Arch Pharm Res Vol 26, No 1, 47-52, 2003

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

The Roles of Kupffer Cells in Hepatocellular Dysfunction after Femur Fracture Trauma in Rats

Woo-Yong Lee and Sun-Mee Lee

College of Pharmacy, Sungkyunkwan University, 300 Cheoncheon-dong, Jangan-gu Suwon, Gyeonggi-do 440-746, Korea

(Received September 25, 2002)

The aim of this study was to investigate the effects of trauma on alterations in cytochrome P450 (CYP 450)-dependent drug metabolizing function and to determine the role of Kupffer cells in hepatocellular dysfunction. Rats underwent closed femur fracture (FFx) with associated soft-tissue injury under anesthesia, while control animals received only anesthesia. To deplete Kupffer cells *in vivo*, gadolinium chloride ($GdCl_3$) was injected intravenously via the tail vein at 7.5 mg/kg body wt., 1 and 2 days prior to FFx surgery. At 72 h after FFx, serum alanine aminotransferase (ALT) activity was increased, and this increase was attenuated by $GdCl_3$ pretreatment. Serum aspartate aminotransferase (AST) and lipid peroxidation levels were not changed by FFx. Hepatic microsomal CYP 450 content and aniline *p*-hydroxylase (CYP 2E1) activity were significantly decreased; decreases that were not prevented by $GdCl_3$. The level of CYP 2B1 activity was decreased by Kupffer cell inactivation, but not by FFx. There were no significant differences in the activities of CYP 1A1, CYP 1A2 and NADPH-CYP 450 reductase among any of the experimental groups. Our findings suggest that FFx trauma causes mild alterations of hepatic CYP 450-dependent drug metabolism, and that Kupffer cells are not essential for the initiation of such injury.

Key words: Femur fracture, Hepatocellular dysfunction, Gadolinium chloride, Cytochrome P450

INTRODUCTION

Although systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) continue to plague critically ill and injured surgical patients with a mortality of 50-80%, the mechanism and available treatment of the sequential injury has not been clearly identified (Deitch, 1992). SIRS can be seen in many conditions such as trauma, pancreatitis, burns, infection or major elective surgery (Bone *et al.*, 1992). Trauma remains one of the important sources leading to SIRS and subsequent multiple organ failure (MOF), and this remote organ injury is mainly associated with the immunologic dissonance of patients themselves (Bone, 1996).

Macrophages play a crucial role in regulating host defense mechanisms after trauma and sepsis (Baker and Huynh, 1996), and their activation initiates inflammatory responses to injury (Nielsen *et al.*, 1994). Kupffer cells constitute 80% of the fixed macrophages and reside at a strategic position in hepatic sinusoids where they interact with hepatocytes, other leukocytes, and variable mediators. Previously, Kupffer cells were shown to mediate responses to endotoxemia (Brown *et al.*, 1997), burns (Wu *et al.*, 1995), ischemia/reperfusion (Bradham *et al.*, 1997), and sepsis (Koo *et al.*, 1999), and to regulate the synthesis of acute phase proteins by hepatocytes. Furthermore, Kupffer cells produce important inflammatory mediators, including tumor necrosis factor (TNF-α), superoxide, nitric oxide (NO), prostaglandin E_2 (PGE_2), and other cytokines (Cutrin *et al.*, 1998). However, few studies have examined the direct effect of trauma on Kupffer cell function *in vivo*.

The activity of the liver in metabolizing and eliminating various drugs often decreases with infectious disease (Renton, 1986). In rodents suffering from bacterial infection (Batra *et al.*, 1987) or challenged with interleukin-1 (IL-1) and other inflammatory cytokines (Shedlofsky *et al.*, 1987), hepatic levels of heme-containing cytochrome P450 (CYP

Correspondence to: Sun-Mee Lee, Ph. D., College of Pharmacy, Sungkyunkwan University, 300 Cheoncheon-dong, Jangan-gu, Suwon, Gyeonggi-do 440-746, Korea
E-mail: sunmee@yurim.skku.ac.kr

450) and drug-metabolizing activity in microsomes both decreased. The liver has high levels of CYP 450 isoforms with different specificity for various substrates. More recently, it was reported that CYP 450 isoforms were differentially modulated by NO in endotoxemic rats after administration of lipopolysaccharide (LPS) (Takemura *et al.*, 1999). However, limited information is available about the effect of trauma on changes in the activities of CYP 450 isozymes.

