Laboratory Investigation

Heat shock protein 70 messenger RNA reflects the severity of ischemia/hypoxia-reperfusion injury in the perfused rat liver

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Abstract

Objectives

To determine whether ischemia-reperfusion and hypoxia-reoxygenation cause cellular damages and stress responses in an isolated perfused rat liver model. To determine whether the increased synthesis of stress protein messenger RNA reflects cellular injury.

Design

Prospective, controlled study.

Setting

Institutional laboratories.

Subjects

Male Sprague-Dawley rats.

Interventions

Isolated rat livers with cell free perfusion were exposed to various periods of ischemia-reperfusion or hypoxia-reoxygenation.

Measurements and Main Results

We measured hepatic oxygen consumption and alanine aminotransferase leakage from liver during perfusion. We analyzed the gene expression of heat shock protein 70, a major stress protein, of the liver by Northern blotting after perfusion. The expression of heat shock protein 70 messenger RNA augmented as the reperfusion period increased. The expression level after graded ischemia or hypoxia significantly correlated with the calculated hepatic oxygen debt ($r^2 = .737$; $p < .001$; $n = 21$), or with the accumulated alanine aminotransferase leakage from the liver ($r^2 = .509$; $p < .001$; $n = 21$).

Conclusions

These results suggest that the accumulation of heat shock protein 70 messenger RNA reflects the severity of ischemia-reperfusion and hypoxia-reoxygenation injuries, and that a stress response in reperfusion can be triggered without formed elements of blood.

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Key Words: ischemia; hypoxia; oxygen; reperfusion; cellular injury; stress protein; heat shock protein 70 (hsp 70); liver; rat; isolated perfusion

Reduction of oxygen supply and blood flow in pathologic conditions such as shock, hypoxia, or surgical maneuver causes temporal or permanent cellular injury in various organs. Functional impairments are reversible unless those insults exceed a certain limit [1,2]. How cells adapt to the pathologic alterations in their environment is a major issue in both physiologic and clinical fields [3]. It has become clear that all cells share a common molecular response to noxious stimuli, such as ischemia, hypoxia, increased temperatures, infection, and toxins, by increasing the synthesis of a family of proteins known as stress proteins or heat shock proteins [4].

The stress-70 protein family, including heat shock protein 70 and heat shock cognate protein 70, are major stress proteins and highly conserved in organisms ranging from prokaryotes to mammalians [5,6]. Some studies [7,8] have demonstrated their importance not only under noxious conditions but also in normal cellular processes such as protein folding, transport, assembly, and degradation. In stressed cells, heat shock protein 70 genes are activated, and increased heat shock protein 70 proteins are believed to stabilize

proteins denatured by various stressors and to repair their conformations [9]. It has also been demonstrated that constitutive over-expression of heat shock protein 70 proteins by introduction of human heat shock protein 70 gene into cultured cells resulted in tolerance against thermal and metabolic insults [10,11]. These observations indicate that heat shock protein 70 proteins have cytoprotective function.

The expression of stress proteins has two important aspects in clinical fields. One is their cytoprotective nature against noxious conditions, and the other is as a marker of cellular injury. Production of heat shock protein 70 against thermal injury is quantitatively correlated with the magnitude of stress and regulated transcriptionally and posttrans-criptionally at the messenger RNA level [12]. Heat shock protein 70 messenger RNAs become stable during injury and are degraded during the recovery period [13]. Therefore, accumulation of heat shock protein 70 messenger RNA may reflect the rate of protein synthesis, and that additional production of heat shock protein 70 proteins is needed for the stressed cells to recover their functions.

Ischemia/hypoxia and reperfusion/reoxygenation are common and serious stressors to the liver as well as other organs. In the present study, we analyzed the expression of heat shock protein 70 messenger RNA in the isolated perfused rat livers, which were exposed to various periods of ischemia or hypoxia. We confirmed stress response could be triggered even in cell-free reperfusion condition, and our results suggest that heat shock protein 70 messenger RNA level may reflect the severity of cellular injury.

MATERIALS AND METHODS

Cell Line.

