Differential mechanisms of hepatic vascular dysregulation with mild vs. moderate ischemia-reperfusion

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Lee SH, Culberson C, Korneszczuk K, Clemens MG. Differential mechanisms of hepatic vascular dysregulation with mild vs. moderate ischemia-reperfusion. Am J Physiol Gastrointest Liver Physiol 294: G1219–G1226, 2008. First published March 6, 2008; doi:10.1152/ajpgi.00527.2007.—Endotoxemia produces hepatic vascular dysregulation resulting from inhibition of endothelin (ET)-stimulated NO production. Mechanisms include overexpression of caveolin-1 (Cav-1) and altered phosphorylation of endothelial nitric oxide (NO) synthase (NOS; eNOS) in sinusoidal endothelial cells. Since ischemia-reperfusion (I/R) also causes vascular dysregulation, we tested whether the mechanisms are the same. Rats were exposed to either mild (30 min) or moderate (60 min) hepatic ischemia in vivo followed by reperfusion (6 h). Livers were harvested and prepared into precision-cut liver slices for in vitro analysis of NOS activity and regulation. Both I/R injuries significantly abrogated both the ET-1 (1 μM) and the ETB receptor agonist (IRL-1620, 0.5 μM)-mediated stimulation of NOS activity. 30 min I/R resulted in overexpression of Cav-1 and loss of ET-stimulated phosphorylation of Ser1177 on eNOS, consistent with an inflammatory response. Sixty-minute I/R also resulted in loss of ET-stimulated Ser1177 phosphorylation, but Cav-1 expression was not altered. Moreover, expression of ETB receptors was significantly decreased. This suggests that the failure of ET to activate eNOS following 60-min I/R is associated with decreased protein expression consistent with ischemic injury. Thus hepatic vascular dysregulation following I/R is mediated by inflammatory mechanisms with mild I/R whereas ischemic mechanisms dominate following more severe I/R stress.

Liver microcirculation; endothelial nitric oxide synthase; endothelin-1; precision-cut liver slices

UNDER INFLAMMATORY OR OXIDATIVE STRESS conditions, hepatic microvascular flow is imbalanced toward a more pronounced vasoconstriction and results in heterogeneous perfusion. Our laboratory has previously shown that hepatic ischemia-reperfusion (I/R) injury increases the contractile response to endothelin (ET)-1, which further sensitizes the portal circulation to elevated ET-1 levels and may contribute to the impairment of liver microcirculation after I/R (8, 34). ET-1, a potent peptide in maintaining physiological liver blood flow, has been shown to play a major regulatory role under stress conditions (1–3). Recently, we reported that endotoxin suppresses the activation of endothelial nitric oxide synthase (eNOS), which is stimulated by exogenous ET-1 treatment in isolated hepatic sinusoidal endothelial cells (SECs). We proposed two different mechanisms for eNOS regulation via the disrupted phosphorylation of eNOS and overexpression of caveolin-1 (Cav-1) between normal and stress conditions in SECs (18). It is widely understood that hepatic I/R is responsible for primary liver dysfunction and ultimate failure after transplantation or hemorrhagic shock. It is also well known that inflammation and oxidative stress are major symptoms of I/R injuries (17). Moreover, the extent of liver injury is dependent upon the presence of a preexisting liver disease and on the duration of ischemia (10), especially during liver transplantation or hepatic surgery with temporal blood occlusion (5). Liver cells most affected by ischemic injury are the hepatocytes and SECs (14, 29). Furthermore, SECs, which are a major cell type involved in the regulation of hepatic microcirculation, suffer severe damage following reperfusion depending on the severity of the ischemic insult. Thus improvement of SEC function during the ischemic period is an important key to increase the success rate in a liver transplantation (28). Experimental data indicate that hepatic microcirculatory failure is a phenomenon of reperfusion injury after ischemia (4, 24) and that imbalanced nitric oxide (NO) production, mainly generated by eNOS, may play an important factor in these damages (32). However, many controversial results have been reported debating the role of NO in a pathophysiological condition (15, 19, 31, 33). Regarding hepatic I/R injury, several studies have shown that eNOS has a more critical role in modulating I/R-induced tissue injury than iNOS (inducible nitric oxide synthase) (13, 22). However, the molecular mechanism for eNOS regulation in I/R injury has not been well defined. Therefore, we investigated the regulatory mechanism of eNOS in hepatic microcirculation using mild and moderate hepatic I/R injuries in an in vivo rat model.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Charles River Laboratories, Fayetteville, NC) weighing 280–320 g were housed in a temperature-controlled animal facility with alternating 12:12-h light-dark cycles and were fasted overnight before surgery but allowed to drink water ad libitum. All procedures for this study were approved by and performed in accordance with the National Institutional Animal Care and Use Committee of the University of North Carolina at Charlotte.

