Regulation of postischemic liver injury following different durations of ischemia

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INTERRUPTION OF BLOOD FLOW to the liver is an unavoidable consequence of liver transplantation and resectional surgery (7). There is a growing body of experimental and clinical data to suggest that the ischemia and reperfusion (I/R) induced by these surgical procedures or pathophysiological events injures the liver and may ultimately lead to tissue dysfunction and possibly liver failure (25). Recent evidence suggests that I/R-induced liver injury occurs in two distinct phases, consisting of an acute phase occurring within the first 6 h of reperfusion followed by a later, subacute phase occurring from 6 to 24 h after ischemia (29). The acute phase is characterized by the polymorphonuclear leukocyte (PMN)-independent activation of resident Kupffer cells, resulting in enhanced production of reactive oxygen species (ROS) in association with alterations in the redox state of the tissue in favor of a more oxidative environment (10, 19, 46). Historically, enhanced ROS production has been thought to injure tissue by virtue of its ability to degrade membrane lipids and/or proteins. However, more recent studies suggest that oxidative degradation of biomolecules is not produced at a time or is produced in insufficient amounts to account for I/R-induced liver injury (34). In fact, it is becoming increasingly appreciated that ROS may mediate the early I/R-induced liver injury via their ability to enhance the expression of certain redox-sensitive genes known to be important in promoting hepatocellular injury. For example, several lines of evidence implicate I/R-induced ROS production in mediating hepatocellular injury by activating the NF-κB and activator protein-1-dependent expression of certain cytokines known to be involved in the pathophysiology of I/R-induced injury (50, 51). Indeed, Kupffer cell- and/or hepatocyte-derived expression of TNF-α, IL-1β, and IL-12 have been implicated as important mediators of reperfusion injury in the liver (3, 28, 42).

In contrast, the late, subacute phase of I/R injury has been shown to be a PMN-dependent process in which I/R-induced ROS generation is associated with cytokine and chemokine expression (16, 33). There is good evidence to suggest that these inflammatory mediators promote the invasion of PMNs into the interstitium via the upregulation of adhesion molecules and formation of chemotactic gradients (33, 43). Adherent PMNs become metabolically activated and transmigrate...
through the sinusoidal and microvascular endothelial cells to the underlying hepatocytes, where they generate additional reactive oxygen metabolites in conjunction with the release of extracellular matrix-degrading enzymes such as collagenase and matrix metalloproteinases (17, 19, 20). The net result is an amplification of the acute responses resulting in extensive inflammatory tissue injury.

Although several different studies have demonstrated the protective effects of antioxidant intervention in I/R-induced liver injury (7, 15, 37, 47, 49, 50), the identity of the specific reactive species, the sources of these oxidants and free radicals, and mechanisms by which these reactive oxygen metabolites promote hepatocellular injury have yet to be defined. Indeed, the use of low-molecular-weight ROS scavengers and/or enzymatic antioxidants has proven problematic given their nonspecific nature and/or short circulating half-life (8). It has been proposed that ROS may be generated during the early and late phases of liver I/R by xanthine oxidase (XO), mitochondrial respiration, and/or Kupffer cell- and PMN-associated NADPH oxidase (16, 26). Although different investigators have implicated mitochondrial metabolism or XO as potential sources for ROS generation in the postischemic liver, there has been little evidence concerning the role of NADPH oxidase. This multimeric O₂⁻-generating complex is composed of several protein subunits, including the membrane-spanning gp91 and p22 subunits, the cytosolic p47 and p67 subunits, and the gp91-associated rac-1 protein (6). Experimental evidence suggests that this enzyme complex is activated in Kupffer cells during a variety of inflammatory stimuli and may contribute extensively to hepatocellular injury (17, 19, 24). In addition, this complex has also been identified in vascular tissue most likely associated with the endothelium (9). Production of even small quantities of ROS at this location following I/R may be important for the initiation of events leading to liver injury as well as cellular adherence and the subacute phase of injury. It is well known that endothelial barrier function and integrity play important roles during I/R (44). Given these data, it appears that this complex, if activated during I/R, would be capable of mediating a significant level of liver injury.

Because the duration of ischemia imposed by the different surgical procedures varies considerably, it is reasonable to assume that the mechanisms responsible for postischemic liver injury may be quite different (7, 15). Thus we wished to define the relationship among Kupffer cells, O₂⁻ production, and TNF-α expression in postischemic liver injury following short and long periods of ischemia.

