Arch Pharm Res Vol 28, No 12, 1386-1391, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

The Roles of Kupffer Cells in Hepatic Dysfunction Induced by Ischemia/Reperfusion in Rats

Joo-Yeon Jung[1] and Sun-Mee Lee

College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea and [1]Narcotic and Neuropharmacological Drug Division, Drug Evaluation Department, Korea Food and Drug Administration, Seoul 122-704, Korea

(Received September 22, 2005)

This study examined the role of Kupffer cells in altering the hepatic secretory and microsomal function during ischemia and reperfusion (Is/Rp). Rats were subjected to 60 min of hepatic ischemia, followed by 1 and 5 h of reperfusion. Gadolinium chloride ($GdCl_3$, 7.5 mg/kg body weight, intravenously) was used to inactivate the Kupffer cells 1 day prior to ischemia. Is/Rp markedly increased the serum aminotransferase level and the extent of lipid peroxidation. $GdCl_3$ significantly attenuated these increases. Is/Rp markedly decreased the bile flow and cholate output, and $GdCl_3$ restored their secretion. The cytochrome P450 content was decreased by Is/Rp. However, these decreases were not prevented by $GdCl_3$. The aminopyrine *N*-demethylase activity was decreased by Is/Rp, while the aniline *p*-hydroxylase activity was increased. $GdCl_3$ prevented the increase in the aniline *p*-hydroxylase activity. Overall, Is/Rp diminishes the hepatic secretory and microsomal drug-metabolizing functions, and Kupffer cells are involved in this hepatobiliary dysfunction.

Key words: Ischemia/reperfusion, Hepatic secretion, Drug metabolism, Gadolinium chloride, Kupffer cells

INTRODUCTION

Ischemia/reperfusion (Is/Rp) injury is responsible for organ damage in a variety of pathological events such as myocardial infarction, stroke, and graft failure after organ transplantation (Thurman *et al.*, 1988). Although the nature of Is/Rp injury has been studied extensively, the mechanisms by which organ damage occurs are unclear. Moreover, the diverse functional sequelae that take place in a multifunctional organ such as the liver are not completely understood.

In patients with chronic liver disease, the elimination of various drugs that are metabolized by the liver is often impaired (Huet and Villeneuve, 1983). Indeed, our recent studies suggest that abnormalities in the microsomal drug-metabolizing function associated with lipid peroxidation occur during hepatic Is/Rp *in vivo* (Lee *et al.*, 2000). Furthermore, studies aimed at preserving donor livers and those examining experimental perfusion models have reported that the secretory function, as indicated by the bile flow rate, is a practical and reliable index of the hepatic function (Lee and Clark, 1977).

Accumulating evidence suggests that reactive oxygen species (ROS) play a major role in producing the microvascular and parenchymal cell damage associated with the reperfusion of ischemic tissues (Drugas *et al.*, 1991). During the acute phase of reperfusion, ROS attack the biological membranes, such as the plasma membrane, mitochondria, and endoplasmic reticulum, which can cause direct cellular damage through protein oxidation and degradation, lipid peroxidation, and DNA damage (Pardini, 1995).

Kupffer cells, which are resident macrophages of the liver, produce several biologically toxic mediators including ROS, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and prostaglandin E_2. Moreover, these cells have been strongly implicated in the pathogenesis of hepatic injury in various animal models (Shiratori *et al.*, 1988). It was reported that the inactivation of Kupffer cells with gadolinium chloride inhibits the early injury phase during reperfusion (Jaeschke and Farhood, 1991). However, few studies have examined the direct effect of Kupffer cell activation on the hepatic I/R injury *in vivo*.

Therefore, this study examined the role of Kupffer cells

Correspondence to: Sun-Mee Lee, College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea
Tel: 82-31-290-7712, Fax: 82-31-292-8800
E-mail: sunmee@skku.edu

in specific abnormalities in terms of the hepatic secretory and microsomal function associated with Is/Rp injury.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 240-300 g were supplied by the Jeil Animal Breeding Company of Korea. The rats were acclimatized to laboratory conditions at Sungkyunkwan University for at least one week, and food and tap water were supplied *ad libitum*. The animals were kept in a temperature and humidity controlled room (25 ± 1°C and 55±5%, respectively) with a 12 h light-dark cycle.

