The Protective Role of Kupffer Cells in the Ischemia-Reperfused Rat Liver*

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Summary. Kupffer cells constitute a major source of the heme-degrading enzyme, heme oxygenase (HO). This study examined the roles of Kupffer cells in the modulation of accelerated heme catabolism in ischemia-reperfused rat livers. Livers from rats treated with or without liposome-encapsulated dichloromethylene diphenylphosphonate, a Kupffer cell-depleting reagent, underwent a 20-min ligation of the portal vein followed by reperfusion. The time course of the biliary output of bilirubin, the terminal heme-degrading product, and the expression of HO-1 mRNA and protein were monitored. HO-1 mRNA levels were elevated 3 to 12 h after ischemia/reperfusion in both control and Kupffer cell-depleted rats. Immunohistochemical analyses of control livers revealed that Kupffer cells expressed high levels of HO-1 while its expression in hepatocytes was low. In Kupffer cell-depleted livers, however, perportal hepatocytes displayed marked HO-1 expression. Under these conditions the two groups exhibited distinct profiles of biliary bilirubin excretion. In the controls, total bilirubin excretion increased 8-fold and peaked at 10 h after ischemia/reperfusion. In contrast, the Kupffer cell-depleting treatment resulted in a significant acceleration of the initial rise in bilirubin production, which peaked at 4 h. However, the total amount of bilirubin excreted within the initial 10 h after reperfusion was reduced by 50% as compared with that of the controls. In Kupffer cell-depleted rats, the levels of GOT and GPT as well as serum endotoxin concentrations were elevated after ischemia/reperfusion. These results suggest that Kupffer cells serve as an ischemia/reperfusion sensor that upregulates heme degradation and bilirubin excretion, and that Kupffer cells protect hepatocytes from gut-derived stressors—including endotoxin—following ischemia/reperfusion.

The liver is a major organ which performs the catabolism of free heme molecules and biliary excretion of their metabolites such as bilirubin. A temporary interruption of blood flow to the liver is often unavoidable during surgery for extensive liver injury or liver transplantation. Following ischemia and reperfusion, transient dysfunction of the liver occurs, probably due to the high susceptibility of the liver to ischemia and injury induced by reperfusion. Liver grafts occasionally cause serious impairment of bile secretion. Still, the bilirubin metabolism in ischemia/reperfusion liver damage is not fully understood. It is known that the physiological degradation of heme to bile pigments is mediated by heme oxygenase (Maines, 1988). Bilirubin is derived mainly from the breakdown of hemoglobin heme and partly from that of other heme proteins including cytochrome P450. The oxidative degradation of these heme molecules is carried out by two distinct isozymes, HO-1 and HO-2 (Shibahara et al., 1985; Maines, 1988). HO-2 is a constitutive form and known to be abundant in the brain, testis and liver of rodents and humans (Cruse and Maines, 1988), whereas HO-1, also known as heat shock protein-32 (Hsp 32), is induced by a variety of stressors such as LPS, hyperthermia (Ewing and

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cytokines (Rizzardini et al., 1993), and heavy metals (Maines and Kappas, 1997). Recent studies on HO-1 gene-targeted mice have revealed impaired heme catabolism and greater susceptibility to endotoxin than in the wild-type mice (Poss and Tonegawa, 1997a,b). The first human case of HO-1 deficiency was shown to display a liver dysfunction (Yachie et al., 1999).

It has recently been suggested that, upon endotoxemia or ischemia-reperfusion, heme oxygenase is markedly induced in the liver (Ito et al., 1997; Bauer et al., 1998). In these studies, HO-1 expression was induced not only in Kupffer cells but also in hepatocytes. However, little is known about the role of HO-1 in Kupffer cells or in hepatocytes during bilirubin production after ischemia/reperfusion.

Kupffer cells act as a potent effector of immune responses and metabolism. To elucidate the role of macrophages in the host defense and metabolism, various in vivo systems have been established. For example, liposome-encapsulated dichloromethylene diphosphonate (liposome-MDP) induces the apoptosis of macrophages (Naito et al., 1996). This could serve as a model for analyzing a broad spectrum of macrophage functions. Intravenous administration of liposome-encapsulated clodronate selectively depleted macrophages in the liver and spleen, but not in other tissues (Van Roojen and Nieuwenhuis, 1984; van Roojen et al., 1999a, b, 1990; Yamamoto et al., 1996). Our previous study showed a significant reduction of bilirubin production in Kupffer cell-depleted mice (Hirano et al., 2001). The present study was thus designed to reveal the intrahepatic expression of HO-1 and metabolism of bilirubin in ischemia/reperfusion liver injury using the Kupffer cell-depletion method.

