expression of the acute-phase reactant inter-α-trypsin inhibitor (α-Ti) was examined.**

Methods: **Pigs were anesthetized with sodium pentobarbital and ketamine hydrochloride, tracheally intubated, and their lungs ventilated. After celiotomy, a hepatic biopsy sample was obtained. Arterial blood pressure, cardiac output, and total hepatic blood flow were measured. Subsequent biopsies were obtained at 1, 2, 3, 4, and 6 h after the initial biopsy. Arterial norepinephrine concentrations were measured using high-pressure liquid chromatography. Nuclear runoff (run on) analysis and Northern blotting were applied to estimate changes in hsp72 and α-Ti gene transcription rates and RNA levels. Western blotting was used to estimate changes in hsp72 levels.**

Results: **Hemodynamic parameters did not change significantly over time. Arterial norepinephrine concentrations were increased at all time points. Hepatic hsp72 RNA levels increased up to sixfold while nuclear runoff assays did not detect significant changes in hsp72 gene transcription rates. The increases in hsp72 RNA levels correlated with accumulation of hsp72 (up to sevenfold). Increases in α-Ti transcription rates up to 42-fold were associated with respective increases in α-Ti RNA levels (up to 17-fold).**

Conclusions: **These data demonstrate that hepatic expression of hsp72 is not confined to conditions that lead to prolonged liver ischemia but is also part of the response of the liver to surgery under general anesthesia. Furthermore, these conditions are permissive for the simultaneous RNA expression of the acute-phase reactant α-Ti. (Key words: Anesthesia, intravenous: ketamine; pentobarbital. Heat-shock response: 72-kd heat-shock proteins. Acute-phase response: inter-α-trypsin inhibitor. Molecular biology: Northern blotting; nuclear runoff analysis; Western blotting.)**

TISSUE injury elicits coordinate changes in the synthesis and secretion of proteins by the liver, leading to a decrease in plasma concentrations of several constitutive proteins and increases in specific procoagulants and antiproteases.[1,2] These proteins, called acute-phase proteins, can contribute to the restoration of systemic homeostasis during generalized inflammatory processes.[3]

However, after prolonged hepatic ischemia followed by reperfusion, a different program of stress gene expression is activated in the liver: the heat-shock response.[4] Many heat-shock proteins seem to function as so-called "molecular chaperons." They bind to nascent polypeptide chains allowing them to gain their normal tertiary and quaternary structure by actively preventing protein malfolding.[5–7] The expression of 72-kd heat-shock proteins (hsp72) marks a major intracellular homeostatic response. Hsp72 seem to be essential for cell survival in the face of stress: heat shock, produced by heating cells to 45°C for 30 min, is lethal to cells microinjected with antibodies against heat-shock proteins whereas control cells survive a similar heat shock.[8]

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The properties of hsp72 could be crucial to maintain the ability of the liver to synthesize functioning proteins (*e.g.*, acute-phase proteins) under stressful conditions.[9] However, in human hepatoblastoma cells *in vitro,* acute-phase and heat-shock gene expression are mutually exclusive and heat-shock gene expression is prioritized over acute-phase protein expression after hypoxia followed by reoxygenation.[9] These observations have been confirmed in a porcine model of hemorrhagic shock where hepatic hsp72 gene transcription excluded the simultaneous transcription of acute-phase response genes.[10]

Because survival after stressful events may ultimately depend on restoration of both systemic (acute-phase response) and intracellular (heat-shock response) homeostasis, it is particularly important to identify and characterize the regulatory mechanisms that control stress gene expression in the liver. Although previous studies have provided some insight into the differential regulation of hepatic stress gene programs after prolonged hepatic ischemia,[10–12] it is not known if hepatic heat-shock protein gene expression is induced under perioperative conditions that do not result in substantial liver ischemia and how the induction of heat-shock genes might affect the regulation of acute-phase gene expression under these conditions. We sought to replicate the clinical constellation of perioperative events (induction and maintenance of anesthesia, tracheal intubation, vascular cannulations, and surgery) in a pig model that allowed us to evaluate their effects on systemic and regional splanchnic hemodynamics, hepatic hsp72 gene-specific transcription rates, steady-state RNA and protein levels and to compare it with the expression of the acute-phase reactant inter-α-trypsin inhibitor (α-Ti).