Hepatic ischemic procedure

The rats were fasted for 24 h before the experiments but were allowed to drink tap water *ad libitum*. The rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), and the abdomen was opened via a midline incision. The left branches of the portal vein and hepatic artery were clamped to induce complete ischemia of the median and left hepatic lobes while the right lobes remained perfused in an attempt to prevent intestinal congestion. After 60 min ischemia, the clip around the left branch of the portal vein was removed, and the branch to the right lobes was ligated. This resulted in flow to all portal and hepatic arterial areas, except for a very small amount to the caudate lobe, which was directed through the previously ischemic lobes. At 0 h (immediately after unclamping), 1 h, and 5 h of reperfusion, PE-50 tubing was inserted into the bile duct to collect bile samples, and blood was taken from the abdominal aorta. The left and the median lobes of the liver were removed and used for the experiments. Sham-operated rats were prepared in a similar manner except that the clip was not placed in the left and the median lobes.

Pretreatment with $GdCl_3$ and experimental groups

In order to deplete the Kupffer cells *in vivo*, gadolinium chloride ($GdCl_3$, 7.5 mg/kg/mL, dissolved in sterilized physiological saline) was injected via the tail vein 24 h prior to Is/Rp (Hisama *et al.*, 1996). In the sham-operated rats, the same volume of physiological saline was injected in the rats. Four experimental groups were examined: (a) vehicle-treated sham, (b) $GdCl_3$-treated sham, (c) vehicle-treated Is/Rp (Is/Rp), and (d) $GdCl_3$-treated Is/Rp ($GdCl_3$ + Is/Rp). Because there were no differences in any of the parameters between vehicle- and $GdCl_3$-treated rats in the sham group, the results of (a) and (b) were pooled, and were referred to as sham.

Preparation of liver microsomes

The liver samples were removed and placed in an ice-cold distilled saline solution. They were then weighed, minced and homogenized with a Teflon pestle homogenizer in 4 volumes of a homogenizing buffer containing 1.15% (w/v) KCl and 50 mM Tris-HCl (pH 7.4). The whole homogenate was centrifuged at 10,000 × g at 4°C for 30 min and the resulting supernatant was then centrifuged at 105,000 × g at 4°C for 60 min. The microsomal pellets were resuspended in 10 volumes of a 1.15% (w/v) KCl solution, pH 7.6, containing 10 mM HEPES and 1 mM EDTA, aliquoted and frozen at -70°C until assayed.

Analytical procedures

The serum alanine aminotransferase (ALT) activity was determined using standard spectrophotometric procedures with a Sigma kit No. 52-UV (Sigma Chemical Co., St. Louis, MO, U.S.A.), and the bile cholate level was determined using the method of Irvin *et al.* (1944). Bilirubin was measured spectrophotometrically using an AM301-K kit (Nipponshaji, Tokyo, Japan). The lipid peroxide level was assayed using the thiobarbituric acid method of Masugi and Nakamura (1976), and 1,1,3,3-tetraethoxypropane was used as the standard. The cytochorome P450 (CYP450) content was calculated using the molar extinction coefficient of 104 mM^{-1} cm^{-1}, and the difference in absorbance between 450 and 500 nm measured using a differential spectrophotometer (Omura and Sato, 1964). The aminopyrine *N*-demethylase and aniline *p*-hydroxylase activity was determined by measuring the level of formaldehyde (Schenkman *et al.*, 1967) and *p*-aminophenol formation (Mieyal and Blumer, 1976), respectively. The protein content was estimated using the dye binding assay of Bradford (1976).

Statistical analysis

All the data are presented as the means ± SEM. Two-way analysis of variance (ANOVA) followed by a Bonferroni correction was used to determine the statistical significance of the differences among the experimental groups. A p value < 0.05 was considered to be significant.