MATERIALS AND METHODS

Animals. Wild-type C57BL/6 mice (18–24 g) were purchased from Harlan Sprague Dawley, and mice genetically deficient in the gp91phox subunit of NADPH oxidase (gp91⁻⁻) were obtained from Jackson Laboratories (Bar Harbor, ME) and generated as described previously (39). All animals were maintained on a standard laboratory diet with free access to food and water until the time of the experiment. All experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (revised 1996), approved by the Council of The American Physiological Society, and with federal and state regulations.

Animal model of partial hepatic I/R. Fasted (16–18 h) male mice were anesthetized with a single intraperitoneal injection of ketamine (150 mg/kg) and xylazine (7.5 mg/kg), and a laparotomy was performed. The intestines were gently lifted from the body cavity to access the liver and portal vein. The quadrate lobe was subsequently dissected from the left lateral lobe to further expose the portal triad. Anatraumatic clip was placed across the portal vein, hepatic artery, and bile duct just above the branch of the right lateral lobe. The intestines were placed back inside the cavity, and 500 μl of 10 U/ml heparinized saline was added to the peritoneal cavity. The animals were then placed under a heating lamp for 45 or 90 min. Sham-operated animals received laparotomy without vessel occlusion. After ischemia or sham laparotomy, the peritoneal cavity was sutured shut, and animals were allowed to recover and live for up to 24 h. Following this period, serum and tissue were collected from each animal and frozen at −70°C for subsequent liver enzyme and cytokine determinations, histopathological assessment, and myeloperoxidase (MPO) content.

Serum alanine aminotransferase measurements. Serum levels of alanine aminotransferase (ALT) were measured from all animals subjected to I/R. Blood was taken from the inferior vena cava following 45 or 90 min of ischemia and 6 h of reperfusion and placed in a serum separator tube (Becton Dickinson, Franklin Lakes, NJ). The samples were allowed to clot on ice for ~10 min, after which they were centrifuged at 4,000 g for 10 min. Serum was then collected, and ALT was measured by using a kit from Sigma (St. Louis, MO). Data are presented as units per liter at 37°C.

Histopathology. Liver tissue was fixed in ice-cold 10% phosphate-buffered formalin (Fisher Scientific, Fair Lawn, NJ) for 24 h at 4°C. The tissue was subsequently partially dehydrated with ethanol and embedded in JB4 plastic mounting media (Polysciences, Warrington PA). Five-micrometer sections were cut and stained with hematoxylin and eosin. Following staining, the sections were scored in a blinded fashion as previously described (12).

MPO measurements. PMN infiltration was assessed by measurement of hepatic MPO activity following 45 or 90 min of ischemia and 6 h of reperfusion by using an established method (27). Briefly, ~100 mg of liver tissue was homogenized in 2 ml of sodium phosphate buffer (pH 7.4). Samples were then centrifuged at 3,000 g for 20 min at 4°C. Pellets were resuspended in 5 volumes of buffer containing EDTA and 0.5% (wt/vol) hexadecyltrimethylammonium bromide (Sigma). Samples were frozen at −20°C, followed by thawing and then sonication on ice. To inactivate any endogenous catalase, samples were heated for 2 h at 60°C. Samples were then centrifuged at 3,000 g at 4°C, and the supernatant was used for MPO measurement. MPO was quantified by measuring the MPO-catalyzed, H₂O₂-dependent oxidation of 3,3’,5,5’-tetrathylbenzidine (Sigma) at 655 nm. MPO content was expressed as optical density (OD) at 655 nm after termination of the reaction with ice-cold 200 mM acetic acid (pH 3.0).

Serum cytokine levels. Serum levels of TNF-α were quantified by using an ELISA kit according to the manufacturer’s specifications (Quantikine M TNF-α; R&D Systems, Abingdon, UK). Serum was collected as described for the ALT measurements and was stored at −80°C until measurements.
could be performed. Data were then presented as picograms per milliliter of serum TNF-α.