Materials and Methods

Experimental Model

Female prepubertal pigs (22–27 kg body weight, n = 4) were fasted for 24 h with free access to water. Anesthesia was induced with intramuscular injections of ketamine hydrochloride (20 mg · kg^{-1} body weight) and sodium pentobarbital (10 mg · kg^{-1} body weight). All surgical procedures were performed under sterile and nonpyrogenic conditions. After cannulation of an ear vein, additional doses of sodium pentobarbital (1–2 mg · kg^{-1} body weight) were administered intravenously every 20–30 min (total dose range of 820–1100 mg). Saline solution was infused at 20 ml · kg^{-1} · h^{-1}. A tracheostomy was performed, and an endotracheal tube was positioned. The lungs were ventilated with oxygen-enriched room air using a constant volume time-cycled ventilator (Harvard Apparatus, South Natick, MA) to keep the PaO_2 greater than 100 mmHg and the $PaCO_2$ between 35 and 45 mmHg. A heating pad maintained body temperature.

A midline celiotomy was rapidly performed and the first hepatic wedge biopsy, consisting of 3.5–5.0 g of tissue, was obtained immediately (time 0 = baseline). After each biopsy, 3 g of liver tissue was rapidly plunged into 0.14 M NaCl and 10 mM Tris-HCl (pH 7.4) at 4°C and washed free of excess blood. Isolation of hepatic nuclei was performed immediately, as described later. The remaining liver tissue was promptly immersed in liquid nitrogen and stored at −80°C for subsequent RNA and protein analysis.

The right carotid artery was cannulated to measure arterial blood pressure continuously and to draw blood samples. The right subclavian vein was catheterized for fluid infusion and drug administration. A thermodilution pulmonary artery catheter was placed *via* the right internal jugular vein to measure right atrial pressure, pulmonary vascular pressures, and cardiac output. Central filling pressures were maintained by adjusting the flow rate of the saline infusion between 20 and 40 ml · kg^{-1} · h^{-1}. Appropriately sized ultrasound flow probes (Transonic, Ithaca, NY) were placed around the common hepatic artery and the portal vein.[13] Ligation of branches of the hepatic artery can result in variable degrees of liver ischemia under conditions in which anatomic variations of the hepatic arterial blood supply are present. Because hepatic ischemia has been shown to affect hsp72 gene expression,[11] these branches were not ligated. Thus, changes in the sum of flow through the two probes can only serve as an estimate of changes in total hepatic blood flow.

Subsequent liver biopsies were obtained after surgical instrumentation was completed at 1, 2, 3, 4, and 6 h after baseline. Biopsy sites were rotated randomly but were in all cases as remote from each other as possible. This technique[14] appears to minimize local effects of biopsy trauma on gene expression.

Arterial blood pressure and heart rate were continuously monitored once available during the study. Arterial blood gases (Radiometer, Copenhagen) and norepinephrine concentrations were measured immediately before each liver biopsy, including the first

biopsy. All other physiologic parameters were measured at 1, 2, 3, 4, and 6 h after baseline.

In two additional experiments, anesthesia was induced with intramuscular injections of midazolam (0.2 $mg \cdot kg^{-1}$ body weight) and sodium pentobarbital (10 $mg \cdot kg^{-1}$ body weight) instead of ketamine hydrochloride/sodium pentobarbital to characterize the potential effect of ketamine on changes in hsp72 and α-Ti steady-state RNA levels.

To evaluate how the extent of surgical maneuvers may affect hepatic hsp72 and α-Ti RNA levels, two other experiments with minimal surgical instrumentation (no central venous catheterization, no flow probes around hepatic artery or portal vein, and size of hepatic wedge biopsy $\leq$500 mg for RNA extraction only) were performed.

After the final biopsy, the animals were killed with an overdose of pentobarbital followed by intravenous potassium chloride. The protocol was approved by the Animal Care and Use Committee of the Johns Hopkins Medical Institutions.

Norepinephrine Measurements

Arterial blood samples were processed for measurements of norepinephrine concentrations using high-pressure liquid chromatography with electrochemical detection after alumina extraction as previously described.[15] The sensitivity of this assay is 20 $pg \cdot ml^{-1}$, and the intraassay and interassay coefficients of variability are 3–5%.

Hepatic Nuclear Runoff Analysis

Nuclear runoff assays were performed as previously described.[14,16] Briefly, hepatic nuclei were isolated by centrifugation through a sucrose gradient and *in vitro* transcription was performed using 1×10^7 nuclei per assay. Radioactively labeled transcripts were purified by the acid guanidinium thiocyanate-phenol-chloroform method.[17] The following porcine cDNA clones were used: heat-shock protein-72 (hsp72), inter-α-trypsin inhibitor (α-Ti), and β-actin. The hsp-72 clone (Genebank accession No. M29506), containing the full length cDNA of the inducible form of heat-shock protein-70 (hsp70), and the α-Ti clone (Genebank accession No. M29507) were cloned from a shocked/resuscitated pig liver library.[18] The linearized DNA (5 μg) was spotted onto nitrocellulose membranes, cross-linked, and hybridized with the radioactively labeled RNA. Autoradiographs exposed in the linear range of film sensitivity were analyzed densitometrically with a two-dimensional scanner (Molecular Dynamics, Sunnyvale, CA). All measured areas were the same size. Background density was measured and subtracted from each raw densitometric value. Transcriptional activity of hsp72 and α-Ti was normalized to the transcriptional activity of β-actin. Results were expressed as relative changes of the dividends of the background-corrected densitometric signals of hsp72/β-actin and α-Ti/β-actin compared to baseline (time 0).