RESULTS

Serum alanine aminotransferase activity and lipid peroxidation

The serum ALT level in the sham-operated rats was 65 ± 10 U/L, and increased to 296 ± 36 U/L 5 h after reperfusion. No changes in serum ALT in ischemic rats were observed after 60 min of ischemia without reperfusion (end of ischemia) compared with the pre-ischemic values (data not shown). However, the serum ALT activity increased to 1134 ± 170 U/L and 3102 ± 470 U/L 1 h and 5 h after reperfusion in the Is/Rp group, respectively. The $GdCl_3$ treatment suppressed the significant increase in

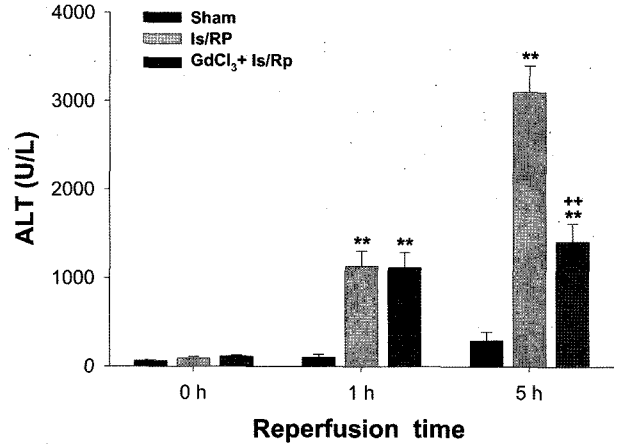

Fig. 1. Effect of $GdCl_3$ pretreatment on the serum ALT release after ischemia and subsequent reperfusion in rats. ** = Significantly different ($p<0.01$) from the sham group. ++ = Significantly different ($p<0.01$) from the Is/Rp group. Values are represented as the means ± SEM for 7-10 rats per group. Is/Rp, ischemia and reperfusion; $GdCl_3$, gadolinium chloride.

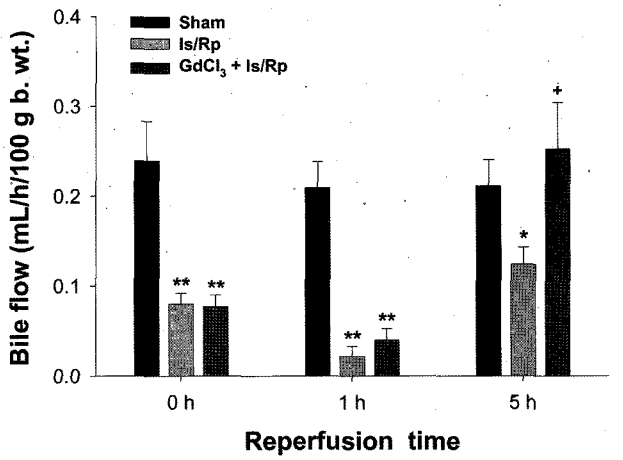

Fig. 3. Effect of $GdCl_3$ pretreatment on the bile flow after ischemia and subsequent reperfusion in rats. *,** = Significantly different ($p<0.05$, $p<0.01$) from the sham group. + = Significantly different ($p<0.05$) from the Is/Rp group. Values are represented as the means ± SEM for 7-10 rats per group. Is/Rp, ischemia and reperfusion; $GdCl_3$, gadolinium chloride.

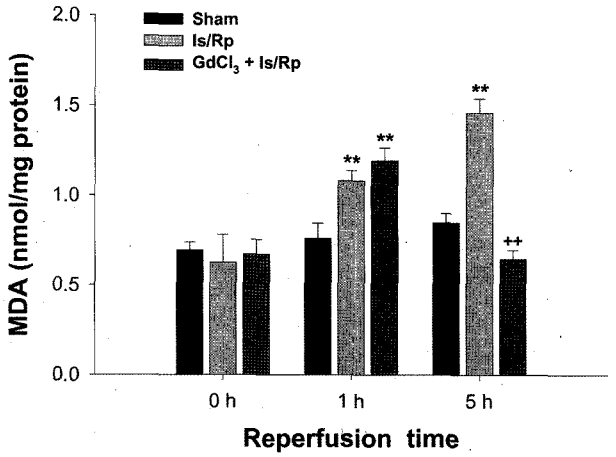

Fig. 2. Effect of $GdCl_3$ pretreatment on the lipid peroxidation in liver microsomes after ischemia and subsequent reperfusion in rats. ** = Significantly different ($p<0.01$) from the sham group. ++ = Significantly different ($p<0.01$) from the Is/Rp group. Values are represented as the means ± SEM for 7-10 rats per group. Is/Rp, ischemia and reperfusion; $GdCl_3$, gadolinium chloride.