A murine T cell hybridoma, 2B4, was cultured in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% fetal calf serum, 4 mM of glutamine, 50 mM of 2-mercaptoethanol, and 100 mg/mL of kanamycin. Cells were maintained at 37 degrees C in a 5% CO_2 incubator (Hirasawa, Tokyo, Japan). For heat shock treatment, cells were incubated at an atmospheric temperature of 42 degrees C or 37 degrees C (to serve as control) in a water bath for 2 hrs and harvested immediately.

Animals.

Male Sprague-Dawley rats, weighing 250 to 310 g, were obtained from Nihon SLC (Shizuoka, Japan). They were provided a standard laboratory chow and water ad libitum at 37 degrees C under a 12 hr-dark and 12 hr-bright cycle. They were fasted for 24 to 32 hr before the experiments. The experiments were approved by Animal Experimentation Committee in our institute, and performed in adherence to the National Institutes of Health guidelines for the use of experimental animals.

Recirculating Perfusion of the Rat Liver.

The recirculating perfusion system has been described previously [14]. The rats were anesthetized with an intravenous injection of sodium pentobarbital at 35 mg/kg through the dorsal penis vein; after a laparotomy, the inferior vena cava was ligated above the left renal vein and a polyethylene catheter (PE-240) was inserted in the portal vein. Nonrecirculating perfusion of the liver with glucose-free Krebs-Ringer-bicarbonate solution was immediately initiated and continued during the following surgical procedure. Thus, an anoxic period never exceeded 1 min. After a thoracotomy, another catheter (PE-260) was placed in the thoracic inferior vena cava. The liver was excised and placed in a modified Miller-type recirculating perfusion-aeration chamber with a temperature control system, by which the temperature of the perfusate was kept at 37 degrees C. The liver was perfused at a constant pressure of 12 cm H_2 O with Krebs-Ringer-bicarbonate solution containing 1% albumin and 10 mM D(+)-glucose equilibrated with 95% oxygen/5% CO_2 atmosphere. The recirculating perfusate volume was 200 mL. Flow rate to the liver and oxygen tensions of the perfusate in inflow and outflow chambers were monitored continuously with an electromagnetic flow transducer (Nihon Koden, Tokyo, Japan) and oxygen electrodes (Intermedical, Nagoya, Japan), respectively.

Hepatic oxygen consumption (VO_2) was calculated as the following formula: VO_2 (mL/min) = 0.0239 x (difference of PO_2 between inflow and out-flow chambers [torr] [kPa]) x (760 torr) [101.3 kPa] sup -1 x flow rate to the liver (mL/min).

Alanine aminotransferase (ALT) activity in the perfusate was determined at 37 degrees C, using an autoanalyzer (736, Hitachi, Tokyo, Japan).

Induction of Heat Stress.

As control experiments, some livers were exposed to heat stress instead of hypoxia and ischemia (n = 3). After the flow and VO_2 became stable, the liver was perfused at 42 degrees C at a constant pressure of 12 cm H_2 O for 90 mins, then harvested.

Induction of Hypoxia or Ischemia.

Livers were perfused with 95% oxygen/5% CO_2 at 37 degrees C at a constant pressure of 12 cm H_2 O for 20 mins, then subjected to various ischemia and hypoxia conditions.

For observation of the expression of heat shock protein 70 messenger RNA after ischemia followed by various reperfusion periods, rat livers were exposed to ischemic insults of 60-min perfusion at a pressure of 2 cm H_2 O at 37 degrees C followed by no, 15-, 30-, 60-, or 90-min reperfusion at a pressure of 12 cm H_2 O at 37 degrees C. These experiments were performed in triplicate, except for the 15-min reperfusion experiment.

For observation of the expression of heat shock protein 70 messenger RNA after various periods of ischemia-reperfusion or hypoxia-reoxygenation, rat livers were exposed to ischemic perfusions for 15, 30, and 60 mins at a pressure of 2 cm H_2 O, or to hypoxia for 15, 30, and 60 mins with 10% oxygen/90% nitrogen at a pressure of 12 cm H_2 O, followed by 60-min reperfusion at a pressure of 12 cm H_2 O with 95% oxygen/5% CO_2 at 37 degrees C. As a control, rat livers were perfused at a pressure of 12 cm H_2 O with 95% oxygen/5% CO_2 for 150 mins at 37 degrees C.

