Ethanol modulates gut ischemia/reperfusion-induced liver injury in rats

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Yamagishi, Yoshiyuki, Yoshinori Horie, Shinzo Kato, Mikio Kajihara, Hironao Tamai, D. Neil Granger, and Hiromasa Ishii. Ethanol modulates gut ischemia/reperfusion-induced liver injury in rats. Am J Physiol Gastrointest Liver Physiol 282: G640–G646, 2002. First published November 28, 2001; 10.1152/ajpgi.00171.2001.—Whereas both ethanol and gut ischemia/reperfusion (I/R) are known to alter hepatic microvascular function, little is known about the influence of ethanol consumption on the hepatic microvascular responses to I/R. The objective of this study was to determine whether acute ethanol administration exacerbates the hepatic microvascular dysfunction induced by gut I/R. Rats were exposed to gut ischemia for 30 min followed by reperfusion. Intravital videomicroscopy was used to monitor leukocyte recruitment and the number of nonperfused sinusoids (NPS). Plasma alanine aminotransferase (ALT), tumor necrosis factor-α (TNF-α), and endotoxin concentrations were monitored. In separate experiments, ethanol was administered 15 min or 24 h before gut ischemia. In control rats, gut I/R increased the number of stationary leukocytes and NPS. It also elevated the plasma ALT, TNF-α, and endotoxin with a corresponding increase in intestinal mucosal permeability. Low-dose ethanol consumption 15 min before gut ischemia blunted the gut I/R-induced leukostasis and elevations in plasma TNF-α and ALT. However, high-dose ethanol consumption aggravated the gut I/R-induced increases in leukostasis and increases in plasma endotoxin and ALT. When ethanol was administered 24 h before, high-dose ethanol aggravated the gut I/R-induced hepatocellular injury, but low-dose ethanol did not have any effects on it. These results suggest that low-dose ethanol consumption shortly before gut ischemia attenuates the hepatic inflammatory responses, microvascular dysfunction, and hepatocellular injury elicited by gut I/R, whereas high-dose ethanol consumption appears to significantly aggravate these gut I/R-induced responses.

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The latter observations are consistent with other reports demonstrating that bacterial endotoxin can induce hepatic microvascular dysfunction and liver injury (11). Furthermore, in endotoxemic animals, even relatively low-dose ethanol that cannot cause hepatic microvascular dysfunction alone induced hepatic microvascular dysfunction (12). Thus interaction between ethanol and endotoxin on hepatic microcirculation has been recognized.

Recently, it was reported that ethanol consumption may alter the tissue responses to I/R (27, 29, 38). In a perfused liver model, ethanol enhanced gut I/R-induced hepatotoxicity (an increase in blood levels of liver enzymes) and enhanced the production of reactive oxygen species (38). Similarly, in an in vivo gut I/R model, ethanol enhanced neutrophil accumulation in the intestinal wall (29). However, other studies indicate that ethanol may attenuate I/R-induced tissue injury. For example, in vivo experiments in the brain indicate that ethanol pretreatment attenuates cerebral I/R injury (27). It has also been reported that both acute and chronic ethanol consumption prevent I/R injury in the rat brain (35). It has recently been shown that rhodamine-6G-labeled leukocytes, was visualized for 90 min after the start of superior mesenteric artery (SMA) occlusion and recorded on a digital video recorder for a 1-min period at 0, 30, 60, and 90 min after reperfusion. The number of stationary leukocytes was determined off line during playback of the videotaped images. A leukocyte was considered stationary within the microcirculation (sinusoids) if it remained motionless for >10 s. Stationary leukocytes were quantified in both the midzonal and pericentral regions of the liver lobule and expressed as the number per field of view (2.1 × 10^4 μm²). The percentage of nonperfused sinusoids was calculated as the ratio of the number of nonperfused sinusoids to the total number of sinusoids per view field.

Experimental protocols. The liver microcirculation was observed for 10 min before ligation of the SMA to ensure that all the parameters measured online were at a steady state. The SMA was then ligated with a snare created from polyethylene tubing for 0 (sham) or 30 min. Estimates of blood flow by laser Doppler flowmetry indicate that ligation of the SMA results in an ~70% reduction of blood flow in mouse liver (13). After the ischemic period, the ligature was gently released. Leukocyte accumulation and number of nonperfused sinusoids were measured before the induction of ischemia immediately after reperfusion and every 15 min for 1 h thereafter. In some experiments, the rats were fed ethanol (10%, 1 g/kg or 40%, 4 g/kg) through a gastric tube 15 min or 24 h before the induction of ischemia. These experiments were performed using five animals in each group.