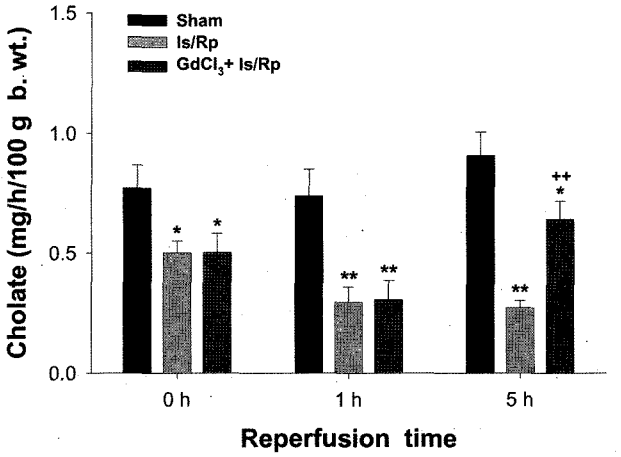

Fig. 4. Effect of $GdCl_3$ pretreatment on the cholate output after ischemia and subsequent reperfusion in rats. *,** = Significantly different ($p<0.05$, $p<0.01$) from the sham group. ++ = Significantly different ($p<0.01$) from the Is/Rp group. Values are represented as the means ± SEM for 7-10 rats per group. Is/Rp, ischemia and reperfusion; $GdCl_3$, gadolinium chloride.

ALT activity observed 5 h after reperfusion in the vehicle-treated ischemic rats (Fig. 1). In the sham-operated animals, the malondialdehyde (MDA) level in the liver ranged from 0.69 ± 0.04 to 0.85 ± 0.12 nmol MDA formed/mg protein throughout the experiments. In the vehicle-treated ischemic rats, the MDA level increased markedly to approximately 1.6 and 2.0 times the sham value 1 h and 5 h after reperfusion, respectively. The $GdCl_3$ treatment attenuated this elevation 5 h after reperfusion (Fig. 2).

Biliary secretion

The bile flow of the sham-operated rats was 0.21 ± 0.03 –0.24 ± 0.04 mL/h/100 g body weight throughout the experiments. Is/Rp reduced the flow to 0.08 ± 0.01, 0.02 ± 0.01, and 0.12 ± 0.02 mL/h/100 g body weight, at 0 h, 1 h, and 5 h after reperfusion, respectively. The $GdCl_3$ treatment significantly increased the level of bile secretion 5 h after reperfusion compared with that of the Is/Rp group (Fig. 3). Similar to bile flow, the cholate output, which is the bile acid-dependent secretion, was significantly reduced by Is/Rp. These decreases were markedly restored by the $GdCl_3$ treatment in the 5 h reperfusion groups (Fig. 4). The total bilirubin secretion was similar in all experimental groups (Fig. 5).

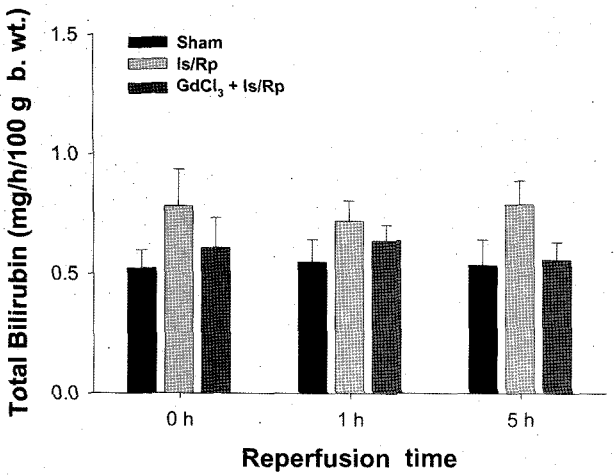

Fig. 5. Effect of $GdCl_3$ pretreatment on total bilirubin content after ischemia and subsequent reperfusion in rats. Values are represented as the means ± SEM for 7-10 rats per group. Is/Rp, ischemia and reperfusion; $GdCl_3$, gadolinium chloride.