After completion of the perfusion, the liver was harvested immediately.

The hepatic oxygen debt during ischemia or hypoxia was calculated as: oxygen debt = duration of insults (mins) x ($VO_{2nor.}$ - $VO_{2isch.}$)/$VO_{2nor.}$, where $VO_{2nor.}$ is the mean oxygen consumption during perfusion with 95% oxygen/5% CO_2 at a pressure of 12 cm H_2 O at 37 degrees C in each experiment; and $VO_{2isch.}$ is mean oxygen consumption during the ischemia, hypoperfusion, or hypoxia period in each experiment.

Isolation of Total RNA and Northern Blot Analysis.

Immediately after harvest, the liver was snap-frozen in liquid nitrogen and stored at -70 degrees C. Total liver RNA was isolated using the acid guanidinium thiocyanate-phenol-chloroform method [15]. The frozen liver (0.1 g) with denaturing solution (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.1 M 2-mercaptoethanol, 0.5% N-lauroyl-sarcosine 1 mL/0.1 g tissue) was homogenized by polytron homogenizer. Then, 0.1 mL of 2 M sodium acetate pH 4, 1 mL of water-saturated phenol, and 0.2 mL of 49:1 chloroform/isoamyl alcohol were added sequentially to the homogenate and mixed thoroughly. The suspension was incubated for 15 mins on ice and centrifuged at 10,000 x g for 20 mins at 4 degrees C. The upper aqueous phase was transferred to a fresh tube and 1 volume of 100% isopropanol was added for RNA precipitation. The solution was placed for 1 hr at -20 degrees C and centrifuged at 10,000 x g for 20 mins at 4 degrees C. After discarding the supernatant, the RNA pellet was dissolved in 0.3 mL of denaturing solution and

transferred into a microcentrifuge tube. One volume of 100% isopropanol was added. The solution was placed for 1 hr at -20 degrees C and centrifuged at 10,000 x g for 10 mins at 4 degrees C. After discarding the supernatant, the RNA pellet was dissolved in 70% ethanol and incubated for 10 mins at room temperature. The solution was centrifuged at 10,000 x g for 10 mins at 4 degrees C. After discarding the supernatant, the RNA pellet was dried in a vacuum for 10 mins and dissolved in 100 micro L diethyl pyrocarbonate-treated water.

RNA from the murine cultured cells was prepared [16]. After washing two times with ice-cold phosphate-buffered saline, cells (1 x 10^7) were resuspended in a lysis buffer (140 mM sodium chloride, 10 mM Tris [centered dot] hydrogen chloride, 1.5 mM magnesium chloride, 250 micro g/mL heparin, 250 micro g/mL spermidine, 4 mL/1 x 10^7 cells), and 160 micro L of 5% nonidet P 40 was added. The suspension was centrifuged for 5 mins at 3000 x g at room temperature. The supernatant was transferred to a new tube with 40 micro L of 10% sodium dodecyl sulfate and 40 micro L of 0.5 M EDTA. Then, extractions with 4 mL of phenol, 2 mL of phenol and 2 mL of 24:1 chloroform/isoamyl alcohol, and 4 mL of 24:1 chloroform/isoamyl alcohol were done sequentially. The upper aqueous phase was transferred to a fresh tube, and 100 micro L of 5 M sodium chloride, 16.3 micro L of 1 M magnesium chloride, and 2.5 volume of 100% ethanol were added for RNA precipitation. The solution was placed for 1 hr at -20 degrees C and centrifuged at 10,000 x g for 20 mins at 4 degrees C. After discarding the supernatant, the RNA pellet was dissolved in 70% ethanol and incubated for 20 mins at -20 degrees C. The solution was centrifuged at 10,000 x g for 10 mins at 4 degrees C. After discarding the supernatant, the RNA pellet was dried in a vacuum for 10 mins and dissolved in 100 micro L diethyl pyrocarbonate-treated water.