Antioxidant and antibody studies. Mice were given a bolus injection in the tail vein of AEOL-10150 (3 mg/kg iv; Aeolus Pharmaceuticals). A mouse was euthanized at 1, 2, and 6 h after injection, and blood was collected in heparin tubes by cardiac puncture. Plasma was analyzed for AEOL-10150 by HPLC-UV detection as previously described (21). The plasma half-life was estimated by using a single exponential term model

\[ \text{concentration (time)} = A_0 e^{-B_1 \times \text{time}} \]

with PK Analyst computer software (Micromath, Salt Lake City, UT). In addition, wild-type mice were treated with a genetically engineered polycationic chimeric form of manganese superoxide dismutase (pcMnSOD) consisting of fusion protein of the mature human MnSOD sequence followed by the 26 COOH-terminal residues of human extracellular SOD (35). This chimeric pcMnSOD binds to the microvascular endothelium and may be displaced with heparin, similar to native extracellular SOD (38). Treated animals received either pcMnSOD (1,000 U/kg iv), native MnSOD (1,000 U/kg iv; Sigma), the membrane-permeable, low-molecular-weight SOD mimetic (AEOL-10150 dissolved in PBS; 3 mg/kg iv), or vehicle (PBS) 15 min before the initiation of either 45 or 90 min of ischemia and 6 h of reperfusion. Serum and tissue were collected and assayed as described for ALT measurements and histopathology, respectively. In addition, some animals were pretreated 15 min before ischemia with a monoclonal antibody (200 μg) directed against TNF-α. This antibody was generated from clone XT22 created at DNAX Research Institute (Palo Alto, CA) and extracted and purified from ascites fluid by Verax (Lebanon, NH) (1).

Inactivation of Kupffer cells. Wild-type or gp91−/− mice were treated with 10 mg/kg ip of GdCl₃ or vehicle (0.9% NaCl) 24 h before the initiation of I/R, as previously described (36). Serum and tissue were subsequently collected following 6 h of reperfusion and analyzed as described above.

Statistical analysis. All values are presented as means ± SE. Data were analyzed by using the Students t-test or analysis of variance, and significance was set at P < 0.05.

RESULTS

Tissue injury and TNF-α expression following different periods of ischemia. Figure 1 demonstrates that 45 or 90 min of ischemia followed by varying times of reperfusion produces significant, time-dependent increases in serum ALT levels. Doubling the duration of ischemia from 45 to 90 min produced an eightfold increase in tissue injury as assessed by serum ALT at 6 h after ischemia. Histopathological evaluation of the livers revealed extensive hepatocellular necrosis in the absence of significant PMN infiltration, which was consistent with the acute phase of liver injury (Fig. 2). Coincident with liver injury, we observed significant elevations in serum protein levels of TNF-α following 45 or 90 min of ischemia and 6 h of reperfusion (Fig. 3). Figure 4 demonstrates that pretreatment of mice with a single injection of TNF-α monoclonal antibody 15 min before ischemia attenuated I/R-induced liver injury following 90 but not 45 min of ischemia and 6 h of reperfusion.
6 h of reperfusion (Fig. 5). When the duration of ischemia was increased to 90 min, MnSOD or AEOL-10150 also significantly attenuated liver injury; however, these protective effects were slightly less than those observed with the longer-lived pcMnSOD (Fig. 5). The protective effect of pcMnSOD correlated well with its ability to significantly reduce serum levels of TNF-α following 45 or 90 min of ischemia (Fig. 6). Interestingly, native MnSOD administration was not effective at reducing serum levels of TNF-α following either period of ischemia, suggesting that its protective effect may be independent of TNF-α generation (Fig. 6). Although AEOL-10150 administration showed a trend toward inhibition of TNF-α production, these differences were also not statistically different compared with vehicle-treated controls (Fig. 6).

**NADPH oxidase and hepatocellular injury.** Having established that O2 plays an important role in postischemic liver injury, we next wished to examine the possible role of the NADPH oxidase complex as a major source of this free radical following both short and long periods of ischemia and 6 h of reperfusion. As shown in Fig. 7, gp91−/− mice were protected by ~50% from the I/R-induced liver injury following 90 but not 45 min of ischemia as assessed by serum ALT. These data suggest that extended periods of ischemia may be required for the activation of this complex in Kupffer cells and/or sinusoidal and microvascular endothelial cells.