Total cytochrome P450 content and drug metabolizing enzyme activity

The hepatic microsomal CYP450 content in the sham-operated rats ranged from 0.33 ± 0.03 to 0.39 ± 0.04 nmol/mg protein throughout the experiments. Is/Rp did not affect the CYP450 content 0 h and 1 h after reperfusion, but markedly reduced it to 0.24 ± 0.04 nmol/mg protein 5 h after reperfusion. The $GdCl_3$ treatment did not prevent this decrease. The aminopyrine *N*-demethylase activity in the sham-operated rats was 29.0 ± 0.8 – 33.5 ± 2.8 nmol HCHO/mg protein/10 min throughout the experiments, but Is/Rp markedly reduced the enzyme activity to 22.4 ± 0.5 and 21.7 ± 1.1 nmol HCHO/mg protein/10 min 1 h and 5 h after reperfusion, respectively. However, this decrease was not prevented by the $GdCl_3$ treatment. In contrast, the aniline *p*-hydroxylase activity in the Is/Rp group was significantly higher than in the sham groups, and this increase was prevented by the $GdCl_3$ treatment (Table I).

DISCUSSION

Kupffer cells preside in a strategic position within the liver sinusoids. They interact with hepatocytes, leukocytes, and various mediators from the gut. Therefore, they represent the first line of defense against microorganisms crossing the gut mucosal barrier and entering the portal circulation. They remove these xenobiotics via phagocytosis and the production of ROS (Bautista *et al.*, 1990). The pathogenic effect of activated Kupffer cells on hepatic cells has been reported but the reported findings are controversial. Bremer *et al.* (1994) have reported that Kupffer cells contribute to a global hepatocellular injury after Is/Rp. More recently, it was reported that the inhibition of Kupffer cell activation by $GdCl_3$ protects the liver from Is/Rp injury via a mechanism that reduces lipid peroxidation (Giakoustidis *et al.*, 2003). However, Imamura *et al.* (1995) demonstrated that Kupffer cell depletion does not reduce the reperfusion injury to sinusoidal endothelial cells, which was confirmed by transplantation experiments, in which there was no improved survival in the rats given the liver grafts depleted of Kupffer cells.

In the vehicle-treated ischemic rats, the ALT level increased 1 h after reperfusion and increased further 5 h after reperfusion. Interestingly, the level of lipid peroxidation also increased 1 h after reperfusion and further increased 5 h after reperfusion. Therefore, there is a temporal association between hepatic injury and lipid peroxidation. A $GdCl_3$ pretreatment prevented the lipid peroxidation and hepatic injury 5 h after reperfusion. These results suggest that Kupffer cell activation is partly related to the hepatocellular damage and free radical generation during ischemia and reperfusion.

Bile secretion is suppressed during ischemia and restored upon reperfusion after a short period of ischemia. These changes in bile secretion have been ascribed to a depression of the cellular ATP level. A correlation between the bile flow rate and the cellular ATP level was reported more than 30 years ago by Slater and Delaney (1970). Kamiike *et al.* (1985) reported that extent of hepatic injury can be assessed simply by monitoring the bile flow rate, which was believed to reflect the cellular ATP level.

In this study, bile secretion decreased rapidly after 60 min of ischemia. In the vehicle-treated ischemic rats, the level of bile secretion increased 5 h after reperfusion, and

Table I. Effect of $GdCl_3$ pretreatment on cytochrome P450 isozyme activities after FFx

Group	Cytochrome P450 (nmol/mg protein)			Aminopyrine *N*-demethylase (nmol HCHO/mg protein/10 min)			Aniline *p*-hydroxylase (nmol PAP/mg protein/15 min)		
	0 h	1 h	5 h	0 h	1 h	5 h	0 h	1 h	5 h
Sham	0.33 ± 0.03	0.34 ± 0.03	0.39 ± 0.04	29.0 ± 0.8	29.7 ± 0.9	33.5 ± 2.8	11.9 ± 1.1	10.0 ± 1.2	11.9 ± 1.4
Is/Rp	0.32 ± 0.03	0.33 ± 0.04	0.24 ± 0.04*	27.4 ± 0.9	22.4 ± 0.5**	21.7 ± 1.1**	12.8 ± 1.2	15.6 ± 1.4*	22.8 ± 2.0**
$GdCl_3$+Is/Rp	0.35 ± 0.05	0.36 ± 0.04	0.23 ± 0.03*	28.5 ± 1.1	21.8 ± 1.2**	23.5 ± 2.3*	11.4 ± 0.8	12.7 ± 0.8	14.7 ± 1.8††