RNA was quantified spectrophoto-metrically and 10 micro g of total RNA was applied to electrophoresis in each lane. Then, the RNA were transferred to nylon membranes (Hybond N sup +, Amersham, Buckinghamshire, UK). Hybridization was performed with^{32} P-labeled complementary DNA probe for human heat shock protein 70 [5] at 58 degrees C. The membranes were rehybridized with a mouse beta-actin complementary DNA probe. Autoradiography and densitometry were performed by Fuji Image Analyzer BAS-2000 system (Fuji Film, Tokyo, Japan).

Data Analysis.

Data were analyzed by one-way analysis of variance and two-way analysis of variance and two-way analysis of variance with repeated measures, followed by Fisher's least significant difference method and contrast test for multiple comparisons, respectively, with Super ANOVA software system (Abacus Concepts, Berkeley, CA). Regression analysis was performed by the least squares method. A $p < .05$ was considered statistically significant.

All the experiments with rat livers and cells in each condition were performed in triplicate unless otherwise mentioned. A representative Northern blotting in each condition was shown.

RESULTS

Isolated liver perfusion in the standard condition did not induce the expression of heat shock protein 70 messenger RNA.

(Figure 1) shows typical recordings of hepatic flow and PO_2 in inflow and outflow chambers.

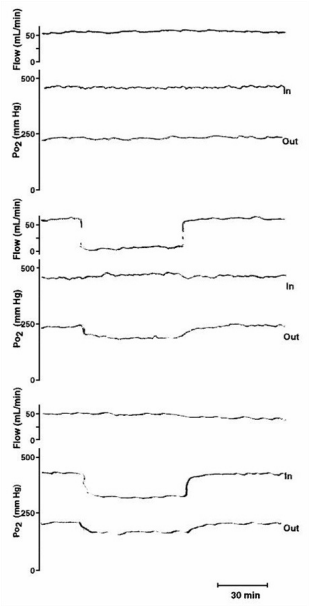

Figure 1:
Typical recordings of hepatic flow, and oxygen tension in inflow and outflow chambers. Top: Standard perfusion at 12 cm H_2 O saturated with 95% oxygen/5% CO_2 gas mixture; middle: ischemic perfusion at 2 cm H_2 O saturated with 95% oxygen/5% CO_2 gas mixture; bottom: hypoxic perfusion at 12 cm H_2 O saturated with 10% oxygen/90% nitrogen gas mixture.

The expression of heat shock protein 70 messenger RNA was examined in the perfused rat liver by Northern blot analysis. We also examined heat shock protein 70 messenger RNA expression in a murine cultured cell line 2B4 as a supportive control (Figure 2). Heat inducible transcripts, a 2.9-kilobase messenger RNA of the murine T cells cultured at 42 degrees C (lane 2), or a 2.6- and a 3.1-kb of the rat liver perfused at 42 degrees C (lane 6) were detected. These transcripts are considered to be heat shock protein 70 messenger RNAs in mouse or rat [16,17]. They were not observed in the standard perfusion condition with a 12 cm H_2 O pressure at 37 degrees C (lane 5) or in sham operations (lanes 3 and 4). The surgical preparation did not affect the expression of heat shock protein 70 messenger RNA.

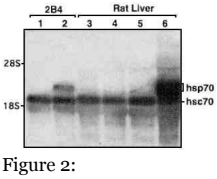

Figure 2:
Northern blot analysis for the expression of heat shock protein (hsp) 70 messenger RNA after heat stress in a murine T cell line 2B4, or in the perfused rat liver with a human heat shock protein 70 complementary DNA probe. Lane 1, 2B4 after 2-hr incubation at 37 degrees C; lane 2, 2B4 after 2-hr incubation at 42 degrees C; lane 3, a rat liver isolated

immediately after laparotomy; lane 4, a rat liver isolated after the surgical preparation without recirculating perfusion; lane 5, a rat liver after a 90-min perfusion at a pressure of 12 cm H_2 O at 37 degrees C; lane 6, a rat liver after a 90-min perfusion at a pressure of 12 cm H_2 O at 42 degrees C. Experiments were performed in triplicate. The positions of the 28S and 18S ribosomal RNAs are indicated at the left margin. Heat shock protein 70 and heat shock cognate protein 70 transcripts are indicated at the right.
A 2.3-kilobase transcript detected in all samples represents the messenger RNA of heat shock cognate gene, hsc70, which is expressed constitutively in all cells and is cross-reactive with heat shock protein 70 complementary DNA probe [17-19].