Therefore, the aim of the present study was to investigate the role of Kupffer cells in altered hepatic drug metabolism after trauma.

MATERIALS AND METHODS

Chemicals

Ethoxyresorufin, methoxyresorufin, pentoxyresorufin, aniline, NADPH, HEPES, thiobarbituric acid, alanine aminotransferase (ALT) kit, and aspartate aminotransferase (AST) kit were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals used were of reagent grades and were locally and commercially available.

Animals

Male Sprague-Dawley rats weighing 240 ± 60 g were supplied by Jeil Animal Breeding Company of Korea and were acclimatized to laboratory conditions at Sungkyunkwan University for at least one week, with food and tap water 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.

Femur fracture

Anesthesia was induced by the intraperitoneal injection of xylazine hydrochloride (20 mg/kg) and ketamine hydrochloride (50 mg/kg). While anesthetized, rats were randomized to receive closed femur fracture (FFx) with associated soft-tissue injury as described by Schirmer *et al.* (1988). Control rats received anesthesia and shaving only. Shortly, two Kelly clamps were applied at the proximal and distal ends of the left femur, and sufficient torsion was given to fracture the femur midshaft. Blunt fracture was ascertained by palpation. After the procedure, sterilized physiological saline (10 ml/100 g of body wt.) was administered subcutaneously in the dorsal wall. Rats had access to food and water *ad libitum* during recovery from anesthesia. At 72 h after FFx, blood was taken from the abdominal aorta. The whole liver was removed and used for the experiment.

Pretreatment with $GdCl_3$ and experimental groups

To deplete Kupffer cells *in vivo*, gadolinium chloride ($GdCl_3$, 7.5 mg/kg/ml, dissolved in sterilized physiological saline) was injected via the tail vein, 1 and 2 days before FFx surgery. In the control rats, physiological saline was injected in the same volume and manner as for $GdCl_3$. Four experimental groups were studied: (a) vehicle-treated control (b) $GdCl_3$-treated control, (c) FFx alone, and (d) $GdCl_3$-treated FFx.

Preparation of liver microsomes

Liver samples were removed and placed in ice-cold distilled saline solution. They were then weighed, minced and homogenized with a teflon pestle homogenizer in 4 volumes of 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 for 30 min at 4°C and the resulting supernatant was then centrifuged at 105,000 × g for 60 min at 4°C. The microsomal pellets were resuspended with 10 volumes of 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. The content of microsomal protein was determined using the Bio-Rad protein assay reagent with bovine serum albumin as a standard.

Serum ALT and AST

ALT and AST activities were determined by spectrophotometric procedures by Sigma kits 51-UV and 52-UV, respectively.

Lipid peroxidation

Lipid peroxidation was estimated by measuring the levels of malondialdehyde (MDA), an end product of lipid peroxidation. MDA was determined by the levels of thiobarbituric acid reactive substances (TBARS) using the method of Buege and Aust (1978). One volume of microsome was mixed with 2 volumes of 0.25 N HCl solution containing 15% (w/v) trichloroacetic acid and 0.375% (w/v) thiobarbituric acid. The mixture was heated for 30 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation at 1,000 × g for 10 min. The absorbance of the supernatant was measured at 535 nm.

Cytochrome P450 content and NADPH-CYP 450 reductase activity

Total CYP 450 content was measured by the method of Omura and Sato (1964) and calculated by using a molar extinction coefficient of 91 $mM^{-1}cm^{-1}$ for the spectral difference between 450 nm and 490 nm in a differential spectrophotometer. The activity of NADPH-CYP 450 reductase was determined by its NADPH-cytochrome *c* reductase activity. Briefly, it was measured by the reduction rate of cytochrome *c* with an extinction coefficient 21 $mM^{-1}cm^{-1}$ at 550 nm following the addition of 0.1 mM NADPH (Vermillion and Coon, 1978).

