Chronic ethanol consumption exacerbates microcirculatory damage in rat mesentery after reperfusion

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Ethanol chronically are significantly correlated with excessive alcohol consumption on subsequent stressful events have long been recognized, pathophysiological mechanisms are incompletely understood. We examined possible roles of oxygen radicals and glutathione content in mesenteric venules of chronically ethanol-fed rats exposed to ischemia-reperfusion. Changes in microvascular hemodynamics, such as red blood cell (RBC) velocity, leukocyte adherence, and albumin extravasation, were monitored in postcapillary venules by intravital fluorescence microscopy. Chronic ethanol feeding significantly exaggerated the magnitude of the decrease in RBC velocity, the number of adherent leukocytes, and increased albumin leakage elicited by 10 min of ischemia followed by 30 min of reperfusion. Oxidative stress in the endothelium of venules monitored by dihydrorhodamine 123 (DHR) fluorescence was more severe in rats fed ethanol chronically. Both superoxide dismutase and N-acetyl-L-cysteine, which is known to increase glutathione content, reduced the ischemia-reperfusion-induced decrease in RBC velocity, the number of adherent leukocytes, and the increase in albumin leakage, as well as oxidative activation of DHR. This suggests that the increased reperfusion-induced microvascular disturbances in the mesenteric venules of rats fed ethanol chronically are significantly correlated with excessive production of oxygen-derived free radicals and decreased glutathione synthesis.

Leukocyte adherence; vascular permeability; mast cell degranulation; oxygen radicals; glutathione

Alcohol-induced injury involves a number of organs of the central nervous system and digestive system (8). Although deleterious effects of excessive alcohol consumption are incompletely understood (2). There have been several reports showing morphological and functional changes in the microcirculation in various organs after chronic ethanol consumption (25, 44). It has been reported that chronic alcohol ingestion impairs dilatation of cerebral arterioles in response to activation of adenylate cyclase or ATP-sensitive potassium channels (26), and chronic ethanol consumption has been found to increase hepatic sinusoidal responsiveness to the vasoconstrictor endothelin-1, which may contribute to the increased susceptibility of ethanol-fed rats to secondary stresses such as endotoxemia (3). We have also demonstrated that chronic ethanol feeding enhances endotoxin-induced hepatic sinusoidal leukocyte adhesion (35). In contrast, regular alcohol consumption has some beneficial effects on cardiac function against ischemia-reperfusion (I/R) due to preconditioning (27, 30–32). However, no one has studied the effect of alcohol consumption on ischemia-reperfusion-induced injury to the microcirculation.

It is now well recognized that reperfusion of ischemic tissues leads to an injury response that is frequently accompanied by endothelial cell injury, enhanced protein efflux, and increased adherence and emigration of leukocytes in postcapillary venules (19, 22, 36). A variety of bioactive compounds and effector cells have been invoked as mediators of reperfusion injury, including reactive oxygen metabolites, lipid mediators, nitric oxide, platelets, mast cells, and granulocytes (13, 15, 18, 19, 42, 47). Both xanthine oxidase-derived and granulocyte-derived active oxygens are known to play a role in the pathogenesis of microvascular injury, and in vitro and in vivo studies have demonstrated that oxygen-derived free radicals promote leukocyte adherence and emigration and subsequent endothelial injury (41, 42, 45). Thus leukocyte adhesion and the release of oxygen radicals after reperfusion are thought to be closely related in the process of microvascular injury. Although acute ethanol ingestion potentiates leukocyte...
migration into small intestine (43), there have been no reports showing the role of oxygen radicals in the process of ischemia-reperfusion-induced microvascular damage in animals after long-term treatment with alcohol.

The tripeptide glutathione (γ-Glu-Cys-Gly) is the major intracellular thiol compound in most mammalian tissues and is known to be involved in a variety of cell functions via participation in metabolic and detoxification reactions (29). Reduced glutathione (GSH) plays a protective role in the metabolism of a large number of toxic agents, reacting either with them directly to form adducts or as a cosubstrate in conjugation and peroxidation systems. Thus decreases in GSH levels have been associated with the increased toxicity of many compounds (12). Chronic ethanol consumption has been demonstrated to increase the liver toxicity of different compounds, and this effect may be related to the changes induced in glutathione metabolism (7, 33). It is unknown, however, whether the changes in glutathione content induced by chronic ethanol consumption modify the extent of microvascular disturbances after ischemia-reperfusion.