Isolation of RNA and Northern Blot Analysis

RNA isolation and Northern blotting were performed as previously described.[14,19] Briefly, total cellular RNA from porcine liver was isolated according to the method of Chomczynski and Sacchi.[17] RNA (20 μg per lane) was electrophoretically resolved in agarose gels, blotted to nylon membranes, probed with a riboprobe generated from the hsp72 cDNA clone, and autoradiographed. The same blot was subsequently stripped and rehybridized with the α-Ti cDNA probe, which was radioactively labeled using the random primer method.[20] After exposure to film, the membranes were restripped and rehybridized as described earlier using a riboprobe generated from a mouse tubulin cDNA clone. Autoradiographs exposed in the linear range of film sensitivity were analyzed densitometrically. Results were expressed as relative changes of the dividends of the background-corrected densitometric values of hsp72/tubulin and α-Ti/tubulin compared to baseline (time 0).

Western Blot Analysis

Western blot analysis of hsp72 was performed as previously described.[19] Briefly, samples of clarified liver homogenates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (100 μg protein per lane), transferred to nitrocellulose membranes, probed with a monoclonal antibody specific for the inducible form of hsp70 (hsp72) C92F3A-5 (StressGen Biotechnologies Corp., Sidney, BC, Canada), and tagged with a ^{125}I-labeled goat-anti-mouse antibody. Autoradiographs exposed in the linear range of film sensitivity were chosen for densitometric analysis. Results were expressed as relative changes of background-corrected densitometric values compared to baseline (time 0).

Data Analysis

All values are presented as mean $\pm$ SEM. Statistical comparisons were done by one-way analysis of variance (ANOVA) for repeated measurements, followed by

Bonferroni corrected t tests when appropriate. Values of $P < 0.05$ were considered to indicate a significant difference.

Results

Physiologic Conditions

At the time of initial measurements, all hemodynamic parameters were within a range that has been previously reported for normal conscious resting pigs.[21] Anesthesia and surgery did not lead to any significant changes in systemic or splanchnic hemodynamic parameters over time; heart rate and mean arterial pressure as well as cardiac output and total hepatic blood flow remained statistically unchanged during the whole experiment (table 1). The mean arterial norepinephrine concentrations at all times were more than 2–3 times greater than basal norepinephrine levels that have been reported in conscious resting pigs[21] (table 1). The blood temperature measured in the pulmonary artery remained unchanged over time (table 1). The liver biopsies produced no measurable hemodynamic effects. Arterial PO_2, PCO_2, and pH were 270.0 mmHg ± 52.4, 37.1 mmHg ± 4.4, and 7.35 ± 0.04, respectively, at baseline and did not change during the course of the experiment.

Gene-specific Transcription Rates of Heat-shock Protein-72 and Inter-α-trypsin Inhibitor

Specific transcription rates of the genes encoding hsp72, the acute-phase reactant α-Ti, and β-actin, which was included as a standard to allow intraindividual and interindividual comparisons of relative hsp72 and α-Ti transcriptional activity were determined by hybridizing the radiolabeled nuclear transcripts to cDNAs coding for these three genes immobilized on filter membranes. Figure 1 shows the typical pattern of hepatic hsp72, α-Ti, and β-actin gene transcription consequent to anesthesia and surgery that was observed under these experimental conditions. Quantitative results of the nuclear runoff transcription assays from all animals are shown in figure 2. The transcription rate of the α-Ti gene showed a steady increase and reached a peak value at 4 h after the initial biopsy (42-fold ± 12-fold increase *vs.* baseline). The α-Ti transcription rate stayed at this level until the end of the observation period. Transcription rates at 4 and 6 h were significantly greater than the transcription rate at baseline. In contrast, nuclear runoff assays did not detect significant changes in hsp72 gene transcription rates in response to anesthesia and surgery.