MATERIALS AND METHODS

Animals

Male Wistar rats (250–300 g) were obtained from Charles River Inc., Japan (Tokyo) and maintained under standard conditions at the Laboratory Animal Center of Niigata University School of Medicine. All animals were allowed free access to laboratory chow and tap water. Rats were fasted 24 h before experiments. After the animals were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), the common bile duct was cannulated with a PE-10 tube for collecting the bile samples. The bile was collected using a fraction collector (ATTO, Tokyo). The ischemia/reperfusion model was produced by clipping the portal vein for 20 min, followed by reperfusion. Control rats received a sham operation. Some animals were splenectomized for 4 weeks. In all experiments, data are expressed as the mean ± SD of three rats.

Antibodies

The mouse monoclonal antibodies GTS-1 and GTS-2 were generated as described previously (Goda et al., 1998). GTS-1 and GTS-2 recognize HO-1 and HO-2, respectively. The mouse monoclonal antibodies Ki-M2R, ED2 (BMA Biomedicals, Augst, Switzerland), and TR-PM3 were used at a dilution of 1:100. They recognize rat macrophages. TR-PM3 was generously supplied by Prof. M. Takeya, Kumamoto University School of Medicine, Japan.

Preparation of liposome-encapsulated dichloromethylene diphosphonate (liposome-MDP)

Multilamellar liposomes were prepared as described previously (Yamamoto et al., 1996). In brief, 58.72 mg of phosphatidylcholine (Nihon Seika Inc., Hyogo, Japan), 8.75 mg of dehexadecyl phosphate and 24.75 mg of cholesterol (Wako, Tokyo) were mixed in chloroform in a round-bottom flask and dried under reduced pressure. The lipids were then hydrated in 5 ml of dichloromethylene diphosphonate (clodronate; kindly provided by Kissei Pharmaceutical Co., Nagano, Japan) and mechanically vortexed at room temperature. The liposomes were extruded through polycarbonate membranes (Nuclepore Co., CA, USA) of pore size 0.8 μm. Liposomes were finally suspended in 5 ml of phosphate-buffered saline (PBS). Aliquots of 0.5 ml of the suspension were injected intravenously 1 day before the experiments to deplete Kupffer cells.

Immunohistochemistry

The rat spleen, liver and testis were fixed for 4 h at 4°C in periodate-lysine-paraformaldehyde (PLP). The samples were sequentially washed for 4 h with PBS containing 10, 15, and 20% sucrose and were embedded in OCT compound (Miles, Elkhart, IN, USA). In some experiments, frozen sections from these organs were prepared and fixed with acetone. The sections (10 μm thick) were treated with avidin, biotin, and normal horse serum to minimize non-specific staining. They were incubated with a monoclonal antibody GTS-1 or GTS-2 dissolved in 1% BSA/PBS at a final concentration of 1 μg/ml for at least 2 h at 25°C or overnight at 4°C. After several washes with PBS, the sections were stained with anti-mouse IgG for 1 h (Vectastain Elite ABC kit, Vector, CA, USA). To prevent endogenous peroxidase reactions, the sections were pretreated with 0.3% H2O2 in cold methanol for 30 min and were subsequently incubated.
with avidin and HRP-conjugated biotin for 30 min. Finally, 0.1 mg/ml of 3,3'-diaminobenzidine (DAB) tetrahydrochloride was applied to sections for 5 min. In order to assign the HO-associated immunostaining, the sections were counterstained with methyl green after fixation with formaldehyde for 20 min, and slides were coverslipped with an aqueous mounting medium. The immunohistochemical staining patterns were examined using samples collected from three individual rats. To confirm the specificity of the immunohistochemical localization, antibodies preabsorbed with an excess of antigens were used. The numbers of HO-1-and ED2-positive cells with nuclei per 1 mm² were counted using a micrometer.