Cytochrome P450 isozyme activities

The hydroxylase activity of CYP 2E1 was measured with aniline as the substrate according to the procedure of Scherkman *et al.* (1967). The reaction mixture containing 50 mM potassium phosphate buffer, 200 mM aniline and microsome was initiated by the addition of 10 mM NADPH and terminated by the addition of ice-cold 20% trichloroacetic acid. After removal of protein by centrifugation at 1,000 × g for 10 min, 1 ml of separated supernatant was mixed with 100 μl of 20% phenol/NaOH. After 30 min, the changed color of *p*-aminophenol was monitored at 630 nm spectrophotometrically. CYP 1A1, CYP 1A2 and CYP 2B1 specific activities were determined by ethoxyresorufin *O*-deethylase (EROD), methoxyresorufin *O*-demethylase (MROD) and pentoxyresorufin *O*-dealkylase (PROD) activities, respectively, according to the methods of Pohl and Fouts (1980) and Burke *et al.* (1985) with slight modifications. The reaction mixture contained 100 mM Tris-HCl buffer, pH 7.5, 25 mM $MgCl_2$, 5 μM substrate (ethoxyresorufin, methoxyresorufin or pentoxyresorufin) and microsome. The reaction was started by the addition of 1 mM NADPH and incubated at 37°C for 10 min. After incubation the reaction was stopped by the addition of ice-cold methanol and the mixture was centrifuged at 2,000 × g for 10 min. Fluorescence of resorufin in the supernatant was measured at excitation and emission wavelengths of 550 nm and 580 nm, respectively.

Statistical analysis

All data are presented as means ± SEM. One-way analysis of variance (ANOVA) followed by Dunnett's *t*-test was used to determine the statistical significance of the differences between experimental groups. A p value < 0.05 was deemed to be significant.

RESULTS

Serum ALT and AST

The serum ALT levels in vehicle-treated control rats and $GdCl_3$-treated control rats were 27.5 ± 2.0 U/L and 32.0 ± 2.9 U/L, respectively. However, the serum ALT activity in FFx rats significantly increased to 37.5 ± 2.7 U/L; an increase which was significantly suppressed by $GdCl_3$ pretreatment (Fig. 1). No significant differences were observed in serum AST activity among any of the experimental groups (Fig. 2)

Lipid peroxidation

As shown in Fig. 3, the liver MDA level was 0.98 ± 0.03 nmol/min/mg protein in the control rats. No changes were observed in MDA levels of FFx rats compared with those of control values. $GdCl_3$ pretreatment did not affect the MDA level after FFx.

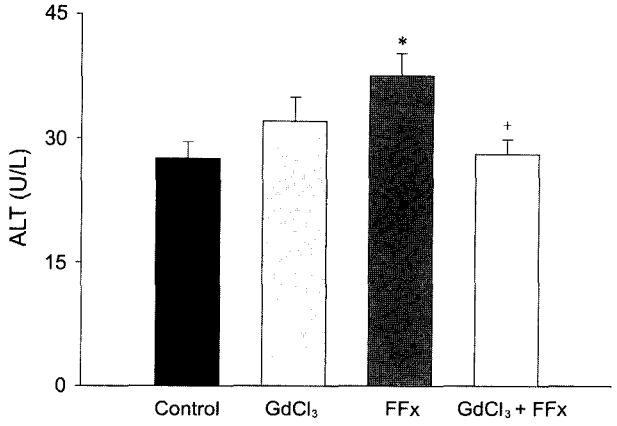

Fig. 1. Effect of $GdCl_3$ pretreatment on serum alanine aminotransferase (ALT) activity after FFx in rats. * = Significantly different ($p<0.05$) from controls. + = Significantly different ($p<0.05$) from FFx alone group. Values are means ± SEM for 9-11 rats per group.

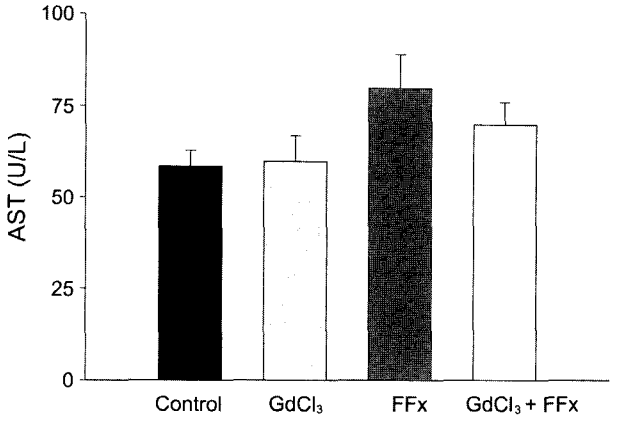

Fig. 2. Effect of $GdCl_3$ pretreatment on serum aspartate aminotransferase (AST) activity after FFx in rats. Values are means ± SEM for 9-11 rats per group.

