Mangafodipir Protects against Hepatic Ischemia-Reperfusion Injury in Mice

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Abstract

Introduction and Aim: Mangafodipir is a contrast agent used in magnetic resonance imaging that concentrates in the liver and displays pleiotropic antioxidant properties. Since reactive oxygen species are involved in ischemia-reperfusion damages, we hypothesized that the use of mangafodipir could prevent liver lesions in a mouse model of hepatic ischemia-reperfusion injury. Mangafodipir (MnDPDP) was compared to ischemic preconditioning and intermittent inflow occlusion for the prevention of hepatic ischemia-reperfusion injury in the mouse.

Methods: Mice were subjected to 70% hepatic ischemia (continuous ischemia) for 90 min. Thirty minutes before the ischemic period, either mangafodipir (10 mg/kg) or saline was injected intraperitoneally. Those experimental groups were compared with one group of mice preconditioned by 10 minutes’ ischemia followed by 15 minutes’ reperfusion, and one group with intermittent inflow occlusion. Hepatic ischemia-reperfusion injury was evaluated by measurement of serum levels of aspartate aminotransferase (ASAT) activity, histologic analysis of the livers, and determination of hepatocyte apoptosis (cytochrome c release, caspase 3 activity). The effect of mangafodipir on the survival rate of mice was studied in a model of total hepatic ischemia.

Results: Mangafodipir prevented experimental hepatic ischemia-reperfusion injuries in the mouse as indicated by a reduction in serum ASAT activity (P<0.01), in liver tissue damages, in markers of apoptosis (P<0.01), and by higher rates of survival in treated than in untreated animals (P<0.001). The level of protection by mangafodipir was similar to that observed following intermittent inflow occlusion and higher than after ischemic preconditioning.

Conclusions: Mangafodipir is a potential new preventive treatment for hepatic ischemia-reperfusion injury.

Introduction

Ethics Statement

Animals received human care in compliance with institutional guidelines under the permit number 75–1302 delivered to Dr Carole Nicco, PhD, the 04/06/2007.

Blood loss and transfusions during liver resection have a deleterious impact on both short and long-term outcomes [1,2]. To minimize intra-operative bleeding, surgical management of hepatocarcinoma requires pedicular clamping [3]. The common drawback of clamping is hepatic ischemia-reperfusion (I/R) injury, especially when the liver is affected by chronic hepatitis or cirrhosis, with a risk of poor postoperative outcome [4]. Two surgical strategies have been developed to minimize I/R injuries: intermittent clamping (IC) and ischemic preconditioning (IP).

IC consists of an intermittent inflow occlusion followed by short periods of reperfusion and has demonstrated a protective effect [5–7]. A drawback inherent to IC is blood loss during each period of reperfusion and the increased operative time [8]. The alternative to IC is IP that consists of a brief period of ischemic reperfusion applied prior to the prolonged ischemic insult. IP has demonstrated a protective effect during liver resection in humans [9]. Studies in animal models of ischemia reperfusion have suggested that the protective effect of IP is mediated by an enhancement in endogeneous anti-oxidative stress mechanisms [10,11].

Liver injury following I/R has a biphasic pattern [12]. In the initial phase, 0.5–2 h after the onset of reperfusion, reactive
oxygen species (ROS) are released and Kupffer cells and hepatocytes are activated. The late phase corresponds to self-aggravating inflammatory injury inducing ROS [12]. Hepatocytes injuries are most likely initiated by ROS and extracellular chemokines [13]. ROS have been shown to exert a central role in contributing to tissue injury after reperfusion of the ischemic liver [14–16].

Superoxide anions ($O_2^-\cdot$) originating from either the mitochondrial respiratory chain or various cytosolic enzymes, such as xanthine oxidase or NADPH oxidase, are generated by ischemic hepatocytes. Superoxide anions are detoxified by superoxide dismutase (SOD) that convert $O_2^-\cdot$ into $H_2O_2$, which is then detoxified by catalase, glutathione peroxidase, or thioredoxin [17,18]. The overproduction of ROS leads to lipid peroxidation, damages of mitochondrial membrane [19], release of cytochrome c into the cytoplasm followed by caspase-3 activation, and finally, to hepatocyte apoptosis [20]. Endogenous antioxidant compounds, such as superoxide dismutase, catalase, and glutathione can limit the effects of ROS but quickly become overwhelmed by the large amounts of ROS produced.