In separate sets of experiments, sections of the liver were double-stained to identify different types of nonparenchymal cells. To this end, anti-rat macrophage monoclonal antibodies, Ki-M2R, TR-PM3, and ED2 (BMA Biomedicals Ltd., Switzerland) were used to stain Kupffer cells. In brief, the sections were immunostained using the first primary antibodies and DAB as described above. After a wash with a glycine/HCl buffer for 1 h, the sections were incubated with the second primary antibodies, biotin-conjugated anti-mouse IgG, and a complex of avidin and HRP-conjugated biotin under the same conditions as those for the primary antibodies. Finally, DAB and nickel chloride were applied to the section to stain single positive cells for second primary antibodies purple blue and double positive cells for both primary antibodies dark brown, as described elsewhere (YAMAMOTO et al., 1996).

For immunocytochemical detection of HO, rat livers were fixed with PLP for 1 h and cut into 50-μm-
thick sections with a vibratome (DTK-1000, Dosaka, Osaka). After the inhibition of endogenous peroxidase activity by the method by ISÖBE et al. (1977), the sections were immunostained with 1 mg/ml of GTS-1 or -2. These samples were incubated with a peroxidase-conjugated anti-mouse IgG (Fab fragment, NA9310, Amersham, UK) and were developed with DAB. Thereafter, they were postfixed in 1% osmium tetroxide for 2 h. After dehydration in a graded series of ethanol solutions, the samples were processed through propylene and embedded in Epon 812 (E. Fullan, Inc., Latham, NY, USA). Ultrathin sections were stained with uranyl acetate and lead citrate, then examined with an electron microscope (H-800, Hitachi, Tokyo). Other reagents were obtained from Sigma Chemical (St. Louis, MO, USA).

Isolation of RNA and analysis of messenger RNA (mRNA) by reverse transcriptase-polymerase chain reaction (RT-PCR)

Total cellular RNA was isolated by guanidinium thiocyanate-phenol-chloroform extraction from the liver (YAMAMOTO et al., 1996). The total RNA (2 mg) was converted to cDNA by reverse transcription using a SuperScript Preamplification kit (Gibco BRL, Gaithersburg, MD, USA) with the oligo (dt) primer. PCR amplification was performed with a Program Temp Control System PC-800 (ASTEC, Tokyo). Amplification was achieved using an initial cycle of 50°C for 2 min and 95°C for 10 min, followed by 26 cycles of 95°C for 15 sec and 50°C for 1 min. PCR was carried out with the following primers: HO-1 forward: 5'-TGGCTTTTTCACCTTCCCG-3', reverse: 5'-TAAAATCCACTGCCCCAGGT-3', smooth muscle actin forward: 5'-TGGAAATCTCTGGCATGAGAC-3', reverse: TAAAACGCGACTCAGTACAGTCC-3'. Primers were made to order by BEX (Tokyo).

Measurements of bilirubin, GOT and GPT

The concentration of bilirubin in bile and serum: The blood was collected from the tail and serum was stored at -20°C for assay. The levels of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were determined by a TALQ kit (Wako, Osaka). Serum bilirubin fractions and bilirubin levels in the bile were measured using the Vitros 950RC (Ortho Clinical Diagnostics, NY, USA). Three samples in each group at each time point were measured.

Measurement of LPS

Blood samples were harvested into a pyrogen-free, heparin-coated plastic syringe by clean cardiac puncture and portal vein puncture. The concentrations of LPS in serum were measured by automated turbidimetry (Toxinometer MT-358; Wako, Osaka) with Limulus amoebocyte lysate containing β-glucan.

Statistics

The significance of the data was evaluated by Student's t-test.

RESULTS

Steady state expression of HO-1 in normal and reperfused liver

In the control rat liver, Kupffer cells were distributed mainly at the periphery of the liver lobules (Fig. 1A). Kupffer cells were completely eliminated in the liver by the administration of lipo-MDP (Figs. 1B, 2). After ischemia/reperfusion, the number and distribution of Kupffer cells were unchanged (Figs. 1C, 2). Kupffer cells were not detected in the Kupffer cell-depleted liver at 12 h after ischemia/reperfusion (Figs. 1D, 2).
A characteristic distribution of the heme oxygenase isozymes was observed in the control liver as reported previously (Godai et al., 1998; Hirano et al., 2001). HO-2 immunolabeling was distributed diffusely among the parenchymal regions in the liver (data not shown), whereas the HO-1 immunostaining was scattered in nonparenchymal regions. The density in the HO-1-positive cells was more predominant in periporal than perivenous regions (Fig. 3A). The HO-1-positively stained cells were polygonal, round, or irregular in shape. Cells expressing HO-1 were completely eliminated in the liver by administration of lipo-MDP (Fig. 3B). In the spleen, HO-1-positive cells were distributed mostly in the red pulp (data not shown). In the splenectomized rat liver, the number of Kupffer cells was similar, but the number of HO-1-positive cells was increased by 10%. In Kupffer cell-depleted rats, splenic red pulp macrophages and HO-1-positive cells were also depleted.