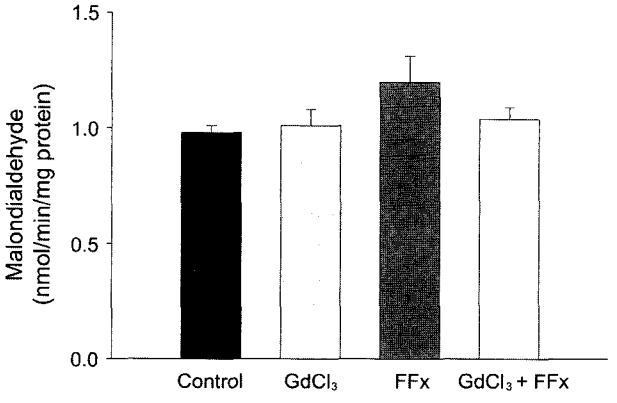

Fig. 3. Effect of $GdCl_3$ pretreatment on hepatic microsomal lipid peroxidation after FFx in rats. Values are means ± SEM for 9-11 rats per group.

Total CYP 450 content and NADPH-CYP 450 reductase activity

The results of total hepatic microsomal CYP 450 content

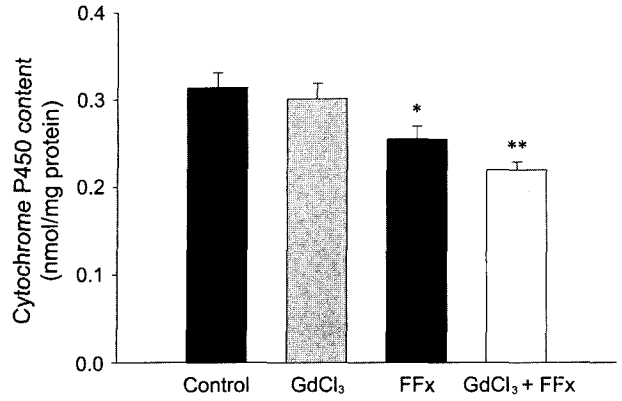

Fig. 4. Effect of $GdCl_3$ pretreatment on total hepatic microsomal cytochrome P450 content after FFx in rats. *,** = Significantly different ($p<0.05$ and $p<0.01$) from controls. Values are means ± SEM for 9-11 rats per group.

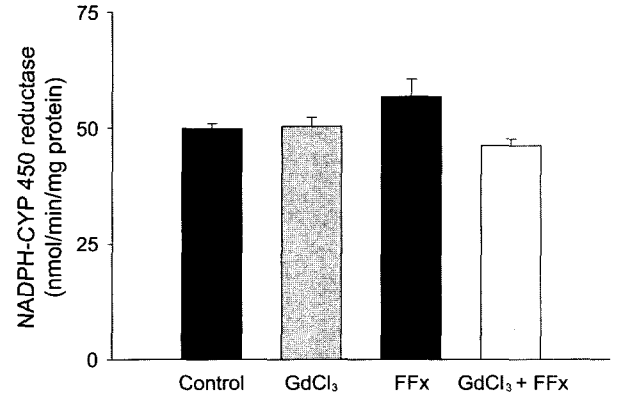

Fig. 5. Effect of $GdCl_3$ pretreatment on hepatic microsomal NADPH-cytochrome P450 reductase activity after FFx in rats. Values are means ± SEM for 9-11 rats per group.

are presented in Fig. 4. In the vehicle-treated control rats and the $GdCl_3$-treated control rats, they were 0.31 ± 0.02 and 0.30 ± 0.02 nmol/mg protein, respectively. Total CYP 450 content was found to significantly decrease in FFx-operated rats and decrease even further in $GdCl_3$-pretreated FFx rats.

As shown in Fig. 5, the NADPH-CYP 450 reductase activity was not significantly different among any of the experimental groups.