Because the oxidative stress plays such an important role in I/R injury, we hypothesized that the administration of a molecule endowed with antioxidative properties could be a valuable treatment of I/R injury. SOD, catalase and GSH reductase have been administered in animal models to counterbalance the endogenous enzymatic depletion during hepatic ischemia-reperfusion [21,22]. Although the targeted delivery of SOD and catalase to Kupffer cells after mannosylation or succinylation could prevent hepatic injury [18], the effectiveness of an exogenous enzymatic supply was controversial probably because of the insufficient delivery to the target sites. Therefore, we chose to test mangafodipir, a non peptidic enzymatic mimic with a high level of liver intracellular penetration. Mangafodipir (MnDPDP), a contrast agent used in magnetic resonance imaging of the liver, [23] the fodipir moiety binds to the pyridoxal 5’ phosphate molecules [23]. The fodipir moiety binds to the pyridoxal 5’ phosphate receptor on hepatocytes and ensures a high intrahepatic concentration of mangafodipir in the liver. In addition to the known capability of fodipir to increase GSH levels under a variety of oxidative conditions [24–26], we have shown that mangafodipir displays pleiotropic antioxidative properties. Indeed, this molecule is endowed with SOD-, catalase-, and glutathione reductase-like activities that allow both detoxification of mitochondrial ROS and regeneration of the GSH pool [27]. Those properties explain the effectiveness of mangafodipir in the treatment of APAP-induced acute liver failure in mice [28]. The aim of our study was, therefore, to identify the therapeutic activity of mangafodipir in a mouse model of hepatic I/R injury, versus IC and IP.

**Methods**

**Chemicals**

All chemicals were from Sigma (Saint Quentin Fallavier, France) except for mangafodipir (MnDPDP, Teslascan®, Amersham Health, Amersham, UK).

**Animals**

BALB/c female mice between 6 and 8 weeks of age were used in all experimental groups (Ilfa Credo, L’Arbresles, France). Animals received human care in compliance with institutional guidelines under the permit number 75–1302 delivered to Dr Carole Nico, PhD, the 04/06/2007. Hepatic ischemia was performed as follow: animals were anesthetized with an intraperitoneal injection of Avertine (10 mg/kg) and laparotomy was performed. Ischemia of the left lateral and left median lobes was induced by a microvascular clamp for 90 minutes. Reperfusion was initiated by removing the clamp [29].

**Experimental Design**

The following groups of mice were studied: Group 1: sham (anesthesia and laparotomy) (n = 9); group 2: control (90 minutes of ischemia) (n = 9); group 3: (as group 2 with intraperitoneal administration of 10 mg/kg of mangafodipir 30 minutes prior to ischemia) (n = 9); group 4: IP (as group 2 but with preconditioning induced by 10 minutes of ischemia followed by 15 minutes of reperfusion [30]) (n = 9); group 5: IC (six cycles of 15 minutes of ischemia followed by 5 minutes of reperfusion [30]) (n = 9).

Mice were injected intraperitoneally with 10 mg/kg of mangafodipir, which corresponds to the concentration used in humans as a contrast agent [31].

Twenty four hours following ischemia, the animals were sacrificed, blood was collected for measurement of serum transaminase activity, and livers were removed from the peritoneal cavities for histopathological and biochemical studies.

**Mitochondrial and Cytosolic Production of O2−**

The effects of mangafodipir on intracellular ROS production were assessed in vivo in human (HepG2) hepatocellular carcinoma cell line. Cytosol and mitochondria from HepG2 cells (15 x 10⁶ cells) were seeded in 24-well plates (Costar, Corning, Inc., Corning, NY) and incubated for 18 hours with mangafodipir or with culture medium alone. Levels of intracellular superoxide anion O2− were assessed spectrofluorometrically (Packard Biotechence, Boston, MA, USA) by oxidation of dihydroethidium (DHE) (Molecular Probes, Leiden, The Netherlands) as previously described [32]. The levels of O2− were calculated in each sample as follows: ROS rate (arbitrary units/2 * 10⁶ cells) = (fluorescence intensity [arbitrary units].