Heat-shock Protein-72 RNA and Inter-α-trypsin Inhibitor RNA Levels

Hepatic hsp72 and α-Ti steady-state RNA levels were analyzed using Northern blotting. Representative autoradiographs are shown in figure 3. Anesthesia and surgery led to a pronounced accumulation of hsp72 RNA within 2–3 h after the initial biopsy. As shown in figure 3, high levels of α-Ti RNA could be detected in the liver after anesthesia and surgery at 4 and 6 h. Quantitative results of Northern blot analyses from all animals are shown in figure 4. Both hsp72 and α-Ti RNA levels increased during the experiment and were significantly different from baseline values at 3, 4, and 6 h. α-Ti RNA levels rose to a peak value at 6 h. This α-Ti RNA level was 17-fold ± 2-fold higher than the respective baseline value. Hepatic hsp72 RNA levels reached a maximum at 4 h (6-fold ± 1-fold increase *vs.* time 0) and remained at this level until the end of the observation period.

Table 1. Physiologic Parameters

Parameter	Time					
	0 h	1 h	2 h	3 h	4 h	6 h
Heart rate (beats · min^{-1})	119 ± 13	128 ± 6	141 ± 17	119 ± 16	128 ± 16	138 ± 14
Body temperature (°C)		38.1 ± 0.5	38.3 ± 0.5	38.4 ± 0.5	38.3 ± 0.2	38.5 ± 0.1
Mean arterial pressure (mmHg)	97 ± 9	100 ± 7	95 ± 8	98 ± 9	95.8 ± 8	88.8 ± 6
Cardiac output (ml · min^{-1} · kg^{-1})		130 ± 10	123 ± 4	101 ± 6	115 ± 9	107 ± 12
Total hepatic blood flow (ml · min^{-1} · kg^{-1})		36 ± 6	33 ± 3	28 ± 2	31 ± 3	30 ± 3
Arterial norepinephrine concentration (pg · ml^{-1})	827 ± 76	1,460 ± 755	1,358 ± 355	1,227 ± 91	1,460 ± 428	410 ± 238

Data are mean ± SEM at the indicated time points (n = 4 pigs).

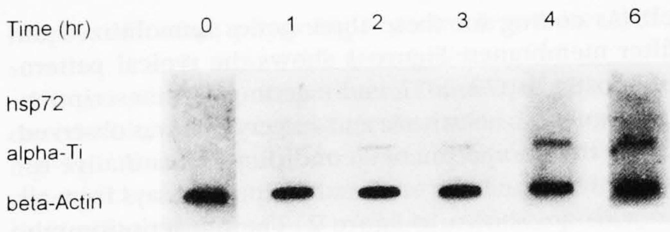

Fig. 1. Hepatic gene transcription of heat-shock protein-72 (hsp72), of the acute-phase reactant inter-α-trypsin inhibitor (α-Ti), and of beta-actin, consequent to anesthesia and surgery. Gene transcription is shown by autoradiographic intensity of ^{32}P-labeled hybrid-selected runoff transcripts purified from isolated pig liver nuclei that had been isolated at baseline (time 0), and at 1, 2, 3, 4, and 6 h after baseline from one animal as described under materials and methods. Analysis of beta-actin gene transcription was included as a standard to allow intra-individual and interindividual comparisons of hsp72 and α-Ti transcriptional activity.

Analyses of changes in acute-phase and heat-shock RNA levels in livers from the two animals that had anesthesia induced without ketamine, and from the two animals that underwent less extensive surgical instrumentation, revealed similar patterns (time course and magnitude) of hsp72 and α-Ti RNA accumulation as compared to the animals in the main body of studies described earlier (data not shown).

Hepatic Heat-shock Protein-72 Levels

To survey the influence of anesthesia and surgery on hsp72 gene expression at the protein level, Western blot analysis of hsp72 was performed. A typical autoradiograph of such a blot probed with a monoclonal antibody specific for the inducible form of hsp72 is shown in figure 5. There was an accumulation of hsp72 starting at 3 h and reaching a peak level at 6 h after baseline. Quantitative results of estimations of changes in hepatic hsp72 levels from all animals using densitometric scanning of autoradiographs are shown in figure 4. Accumulation of hsp72 was detectable at 4 and 6 h. Peak values were reached at 6 h and were sevenfold ± twofold greater than the respective baseline value. There was an interval of 1 h (one data point) between the time that hsp72 RNA levels had increased significantly (3 h after baseline) until a significant accumulation of hsp72 protein occurred (4 h after baseline).