The distribution of HO-1 in Kupffer cells was confirmed by immunohistochemical double staining using GTS-1 and ED2, as reported previously (Godai et al., 1998; Hirano et al., 2001). However, the number of HO-1-positive cells was only about 10% that of ED2-positive cells (Figs. 1A, 3A). Localization of HC-1 in Kupffer cells was also confirmed by immunoelectron microscopy. The immunoreactivity in the endolasmic reticulum and nuclear envelope in Kupffer cells (Fig. 5A) was consistent with the biochemical detection of HO-1 in microsomal fractions. These findings indicate that Kupffer cells constitute a major cellular component responsible for the intra-

Fig. 3. Immunostaining for HO-1 in the rat liver. A. Small numbers of cells expressing HO-1 are distributed in the liver lobules in the control rat liver. B. HO-1-positive cells are not observed after Kupffer cell depletion. C. The number of HO-1-expressing Kupffer cells is increased at 12 h after ischemia/reperfusion. A few hepatocytes also express HO-1. D. Cells expressing HO-1 are detected in hepatocytes of the Kupffer cell-depleted rat liver at 12 h after ischemia/reperfusion. Immunohistochemistry using anti-HO-1 monoclonal antibody GTS-1. c Central vein. ×100
Expression of HO mRNAs in ischemia/reperfusion liver injury

Figure 6 shows levels of the HO-1 mRNA expression in control and Kupffer cell-depleted rat livers after ischemia/reperfusion. The expression was augmented in both groups of rats after ischemia/reperfusion. HO-1 mRNA levels paralleled the increases in HO-1 immunoreactivity. The expression of HO-2 in the liver of both groups of rats was unchanged before and after ischemia/reperfusion (data not shown).

Increased biliary function after ischemia/reperfusion

Figure 7 shows the time course of bile output after ischemia/reperfusion. Bile output was almost completely impaired by portal ischemia, but rapidly recovered after reperfusion (Fig. 7A). Bilirubin concentrations in bile and the amount of bilirubin produced in non-ischemia/reperfusion rats fell within a narrow range but increased after ischemia/reperfusion. The level of bilirubin production (bile flow × bilirubin concentration) increased remarkably up to 10 h after reperfusion in Kupffer cell non-depleted rats, whereas it was maximal at 6 h after reperfusion in Kupffer cell-depleted rats (Fig. 7B, C).

In splenectomized rats, bile production was greater by 10% before and after ischemia/reperfusion compared with the control (data not shown).

Serum bilirubin and GOT and GPT levels after ischemia/reperfusion

Serum GOT levels were within the normal range in normal and Kupffer cell-depleted rats. However, the levels of serum GOT in Kupffer cell-depleted rats increased by some ten times more than those at 12 h after reperfusion (Fig. 8A). Serum GPT levels were high in Kupffer cell-depleted rats, especially after ischemia/reperfusion (Fig. 8B). Serum bilirubin levels were slightly higher in Kupffer cell-depleted and non-depleted rats after ischemia/reperfusion compared with those without ischemia/reperfusion treatment, but these were not significantly different (Fig. 8C).

Serum endotoxin levels after ischemia/reperfusion

Serum levels of endotoxin were below the detectable level in control rats with or without ischemia/reperfusion. Endotoxin was not detected in the serum of Kupffer cell-depleted rats. However, it was detected in both portal and arterial blood obtained from hepatic HO-1 expression under normal physiological conditions. In the Kupffer cell-depleted rat liver, there were no HO-1-positive cells for more than 5 days (data not shown).