Ischemia/hypoxia and reperfusion induced expression of heat shock protein 70 messenger RNA.

(Figure 3) (top) shows expression of heat shock protein 70 transcripts in total RNAs isolated from the rat livers after different periods of reperfusion following ischemic perfusion at a pressure of 2 cm H_2 O for 60 mins at 37 degrees C. Even at 15 mins after reperfusion, heat shock protein 70 messenger RNAs were detected. No heat shock protein 70 transcripts were observed without reperfusion.

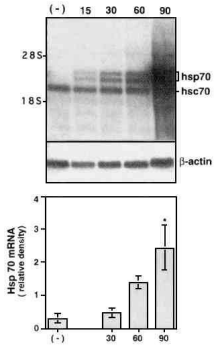

Figure 3:
Expression of heat shock protein (hsp) 70 messenger RNA after ischemia followed by various reperfusion periods in the perfused rat liver. No reperfusion (lane -), or 15 to 90-min reperfusion (lanes 15, 30, 60, and 90) (top). The membranes were rehybridized with a mouse beta-actin complementary DNA probe (middle). The relative amount of heat shock protein 70 messenger RNA (bottom). Values represent mean +/- SEM. *Value significantly ($p < .05$) different from the value at lane (-). Experiments were performed in triplicate, except for the 15-min reperfusion experiment.
To assess the expression of heat shock protein 70 gene quantitatively, we compared the relative amount of heat shock protein 70 transcripts to that of hsc70 by densitometric analysis because the expression of hsc70 messenger RNA is constitutive and less affected by heat or ischemia [6]. The results indicated that the amount of heat shock protein 70 transcripts increased gradually after reperfusion, and increased significantly higher after 90 mins compared with the amount of the transcripts without reperfusion (Figure 3, bottom). Similar results were obtained by the normalization of heat shock protein 70 messenger RNA to beta-actin.

(Table 1) shows parameters of control and ischemic perfusions of the liver. The perfusion with 95% oxygen/5% CO_2 at 37 degrees C at a constant pressure of 12 cm H_2 O provides a stable condition, in which the flow rate to and oxygen consumption of the liver are minimally affected. However, an earlier study [20] recommended that the perfused liver should be considered a useful system with a life span of [approximately]3 to 4 hrs. After 60-min ischemic perfusions (time 0 to 60 mins in Table 1), flow rate and VO_2 gradually became decreased and unstable. Therefore, we decided not to prolong perfusions beyond 120 mins from time 0 to exclude experimental variations.

	Flow (mL/min)		VO_2 (mL/min)	
Time (min)	Control	Ischemia	Control	Ischemia
0	54.7 ± 0.882	56.0 ± 1.732	0.477 ± 0.021	0.436 ± 0.003
60	51.7 ± 0.667[a]	2.0 ± 0.0[a,b]	0.411 ± 0.051[a]	0.026 ± 0.001[a,b]
90	50.7 ± 0.882	46.3 ± 2.028	0.423 ± 0.041	0.368 ± 0.019
120	49.2 ± 0.882	42.0 ± 4.359[a]	0.412 ± 0.041	0.341 ± 0.031
150	45.7 ± 2.728[a]	35.0 ± 8.718[a,b]	0.395 ± 0.048	0.316 ± 0.057[a]

[a]Values significantly ($p < .05$) different between the two groups; [b]values significantly ($p < .05$) different from baseline at time 0.
Ischemic perfusions at a pressure of 2 cm H_2O were performed in the ischemia group from time 0 to 60 mins.

Table 1:
Flow rate and oxygen consumption (VO_2) in isolated perfused rat liver (n = 3) (mean +/- SEM)
We next analyzed the effect of reduced oxygen transport on the expression of heat shock protein 70 messenger RNA in the liver by altering the perfusion pressure and oxygenation status of the perfusate (Figure 4). Ischemic perfusions at a pressure of 2 cm H_2 O (lanes 1 to 3) reduced oxygen transport to [approximately]7.4% of the control value at 12 cm H_2 O. Hypoxic perfusions with 10% oxygen/90% nitrogen (lanes 4 to 6) resulted in 4.8% oxygen transport of the control value with 95% oxygen. These perfusion conditions with reduced oxygen content were followed by reperfusion with 95% oxygen/5% CO_2 at a pressure of 12 cm H_2 O for 60 mins. These reoxygenations induced expression of heat shock protein 70 messenger RNA, which increased as the duration of insults became longer (lanes 1 to 3 and 4 to 6).