Analysis of intestinal mucosal permeability. After laparotomy, a plastic catheter was secured in the lumen of the duodenum and the bowel was occluded just proximal to the opening of the plastic tube. The ileal end was transected, and the small intestine was gently lavaged with 10 ml saline. After ligation of the ileal end, ethanol (10%, 1 g/kg or 40%, 4 g/kg) or water (10 ml/kg body) was placed in the intestine. After 30 min, the small intestine was gently lavaged with 10 ml saline. Forty-five minutes after the administration of ethanol or water, the intestine was loaded with 5 ml of a solution (25 mg/ml) containing fluorescein isothiocyanate-dextran (FD4) with an average molecular mass 4,000 kDa (Sigma, St. Louis, MO). Blood samples were obtained from a femoral vein at 0, 15, 30, and 60 min after the administration of FD4 solution. The concentration of FD4 in plasma was determined using a fluorescence spectrophotometer (model RF-5300 PC; Shimadzu, Tokyo, Japan). In these experiments, both renal pedicles were ligated to prevent urinary excretion of the fluorescent probe (34).

Liver enzyme, TNF-α, endotoxin, and ethanol assays. Plasma samples were collected (105 min after the ethanol or saline administration) from a catheter placed in the inferior vena cava at a point distal to the hepatic veins with ligation of inferior vena cava at a point proximal to the entry of the renal veins. This procedure allowed only blood passing through the liver to be collected. The plasma TNF-α concentration was also determined in a microtiter plate using a commercial TNF-α assay kit (BioSource International, Camarillo, CA), based on an ELISA. In another set of experiments, alanine aminotransferase (ALT) activity was determined in the samples collected 1 and 6 h after reperfusion by
a spectrophotometric assay using a commercial kit (Sigma). The plasma endotoxin concentration was measured with endospec, an endotoxin-specific chromogen (Seikagaku, Tokyo, Japan), as described previously (31). In some experiments, plasma ethanol concentration was measured with a gas chromatograph (Perkin-Elmer, Yokohama, Japan), according to a previous report (32).

**Statistical analysis.** All values were expressed as means ± SE. Statistical significance was determined by one-way ANOVA and Scheffé’s post hoc test. Statistical significance was set at $P < 0.05$.

**RESULTS**

Plasma ethanol concentration was $14.8 ± 1.7$ mM at 45 min after low-dose (1 g/kg) ethanol administration, and $36.4 ± 1.7$ mM at 45 min after high-dose (4 g/kg) ethanol administration.

Figure 1 summarizes the changes in hepatic leukosequestration (number of stationary leukocytes) elicited by gut I/R in untreated rats and in rats consuming either low-dose (1 g/kg) or high-dose (4 g/kg) ethanol. Although low-dose ethanol did not change the number of stationary leukocytes under basal conditions, a significant (277%) increase was noted after high-dose ethanol. A more dramatic increase in hepatic leukosequestration (8-fold) was elicited by gut I/R in untreated rats. After consumption of low-dose ethanol, the leukosequestration response to gut I/R was profoundly attenuated. However, high-dose ethanol consumption exerted the opposite effect, i.e., it exacerbated the leukocyte accumulation induced by gut I/R. Changes in the number of nonperfused sinusoids (NPS) (Fig. 2) that resulted from gut I/R followed a pattern similar to that observed for leukocyte adhesion (Fig. 1). That is, low-dose ethanol attenuated, whereas high-dose ethanol exacerbated, the %NPS response to gut I/R. Furthermore, high-dose ethanol produced a small but significant increase in %NPS under basal conditions, i.e., in the absence of gut I/R.