Ischemia-reperfusion. The surgical procedure was performed on pentobarbital (50 mg/kg ip, Abbott Laboratories, North Chicago, IL) anesthetized animals with body temperatures maintained at 37°C. Briefly, a midline incision was made to the abdomen, and the left branches of the portal vein, hepatic artery, and bile duct were clamped to induce complete ischemia of the median and left hepatic lobes. The right branches maintained a normal circulation to prevent intestinal congestion. Following 30 (mild) and 60 (moderate) min of ischemia, livers were reperfused by removing the atrumatic clip; the peritoneal cavity was sutured closed for 6 h. The sham-operated animals were prepared in an identical manner but without portal vein clamping. After 6 h of reperfusion, blood samples were taken to measure the alanine aminotransferase (ALT) and lactate dehydrogenase (LDH).

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activities (Point Scientific Canton, MI). The median and left lobes of the liver were removed to prepare liver slices and a piece of liver tissue was snap frozen in liquid nitrogen for immunoblotting analysis.

**Preparation of PCLS.** After flushing the exposed liver in situ with ice-cold, oxygenated, sterile Krebs-Henseleit solution to remove excess blood, the liver was carefully excised, the lobes separated, and cored into 8-mm-diameter slices. Using the tissue slicer (Vitron, Tucson, AZ), we the liver cores sliced into the ice-cold Krebs-Henseleit buffer at ~200–250 μm/slice. Precision-cut liver slices (PCLS) were transferred to a nutrient medium composed of Williams’ E medium (GIBCO-BRL, Gaithersburg, MD) supplemented with insulin (500 units/l), glucagon (7 μg/l), EGF (20 μg/l), hydrocortisone (7.5 mg/l), penicillin (100,000 units/l), streptomycin (100,000 μg/l), amphotericin B (250 μg/l), and 10% fetal bovine serum. Two liver slices per well were loaded in six-well plates in 2 ml of medium and were incubated for 40 min at 37°C under 70% O2/5% CO2/25% N2. At 40 min postloading, the medium was replaced by fresh culture media or arginine-free HEPES buffer for further experiments.

**NOS activity.** The conversion of [3H]-arginine (Sigma, St. Louis, MO) to [3H]-citrulline was used to assess nitric oxide synthase (NOS) activity in PCLS. All experiments were conducted with Norvaline (15 mM), an inhibitor of arginase, to maximize the NOS reaction, whereas the Nω-nitro-L-arginine (15 mM) was used as a negative control to measure background levels. PCLSs were carefully moved to new six-well plates and incubated in 1 ml of warm arginine-free HEPES buffer (10 mM HEPES, 145 mM NaCl, 5 mM KCl, 5 mM, MgSO4, 10 mM glucose, 1.5 mM CaCl2, 0.25% bovine serum albumin, pH 7.4) for 30 min. At the end of the incubation time, 2 μCi/μl [3H]-arginine were added to each well and incubated for 2 min, after which ET-1 (1 μM) or IRL-1620 (0.5 μM) (American Peptide, Sunnyvale, CA) was added for a further 30-min stimulation. The reaction buffer was then removed and the PCLSs were washed three times with cold arginine-free HEPES buffer to remove the extracellular radioactive arginine. The reaction was terminated by adding 350 μl of ice-cold PBS (5 mM L-arginine, 4 mM EDTA, and 4 mM EGTA, 6 mM L-citrulline), after which the cells were lysed with 350 μl of lysis buffer (5 mM Tris (pH 7.4), 20 mM EDTA, 0.5% Triton X-100). The liver slices were disrupted by sonicator and centrifuged at 10,000 g to remove the debris. Aliquots from each sample were run through a cation-exchange chromatography resin (Dowex AG 50W-X8, Molecular Biology Grade, 200–400 mesh sodium form, Bio-Rad Laboratories, Hercules, CA) to separate the [3H]-arginine from the [3H]-citrulline. The resins were then washed with stop buffer (20 mM HEPES, 5 mM EDTA, 5 mM EGTA, pH 5.5), and the flowthrough was collected in glass vials to which 6 ml of scintillation fluid (SX20-5 Scinti-Safe Econo-1, Fisher Scientific, Springfield, NJ) was added. The presence of radioactive [3H] L-citrulline was detected with a liquid scintillation counter (Beckman LS 6000 series, Beckman Instruments, Fullerton, CA). Background levels were subtracted from all the sample levels, which were expressed as ratio of stimulated over nonstimulated samples.