CYP 450 isozyme activities

The results of the activities of CYP 450 isozymes are shown in Table I. There were no significant differences in ethoxyresorufin *O*-deethylase (CYP 1A1) and methoxyresorufin *O*-demethylase (CYP1A2) activities among any of the experimental groups. However, aniline hydroxylase (CYP 2E1) activity was significantly decreased in the FFx alone group (0.184 ± 0.023 nmol/min/mg protein) compared with that of the vehicle-treated control group (0.258 ± 0.020 nmol/min/mg protein); a decrease that was not prevented by $GdCl_3$ pretreatment. Pentoxyresorufin *O*-dealkylase (CYP 2B1) activity was decreased by $GdCl_3$ pretreatment, but not by FFx.

DISCUSSION

Kupffer cells are the first line of defense against external microorganisms that cross the mucosal barrier of the gastrointestinal tract and enter portal blood. They remove these xenobiotics by phagocytosis and the production of reactive oxygen species (Bautista *et al.*, 1990). Kupffer cells also modulate synthesis of acute phase proteins by hepatocytes and release powerful inflammatory mediators including tumor necrosis factor (TNF-α), nitric oxide (NO) and a variety of other cytokines (Cutrin *et al.*, 1998). Brock *et al.* (1999) have shown that Kupffer cells contribute to global hepatocellular injury after trauma, i.e. limb ischemia/reperfusion. More recently, it was reported that FFx trauma increased the phagocytosis and superoxide formation by Kupffer cells, whereas it decreased the release of TNF-α and NO from Kupffer cells (Huynh *et al.*, 1997). In addition, FFx increased the production of PGE_2, associated with the suppression of inflammatory response. Such changes suggest an adaptation of Kupffer cells to a more antimicrobial and less proinflammatory phenotype after tissue trauma (Huynh *et al.*, 2000). Despite numerous investigations, a clear pattern of Kupffer cell alteration after injury remains to be elucidated. Accordingly, we tested the hypothesis that blunt femur fracture activates Kupffer cells *in vivo*, leading to altered drug metabolism.

Liver failure is one of the hallmarks of multiple organ failure (MOF) syndrome. To study the effect of trauma on

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

Group	Ethoxyresorufin *O*-deethylase (pmol/min/mg protein)	Methoxyresorufin *O*-demethylase (pmol/min/mg protein)	Pentoxyresorufin *O*-dealkylase (pmol/min/mg protein)	Aniline *p*-hydroxylase (nmol/min/mg protein)
Control	45.0 ± 1.3	18.7 ± 0.7	13.0 ± 1.8	0.26 ± 0.02
$GdCl_3$	46.3 ± 4.3	19.9 ± 1.1	7.3 ± 0.9*	0.23 ± 0.02
FFx	40.9 ± 5.5	18.7 ± 1.5	10.2 ± 1.4	0.18 ± 0.02*
$GdCl_3$ + FFx	38.2 ± 4.8	16.0 ± 1.1	6.5 ± 0.7**	0.13 ± 0.01**

*,**=Significantly different ($p<0.05$ and $p<0.01$) from controls. Values are means ± SEM for 9-11 rats per group.

remote organ injury, Schirmer *et al.* (1988) developed a femur fracture model and showed that blunt fracture caused a sustained and pathologic reduction in hepatic perfusion. When femur fracture was associated with soft-tissue trauma, the elevated cardiac output was normalized at 48 hrs, but the hepatic perfusion defect remained. In fact, previous studies have suggested that such perfusion deficits were an essential first step toward injury to the liver parenchyma (Chun *et al.*, 1994). In the present study, FFx increased serum ALT activity and this increase was attenuated by pretreatment with $GdCl_3$. However, no significant changes were seen in serum AST and lipid peroxidation levels among any of the experimental groups, even though the tendency was similar to ALT activity. A possible explanation for the increases in ALT without associated increases in AST and microsomal lipid peroxidation is that the FFx-induced hepatocellular damage is mild and diffuse at 3 days after FFx. Our results also suggest that Kupffer cell activation is in part related to hepatocellular damage after FFx. It should be pointed out that the term hepatocellular dysfunction has been frequently used to reflect hepatocellular damage (e.g. increased circulating levels of ALT and AST). Because the elevated serum liver enzymes do not reflect hepatocellular dysfunction but rather hepatocellular damage, it is encouraging that some of the subtle alterations in cellular functions that occur during trauma are identified, consequently leading to better management of the traumatic patient.