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Fig. 2. Histopathology of livers subjected to 45 or 90 min of ischemia and 6 h of reperfusion. A: sham-operated control for 45 min of ischemia and 6 h of reperfusion. B: liver subjected to 45 min of ischemia and 6 h of reperfusion. Note the absence of polymorphonuclear leukocyte (PMN) infiltration but the presence of hepatocellular necrosis as evidenced by pyknotic nuclei (arrows), cytoplasmic blanching, and loss of distinct hepatocellular borders. C: sham-operated control for 90 min of ischemia and 6 h of reperfusion. D: liver subjected to 90 min of ischemia and 6 h of reperfusion. Enhanced hepatocellular necrosis (e.g., pyknotic nuclei, cytoplasmic blanching, and loss of distinct hepatocellular borders) is evident in these livers. Representative 5-μm sections stained with hematoxylin and eosin are shown at ×400 magnification.

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and that this complex contributes to approximately half of the O$_2$-mediated postischemic liver injury in this model. In addition, we found that, although deficiency in gp91$^{phox}$ did not protect mice from shorter periods of ischemia (i.e., 45 min ischemia), it did result in complete elimination of serum TNF-α at both 45 and 90 min of ischemia (Fig. 8).

**Role of Kupffer cells in postischemic injury following different periods of ischemia.** Several different studies have implicated Kupffer cells as important effector cells in the pathophysiology of postischemic liver injury via their ability to elaborate potentially injurious ROS and cytokines (14, 19, 41). However, few studies have systematically evaluated the importance of these resident phagocytes following different durations of ischemia within the same model. Therefore, we examined the role of Kupffer cells following 45 or 90 min of ischemia and 6 h of reperfusion. We found that inactivation of Kupffer cell function by pretreatment of mice with 10 mg/kg of GdCl$_3$ 24 h before the induction of ischemia resulted in dramatic inhibition (80%) of I/R-induced injury following 45 min of ischemia and 6 h of reperfusion (Fig. 9). This same protocol reduced liver injury by only 45% following 90 min of ischemia and 6 h of reperfusion (Fig. 9). Because we and others (3, 40) have demonstrated that TNF-α is responsible for some of the I/R-induced hepatocellular injury, coupled with the fact that Kupffer cells are well-known sources of this potentially injurious cytokine (14), we investigated how Kupffer cell inactivation affected cytokine expression following I/R. Figure 10 shows that inactivation of Kupffer cell function by pretreatment with GdCl$_3$ decreased I/R-induced TNF-α release following both 45 and 90 min of ischemia. In the final series of experiments, we assessed the NADPH oxidase-independent effects of Kupffer cells in our model of liver I/R by inactivating Kupffer cells in gp91$^{-/-}$ mice by using GdCl$_3$. We found that Kupffer cell inactivation attenuated I/R-induced liver injury following 90 min of ischemia and 6 h of reperfusion by ~60% compared with gp91$^{-/-}$ mice containing Kupffer cells (Fig. 11). Compared with wild-type mice containing Kupffer cells, I/R-induced liver injury was reduced by ~85% in Kupffer cell-inhibited gp91$^{-/-}$ mice (Fig. 11). Not surprisingly, this treatment protocol did not reduce further the serum levels of TNF-α compared with gp91$^{-/-}$ mice with intact Kupffer cells (data not shown).

**DISCUSSION**

I/R-induced liver injury is a consequence of different surgical manipulations, including transplantation and resectional surgery (7). The duration of ischemia varies considerably in each of these surgical procedures and pathophysiological situations, suggesting that the mechanism(s) responsible for postischemic liver injury may be quite different (7, 15). Because there has been little attempt to systematically evaluate the mechanisms of postischemic liver damage following different periods of ischemia, we wished to define the relationship among Kupffer cells, O$_2$ generation, and proinflammatory cytokine production in postischemic liver injury following short or long periods of ischemia within the same model. Data obtained in the present study clearly demonstrate that O$_2$ generation represents an early, initiating event responsible for the PMN-independent phase of tissue injury regardless of the duration of ischemia. In addition, we provide evidence showing that the sources for O$_2$ formation as well as the relative importance of TNF-α in the pathophysiology of I/R-induced hepatocellular injury differ depending on the duration of ischemia.

One of the most consistent findings in our present study was the rather remarkable protective effects provided by the three different SODs with varying circulating half-lives in vivo (Fig. 5). Although our data

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**Figure 3.** Serum TNF-α levels following 45 or 90 min of ischemia and varying periods of reperfusion. No significant differences in time-matched, sham-operated controls were observed, and these mice were subsequently grouped. *$P < 0.05$ vs. sham-operated control; $n \geq 5$ /group.