The present experiment was therefore undertaken to assess ischemia-reperfusion-induced alteration of microvascular hemodynamics, albumin leakage, and leukocyte-endothelial cell interactions in the postcapillary venules of rats chronically fed ethanol. These issues are addressed in a rat mesentery model, which allows for simultaneous measurement of leukocyte adherence, albumin leakage, and release of oxygen-derived free radicals in discrete segments of postcapillary venules exposed to ischemia, and subsequent reperfusion (29, 45). N-acetyl-L-cysteine (NAC), maleic acid diethyl ester, and superoxide dismutase (SOD) were used to assess the relative contribution of GSH concentration or oxygen radical production to the leukocyte-endothelial cell interactions and albumin leakage observed in postcapillary venules exposed to ischemia-reperfusion.

MATERIALS AND METHODS

Animals and chronic ethanol feeding. All animals were handled according to the guidelines of the Keio University Animal Research Committee. Male Wistar rats (200–250 g; Charles River Breeding Laboratories, Tokyo, Japan) were pair fed for 8 wk with a liquid diet containing ethanol that provided 36% of their total energy or an isocaloric control diet according to the methods of Lieber and DeCarli (21).

Experimental protocol for I/R injury. Rats were anesthetized by intraperitoneal injection of sodium pentobarbital, 30 mg/kg body wt. The right jugular vein was cannulated with a polyethylene catheter. The abdomen was opened via a midline incision 20–30 mm long. The ileocecal portion of the mesentery 20 cm caudal was gently drawn out, exteriorized, and mounted on a transparent plastic stage specially designed for the rat. The mesentery was kept warm and moist by continuous superfusion with Krebs-Ringer-bicarbonate-buffered solution at 37°C. Microcirculatory hemodynamics in the mesentery were observed by a transillumination method by using an inverted microscope (Diaphot TMD-2S; Nikon, Tokyo, Japan). The mesentery was transilluminated with a 12-V, 100-W, direct current-stabilized light source. A video camera mounted on the microscope projected the image onto a color monitor, and the images were recorded with a videocassette recorder. A video time-date generator projected the time and stopwatch function onto the monitor. Single unbranched venules with diameters ranging between 25 and 35 μm and length >200 μm were selected for study.

After 10 min of basal observation of the hemodynamics in the rat mesenteric microvasculature, the I/R was performed by ligation of the mesenteric artery of the feeding branch of the anterior mesenteric artery and the corresponding vein simultaneously with a snare created from a polyethylene tube for 10 min and subsequent release of the blood flow occlusion (18). Because red blood cell (RBC) velocity of the vessels during the ischemia was not zero, there may have been collateral perfusion to the observed area. Thus both artery and vein were ligated to stop blood supply and to induce venular congestion to enhance ischemia. Earlier study showed that 10 min of ischemia followed by reperfusion was enough to induce mesenteric microcirculatory disturbance and minimize intestinal tissue damage (15). Sham-operated rats without I/R were used as controls.

Measurement of microvascular parameters. The images of arterioles and venules were obtained through a charge-coupled device (CCD) color video camera system (CC-090; Flovel, Tokyo, Japan). The diameters of microvessels were determined time dependently using a video measuring gauge (IV-560; Hoei, Tokyo, Japan). The leukocytes that had adhered to the venules walls were identified by reviewing the dynamic images of leukocytes recorded through this system. Adherent leukocytes were defined as cells that attached to the same site for more than 10 s judging from the replayed video images. The number of adherent leukocytes was counted along venules (25–35 μm in diameter, 200 μm in length) randomly selected from the videotape images recorded (22, 40).

The velocity of RBCs in the venules was simultaneously recorded at a rate of 1,000 frames/s by changing the monitor from CCD to a high speed video camera system (Ektapro 1000; Kodak, San Diego, CA), and the recordings were replayed from the high speed stored images at a rate of 30 frames/s. RBC velocity in the venule was measured with a temporal correlation velocimeter (CapiFlow, Kista, Sweden) (40).