Stimulation of the immune system during infection or inflammation results in an impairment of CYP 450 content (Morgan, 1997). *In vivo* and *in vitro* studies have shown that the cytokines IL-1, IL-6 and TNF-α can mimic the down-regulation of CYP 450 gene product seen during infection or inflammation (Chen *et al.*, 1995). The nitric oxide released during inflammation has been implicated as the mediator of the decreased catalytic activity of CYP 450. In FFx rats, total CYP 450 content was significantly decreased; a decrease not inhibited by $GdCl_3$ treatment. Such a decrease in the total content of CYP 450 suggests that the overall activity of the CYP 450-dependent oxidases was similarly decreased. However, the loss of CYP 450 may not be mediated by the Kupffer cells. The liver has high levels of CYP 450 isoforms with different specificity for various substrates. Our previous study has shown that the activity of aniline *p*-hydroxylase was increased, whereas the activity of aminopyrine *N*-demethylase was decreased during ischemia/reperfusion (Lee *et al.*, 2000). In our present study, reduction of CYP 2E1 occurred in parallel to the reduction in total CYP 450 content. The activities of CYP 1A1, 1A2 and 2B1 were unchanged during traumatic injury. Even though the mechanisms of these inconsistent alterations in drug metabolizing systems have not been identified, the individual CYP 450 isozymes seem to be differentially affected by traumatic injury. These results are similar to those of Sewer and Morgan (1998), who found that endotoxin (LPS) treatment suppressed both total CYP 450 content and mRNA expression of CYP 2E1. In contrast, the up-regulation of CYP 2E1 has been reported in variable experimental pathological conditions, including CCl_4-induced hepatic fibrosis, alcohol-induced liver disease, and hepatic ischemia-reperfusion injury, which was implicated with the activation of Kupffer cells (Rivera *et al.*, 2001; Kono *et al.*, 2000). Our data indicate that the decrease in CYP 2E1 activity is not due to activation of Kupffer cells, because $GdCl_3$ did not prevent the decrease in CYP 2E1 activity. The possibility should also be considered that Kupffer cells *in vivo* could affect the catalytic activity of CYP 450 isozymes indirectly, by regulating the formation of some other modulating factor in hepatocytes or non-parenchymal cells. In the clinical situation, unexpected alterations in drug metabolism could occur in trauma patients, thereby necessitating more careful administration of drugs to patients.

In summary, blunt trauma resulted in abnormalities in the microsomal drug-metabolizing function *in vivo*. Our findings suggest that activation of Kupffer cells is not required for trauma-induced hepatocellular dysfunction.

ACKNOWLEDGEMENT

This work was supported by grant R04-2001-000-00011-0 from the Basic Research Program of the Korea Science and Engineering Foundation.

REFERENCES

Baker, C. C. and Huynh, T., Immunologic response to injury, In Moore, E. E., Mattox, K. L., Feliciano, D. V. (Eds.). *Trauma*. Appleton & Lange, Norwalk, CT, pp. 1177-1191 (1996).

Batra, J. K., Venkitasubramanian, T. A., and Raj, H. G., Drug metabolism in experimental tuberculosis. I. Changes in hepatic and pulmonary monooxygenase activities due to infection. *Eur. J. Drug Metab. Pharmacol.*, 12, 109-114 (1987).

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).

Bone, R. C., Bark, R. A., Cerra, F. B., Dellinger, R. P., Fein, A. M., and Knaus, W. A., Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. ACCP/SCCM consensus conference committee. *Chest*, 101, 1644-1655 (1992).

Bone, R. C., Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). *Ann. Intern. Med.*, 125, 680-687 (1996).

Bradham, C. A., Stachlewitsz, R. F., Gao, W., Qian, T., Jayadev,

S., Jenkins, G., Hannun, Y., Lemasters, J. J., Thurman, R. G., and Brenner, D. A., Reperfusion after liver transplantation in rats differentially activates the mitogen-activated protein kinases. *Hepatology*, 25, 1128-1135 (1997).

Brock, R. W., Lawlor, D. K., Harris, K. A., and Potter, R. F., Initiation of remote hepatic injury in the rat: interactions between Kupffer cells, tumor necrosis factor-alpha, and microvascular perfusion. *Hepatology*, 30, 137-142 (1999).

Brown, A. P., Harkema, J. R., Schultze, A. E., Roth, R. A., and Ganey, P. E., Gadolinium chloride pretreatment protects against hepatic injury but predisposes the lungs to alveolitis after lipopolysaccharide administration. *Shock*, 7, 186-192 (1997).