**Figure 4.** Effect of a monoclonal antibody (MAb) to mouse TNF-α (200 μg/mouse) on serum ALT levels following 45 or 90 min of ischemia and 6 h of reperfusion. *$P < 0.05$ for MAb-treated vs. vehicle-treated control; $n \geq 5$ /group.
agree well with those of other investigators (18, 47–49) who used native and modified forms of the cytosolic isoform of SOD (e.g., CuZnSOD), the present study demonstrates more dramatic protective effects in that pcMnSOD decreases liver injury (60–80%) following both 45 and 90 min of ischemia. Addition of a 26-amino acid polycationic tail to human MnSOD makes this enzyme capable of binding to the negatively charged heparan sulfate residues found on the surface of sinusoidal and microvascular endothelial cells, thereby extending its half-life in vivo from 6 min for native SOD to 30 h for pcMnSOD (38). Previous studies required the use of large quantities of enzymatic scavengers to achieve even more modest protection (18), whereas we attained a much greater degree of protection by using ~10-fold less SOD as in the other studies (47, 49). The reasons for these differences are not readily apparent but are most likely due to the localization of pcMnSOD to the sinusoidal endothelial cells (SECs) or the microvascular endothelium. Previous studies have utilized genetically or chemically modified forms of CuZnSOD and catalase to target these antioxidants to specific cells within the liver, where they are endocytosed and maintained within the cell (18). The polycationic MnSOD used in the present study has been engineered to possess binding characteristics similar to native extracellular CuZnSOD, which readily binds to the heparin sulfate-binding regions on the surface of endothelial cells (38). Localization to the surface of SECs, for example, would increase the effective concentration of the antioxidant in an environment (e.g., extracellular space) deficient in antioxidant enzymes. Indeed, our data suggest that extracellular O$_2^-$ represents an important pathway for postischemic liver injury.

Another interesting aspect of the data obtained in the present study was that both short-lived SODs (e.g., native MnSOD and AEOL-10150) afforded protection equal to that of long-lived pcMnSOD following 45 min of ischemia and 6 h of reperfusion (Fig. 5). These data agree with other investigators (5, 31) who demon-
strated protective effects using similar low-molecular-weight SOD mimetics in models of intestinal or brain ischemia. Since the circulating half-lives of AEOL-10150 and MnSOD are ~22 and 6 min, respectively, our data suggest that O$_2^-$ produced very early during reperfusion is directly or indirectly responsible for initiating much of the neutrophil-independent postischemic liver injury. Production of O$_2^-$ during the first several minutes of reperfusion may damage mitochondrial membrane lipids and proteins, leading either directly or indirectly to the loss of inner membrane potential and ATP-generating capacity (32). In addition, O$_2^-$ is known to inhibit certain enzymes within the mitochondria, including aconitase, which may lead to substantial reductions in ATP formation and ultimately cell death (11). However, it should be emphasized that extracellular O$_2^-$ may also interact with other hepatoprotective molecules such as nitric oxide. Although this interaction may lead to the production of the strong oxidant ONOO$^-$, our data as well as those of other investigators (13, 22, 23, 30) suggest that NO production is protective and not a component of a much more damaging species and that its removal leads to substantial increases in postischemic liver injury. Together, our data suggest that the early generation of O$_2^-$ is a critical event in the pathophysiology of liver I/R-induced injury. The cellular sources for O$_2^-$ production following 45 min of ischemia are not known with certainty, but data obtained in the present study demonstrate NADPH oxidase-independent sources. As mentioned above, we show that, although short- and long-lived SODs protect equally well following 45 min of ischemia and 6 h of reperfusion, NADPH oxidase does not appear to represent a major source of this free oxygen radical.
radical (Fig. 7). Potential sources for $O_2^-$ following 45 min of ischemia include mitochondria, XO, and NADPH cytochrome P-450 reductase to name just a few. Preliminary data from our laboratory, however, suggest that I/R-induced liver injury is not mediated by XO (data not shown).