Discussion

Hepatic Heat-shock Protein 72 Gene Expression

The results of the current study demonstrate that anesthesia and surgery lead to an increase in the hepatic hsp72 RNA level. This increase in hsp72 RNA correlated with a subsequent accumulation of hsp72 protein. This confirms that the signal on the Northern blot represents translatable message and is in agreement with previous results showing that translation of hsp72 is proportional to the steady-state levels of hsp72 mRNA *in vitro*[22] and *in vivo*.[19] It has been shown that prolonged hepatic warm ischemia (>60 min) followed by reperfusion leads to an accumulation of hsp72 RNA and protein in the liver.[11,19] However, the results presented here clearly indicate that hepatic expression of hsp72 is not confined to these conditions but is also part of the response of the liver to events (surgery under general anesthesia) that are not associated with significant changes in systemic hemodynamics or reductions in total hepatic blood flow. This new observation raises

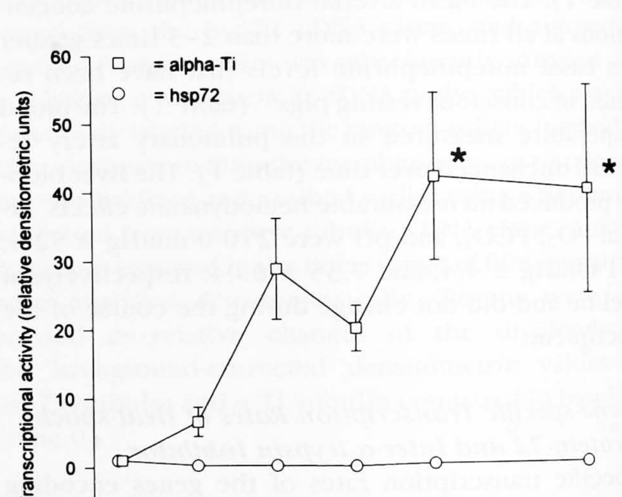

Fig. 2. Changes in gene-specific transcription rates for heat-shock protein-72 (hsp72), and inter-α-trypsin inhibitor (α-Ti) consequent to anesthesia and surgery. Hepatic nuclear runoff assays were performed using ^{32}P-labeled hybrid-selected runoff transcripts purified from isolated pig liver nuclei that had been isolated at baseline (time 0), and at 1, 2, 3, 4, and 6 h after baseline. Autoradiographs exposed in the linear range of film sensitivity were analyzed densitometrically with a two-dimensional scanner. Transcriptional activity of hsp72 and α-Ti was normalized to the respective beta-actin gene transcription rate of each animal at each time point. Results were expressed as relative changes of the dividends of the background corrected densitometric signals of hsp72/beta-actin and α-Ti/beta-actin compared to baseline (relative densitometric units). Data represent mean ± SEM (n = 4 pigs; one tissue sample from each animal analyzed at each time point). SEM of hsp72 transcriptional activity was so small at all time points, that error bars are hidden by the open circle symbols. *Significant difference compared to baseline by one-way analysis of variance followed by Bonferroni corrected *t* tests.