After ischemia/reperfusion, the number of HO-1-positive Kupffer cells increased along the sinusoid of the Kupffer cell non-depleted rat liver (Fig. 3C). HO-1-positive Kupffer cells increased in zones 1 to 2, and in zone 3 with time. A few hepatocytes expressed HO-1 with time. In Kupffer cell-depleted rats, no Kupffer cells were detected immunohistochemically, but HO-1 immunoreactivity was markedly upregulated in hepatocytes in zone 1 from 4 h after ischemia/reperfusion. With time, the HO-1-positive area was expanded in zones 2 and 3 (Rappaport classification), resulting in a predominant periportal expression of HO-1 in hepatocytes in Kupffer cell-depleted rats (Figs. 3D, 4). By immunoelectron microscopy, localization of HO-1 immunoreactivity was observed in the endoplasmic reticulum and nuclear envelope in hepatocytes (Fig. 5B). A sham operation or splenectomy did not influence the immunostaining.
Kupffer cell-depleted rats at 3 h after ischemia/reperfusion (Fig. 9).

DISCUSSION

The present study provided evidence that HO-1 expression was enhanced in Kupffer cells by ischemia/reperfusion, and that Kupffer cell-depletion resulted in a marked induction of HO-1 in hepatocytes after ischemia/reperfusion. Although bilirubin production was increased in both groups of rats, the time course of the production differed. Hepatocyte damage and transient endotoxemia following ischemia/reperfusion were observed in Kupffer cell-depleted rats. These results suggest that enhanced HO-1 expression in Kupffer cells and hepatocytes contributes to bilirubin overproduction, and that Kupffer cells play a protective role in limiting endotoxin and the oxidative impact to hepatocytes in ischemia/reperfusion liver injury.

HO-1 expression in Kupffer cells in normal liver after ischemia/reperfusion

An earlier study demonstrated that it takes only a few hours for unconjugated bilirubin to be metabolized and secreted into bile as conjugated bilirubin (OSROW et al., 1962). Another showed the levels of HO-1 expression in Kupffer cells were elevated immediately after the phagocytosis of heat-denatured erythrocytes and total bilirubin excretion increased about 2-fold at 5 h after the administration of heat-denatured erythrocytes (HIRANO et al., 2001). In the ischemia/reperfusion model, the hepatic HO-1 mRNA level reached a maximum after 4 h and HO-1 activity began to rise at this time while remaining high until 24 h postperfusion (YAMAGUCHI et al., 1995). In the present study, HO-1 mRNA and protein expression levels were rapidly enhanced and bilirubin excretion
increased by 8-fold at 10 h after ischemia/reperfusion. Immunohistochemical observations demonstrated that the number of HO-1-positive Kupffer cells increased in the control rat liver after ischemia/reperfusion. Because the number of Kupffer cells did not change after ischemia/reperfusion, the increased number of HO-1-positive cells reflects the enhanced expression of HO-1 in Kupffer cells. These results indicate that bilirubin metabolism is a rapid process associated with the expression of HO-1 in Kupffer cells.

**Bilirubin production and expression of HO-1 in hepatocytes**

It has been shown that about 75% of bilirubin is derived from the breakdown of senescent erythrocytes by macrophages (Maines, 1988). The importance of macrophages in bilirubin metabolism is demonstrated by the fact that the biliary output of bilirubin was reduced by 40% after macrophage depletion and the loading of heat-denatured erythrocytes did not increase the production of bilirubin in Kupffer cell-depleted rats (Hirano et al., 2001). Unconjugated bilirubin is released from macrophages into the circulation, enters the hepatocytes, and is processed to become conjugated. Besides macrophage-derived bilirubin, a fraction of the bilirubin is derived from the breakdown of heme proteins in hepatocytes. Therefore, heme protein-derived and macrophage-independent bilirubin may be a predominant source of bilirubin in the macrophage-depleted rats. Accordingly, the enhanced expression of HO-1 in hepatocytes may accelerate heme protein catabolism and bilirubin production in Kupffer cell-depleted ischemia/reperfusion rat livers. In contrast, the biliary

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**Fig. 7 A.** Volume of bile flow after ischemia/reperfusion. Bile flow is constant in both control and Kupffer cell-depleted rat livers for 12 h. Bile output is impaired by portal ischemia, but recovers after reperfusion in both groups of rats. **B.** Concentrations of bilirubin in bile. Bilirubin concentrations are increased remarkably in Kupffer cell non-depleted rats. Kupffer cell-depleted rats show mild increases in bilirubin concentration. **C.** Total amount of bilirubin in bile. Bilirubin production is increased remarkably in Kupffer cell non-depleted rats. Kupffer cell-depleted rats show mild increases in bilirubin production. *P < 0.01
Fig. 8. Bilirubin, GOT and GPT levels. GOT or GPT levels are increased in Kupffer cell-depleted rats after ischemia/reperfusion. Bilirubin concentrations do not show significant changes. *p<0.01