*,** = Significantly different ($p<0.05$, $p<0.01$) from sham. †† = Significantly different ($p<0.01$) from Is/Rp.
Values are means ± SEM for 7-10 rats per group.

was found to be approximately 60% of those in the sham-operated rats. Bile is secreted from the hepatocytes via mechanisms that depend on the active transport of bile salts and a bile salt-independent pump. The transport of electrolytes (i.e., sodium, bicarbonate) and nonbile acid organic ions (i.e. glutathione, bilirubin) contribute to the bile salt-independent fraction of bile secretion (Forker, 1977). This study showed that the decrease in the cholate output until 5 h after reperfusion was paralleled by the inhibition of bile excretion. This suggests that the bile salt-dependent flow is more sensitive to the decrease in bile excretion that occurs during ischemia and reperfusion than to the bile salt-independent flow. A pretreatment with $GdCl_3$ attenuated the decrease in bile secretion 5 h after reperfusion.

Because CYP450 is strongly associated with the lipid of the endoplasmic reticulum, the lower levels of the cytochrome and its associated activities subsequent to the reperfusion of a previously ischemic liver tissue may be the result of the destruction of the cytochrome apoprotein, heme moiety, or lipid environment (Lee and Clemens, 1992). *In vivo* and *in vitro* studies have shown that the cytokines IL-1, IL-6, and TNF-α can mimic the down-regulation of the CYP450 gene product observed during infections or inflammation (Chen *et al.*, 1995). In the vehicle-treated ischemic rats, the CYP450 level was not changed until 1 h after reperfusion but was significantly lower 5 h after reperfusion. Pretreating the animals with $GdCl_3$ had no influence on the decrease in the CYP450 level. Such a decrease in the total content of CYP450 suggests that the overall activity of the CYP450-dependent oxidases would also decrease. As expected, the *N*-demethylase activity in the Is/Rp groups was significantly lower 1 h and 5 h after reperfusion, but $GdCl_3$ did not prevent it. In contrast, the aniline *p*-hydroxylase activity was elevated as a result of the ischemia and reperfusion. These increases were prevented by the $GdCl_3$ treatment, which is similar to the report by Kono *et al.* (2000), who found that the hydroxylation of the CYP2E1-specific substrate *p*-nitrophenol was higher in alcohol-induced liver disease, which was implicated with the activation of Kupffer cells. Even though the mechanisms responsible for these inconsistent alterations in the drug-metabolizing system have not been identified, the individual CYP450 isozymes appear to be affected differentially by Is/Rp injury, and Kupffer cells play a significant role.

These results suggest that the activation of Kupffer cells contributes to the hepatobiliary dysfunction after ischemia and reperfusion.

ACKNOWLEDGEMENT

This work was supported by the Brain Korea 21 project in 2005.

REFERENCES

Bautista, A. P., Meszaros, K., Bojta, J., and Spitzer, J. J., Superoxide anion generation in the liver during the early stage of endotoxemia in rats. *J. Leukoc. Biol.*, 48, 123-128 (1990).

Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72, 248-254 (1976).

Bremer, C., Bradford, B. U., Hunt, K. J., Knecht, K. T., Connor, H. D., Mason, R. P., and Thurman, R. G., Role of Kupffer cells in the pathogenesis of hepatic reperfusion injury. *Am. J. Physiol.*, 267, G630-G636 (1994).

Chen, J. Q., Strom, A., Gustafsson, J. A., and Morgan, E. T., Suppression of the constitutive expression of cytochrome P-450 2C11 by cytokines and interferons in primary cultures of rat hepatocytes: comparison with induction of acute-phase genes and demonstration that CYP2C11 promoter sequences are involved in the suppressive response to interleukins 1 and 6. *Mol. Pharmacol.*, 47, 940-947 (1995).

Drugas, G. T., Paidas, C. N., Yahanda, A. M., Ferguson, D., and Clemens, M. G., Conjugated desferoxamine attenuates hepatic microvascular injury following ischemia/reperfusion. *Circ. Shock*, 34, 278-283 (1991).

Forker, E. L., Mechanisms of hepatic bile formation. *Annu. Rev. Physiol.*, 39, 323-347 (1977).