Figure 3 summarizes the changes in plasma FD4 concentration, a measure of intestinal mucosal permeability (IMP), elicited by gut I/R in untreated rats and in rats consuming either low-dose (1 g/kg) or high-dose (4 g/kg) ethanol. Although low-dose ethanol did not alter plasma FD4 under basal conditions, high-dose ethanol elicited a substantial (17-fold) increase in IMP in the absence of gut I/R. Indeed, the magnitude of the increase in plasma FD4 produced by gut I/R in untreated rats was comparable with that caused by high-dose ethanol in the absence of gut I/R.

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**Fig. 1.** Effects of ischemia/reperfusion (I/R) and/or ethanol (EtOH) administration on number of stationary leukocytes. $n = 5$. *$P < 0.05$ vs. control. +$P < 0.05$ vs. I/R. $#P < 0.05$ vs. same amount of EtOH without I/R.

**Fig. 2.** Effects of I/R and/or EtOH administration on percentage of nonperfused sinusoids (%NPS). $n = 5$. *$P < 0.05$ vs. control. +$P < 0.05$ vs. I/R. $#P < 0.05$ vs. same amount of EtOH without I/R.

**Fig. 3.** Effects of I/R and/or EtOH administration on intestinal mucosal permeability [plasma fluorescein isothiocyanate-dextran (FD4) concentration]. $n = 5$. *$P < 0.05$ vs. control. +$P < 0.05$ vs. EtOH (1 g/kg)
consumption, at either dose, did not alter the mucosal permeability response to gut I/R.

Plasma endotoxin and TNF-α concentrations were measured at 105 min after ethanol or saline administration (Figs. 4 and 5). Low-dose ethanol under basal conditions did not affect plasma endotoxin and TNF-α concentrations; however, the concentration of both inflammatory mediators was increased after high-dose ethanol consumption in otherwise normal rats. Gut I/R elicited significant increases in plasma endotoxin and TNF-α concentrations in untreated rats. Whereas consumption of low-dose ethanol did not alter the gut I/R-induced increase in plasma endotoxin, this treatment blunted the gut I/R-induced increase in plasma TNF-α. High-dose ethanol consumption had the opposite effect on the responses of these mediators to gut I/R, i.e., it aggravated the increase in plasma endotoxin concentration but did not alter the elevation in plasma TNF-α concentration.

Plasma ALT activity was measured 1 and 6 h after reperfusion or ethanol administration (Figs. 6 and 7). Neither low-dose nor high-dose ethanol per se affected the plasma ALT activities in otherwise normal animals. However, gut I/R elicited a significant increase in the plasma ALT activities 6 h after reperfusion. Consumption of low-dose ethanol blunted the gut I/R-induced increase in plasma ALT, whereas high-dose exacerbated the increment the plasma ALT at 1 h after the gut I/R.

Figure 8 shows plasma ALT activities 6 h after reperfusion in rats pretreated with ethanol. Pretreatment with low-dose ethanol 24 h before gut ischemia did not affect gut I/R-induced increase in plasma ALT activities. On the other hand, pretreatment with high-dose ethanol aggravated them.

**DISCUSSION**

In the present study, gut I/R was shown to cause leukosequestration in the liver, increase the %NPS, and result in hepatocellular injury (as reflected in an elevated serum ALT). These responses to gut I/R are consistent with previous studies of rat and mouse liver (13–15). Accompanying these changes were an increased IMP, and elevated plasma concentrations of endotoxin and TNF-α. On the basis of the latter observations, it is tempting to speculate that the increased
The major objective of the present study was to determine whether and how ethanol consumption alters the hepatic microvascular and inflammatory responses to gut I/R. Our findings indicate that ethanol consumption exerts a profound influence on the liver responses to gut I/R and that the amount of ethanol consumed determines the nature (protection vs. exacerbation) of these responses. We observed that low-dose ethanol consumption, which did not affect the hepatic microcirculation in otherwise normal animals, significantly blunted the gut I/R-induced hepatic microvascular dysfunction and concomitant hepatocellular injury (elevation of serum ALT levels). It also attenuated the gut I/R-induced increase in the plasma TNF-α concentration but not the increase in IMP or blood endotoxin concentration. These observations, coupled with previously reported protection of a TNF-α mAb against gut I/R-induced hepatic dysfunction (10), suggest that low-dose ethanol may exert its beneficial effects in this model by attenuating TNF-α production after gut I/R. Of potential relevance in this regard is a report describing an attenuated sensitivity of Kupffer cells to endotoxin after ethanol treatment in vivo in early period (7). On the other hand, pretreatment with low-dose ethanol 24 h before the gut I/R did not affect the gut I/R-induced hepatocellular injury (increases in plasma ALT activities). This result suggests that the low-dose ethanol administration did not cause tolerance of Kupffer cells 24 h before the gut I/R.