**Serum Aminotransferase activity**

Serum activity of aspartate aminotransferase (ASAT) was used as a marker of hepatocyte cytolysis. ASAT activity was quantified using a standard clinical automatic analyser (Hitachi type 747, Roche Diagnostics, Meylan, France).

**Histological examination of livers**

Livers were fixed overnight at 4°C in 4% paraformaldehyde in PBS, dehydrated, paraffin-embedded, and cut at 4-µm thickness. Hematoxylin-eosin-stained sections were used for histopathological evaluation of hepatic injuries. Two pathologists who were not aware of which animals received which treatment examined slides.

**Lipid peroxidation assay**

The concentrations of 4-hydroxyalkenals (4-HNE) and malondialdehyde (MDA) were measured in whole liver homogenates using the lipid peroxidation kit from Calbiochem (Calbiochem, Paris, France); whole liver homogenates were mixed with methanol: acetonitrile and N-methyl-2-phenylindole to yield a purple chromophore, which was measured spectrophotometrically at 586 nm. The level of lipid peroxidation was expressed as the amounts of 4-HNE+MDA per mg of proteins.

**Measurement of hepatic GSH content**

GSH levels in liver tissue were measured by the method of Baker et al [33]. Briefly, 50 µL of whole liver extract (1 mg/mL) or of reduced GSH (Sigma) as standard were added to 100 µL of a
reaction mixture containing 5 mL of 1 mM DNTB, 5 mL of 1 mM NADPH, 5.75 mL of 100 mM NaPO₄, 1 mM EDTA buffer, pH 7.5 and 0.1 mL of GSH reductase (200 U/mL). GSH levels were determined by measuring absorbances at 405 nm.

**Cytochrome c determination**

Cytochrome c concentration was determined on cytosolic fractions or enriched mitochondrial fractions from livers of mice. The amount of proteins in each lysate was measured using the BSA microbiuret assay (Pierce, Bezons, France). The rat/mouse Cytochrome c ELISA from R&D (Abingdon, Oxon, UK) was used to determine the levels of cytochrome c in each fraction. Results were expressed as pg of cytochrome c per μg of proteins.

**Measurement of caspase-3 activity**

Caspase-3 activity was measured on cytosolic fractions from livers of mice using 400 μM of chromogenic substrate Ac-DEVD-pNA (Calbiochem) and 200 μM of cytosolic fraction. Caspase-3 activity was calculated as the mean of the duplicate test wells minus the value obtained for the control wells containing 200 μM specific inhibitor.

**Animal Survival**

The rate of mouse survival was evaluated in groups 2 and 3 after total hepatic ischemia (22). Segmental hepatic ischemia (70%) was followed by the resection of the non ischemic liver lobes (30%). For the survival time courses, animals were observed for 30 days and sacrificed when they appeared moribund. The moribund state has been appreciated in a blinded manner by two observers who were not aware of which animals had received which treatment.

**Statistical analysis**

Results were expressed as means ± standard error (SEM). The statistical significance of differences in the groups was analysed by chi-square tests for incidence data. Paired Student’s t-test was used for comparison of means between two groups. A level of P<0.05 was accepted as significant. * p<0.05; ** p<0.02; *** p<0.01; **** p<0.001 vs untreated ninety minutes’ ischemia controls.

**Results**

**Serum enzymatic activities**

Ninety minutes’ ischemia (control) induced a 40-fold increase in serum transaminase activities compared to the sham-operated group. Compared to the untreated ninety minutes’ ischemia control group, ASAT activities were significantly lower after administration of mangafodipir and IC (P<0.01 and p = 0.01, respectively), and not significantly different from the basal level observed in the sham-operated group. ASAT activities in the IP group were lower than in the control group, but the difference between the two groups did not reach significance (Fig. 1).