Figure 4:
Expression of heat shock protein (hsp) 70 messenger RNA after various periods of ischemia-reperfusion or hypoxia-reoxygenation in the isolated perfused rat livers. Lane C, control; lanes 1, 2, and 3, ischemic perfusions for 15, 30, and 60 mins respectively; lanes 4, 5, and 6, hypoxic perfusions for 15, 30, and 60 mins, respectively (top). The membranes were rehybridized with a mouse beta-actin complementary DNA probe (middle). The relative amount of heat shock protein 70 messenger RNA (bottom). Values represent mean +/- SEM. *Values are significantly ($p < .05$) different from the control; *values are significantly ($p < .05$) different between ischemia and hypoxia groups. Experiments were performed in triplicate.

The expression level of heat shock protein 70 messenger RNA correlates with the severity of hepatic damage.

Shortage of oxygen supply causes serious cellular injury in the liver as well as other organs. To evaluate the significance of the heat shock protein 70 messenger RNA level as a marker of cellular damage, we analyzed the relationships between the expression level of heat shock protein 70 messenger RNA and the amount of oxygen debt during ischemia and hypoxia, or accumulated ALT leakage from the liver in the perfusate. Oxygen debts represent the severity of the insult to the liver, and ALT leakage represents the damage of hepatocytes. Ischemia and hypoxia perfusions caused transient ALT leakage, which reduced to the same amount in control perfusions at time 120 mins (Figure 5). Therefore, we considered the accumulated ALT leakage in the perfusates reflected the liver damage at that point. The relative amounts of hsp 70 messenger RNA significantly correlated with both oxygen debts of the perfused liver (Figure 6, top) ($r^2 = .737$; $p < .001$; $n = 21$) and accumulated ALT activity (Figure 6, bottom) ($r^2 = .509$; $p < .001$; $n = 21$).

Figure 5:
Alanine aminotransferase (ALT) leakage in the perfusate. Open circles, control condition; solid squares, a 60-min ischemic condition; solid triangles, a 60-min hypoxic condition. Experiments were performed in triplicate, and values represent average alanine aminotransferase leakage for every 15 mins mean +/- SEM. *Values are significantly ($p < .05$) different from the value at time 0; #values are significantly ($p < .05$) different from the control group.

Figure 6:
Relationship between the expression level of heat shock protein (hsp) 70 messenger RNA and oxygen debt during ischemia and hypoxia (top) (r^2 = .737; p < .001; n = 21), or accumulated alanine aminotransferase leakage from the liver (bottom) (r^2 = .509; p < .001; n = 21). Samples are from ischemia and hypoxia experiments described in Figure 4. Oxygen debt was calculated as described in the Materials and Methods section.

DISCUSSION

Induction of stress proteins against transient ischemia has been reported in several animal models [21-24]. In these studies, temporal occlusion of blood vessels in vivo provided ischemic insults to the concerned organs. However, the degree of damage by the vascular occlusion is difficult to quantify because of the existence of collateral blood flow and the uncertainty of reestablishment of blood stream after release in vivo.

In the present study, we applied the isolated perfusion of rat liver to examine the relationship between the expression of heat shock protein 70 messenger RNA and the severity of cellular injury. The isolated perfused rat liver has been used to explore the physiology and pathophysiology of the liver [20], and enables us to expose a liver to different extents of stress by alternating the perfusion pressure, oxygenation status, and temperature of the perfusate. When a liver is perfused at a constant pressure of 12 cm H_2 O with Krebs-Ringer-bicarbonate solution equilibrated with 95% oxygen/5% CO_2 atmosphere at 37 degrees C as a standard condition in the present study, oxygen supply should exceed the oxygen demand in the perfused liver [20]. Since neither the surgical procedure nor the perfusion at the standard condition induced expression of heat shock protein 70 gene, we can estimate the effect of various stressors on gene activation of heat shock protein 70 in this model.