 Mast cells were identified in the same animals by vital staining with topical application of 0.1% toluidine blue to the mesentery 30 min after the onset of I/R. The numbers of both nondegranulated mast cells and degranulated mast cells were counted from the CCD video images, and the ratio of the number of degranulated mast cells to the total number of mast cells is expressed as the degranulated mast cell ratio (40). To quantify albumin leakage across mesenteric venules, the animals were intravenously injected with 50 μg/kg of FITC-labeled bovine serum albumin (Sigma Chemical) 30 min before each experiment as described previously (17). Fluorescence intensity (excitation 420 to 490 nm; emission 520 nm) was detected using a silicon-intensified target camera (C-2400–08; Hamamatsu Photonics, Hamamatsu, Japan). The fluorescence intensity of FITC-albumin in three segments of the venule under study (L1) and in three contiguous areas of perivenular interstitium (L2) area were measured at various times after the administration of FITC-albumin with a computer-assisted digital imaging processor (NIH Image 1.35 on a Macintosh computer), with background fluorescence intensity subtracted from each value. The windows to measure average fluorescence intensity within and
along the venule were set at 50 μm long and 25 μm wide (16, 18). An index of vascular albumin leakage was determined by dividing Iₜ by Iᵦ and expressing this ratio as a percentage (i.e., a value of 50% would indicate that Iₜ is one-half that of Iᵦ). This tissue-to-plasma index is referred to as “albumin leakage” and is reported during the baseline period as well as during specific times of reperfusion.

In another set of experiments, the oxidant-sensitive fluorescent probe dihydrorhodamine 123 (DHR; Molecular Probes) was added to the mesenteric superfusate (10 μmol/l) to monitor oxidant stress in venular walls as described previously (16). The fluorochrome was visualized by using the same microscope and image analysis system described above. DHR fluorescence intensity was monitored in a region of mesentery equivalent to twice the area of the venule being examined. An image processor was used to monitor fluorescence intensity just before (baseline value; Iₜₖₑₜₜₑₜ) and after (Iₜ) I/R. Since Iₜₖₑₜₜₑₜₑ varied depending on DHR loading into the cells, the ratio of Iₜₖₑₜₜₑₜₑ to Iₜₖₑₜₜₑₜₑ was defined as an index of oxidant stress in the mesenteric tissue and expressed as DHR fluorescence ratio (16), in which DHR was oxidized to fluorescent rhodamine 123 by intracellular and extracellular oxidant stress in the mesenteric tissue and expressed as a percentage (i.e., a value of 50% would indicate that Iₜ is one-half that of Iᵦ).

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Agents studied. In a separate group, SOD, a scavenger of superoxide anion, was continuously infused (12,000 units·kg⁻¹·h⁻¹) via the jugular vein from 30 min before ischemia until the end of the experiment. In some experiments, we assessed the microvascular parameters described above after superfusion of rat mesentery with 5 mmol/l NAC, a stimulator of glutathione synthesis (34) or 100 μmol/l maleic acid diethyl ester (DMA), a depletor of cell glutathione (34).

Statistical analysis. Data were analyzed by standard statistical methods, i.e., one-way ANOVA and Fisher’s post hoc test. All values are reported as means ± SD of values from 4 to 6 rats, and statistical significance was set at P < 0.05. In the experiments of RBC velocity, leukocyte adhesion, and mast cell degranulation, pair-fed rats (dextrin and alcohol groups) were used in the sham-operated group (n = 6), I/R alone group (n = 6), I/R + SOD group (n = 6), I/R + NAC group (n = 5), and I/R + DMA group (n = 6). Albumin leakage was measured in the pair-fed rats from the sham-operated group (n = 4), I/R alone group (n = 6), I/R + SOD group (n = 4), I/R + NAC group (n = 4), and I/R + DMA group (n = 4). DHR fluorescence was examined in the pair-fed rats from the sham-operated group (n = 4), I/R alone group (n = 5), I/R + SOD group (n = 4), I/R + NAC group (n = 4), and I/R + DMA group (n = 4).

RESULTS

Changes in vascular diameter and RBC velocity. The diameter of arterioles and venules under baseline conditions was 22.5 ± 2.9 and 35.9 ± 3.5 μm, respectively, in the control dextrin-fed group (n = 6) and 22.3 ± 2.5 and 36.1 ± 3.0 μm in the ethanol-fed group (n = 6). The values in the two groups were not significantly different. On the basis of a morphometric analysis, the diameters of the mesenteric arterioles and venules were increased in the I/R group, possibly as a result of congestion, whereas the diameter of the arterioles and venules was almost completely maintained under baseline conditions.