Data obtained in the current study also suggest interesting relationships among $O_2^-$ production, TNF-α expression, and hepatocellular injury following different periods of ischemia. A number of previous studies have implicated TNF-α in the generation of posts ischemic liver injury, especially that associated with the neutrophil-dependent subacute phase (4). Only recently has there been data to suggest that this cytokine is an important component of the neutrophil-independent liver injury (40). Using a monoclonal antibody directed against mouse TNF-α, we demonstrated that this cytokine is an important mediator of tissue injury following 90 but not 45 min of ischemia, despite the fact that both periods of ischemia produce similar increases in serum levels of TNF-α (Figs. 3 and 4). Our observation that short periods of ischemia (45 min) involve an $O_2^-$-dependent but TNF-α-independent mechanism of liver injury is further supported by data demonstrating that, whereas short-lived SODs (e.g., MnSOD and AEOL-10150) attenuate reperfusion injury, they do not significantly reduce serum levels of TNF-α following 45 min of ischemia (Figs. 5 and 6). However, when the duration of ischemia is increased, we observed a more direct relationship among $O_2^-$ production, TNF-α expression, and liver injury. For example, we observed that ~40–50% of the total postischemic liver injury is dependent on $O_2^-$ generation, NADPH oxidase activity, and TNF-α expression following 90 min of ischemia (Figs. 7 and 8). These data are consistent with the hypothesis that NADPH oxidase-derived $O_2^-$ promotes the expression of TNF-α, which directly or indirectly mediates the I/R-induced liver damage. Indeed, pretreatment of gp91$^-/-$ mice with TNF-α monoclonal antibody offered no additional protection compared with gp91$^-/-$ mice treated with IgG control antibody (data not shown). If one assumes that the mechanisms responsible for liver injury following 90 min of ischemia represents the sum of both NADPH oxidase-dependent and -independent sources of $O_2^-$, then one would predict that approximately half the liver injury is mediated by NADPH oxidase-derived $O_2^-$, with the remaining 50% generated by NADPH oxidase-independent sources of $O_2^-$.

Since we show that posts ischemic liver injury occurs in the absence of a significant neutrophil infiltration, the most likely cellular sources of NADPH oxidase are the Kupffer cells and possibly the sinusoidal and/or microvascular endothelial cells (9). Indeed, we demonstrated that Kupffer cells may account for as much as 60–80% of the reperfusion injury, depending on the duration of ischemia (Fig. 9). In this study, we did choose to use GdCl3 to inactivate Kupffer cells. It should be noted that this maneuver may not completely eliminate Kupffer cells from the liver but may simply serve to inactivate them. Thus we may actually be underestimating the role of Kupffer cells within the posts ischemic liver in our model. On the basis of work by other investigators, one would predict that Kupffer cell-associated NADPH oxidase plays a major role in the pathophysiology of liver injury induced by longer periods of ischemia (2, 18, 36). The mechanisms by which longer periods activate the NADPH complex are incompletely understood at the present time. Our data would suggest that factors released during extended ischemic periods are capable of activating this complex, whether it be located within the Kupffer cells and/or endothelial cells. One possible mechanism for this time-dependent activation may be related to portal congestion-induced intestinal ischemia. It is known that serum levels of bacterial products increase following intestinal hypoperfusion, either through hemorrhagic shock or direct interruption of arterial flow (45). In our studies, we took care to provide adequate portal flow through the nonischemic lobes. However, this reduced volume (~30%) of liver could lead to at least partial portal congestion. Extended periods of impaired flow through the intestine may then lead to altered intestinal barrier function and subsequent bacterial translocation, activation of Kupffer cell and/or endothelial cell-associated NADPH oxidase, and production of $O_2^-$. Preliminary studies however, would argue against this possibility because portal pressure remains relatively constant, with the exception of an early and transient increase upon clamping during 70% liver ischemia in the mouse. These data do, however, emphasize the important differences in the liver’s response to short vs. extended periods of I/R.
In summary, this study has identified significant differences in the mechanisms of postischemic liver injury following short vs. long periods of ischemia. Although hepatocellular injury induced by short periods of ischemia appears to require Kupffer cells and the NADPH oxidase-independent production of O$_2^·$, liver injury induced by extended periods of ischemia appear to be dependent on Kupffer cells, NADPH oxidase-derived O$_2^·$, and TNF-α. Data generated from this study may provide for a more rational approach to the design of new drug therapies to treat postischemic liver injury.

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