**Liver histological examination**

After 90 minutes of continuous ischemia, large confluent areas of tissue lysis with blood congestion in the sinusoids and leukocyte infiltrates were observed. In the IP liver, limited and focal areas of hepatocyte necrosis were also observed (Fig. 2), whereas the parenchyma was almost normal after mangafodipir or IC treatment.

**Lipid Peroxidation assay**

Lipid peroxidation has been used as an indirect measurement of oxidative damage induced by ROS (24). Levels of 4-HNE and MDA were significantly higher in the ninety minutes’ ischemia control group than in the sham-operated group (13.3±1.7 μM/mg versus 5.4±0.5 μM/mg, P<0.001) after liver reperfusion. In animals treated by IC or IP, lipoperoxidation was not significantly reduced (9.8±1.5 μM/mg with IC and 10.2±1.1 μM/mg with IP. Only mangafodipir significantly reduced lipoperoxidation versus the ischemia control group (7.6±0.3 μM/mg, P<0.01) (Fig 3).

![Figure 1. Mangafodipir and intermittent clamping prevent the increase in transaminase activities following reperfusion injury.](image-url)
Measurement of hepatic GSH content

In the control group with continuous inflow occlusion, the cytosolic GSH content was decreased by 55% versus the sham-operated group. Treatment with mangafodipir, IC or IP, decreased mitochondrial GSH content by only 4%, 14% and 30%, respectively (P<0.001, 0.001 and 0.05, respectively versus the ninety minutes’ ischemia control group). The GSH content in the mitochondria of liver cells was also modified following continuous inflow occlusion and was significantly lower than in the sham-operated group (P<0.001). Mangafodipir, IC and IP increased the mitochondrial...
Figure 4. Prevention of glutathione depletion in the mitochondria (A) and in the cytoplasm (B) of hepatocytes following reperfusion injury. The concentrations of glutathione in cytoplasm and mitochondria of mouse liver were measured spectrophotometrically in whole liver homogenates. The levels of reduced glutathione were expressed as pmol per 10 μg of proteins. Bars represent means ± SEM, nine mice in each group. The O2⁺ depletion in the mitochondria (C) of HepG2 cells induced by mangafodipir (400 μM) was assessed in a kinetic experiment. Immunofluorescence microscopy of HepG2 cells stained with oxidation of dihydroethidium (DHE) and treated for 18 h or not with mangafodipir. (D–E) A decreased intensity of cytosol with mangafodipir in mangafodipir treated cells was observed.

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**GSH content** versus the ninety minutes' ischemia control group (P<0.01, 0.001 and 0.02, respectively) (Fig 4A and 4B).

**Cellular Mediators of Apoptosis**

To further evaluate that mangafodipir inhibit cystolic and mitochondrial ROS, levels of intracellular superoxide anion O$_2^-$ in mitochondria and cytosol from HepG2 cells were assessed and confirm a 29% and 28% ROS level decreases in mitochondria and cytosol, respectively. (figure 4C–D)

**Cellular Mediators of Apoptosis**

To further evaluate the mechanisms leading to liver cell injuries, we evaluated apoptotic liver cell death by measuring the cytochrome c released from mitochondria into the cytosol, and by assaying cystolic caspase-3 activity. Ischemia/reperfusion injuries were associated with mitochondrial collapse, as revealed by the significant 35% decrease in the mitochondrial/cytosolic cytochrome c ratio in the ninety minutes' ischemia control group versus the sham-operated group (P<0.001). The decrease in the mitochondrial/cytosolic ratio was reduced by IP (24%), IC (16%) and mangafodipir (9%) versus the ninety minutes' ischemia control group (P<0.001, 0.001 and 0.01, respectively) (Fig 5A). The mitochondrial collapse following reperfusion injury is followed by an increase in caspase-3 activation, as exemplified by the 9-fold increase in caspase-3 activity in the ischemia control group versus the sham-operated group (P<0.001). Mangafodipir, IC and IP increased by 2.3-, 3.2- and 4.8-fold the caspase-3 activity (P<0.001, 0.01 and 0.01, respectively) versus the ischemia control group (Fig 5B).