Perfusions not only with heat condition but also with ischemic conditions induced the expression of heat shock protein 70 messenger RNA in the isolated liver. When the perfusion pressure was reduced to 2 cm H_2 O, the flow rate to the liver decreased to <10% of control value at 12 cm H_2 O. Furthermore, an increase in oxygen extraction rate of the liver and an accumulation of lactate in the perfusate were also observed. These results imply that the perfused liver suffered from an ischemic insult.

In Figure 3, after ischemic perfusions, heat shock protein 70 transcripts were not seen without reperfusion, and accumulated gradually after reperfusion. Reperfusion and reoxygenation may have two opposite effects on stress responses. Reperfusion may supply metabolites for the synthesis of stress proteins, which function cytoprotectively. On the other hand, reperfusion generates reactive oxygens, which may cause cellular damage and accelerate the gene activation of stress proteins. Reactive oxidants that may injure the hepatocytes come from both intracellularly and extracellularly. While xanthine oxidase in the cytosol and electron transport system in the mitochondria provide sources of the intracellular reactive oxidants in the liver, it has been demonstrated that the hepatocyte has a high antioxidant capacity sufficient to detoxify their adverse effects [23]. Extracellular oxidants are generated by neutrophils and Kupffer cells. In the initial reperfusion phase, Kupffer cells are the most responsible for the oxidative injury in hepatocytes [25] and in the later phase, neutrophils induce hepatocellular damages [26]. In the present study, we applied blood-free perfusion system, which demonstrated that stress responses could be triggered in the reperfusion condition without blood cell components. In this condition, neutrophils were excluded. Therefore, we can focus on the initial phase of reperfusion injury. Cellular damages might arise mainly from metabolic disorders due to shortage of oxygen supply during ischemic perfusion, and reactive oxygens generated by Kupffer cells in the initial reperfusion phase.

Since we intended to clarify the significance of the expression of stress proteins as a marker of cellular injury, we analyzed the gene expression of heat shock protein 70 after various periods of ischemic or hypoxic insults followed by 60-min reperfusion in the perfused rat liver. The heat shock protein 70 messenger RNA level significantly correlated with the calculated hepatic oxygen debt and the amount of ALT, a specific liver enzyme leaking from damaged hepatocytes. These results suggest that expressed level of heat shock protein 70 messenger RNA reflects the severity of cellular injury.

Many kinds of stressors including hypoxia and oxidants induce the synthesis of stress proteins [19,27]. Adenosine triphosphate depletion and accumulation of denatured proteins due to damaging conditions make these stress responses active, and accumulated heat shock protein 70 proteins terminate the response though inactivation of heat shock transcription factor [28]. Thus, stress responses and induction of stress proteins are controlled under autoregulatory mechanism [28].

The heat shock protein 70 family seems to be a kind of sensor for cellular injury. The synthesis of heat shock protein 70 proteins is regulated both transcriptionally and posttranscriptionally through induction of the messenger RNA synthesis and degradation of the transcripts [12,13]. Therefore, accumulated heat shock protein 70 messenger RNA may reflect the shortage of hsc/heat shock protein 70 pool for adaptation against injurious conditions at real time rather than the amount of heat shock protein 70 protein. On the other hand, protein accumulation represents the consequences during a relatively long period. Stress response is a common and essential response for every cell in all organisms to maintain their life. If it can be estimated properly, it would be an ideal indicator for cellular injury, which could be applied to every cell in all organs. Therefore, we are able to find those advantages in measuring heat shock protein 70 messenger RNA as a marker of cellular injury.

We intended to show the possibility that heat shock protein 70 messenger RNA level might reflect the stress response at real time. In this study, we present evidence that stress response in liver against reperfusion/oxygenation can be triggered in cell free perfusion system and heat shock protein 70 messenger RNA may reflect the cellular injury. Although the method in this study is too complicated, in some clinical situations, such as surgical operations especially when blood vessels are reconstructed, impairments of blood flow could be estimated by the expression of heat shock protein 70 messenger RNA at real time. Moreover, manipulation of stress response would provide a new strategy for the treatment of cellular injury.

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