Giakoustidis, D. E., Iliadis, S., Tsantilas, D., Papageorgiou, G., Kontos, N., Kostopoulou, E., Botsoglou, N. A., Gerasimidis, T., and Dimitriadou, A., Blockade of Kupffer cells by gadolinium chloride reduces lipid peroxidation and protects liver from ischemia/reperfusion injury. *Hepatogastroenterology*, 50, 1587-1592 (2003).

Hisama, N., Yamaguchi, Y., Ishiko, T., Miyanari, N., Ichiguchi, O., Goto, M., Mori, K., Watanabe, K., Kawamura, K., Tsurufuji, S., and Ogawa, M., Kupffer cell production of cytokine-induced neutrophil chemoattractant following ischemia/reperfusion injury in rats. *Hepatology*, 24, 1193-1198 (1996).

Huet, P. M. and Villeneuve, J. P., Determinants of drug disposition in patients with cirrhosis. *Hepatology*, 3, 913-918 (1983).

Imamura, H., Sutto, F., Brault, A., and Huet, P. M., Role of Kupffer cells in cold ischemia/reperfusion injury of rat liver. *Gastroenterology*, 109, 189-197 (1995).

Irvin, J. L., Johnston, C. G., and Kopara, J., A photometric method for the determination of cholate in bile and blood. *J. Biol. Chem.*, 153, 439-444 (1944).

Jaeschke, H. and Farhood, A., Neutrophil and Kupffer cell-induced oxidant stress and ischemia-reperfusion injury in rat liver. *Am. J. Physiol.*, 260, G355-G362 (1991).

Kamiike, W., Nakahara, M., Nakao, K., Koseki, M., Nishida, T., Kawashima, Y., Watanabe, F., and Tagawa, K., Correlation

between cellular ATP level and bile excretion in the rat liver. *Transplantation*, 39, 50-55 (1985).

Kono, H., Bradford, B. U., Rusyn, I., Fujii, H., Matsumoto, Y., Yin, M., and Thurman, R. G., Development of an intragastric enteral model in the mouse: studies of alcohol-induced liver disease using knockout technology. *J. Hepatobiliary Pancreat. Surg.*, 7, 395-400 (2000).

Lee, D. and Clark, D. G., Influence of ischaemic time on the production of bile by perfused rat liver. *Cryobiology*, 14, 37-44 (1977).

Lee, S.-M. and Clemens, M. G., Effect of alpha-tocopherol on hepatic mixed function oxidases in hepatic ischemia/reperfusion. *Hepatology*, 15, 276-281 (1992).

Lee, S.-M., Park, M.-J., Cho, T.-S., and Clemens, M. G., Hepatic injury and lipid peroxidation during ischemia and reperfusion. *Shock*, 13, 279-284 (2000).

Masugi, F. and Nakamura, T., Effect of vitamin E deficiency on the level of superoxide dismutase, glutathione peroxidase, catalase and lipid peroxide in rat liver. *Int. J. Vitam. Nutr. Res.*, 46, 187-191 (1976).

Mieyal, J. J. and Blumer, J. L., Accleration of autooxidation of human oxyhemoglobin by aniline and its relation to hemoglobin-catalyzed aniline hydroxylation. *J. Biol. Chem.*, 251, 3442-3446 (1976).

Omura, T. and Sato, R., The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.*, 239, 2370-2378 (1964).

Pardini, R. S., Toxicity of oxygen from naturally occurring redox-active pro-oxidants. *Arch. Insect. Biochem. Physiol.*, 29, 101-118 (1995).

Schenkman, J. B., Remmer, H., and Estabrook, R. W., Spectral studies of drug interaction with hepatic microsomal cytochrome. *Mol. Pharmacol.*, 3, 113-123 (1967).

Shiratori, Y., Kawase, T., Shiina, S., Okano, K., Sugimoto, T., Teraoka, H., Matano, S., Matsumoto, K., and Kamii, K., Modulation of hepatotoxicity by macrophages in the liver. *Hepatology*, 8, 815-821 (1988).

Slater, T. F. and Delaney, V. B., Liver adenosine triphosphate content and bile flow rate in the rat. *Biochem. J.*, 116, 303-308 (1970).

Thurman, R. G., Marzi, I., Seitz, G., Thies, J., Lemasters, J. J., and Zimmerman, F., Hepatic reperfusion injury following orthotopic liver transplantation in the rat. *Transplantation*, 46, 502-506 (1988).