**Effect of mangafodipir on the Survival Rate of Mice**

In the ischemia control group, 8 mice of 9 survived no longer than 7 days and only one mouse survived longer than 30 days (Table 1). By contrast, all animals survived more than 30 days in the group treated by mangafodipir (P<0.001).

### Discussion

The damage caused to the liver by I/R is a limiting factor in many clinical settings such as liver surgery, transplantation, and low-flow states. Because ROS and oxidative stress have been shown to play a major role in organ I/R injury, the purpose of this study was to test the hypothesis that mangafodipir, an antioxidant molecule with pleiotropic antioxidative properties, would be effective in preventing hepatic I/R injury.

I/R injuries were generated in a model of partial hepatic ischemia in mice. This method produces severe hepatic I/R injuries without mesenteric venous hypertension. Mesenteric congestion is avoided by allowing intestinal blood flow through right and caudate lobes, which leads to a satisfactory survival rate with substantial I/R injuries.

**Table 1.** Increasing the survival rate of mice by mangafodipir following reperfusion injury of the liver.

| 90 minutes of ischemia          | 7 days overall survival | 30 days overall survival |
|---------------------------------|-------------------------|--------------------------|
| Continuous inflow occlusion (control) | 11%                     | 0%                       |
| Mangafodipir therapy prior to ischemia | 100%                    | 100%                     |

*Animal survival was evaluated using the model of total hepatic ischemia (37). The rate of mouse survival was evaluated in control group (90 minutes of ischemia; n = 9) and in control group with intraperitoneal administration of 10 mg/kg of mangafodipir 30 minutes prior to ischemia (n = 9) [40]. For the survival time courses, animals were sacrificed when they appeared moribund.

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We chose to test mangafodipir for the prevention of I/R injuries because this molecule is known to abrogate ROS-mediated apoptosis/necrosis of hepatocytes in the murine model of acetaminophen-induced acute liver failure [27]. Superoxide anion has been suspected for a long time to be one of the principal actors of liver lesions induced by I/R. Superoxide anion originates from mitochondria or from a cytosolic enzymatic system such as NAPDH oxidase or xanthine/xanthine oxidase system. The protective effects of SOD against experimental I/R in transgenic mice overexpressing SOD, have established the role of superoxide anion in I/R injuries of the liver. However, superoxide anion is not the only ROS implicated in I/R. Indeed, high levels of hydrogen peroxide (H$_2$O$_2$) are produced by Kupffer cells and by parenchymal liver cells following I/R. H$_2$O$_2$ detoxification by post-ischemic intravenous administration of GSH prevents reperfusion injury in the rat liver after prolonged warm ischemia [22]. Treatment with GSH prevents microcirculatory failure and damages to hepatocytes and improves animal survival. The protection was associated with an increased formation of plasma GSSG, providing evidence of an accelerated detoxification of ROS by intravenously applied GSH [22].

In our model of partial hepatic ischemia, the administration of mangafodipir reduced serum ASAT activities and histological lesions of the liver, diminished the leakage of mitochondrial cytochrome c into the cytosol, and aborted caspases-3 activation. Altogether, data indicates that mangafodipir ultimately inhibits the apoptosis of hepatocytes that occurs during I/R. Beneficial effects are correlated with the inhibition of ROS production and the preservation of the GSH pool. Mangafodipir, endowed with SOD-like and Glutathione reductase-like activities, allows the detoxification of mitochondrial ROS, and the regeneration of the GSH pool. Thus, the pleiotropic antioxidative properties of mangafodipir can explain its beneficial effects in experimental liver I/R.

The effectiveness of mangafodipir in controlling those parameters is higher than that of IP, which probably acts through other undefined mechanisms. The mild burst of oxidative stress generated during IP activates transcription factors, such as nuclear factor-kappaB and activating protein -1 [11,34,35] that might trigger the activity of antioxidative systems [36–38]. However, increasing antioxidative systems through IP are very versatile and do not allow the control of the antioxidative status of the liver as well as by a chemical drug.