Buege, T. A. and Aust, S. D., Microsomal lipid peroxidation. *Methods Enzymol.*, 52, 302-310 (1978).

Burke, M. D., Thompson, S., Elcombe, C. R., Halpert, J., Haaparanta, T., and Mayer, R. T., Ethoxy-, pentoxy-, and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P-450. *Biochem. Biopharmacol.*, 34, 3337-3345 (1985).

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).

Chun, K., Zhang, J., Biewer, J., and Clemens, M. G., Microcirculatory failure determines lethal hepatocyte injury in ischemic/reperfused rat livers. *Shock*, 1, 3-9 (1994).

Cutrin, J. C., Llesuy, S., and Boveris A., Primary role of Kupffer cell-hepatocyte communication in the expression of oxidative stress in the post-ischemic liver. *Cell Biochem. Funct.*, 16, 65-72 (1998).

Deitch, E. A., Multiple organ failure. *Ann. Surg.*, 216, 117-134 (1992).

Huynh, T., Baker, C. C., Bracey, L. W., and Lemasters, J. J., Adaptive Kupffer cell alterations after femur fracture trauma in rats. *Am. J. Physiol.*, 272, G1457-G1462 (1997).

Huynh, T., Lemasters, J. J., Bracey, L. W., and Baker, C. C., Proinflammatory Kupffer cell alteration after femur fracture trauma and sepsis in rats. *Shock*, 14, 555-560 (2000).

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).

Koo, D. J., Chaudry, I. H., and Wang, P., Kupffer cells are responsible for producing inflammatory cytokines and hepatocellular dysfunction during early sepsis. *J. Surg. Res.*, 83, 151-157 (1999).

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).

Morgan, E. T., Suppression of constitutive cytochrome P450 gene expression in liver of rats undergoing an acute phase response to lipopolysaccharide. *Mol. Pharmacol.*, 36, 699-707 (1989).

Nielsen, B. W., Mukaida, N., and Matsushima, K., Macrophages as producers of chemotactic proinflammatory cytokines. *Immunol. Ser.*, 60, 131-142 (1994).

Omura, T. and Sato, R., The carbon monoxide binding pigment of liver microsomes. *J. Biol. Chem.*, 239, 2370-2379 (1964).

Pohl, R. J. and Fouts, J. R., A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Anal. Biochem.*, 107, 150-155 (1980).

Renton, K. W., Factors affecting drug biotransformation. *Clin. Biochem.*, 19, 72-75 (1986).

Rivera, C. A., Bradford, B. U., Hunt, K. J., Adachi, Y., Schrum, L. W., Koop, D. R., Burchardt, E. R., Rippe, R. A., and Thurman R. G., Attenuation of CCl_4-induced hepatic fibrosis by $GdCl_3$ treatment or dietary glycine. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 281, G200-G207 (2001).

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

Schirmer, W. J., Schirmer, J. M., Townsend, M. C., and Fry, D. E., Femur fracture with associated soft-tissue injury produces hepatic ischemia. *Arch. Surg.*, 123, 412-415 (1988).

Sewer, M. B. and Morgan, E. T., Down-regulation of the expression of three major rat liver cytochrome P450s by endotoxin *in vivo* occurs independently of nitric oxide production. *J. Pharmacol. Exp. Ther.*, 287, 352-358 (1998).

Shedlofsky, S. I., Swim, A. T., Robinson, J. M., Gallichio, V. S., Cohen, P. A., and McClain, C. J., Interleukin-1 (IL-1) depresses cytochrome P-450 levels and activities in mice. *Life Sci.*, 40, 2331-2336 (1987).

Takemura, S., Minamiyama, Y., Inaoka, S., Funae, Y., Hirohashi, K., Inoue, M., and Kinoshita, H., Hepatic cytochrome P450 is directly inactivated by nitric oxide, not by inflammatory cytokines, in the early phase of endotoxemia. *J. Hepatol.*, 30, 1035-1044 (1999).

Vermillion, J. and Coon, M. J., Purified liver microsomal NADPH-cytochrome P-450 reductase. *J. Biol. Chem.*, 253, 8812-8819 (1978).

Wu, J. Z., Ogle, C. K., Fischer, J. E., Warden, G. D., and Ogle, J. D., The mRNA expression and in vitro production of cytokines and other proteins by hepatocytes and Kupffer cells following thermal injury. *Shock*, 3, 268-273 (1995).