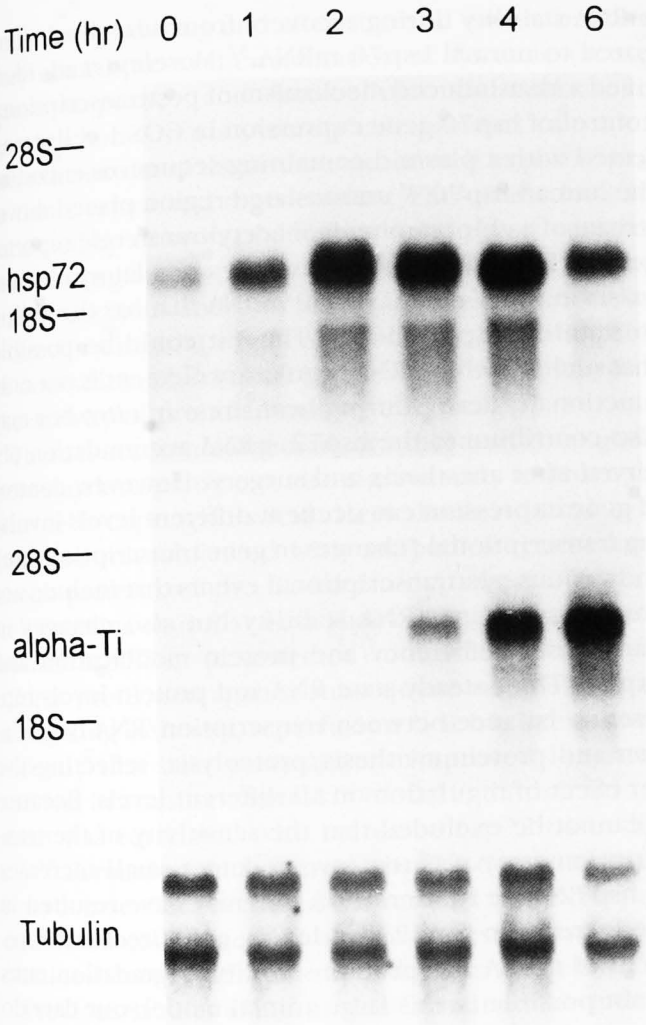

Fig. 3. Autoradiographs of a Northern blot showing the effect of anesthesia and surgery on hepatic heat-shock protein-72 (hsp72), inter-α-trypsin inhibitor (α-Ti), and tubulin steady-state RNA levels. Total RNA (20 μg/lane) was isolated from serial pig liver biopsy samples at baseline (time 0), and at 1, 2, 3, 4, and 6 h after baseline. The RNA was electrophoretically resolved, blotted, probed with hsp72 cDNA, and autoradiographed. The same blot was subsequently stripped and reprobed twice to determine α-Ti and tubulin RNA levels. The positions of the 18 S and 28 S ribosomal RNA subunits derived from visual inspection of the ethidium-bromide-stained gel are indicated. Analysis of the tubulin RNA level was included to confirm the lane-to-lane uniformity of RNA loading and was used as a standard to allow formal normalization of the hsp72 and α-Ti signals. RNA processed for this Northern blot and the hepatic nuclei used for the runoff analysis shown in figure 1 were obtained from the same animal.

the question of how the induction of hsp72 is regulated under these experimental conditions.

Previous studies suggest that transcriptional mechanisms may play an important role in the regulation of heat-shock protein gene expression. Binding of transcriptional activators (heat-shock factors) to short, highly conserved DNA sequences known as heat-shock elements has been shown to modulate heat-shock gene transcription.[7,23] The hsp70 promoter has a number of such 5′ regulatory elements that can bind transacting factors and thereby augment heat-shock gene transcription.[24] The intracellular activity of these transacting factors correlates with the rate of transcription of the hsp70 gene.[25] Expression of the inducible form of the hsp70 gene in the postischemic rat liver is largely dependent on activation of transcription.[11] Binding of activated heat-shock factors to a synthetic oligonucleotide containing the heat-shock consensus sequence and

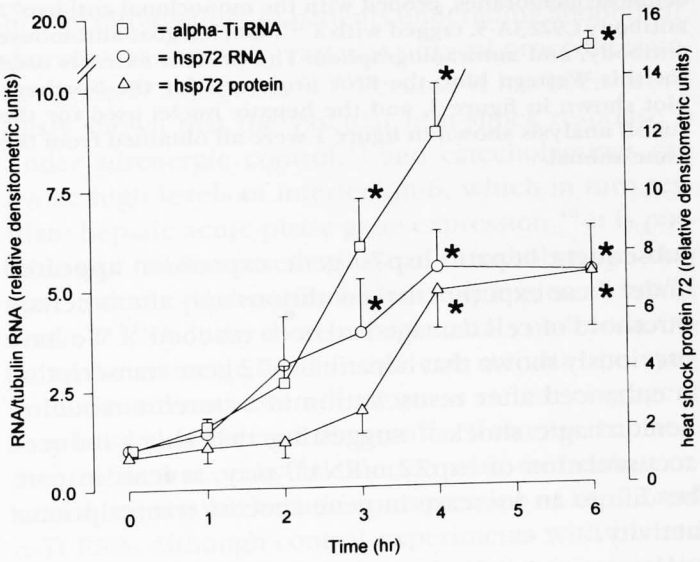

Fig. 4. Effect of anesthesia and surgery on hepatic steady-state levels of inter-α-trypsin inhibitor (α-Ti) RNA, heat-shock protein-72 (hsp72) RNA, and of hsp72 protein. Total cellular RNA was isolated from serial pig liver biopsy samples at baseline (time 0), and at 1, 2, 3, 4, and 6 h after baseline, electrophoretically resolved, blotted, probed with hsp72 cDNA, autoradiographed, reprobed with α-Ti and tubulin cDNA, and reexposed to film. Liver homogenates were prepared from the same pig liver biopsy samples, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to membranes, probed with the monoclonal anti-hsp72 antibody C92F3A-5, tagged with a ^{125}I-labeled goat-anti-mouse antibody, and autoradiographed. Changes in hepatic α-Ti RNA and hsp72 RNA and hsp72 protein levels were estimated using two-dimensional densitometric scanning of Northern and Western blot autoradiographs exposed in the linear range of film sensitivity. Hepatic RNA levels were expressed as relative changes of the dividends of the background-corrected densitometric signals of hsp72/tubulin and α-Ti/tubulin compared to baseline. Hepatic hsp72 protein level is plotted as relative change of the background-corrected densitometric hsp72 protein signal compared to baseline. Data represent mean ± SEM (n = 4 pigs; one tissue sample from each animal analyzed at each time point). *Significant difference compared to baseline by one-way analysis of variance followed by Bonferroni corrected *t* tests.