Mangafodipir has SOD-, catalase-, glutathione reductase-like activities, which contribute to its protective effect against hepatic I/R injury. In a recent study by Llacuna et al [39], I/R of the liver was performed on mice treated with MnTBAP, with BSO or S-adenosylmethionine and demonstrated that SOD mimics present I/R lesion. Moreover, acting on the GSH metabolism by GSH, up-regulation by S-adenosylmethionine decrease I/R injuries while GSH depletion by glutathione reductase inhibitor, BSO, potentiate oxidative stress and increase I/R lesions. Since mangafodipir is endowed with both SOD and glutathione reductase-like activity, data indicates that the two enzymatic activities could add an effect to prevent I/R lesions.

In addition to its pharmacological properties, mangafodipir presents pharmacoconomic advantages. Indeed, Mangafodipir trisodium is a tissue-specific imaging agent that concentrates in the liver following in vivo administration. Mangafodipir trisodium is a chelate of manganese (II) and of fodipir (DPDP), a vitamin B6 derivate. The fodipir moiety ensures high intra-hepatic concentration of mangafodipir through its specific binding to vitamin B6 receptors borne by hepatocytes. As a contrast agent for liver MRI, mangafodipir has already been safely used in human.

In conclusion, mangafodipir is a major candidate as a molecule used for the prevention of ischemia-reperfusion injury during liver surgery: it exerts a protective effect through its beneficial pharmacological properties and pharmacokinetics. In addition, compared to IC, it reduces blood loss and duration of surgery. Furthermore, mangafodipir is readily available for clinical trials since it has been used for decades as a contrast agent for liver MRI in human.

**Author Contributions**

Conceived and designed the experiments: FB AL NK. Performed the experiments: CN SB CG. Analyzed the data: CN RC SG. Contributed reagents/materials/analysis tools: RC NK ML CN SB CC FB. Wrote the paper: RC NK ML SB CN BW AL FB CG. Histology: SB CN.

**References**

1. Gozzetti G, Maziotti A, Grazi GL, Jovine E, Gallucci A, et al. (1995) Liver resection without blood transfusion. Br J Surg 82: 1105–1110.

2. Matsunaga T, Ikeda Y, Hayashi H, Nakamura T, Taketomi A, et al. (1993) The association between transfusion and cancer-free survival after curative resection for hepatocellular carcinoma. Cancer 72: 1866–1871.

3. Man K, Fan ST, Ng IO, Lo CM, Liu CL, et al. (1997) Prospective evaluation of ischemic preconditioning following partial hepatic ischemia. Eur J Surg 161: 181–186.

4. Nagaosa N, Uchida M, Kubota H, Hayashi T, Kohno H, et al. (1995) Carnitine: livers can tolerate 30 minutes ischaemia at normal environmental temperature. Eur J Surg 161: 181–186.

5. Makaschi M, Mori T, Grimm P, Yamaoka S, Hasegawa H (1987) Safety of hepatic vascular occlusion during resection of the liver. Surg Gynecol Obstet 164: 155–158.

6. Ezaki T, Sato Y, Tomoda H, Furusawa M, Kanematsu T, et al. (1992) Partial hepatic resection under intermittent hepatic inflow occlusion in patients with chronic liver disease. Br J Surg 79: 224–229.

7. Perales C, Hotter G, Closa D, Gelpi E, Bulbena O, et al. (1997) Protective effect of preconditioning on the injury associated to hepatic ischemia-reperfusion in the rat: role of nitric oxide and adenosine. Hepatology 25: 934–937.

8. Belgatti J, Noun R, Malafosse R, Jagot P, Sauvanet A, et al. (1999) Continuous versus intermittent portal triad clamping for liver resection: a controlled study. Ann Surg 229: 369–375.

9. Clavien PA, Schiller M, Rudiger HA, Graf R, Kasl Y, et al. (2003) A prospective randomized study in 100 consecutive patients undergoing major liver resection with versus without ischemic preconditioning. Ann Surg 238: 845–850; discussion 851–842.

10. Peralta C, Bulbena O, Xaus C, Prats N, Curtin JC, et al. (2002) Ischemic preconditioning: a defense mechanism against the reactive oxygen species generated after hepatic ischemia reperfusion. Transplantation 73: 1203–1211.