**Western blot assay.** The whole-liver tissue and slices were homogenized with Pro-Prep solution (iNtRON Biotechnology, Sungnam, Kyungki-Do, Korea) which included protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 0.7 μg/ml pepstatin A, 0.5 μg/ml leupeptin, and 2 μg/ml aprotinin) and added phosphatase inhibitors (2 mM sodium orthovanadate, 5 mM sodium fluoride). The tissue lysate was centrifuged at 13,000 rpm for 10 min to remove the insoluble fraction. Total protein concentration for each sample was measured by use of the Micro BCA protein assay kit (Pierce, Rockford, IL). Fifty-microgram protein samples were boiled for 5 min in Laemmli loading buffer and separated by SDS-PAGE on 8–12% acrylamide gels. Nitrocellulose membranes were used to electroblot the proteins and then stained with Ponceau S to confirm equal protein loading and transfer. Membranes were washed in Tris buffered saline (TBS) with 0.1% vol/vol Tween 20 (TBS-T), blocked in 5% wt/vol nonfat milk or BSA in TBS-T buffer for 1 h, and then incubated with anti-Ser1177 (1:1,000), anti-Thr495 (1:1,000), anti-eNOS (1:1,000), heat shock protein 90 (HSP90; 1:1,000), calmodulin (1:2,000), anti-ETA receptor (anti-ETAR R; 1:2,000), anti-ETAR R (1:2,000), anti-Cav-1 (1:2,000), and β-actin (1:5,000) antibodies at 4°C overnight. Polyclonal antibodies recognizing eNOS phosphorylation site serine 1177 (eNOS pSer1177) and threonine 495 (eNOS pThr495) were purchased from Cell Signaling Technology (Beverly, MA), and a polyclonal antibody against ETAR was acquired from Abcam (Cambridge, MA). Anti-Cav-1 polyclonal and β-actin monoclonal antibody were purchased from Sigma. All other monoclonal antibodies and ETAR polyclonal antibody were from BD Transduction Laboratories (San Diego, CA). Membranes were then washed repeatedly in TBS-T and incubated for 1 h at room temperature with a horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). Immunoreactive bands were detected by use of the enhanced chemiluminescence reagent (GE Healthcare, Piscataway, NJ) and were exposed to CL-X Posure film (Pierce, Rockford, IL).
The quantification of band intensity was performed via a densitometric analysis program (Quantity One, Bio-Rad Laboratories, Hercules, CA). All signal intensities were normalized with β-actin.

Statistical analysis. Data are presented as means ± SE. Statistical differences between groups were determined by one-way ANOVA followed by post hoc Student-Newman-Keuls test. A *P* < 0.05 was considered significant. All statistical analyses were performed with Sigma-Stat software.

RESULTS

Effect of ischemia-reperfusion on serum ALT and LDH activities. Serum ALT and LDH were measured to estimate the extent of liver damage induced by I/R injury. In Fig. 1, the level of ALT was significantly elevated by 30 min (mild injury) ischemia-6 h reperfusion (237 ± 7 IU) and even higher levels by 60 min (moderate injury) I/R (439 ± 51 IU) compared with the sham group (46 ± 7 IU) and 30-min ischemia group. In addition, mild I/R injury did not show a difference in LDH activity; however, moderate I/R injury maintained significant higher LDH levels after the 6-h reperfusion period compared with sham (648 ± 85 vs. 180 ± 38 IU).