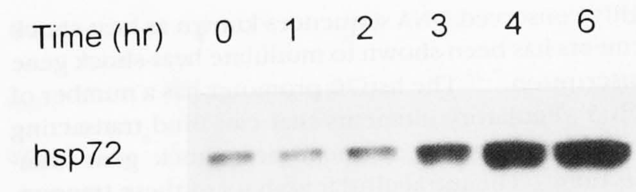

Fig. 5. Autoradiograph of a Western blot showing the effect of anesthesia and surgery on hepatic steady-state heat-shock protein-72 (hsp72) levels. Total cellular proteins were isolated from serial pig liver biopsy samples that had been obtained at baseline (time 0), and at 1, 2, 3, 4, and 6 h after baseline, and were probed with a monoclonal antibody specific for the inducible form of heat-shock protein-70 (heat-shock protein-72; hsp72). Samples of clarified liver homogenates were subjected to sodium dodecyl sulfate-polyacrylamide (10%) gel electrophoresis (100 μg protein/lane), transferred to nitrocellulose membranes, probed with the monoclonal anti-hsp72 antibody C92F3A-5, tagged with a ^{125}I-labeled goat-anti-mouse antibody, and autoradiographed. The protein extracts used for this Western blot, the RNA processed for the Northern blot shown in figure 3, and the hepatic nuclei used for the runoff analysis shown in figure 1 were all obtained from the same animal.

subsequent hepatic hsp70 gene expression appeared under these experimental conditions only after a certain threshold of cell damage had been reached.[12] We have previously shown that hepatic hsp72 gene transcription is enhanced after resuscitation in a porcine model of hemorrhagic shock,[10] suggesting that shock-induced accumulation of hsp72 mRNA[18] may, at least in part, be due to an increase in gene-specific transcriptional activity.

However, in the current study, accumulation of hsp72 RNA occurred, although nuclear runoff assays showed no detectable increase in hsp72 gene-specific transcription rates. An increase in steady-state RNA levels despite unchanged gene transcription rates would suggest that posttranscriptional mechanisms such as reduced degradation and/or increased stabilization of mRNA contribute to the RNA accumulation. Recently, regulatory motifs have been described in the 3′ untranslated region of several transiently expressed genes that are important in their posttranscriptional regulation, influencing mRNA translation[26] and degradation.[27–29] Theodorakis and Morimoto showed that hsp70 mRNA stability increased more than tenfold upon heat-shock of human HeLa cells in culture.[30] Peterson and Lindquist created a series of Drosophila hsp70 3′ deletion mutants that resulted in a marked alteration in mutant mRNA stability in transfected Drosophila cells.[31] An x-ray-induced 3′ end deletion mutant of the Drosophila melanogaster hsp70 gene showed increased mRNA stability during recovery from heat-shock compared to normal hsp70 mRNA.[32] Moseley *et al.* identified a heat-induced mechanism of posttranscriptional control of hsp70 gene expression in COS-1 cells transfected with a plasmid containing sequences encoding the human hsp70 3′ untranslated region placed downstream of a chloramphenicol acetyltransferase reporter gene.[33] These findings suggest that a regulatory element exists in the 3′ end of hsp70 mRNA that has the ability to stabilize hsp70 mRNA. Thus, it could be possible that such a 3′ end mRNA regulatory element is not only functionally active during heat stress *in vitro* but may also contribute to the hsp72 mRNA accumulation observed after anesthesia and surgery. However, control of gene expression can occur at different levels involving transcriptional (changes in gene transcription rate) and various posttranscriptional events that include not only changes in mRNA stability but also changes in translational efficiency and protein modification and export. Thus, steady-state RNA and protein levels represent a balance between transcription/RNA degradation and protein synthesis/proteolysis, reflecting the net effect of regulation on all different levels. Because it cannot be excluded that the sensitivity of the transcription assay was too low to detect small increases in hsp72 gene transcription that may have resulted in the increase in hsp72 RNA levels, and direct measurement of mRNA and protein stability/degradation rates is not possible in this large animal model, our data do not allow us to identify definitively where hsp72 gene expression was primarily regulated under these experimental conditions.