In a manner similar to gut I/R, high-dose ethanol administration per se induced an increase in IMP and blood endotoxin levels, but it did so without causing corresponding hepatocellular dysfunction or an elevated plasma TNF-α. These observations suggest that high-dose ethanol- and gut I/R-induced hepatic microvascular dysfunction may involve both common and distinct mechanisms. Both high-dose ethanol administration and gut I/R have been reported to cause hepatic microvascular dysfunction via inducing oxidative stress. There is an oxidative stress that results from alcohol metabolism via cytochrome P-450 2E1 (4, 16). This enzyme, as well as others involved in ethanol metabolism, such as alcohol dehydrogenase, aldehyde...
dehydrogenase, and cytochrome P-450 2E1, are localized in the pericentral region of the liver lobule (18, 33). Hence, our findings that high-dose ethanol administration alone could cause an increase in NPS, and that high-dose ethanol-induced leukostasis occurred to a greater extent in the pericentral region, compared with the midzonal region, of the liver lobule is consistent with the possibility that ethanol metabolism (oxidation)-induced hypoxia and oxidative stress in the hepatic sinusoids contributes to ethanol (high dose)-induced hepatic microvascular dysfunction.

Another interesting finding of the present study is that high-dose ethanol consumption aggravated gut I/R-induced hepatic microvascular and hepatocellular dysfunction, with an accompanying profound elevation in blood endotoxin concentration. There is no significant difference in IMP after gut I/R with or without ethanol administration. Either gut I/R or high-dose ethanol administration substantially increased IMP. Because either one already has substantial effects on IMP, it is not surprising that gut I/R with high-dose ethanol administration did not have synergical enhancement of an increase in IMP. Because the gut I/R-induced increase in IMP was not further increased by high-dose ethanol, it appears unlikely that more mucosal injury accounts for the higher blood endotoxin concentration. It has been reported that administration of high-dose ethanol decreases the clearance of endotoxins (8). Reticuloendothelial function disturbed by high-dose ethanol can decrease elimination capacity of endotoxin, which causes the enhanced increase in plasma endotoxin levels by high-dose ethanol administration (30). It is also noteworthy that despite the more pronounced elevation in blood endotoxin noted in our gut I/R model receiving high-dose ethanol, the plasma TNF-α concentration was not further elevated. This attenuated TNF-α response likely results from the reduced sensitivity of Kupffer cells to endotoxins (7) mentioned above. Irrespective of why TNF-α was not further elevated after gut I/R in the high-dose, ethanol-treated animals, this observation suggests that mediators other than TNF-α contribute to the exacerbation of gut I/R injury when high-dose ethanol is consumed.

It was reported that after ethanol treatment, isolated Kupffer cells exhibited tolerance to endotoxin early, whereas sensitization was observed later (7, 36). Pretreatment with high-dose ethanol aggravated gut I/R-induced increases in plasma ALT activities. Previous evidence and this result raise a possibility that pretreatment with high-dose ethanol induces tolerance of Kupffer cells at early points and their sensitization at later points. Although gut I/R-induced increases in plasma endotoxin concentration were aggravated, the high-dose, ethanol-induced tolerance of Kupffer cells at early points may compensate for endotoxin-induced elevation of plasma TNF-α concentration at early points. The high-dose ethanol-induced sensitization of Kupffer cells at later points may aggravate hepatocellular injury at later points.

In conclusion, the results of this study provide compelling evidence that ethanol consumption modifies the liver injury response to gut I/R, with low-dose ethanol providing protection against injury and high-dose ethanol exacerbating the injury response. The mechanisms that underlie these dose-dependent responses to gut I/R remain unclear. Our findings suggest that whereas gut I/R-induced changes in plasma endotoxin and TNF-α concentrations are affected by ethanol consumption, these factors alone cannot explain the differential responses to low- and high-dose ethanol. Identification of these factors may lead to improved therapeutic approaches for ischemic liver diseases.

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