Effect of mild and moderate I/R injury on ET-1 response of NOS activity in PCLS. Previously, we reported that endotoxemia inhibits ET-1 induced eNOS activity in hepatic SECs (18). To investigate whether in vivo liver injury exerts a similar

![Graphs and images showing changes in eNOS and phosphorylation levels](http://ajpgi.physiology.org/)

Fig. 3. Effects of postischemic injury following mild or moderate ischemia on total endothelial NOS (eNOS) and eNOS phosphorylation in whole liver tissue without (-) or with (+) ET-1. The protein level of total eNOS (A) and phosphorylation of eNOS at Ser1177 (B) and at Thr495 (C) residue were investigated in whole hepatic tissue after 6-h reperfusion. Protein extracts from all experimental groups were analyzed by Western blot and normalized over β-actin. Data are means ± SE for 5 or 6 animals per group and are presented as comparable ratios with its density of sham group. *P* < 0.05 vs. sham group of each I/R experiment.

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response, we tested NOS activity using PCLS after I/R injury. In the previous studies we were able to model the in vivo stress by direct exposure of the SECs to LPS; however, I/R injury in the liver is not easily mimicked by purely in vitro models. Isolation of SECs following I/R injury in vivo is problematic because the time required for isolation and recovery from the enzymatic digestion period allows the cells to lose their in vivo phenotype. PCLS offers the advantage of minimal intervention and preparation time so that the tissue can be evaluated in vitro within minutes of harvesting. Preliminarily, we conducted a dose–response study to assess the optimal ET-1 and IRL-1620, a specific ETB receptor agonist, concentrations in PCLS. Four different doses ranging from 0.1 to 1 μM of each reagent were tested for NOS activity with normal liver slices. The maximal response was determined to be 1 and 0.5 μM from ET-1 and IRL-1620, respectively (data not shown). In sham-operated PCLS, 30 min of treatment with ET-1 led to a significant induction of NOS activity up to 50 and 70% at each tested condition, whereas mild and moderate I/R injuries totally inhibited these effects (Fig. 2). As expected, elevation of NOS activity by IRL-1620 in the sham group was also blocked by 30- and 60-min ischemia with 6-h reperfusion (Fig. 2).

Effect of mild and moderate I/R injury on eNOS phosphorylation in whole liver tissue and PCLS. Several studies have shown that phosphorylation at Ser1177 of eNOS is an activation site (27), whereas Thr495 residue is an inhibitory site of eNOS (7). Thus the phosphorylation patterns of eNOS were investigated in whole hepatic tissue immediately following 6-h reperfusion after mild or moderate ischemia and after ET-1 stimulation in PCLS. As shown in Fig. 3A, eNOS expression level in whole liver tissue was significantly decreased in mild I/R injury but not in moderate I/R compared with the sham group. However, neither 30- nor 60-min ischemia followed by 6-h reperfusion affected eNOS Ser1177 or Thr495 phosphorylation in whole liver tissue (Fig. 3, B and C). In addition, we observed the effect of ET-1 on phosphorylated eNOS in sliced liver tissue to investigate the mechanism behind I/R inhibition of ET-1 induced eNOS activation. ET-1 treatment in sham increased eNOS Ser1177 phosphorylation levels compared with non-ET-1 treated sham PCLS. However, this upregulation of eNOS pSer1177 was impaired by mild I/R induced hepatic injury compared with ET-1-stimulated sham PCLS. In contrast, phosphorylated Ser1177 levels in 60 min ischemia/6 h reperfusion were slightly lower than in the sham group, but this difference did not reach statistical significance (Fig. 4A). In addition, phosphorylation of Thr495, an inhibitory site of eNOS, was not changed with 30 min ET-1 treatment in PCLS following sham or either I/R operation (Fig. 4B).

Effect of mild and moderate I/R injury on expression of ET receptor subtypes in whole liver tissue. The change in ET receptor subtype expression was confirmed at the protein level by Western blot analysis. Mild (30 min) or moderate (60 min) I/R injury did not affect the protein levels of ETA receptors, which mediate the vasoconstrictive effects of ET-1 compared with sham group (Fig. 5A). However, the density ratio of ETB receptors, which mediate the vasodilatory effects, was slightly decreased with mild I/R injury and significantly decreased in moderate I/R compared with sham, as shown in Fig. 5B.