11. Tejima K, Arai M, Ikeda H, Tomiya T, Yanase M, et al. (2004) Ischemic preconditioning protects hepatocytes via reactive oxygen species derived from Kupffer cells in rats. Gastroenterology 127: 1488–1496.

12. Hassan-Khabbar S, Vany M, Contart CH, Wendum V, Viheer F, et al. (2010) Protective effect of post-ischemic treatment with trans-resveratrol on cytokine production and neutrophil recruitment by rat liver. Biochimie 92: 405–410.

13. Nieuwenhuys VB, De Brujin MT, Padbury RT, Barring GJ (2006) Hepatic ischemia-reperfusion injury: roles of Ca2+ and other intracellular mediators of impaired bile flow and hepatocyte damage. Dig Dis Sci 51: 1087–1102.

14. Jaeschke H (1991) Reactive oxygen and ischemia/reperfusion injury of the liver. Chem Biol Interact 79: 115–136.

15. McCoed JM (1983) Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 312: 159–163.

16. Sasaki H, Matsuno T, Nakagawa K, Matsuoka J, Tanaka N (1998) Superoxide induces hepatocyte apoptosis during the early phase of reperfusion after murine liver ischemia. Transplant Proc 30: 2958–2959.

17. Stein HJ, Oosthuizen MM, Hindel RA, Lamprechts H (1991) Oxygen free radicals and glutathione in hepatic ischemia/reperfusion injury. J Surg Res 50: 390–402.

18. Yaffe Y, Kobayashi N, Nishihashi T, Takahashi R, Nishikawa M, et al. (2001) Prevention of neutrophil-mediated hepatic ischemia/reperfusion injury by superoxide dismutase and catalase derivatives. J Pharmacol Exp Ther 298: 894–899.
19. Nguyen WD, Kim DH, Alam HB, Provost HS, Kirkpatrick JR (1999) Polyethylene glycol-superoxide dismutase inhibits lipid peroxidation in hepatic ischemia/reperfusion injury. Crit Care 3: 127–130.

20. Hirakawa A, Takeyama N, Nakatani T, Tanaka T (2003) Mitochondrial permeability transition and cytochrome c release in ischemia-reperfusion injury of the rat liver. J Surg Res 111: 240–247.

21. Atalla SL, Toledo-Pereyra LH, MacKenzie GH, Cederna JP (1985) Influence of oxygen-derived free radical scavengers on ischemic livers. Transplantation 40: 584–590.

22. Schauer RJ, Gerbes AL, Vonier D, Meissner H, Michl P, et al. (2004) Glutathione protects the rat liver against reperfusion injury after prolonged warm ischemia. Ann Surg 239: 229–231.

23. Elizondo G, Fretz CJ, Stark DD, Rocklage SM, Quay SC, et al. (1991) Preclinical evaluation of MnDPDP, new paramagnetic hepatobiliary contrast agent for MR imaging. Radiology 178: 73–78.

24. Ali BH, Bashir AA (1993) Comparative modulating effects of captopril, diltiazem, dietary calcium and pyridoxal-5-phosphate on gentamicin-induced nephrotoxicity in the rat. Gen Pharmacol 24: 1279–1283.

25. Calabrese V, Calderone A, Ragusa N, Rizza V (1998) Long-term ethanol administration enhances age-dependent modulation of redox state in central and peripheral organs of rat: protection by metadoxine. Drugs Exp Clin Res 24: 85–91.

26. Bedda S, Laurent A, Conti F, Chereau C, Tran A, et al. (2003) Mangafodipir prevents liver injury induced by acetaminophen in the mouse. J Hepatol 39: 765–772.

27. Ferret PJ, Hammond R, Talliez M, Tran A, Trebeden H, et al. (2001) Detoxification of reactive oxygen species by a nonpeptidyl mimic of superoxide dismutase cures acetaminophen-induced acute liver failure in the mouse. Hepatology 33: 1173–1180.

28. Koo A, Komatsu H, Tao G, Inoue M, Guth PH, et al. (1992) Contribution of no-reflow phenomenon to hepatic injury after ischemia-reperfusion: evidence for a role for superoxide anion. Hepatology 15: 307–314.