Hepatic Inter-α-trypsin Inhibitor Gene Expression

Increases in gene transcription rates of the acute-phase reactant α-Ti have been shown to occur under comparable conditions of celiotomy under general anesthesia.[14] The results of the current study that showed an accumulation of high levels of α-Ti RNA that were associated with pronounced increases in α-Ti gene transcription, up to 42-fold compared to baseline values, are consistent with this earlier observation and support the concept that increases in hepatic acute-phase gene expression are, at least in part, mediated by increases in gene transcription rates.[34,35]

Potential Implications of the Simultaneous Expression of Hepatic Heat-shock and Acute-phase Genes after Anesthesia and Surgery

There are several lines of evidence that suggest that both hepatic stress gene programs may be essential for

cell survival during and after stressful events because they contribute to the restoration of systemic (acute-phase response) and intracellular (heat-shock response) homeostasis. Acute-phase proteins can minimize the extent of tissue damage, as well as participate in tissue repair and regeneration. These functions are mediated by a variety of different acute-phase reactants, such as coagulation proteins that control blood clotting and fibrinolysis, and play essential roles in promoting wound healing, or proteinase inhibitors that are capable of neutralizing lysosomal hydrolases, thus controlling the activity of proinflammatory enzymes.[3,36]

There is accumulating evidence that activation of the heat-shock response can also significantly improve the way cells cope with stress. Heat shock is lethal to fibroblasts microinjected with antibodies against heat-shock proteins of the 70-kd family, whereas control cells survive a similar heat shock.[8] Overexpression of human hsp72 in (transfected) cultured murine cells affords protection against subsequent metabolic stress mimicking ischemia/reperfusion.[37] It has been shown recently that heat pretreatment improved long-term survival in a mouse endotoxin model and this improvement was correlated with increased hsp72 tissue levels.[38] Formation and decay of hsp72 demonstrated a time course that paralleled the survival curve from the endotoxin challenge.[38] Comparable results have been obtained in two different rat endotoxin models.[39–42]

Previous studies done in this laboratory have demonstrated that hemorrhagic shock followed by resuscitation triggered either hepatic acute-phase or heat-shock gene transcription, and that hsp72 gene transcription occurred at the expense of acute-phase gene transcription.[10] It has been postulated that a competition between two mutually exclusive patterns of hepatic stress gene expression, with the consequent shutdown of the expression of acute-phase genes seemingly essential to the restoration of systemic homeostasis after stressful events, could provide a basis for the development of multiple organ dysfunction syndrome.[10]

The results of the current study appear to demonstrate that general anesthesia and celiotomy are permissive for the simultaneous accumulation of hsp72 and α-Ti RNA. Simultaneous expression of heat-shock and acute-phase gene products in the liver under clinically relevant experimental conditions (because they mimic those of elective human surgical procedures) could well contribute to the restoration of both intracellular and systemic homeostasis and thus promote survival. Although Western blotting revealed increases in steady-state hsp72 protein levels, the absence of a specific anti-pig α-Ti antibody prevented testing whether co-expression of hsp72 and inter-α-trypsin inhibitor occurred also on the protein level in this experimental model.

Several factors may have influenced the simultaneous expression of hsp72 and α-Ti RNA in the liver. The combination of events including anesthesia (fasting, anesthetic agents, level of anesthesia) as well as all surgical maneuvers (vascular cannulations, tracheostomy, tracheal intubation, celiotomy) resulted in increased arterial norepinephrine concentrations, which agrees with clinical studies that showed two- to threefold increases in norepinephrine levels compared to preoperative values in patients undergoing abdominal, thoracic, or extremity vascular surgical procedures under general anesthesia.[43,44] Because it has been previously shown that the vascular heat-shock response is under adrenergic control,[45] and catecholamines can evoke high levels of interleukin-6, which in turn regulate hepatic acute-phase gene expression,[46] it is possible that the increased release of catecholamines may have influenced the joint hepatic expression of hsp72 and α-Ti RNA under these experimental conditions. Because ketamine hydrochloride can activate the sympathetic nervous system,[47] its use as an inducing agent may have also affected the results. However, replacement of ketamine hydrochloride with midazolam resulted in a similar hepatic co-expression of hsp72 and α-Ti RNA. Although control experiments with limited surgical instrumentation showed similar increases in RNA levels of the two genes, these data do not allow us to define the relative contributions of the various factors that may have influenced this response. Therefore, further *in vitro* and *in vivo* studies will be needed to precisely determine the specific effects of anesthesia (*e.g.*, anesthetic agents, techniques, level of anesthesia, duration) and the surgical maneuvers (*e.g.*, type, extent) on the simultaneous accumulation of hsp72 and α-Ti RNA in the liver that have been described here. This could ultimately lead to the development of prophylactic or therapeutic (*e.g.*, pharmacologic) strategies that induce hepatic stress gene expression to improve the aggregate response to injury.

In conclusion, celiotomy under general anesthesia elicits a novel pattern of hepatic stress gene expression in the pig that includes the simultaneous expression of hsp72 and α-Ti RNA. These findings could be of particular importance, because the coordinate induction of hepatic acute-phase and heat-shock genes may con-

tribute to the restoration of both intracellular and systemic homeostasis after anesthesia and surgery.

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