Effect of mild and moderate I/R injury on eNOS-related proteins in whole liver tissue. We also detected several proteins that interact with eNOS to either activate or inhibit its activity. Neither mild or moderate I/R affected the expression of HSP90

![Fig. 4. Effects of mild or moderate I/R injury on eNOS phosphorylation in PCLS.](image-url)
or calmodulin (Fig. 6, A and B). However, Cav-1 protein expression was significantly increased after mild I/R injury compared with the sham group, but not in moderate I/R injury (Fig. 6C).

**DISCUSSION**

Clinically, hepatic I/R occurs during liver transplantation and in hepatic surgery with temporal blood occlusion. This imbalanced ratio between demand and supply of oxygen by I/R injury is known to result in microcirculatory dysfunction in endothelial cells. Usually, the major factors of I/R injury include direct cellular damage due to the ischemic insult as well as a prolonged malfunction resulting from activation of the liver inflammatory pathways. Also, the proinflammatory cytokines and reactive oxygen species (ROS), which are mainly produced during the reperfusion period, may play a critical role in the initiation of I/R-mediated parenchymal and nonparenchymal cell damage. In addition to I/R damage, an imbalance between NO and ET production could cause microcirculatory disturbances in liver, which consequently incur damage to its structure and function. Moreover, several studies have shown that NO may be involved in ROS-mediated reactions, thereby modulating the inflammatory response (9, 16) and further supporting the premise that I/R injury is closely associated with a decrement in the bioavailability of NO (11, 22, 26). Although these results indicate that altered NO production in these situations contributes to liver microcirculatory dysfunction, the molecular mechanisms underlying its effects are still not elucidated.

Therefore, in the present study, we investigated the change of ET-1-stimulated eNOS activity following reperfusion injury using different ischemic times in a PCLS model. This model is extensively used in drug metabolism and toxicological studies since it bridges the in vitro and in vivo conditions. Here, we tested for factors involved in eNOS regulation previously suggested from our in vitro studies (18, 21). To confirm the hepatocellular injury by I/R operations, ALT and LDH serum levels were measured as representative indicators. Detrimental changes in ALT after 6-h reperfusion following mild (30 min) or moderate (60 min) ischemic injury was observed and, as expected, the moderate ischemic group caused a more severe cellular damage than the mild I/R (12). Additionally, a significant increase in LDH activity was found only in the moderate I/R injury. These data suggest that a 30- and 60-min ischemia period followed by 6-h reperfusion provide different levels of hepatic damage.

Under normal physiological conditions, NO is produced through the activation of eNOS in SECs and then diffuses a short distance to hepatic stellate cells, causing their relaxation and subsequently sinusoidal dilation. Previously, our laboratory has shown that endotoxin desensitizes ET-1-stimulated eNOS activation in SECs, but its effect on hepatic impairment in an in vivo injured animal model is unclear. To investigate this, we used a PCLS technique, a model having the advantage of in vitro and in vivo conditions. Here, we tested for factors involved in eNOS regulation previously suggested from our in vitro studies (18, 21). To confirm the hepatocellular injury by I/R operations, ALT and LDH serum levels were measured as representative indicators. Detrimental changes in ALT after 6-h reperfusion following mild (30 min) or moderate (60 min) ischemic injury was observed and, as expected, the moderate ischemic group caused a more severe cellular damage than the mild I/R (12). Additionally, a significant increase in LDH activity was found only in the moderate I/R injury. These data suggest that a 30- and 60-min ischemia period followed by 6-h reperfusion provide different levels of hepatic damage.

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dependent dilation will result in a net increase in vasoconstriction. On the basis of this hypothesis, we previously showed that after oxidative stress, exogenous ET-1 stimulation failed to activate eNOS, which could lead to increased ET-1-mediated vasoconstriction as seen in vivo (2). However, it is still unclear which inhibitory mechanisms for eNOS activation participate in various postischemic injury states depending on different ischemic times.