Fig. 9. Serum endotoxin levels. Serum levels of endotoxin are elevated in both portal and arterial blood in Kupffer cell-depleted rats at 3 h after ischemia/reperfusion. *p<0.01
output of bilirubin was similar between Kupffer cell-depleted and non-depleted rat livers up to 6 h after ischemia/reperfusion. This finding suggests that the intrahepatocyte heme protein pathway compensates for the loss of Kupffer cell-derived bilirubin for a limited period coupled with the enhanced expression of HO-1 in hepatocytes. A gradual decline in bilirubin production may represent a decreased pool of heme protein in hepatocytes in Kupffer cell-depleted liver. In the control rat liver, Kupffer cells are responsible for the production of larger amounts of bilirubin after ischemia/reperfusion.

Inductive mechanism of HO-1 and roles of Kupffer cells in ischemia/reperfusion liver injury

Kupffer cells are thought to constitute a major source of cytokines and pro-inflammatory mediators involving oxygen free radicals, and have been considered to play a primary role in the pathogenesis of liver disease resulting from ischemia/reperfusion. However, several recent reports have failed to show any beneficial effect of the depletion of Kupffer cells on organ function. Our previous study has shown that, at least initially after reperfusion under *ex vivo* conditions, Kupffer cells contribute little to oxidative tissue damage (Kumamoto et al., 1999). We confirmed that liver transaminase levels were elevated and HO-1 expression was induced in the hepatocytes of Kupffer cell-depleted rats. HO-1 is also referred to as heat shock protein 32 and is known to be induced in the liver by a variety of stressor stimuli such as cytokines, hypoxia, reactive oxygen species, NO, and bacterial toxins. It has been shown that the expression of HO-1 in hepatocytes is induced by endotoxin (lipopolysaccharide [LPS]), glutathione depletion, and hemorrhagic hypotension (Bauer et al., 1998). Hepatic ischemia/reperfusion inevitably results in intestinal congestion and may induce endothelial damage in the intestinal mucosa and alter the permeability for various molecules, including LPS. Reperfusion of the ischemic intestine is associated with acute hepatic injury characterized by the release of hepatocellular enzymes, a reduced bile flow, and metabolic dysfunction (Turnage et al., 1996). Olcay et al. (1974) demonstrated that systemic endotoxemia occurred during occlusion of the portal vein. Because levels of serum LPS and liver transaminase were elevated in Kupffer cell-depleted rats, it is likely that the enhanced absorption of LPS from the congested and reperfused intestine induced HO-1 expression in hepatocytes. Kupffer cells take up LPS and other agents primarily from the portal blood (Mathison and Ulevitch, 1979; Freudenberg et al., 1982; Van Bossuyt and Wisse, 1988), protecting hepatocytes from exposure to high levels of LPS and other oxidative stresses. In contrast, LPS-stimulated Kupffer cells produce TNF-α and lead to hepatocyte and endothelial damage (Arai et al., 1993), indicating that the activation of Kupffer cells is responsible for liver damage. However, it is not probable that the serum levels of endotoxin detected in the portal blood of Kupffer cell-depleted rats after ischemia/reperfusion are high enough to activate Kupffer cells, because hepatocyte damage was not observed in Kupffer cell non-depleted rats even after ischemia/reperfusion. Thus, the present results suggest that hepatocytes are exposed to various noxious agents in the portal blood in the Kupffer cell-depleted liver after ischemia/reperfusion and that Kupffer cells may play a protective role for hepatocytes by clearing away various gut-derived substances in ischemia/reperfusion liver injury.

In summary, ischemia/reperfusion enhances HO-1 expression and bilirubin production in the liver. The analysis of HO-1 expression will provide insight into Kupffer cell-hepatocyte interaction in damaged liver after ischemia/reperfusion. This model should prove useful for analyzing Kupffer cell-dependent and independent metabolism of bilirubin in vivo.

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REFERENCES


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