Recently, from in vitro studies, we proposed three major molecular mechanisms involved in endotoxin-mediated inhibition of ET-1 induction of eNOS: disruption of eNOS phosphorylation, increased Cav-1 expression, and inhibition of eNOS translocation (21, 30). In our present study, only mild I/R injury significantly abrogated the increased phosphorylated Ser1177 of eNOS as an activation site by treatment of exogenous ET-1 in PCLS, but this injury had no effect on eNOS Thr495 phosphorylation for inhibition. These findings suggest a decreased response of eNOS to ET-1 after mild I/R damage due to the downregulated phosphorylation of eNOS Ser1177 in PCLS. However, our previous in vitro results have shown a more predominant change of the eNOS Thr495 phosphorylative site than at the Ser1177 site (18). This disparity in the results is likely due to differences between single endothelial cells in vitro and liver tissue containing the complex network between cell types but obviously requires further work to clarify. We also determined the effect on phosphorylation of eNOS in whole liver tissue following two different ischemic times and 6-h reperfusion, but there are no differences compared with sham animal. However, total eNOS protein expression was decreased by mild I/R injury, which is consistent with

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**Fig. 6. Effects of postischemic injury following mild and moderate ischemia on eNOS signaling proteins in whole liver tissue.** Protein extracts from all experimental groups were analyzed with Western blot technique. The alteration of protein expressions of heat shock protein 90 (HSP90) (A), calmodulin (B), and caveolin-1 (Cav-1; C) were investigated in whole hepatic tissue after 6-h reperfusion following mild (30 min) or moderate (60 min) ischemia condition. Each blot was quantified and normalized by β-actin. Data represent means ± SE for 5 or 6 animals per group and are presented as relative ratios with its density of sham group. *P < 0.05 vs. sham group of each I/R experiment.
our previous results shown in chronic alcohol consumption experiments (20). Therefore, these findings suggest that the impairment of NO production by ET-1 stimulation in postischemic injuries following different ischemic times also could be explained with other possibilities, such as receptor mediation and/or alteration of protein-protein interaction, as well as disruption of eNOS phosphorylation.

To further explore whether different postischemic injuries alter the receptor-mediated eNOS activation, we measured the protein expressions of ETA and ETB receptors. These receptors have been reported to mediate the vasoconstrictive and vaso-dilative effects through ET-1 binding in the liver. Although ETA protein levels were not changed in either postischemic injury model, ETB receptor expression was somewhat decreased in the mild I/R injury but significantly so in the moderate I/R model. These results are supported by other hepatic injury models (20), but the proportion of ETB receptors and mRNA expression of ETB after I/R injury is still controversial (34, 35). This discrepancy could be explained by the reports that ETB receptors exist in two putative subtypes. ETB1 receptors are expressed on endothelial cells and are related to NO production, whereas ETB2 receptors are mainly expressed on vascular smooth muscle cells and are linked to vasoconstriction. Thus the differences in the ETB expression pattern are likely to be related to differential expression of these receptor types.

For signaling to eNOS, several known proteins are involved that act as activators (calmodulin and HSP90) or inhibitors (Cav-1). Previously, our laboratory has shown in experiments with SECs that endotoxin alters the expression and regulation of regulatory proteins to inhibit the activation of eNOS and suggests that overexpression of Cav-1, the major structural protein of caveolae, has a critical role in this downregulation of eNOS (18). In the present study, mild and moderate I/R injury did not affect the protein levels of calmodulin or HSP90 protein levels. However, mild I/R injury results in an increase in Cav-1 protein expression. Cav-1 has been shown to bind to eNOS, resulting in a potent inhibition of NO synthesis (6). In contrast, moderate I/R injury did not change the protein level of Cav-1 compared with sham group. These results indicate that postischemic damage induced by a mild ischemic period results in the alteration of signaling pathways related to eNOS, thus leading to an impairment of NO production by ET-1 upregulation.

Taken together, although postischemic injury following mild or moderate ischemia leads to similar effects on NO production by eNOS following ET-1 stimulation, they likely exert their effects through different regulatory mechanisms. The mild (30 min) I/R, which can produce oxidative stress and an inflammatory injury, altered the pattern of eNOS phosphorylation and increased the expression of Cav-1, results also observed in vitro in endotoxin-treated SECs. Extended injury following moderate (60 min) I/R, which evokes a more severe damage to the cellular membrane, appeared to decrease the binding of ET-1 because of further degradation of ETB receptor proteins than mild I/R. In conclusion, this study suggests that eNOS-related hepatic vascular imbalance is a common pathway following different durations of ischemia is mediated by oxidative stress and inflammatory mechanisms with mild I/R, whereas different ischemic mechanisms dominate the disorder of microcirculation during extended periods of ischemia. Although mechanisms involving phosphorylation of eNOS and protein interactions have been described in this in vivo study, further mechanistic studies are needed to assess the contribution of each regulatory pathway.

GRANTS

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