Figure 1A compares the time course of changes in RBC velocity in venules after I/R treatment in the dextrin-fed and ethanol-fed groups. There was no sig-
nificant difference in baseline RBC velocity between the two groups. Just after the ischemic period, a significant decrease in RBC velocity was observed in both the dextrin-fed and ethanol-fed groups, and during the subsequent reperfusion period, RBC velocity decreased in both the dextrin-fed and ethanol-fed groups at 10 min. In the ethanol-fed group, RBC velocity further decreased to 38.0 ± 2.4% (n = 6) of the baseline at the end of observation period, whereas in the dextrin-fed group RBC velocity maintained its value at 70.7 ± 7.3% (n = 6) after 30 min. There were no significant changes in RBC velocity of the sham-operated rats in either the dextrin-fed or ethanol-fed group.

Figure 1B shows the effect of pretreatment with SOD, NAC, or DMA on changes in RBC velocity in venules at 30 min after reperfusion in the dextrin-fed and ethanol-fed groups. Both SOD and NAC treatment effectively attenuated the I/R-induced decline in RBC velocity of the ethanol-fed group. There were no significant differences in RBC velocity between the dextrin-fed and ethanol-fed groups after SOD and NAC treatment. DMA treatment did not alter RBC velocity compared with I/R alone in either group.

Changes in the number of leukocytes adhering to venular walls. Figure 2A compares the time course of changes in the number of leukocytes adhering to venular walls before and after the I/R period in the dextrin-fed and ethanol-fed groups. Before ischemia, no leukocytes adhered to the venular walls in either the dextrin-fed or ethanol-fed group. In the sham-operated groups, a small number of leukocytes was found, and their number gradually increased to 3.5 ± 2.2 (per 100 μm venules; n = 6) in the dextrin-fed group and 3.9 ± 2.3 (per 100 μm venules; n = 6) in the ethanol-fed group. In the I/R animals, an initial and dramatic increase in the number of adherent leukocytes was observed in both the dextrin-fed and ethanol-fed groups immediately after the reperfusion. Although the number of adherent leukocytes further increased thereafter in the ethanol-fed group and reached to 21.3 ± 2.6 (per 100 μm venules; n = 6) at 30 min after reperfusion, the number in the dextrin-fed group showed no significant further increase from 10 to 30 min after the reperfusion.

Figure 2B shows the effect of pretreatment with SOD, NAC, or DMA on the number of leukocytes adhering to venules at 30 min after reperfusion in the dextrin-fed and ethanol-fed groups. Both SOD and NAC treatment significantly attenuated the I/R-induced increase in leukocyte number in the ethanol-fed group, but these attenuating effects were not significantly demonstrated in the dextrin-fed group. Although not shown in this figure, SOD treatment did not affect reperfusion-induced leukocyte adherence at 10 min even in the ethanol-fed group. DMA treatment, on the other hand, significantly enhanced the I/R-induced increase in leukocyte number in the ethanol-fed group. DMA treatment in the dextrin-fed group slightly, but not significantly, enhanced the I/R-induced increase in leukocyte adherence.

Changes in albumin leakage from venular walls. Figure 3, A and B, shows representative fluorographic images of albumin leakage in the mesentery of the ethanol-fed animal at baseline and 30 min after the reperfusion, respectively. Figure 4 demonstrates significant adherence of leukocytes to venular walls with...
degranulation of mast cells in the interstitium by using a CCD camera and toluidine blue staining in the same microvasculature at 30 min in the higher magnification. Figure 5A illustrates the time course of changes in albumin leakage from the venular walls before and after the I/R period in the dextrin-fed and ethanol-fed groups. Before ischemia, no detectable albumin leakage was observed in either the dextrin-fed or ethanol-fed group. In the sham-operated groups, a small amount of albumin leakage was found during the observation period (1.8 ± 0.8% in dextrin-fed group (n = 4) and 2.0 ± 0.7% in the ethanol-fed group (n = 4) at the end of experiments). In the I/R groups, the extent of albumin leakage from venules initially increased immediately after reperfusion in both the dextrin-fed and ethanol-fed groups. A further and significant increase in albumin leakage was observed from 10 to 30 min after reperfusion in the ethanol-fed groups, but no such increase was observed in the later period in the dextrin-fed group.

As shown in Fig. 5B, treatment with SOD or NAC significantly attenuated the I/R-induced increase in albumin leakage from venules 30 min after reperfusion in both the dextrin-fed and ethanol-fed groups. SOD treatment already significantly inhibited the reperfusion-induced albumin leakage at 10 min in both groups (data not shown). DMA treatment, on the other hand, significantly aggravated the I/R-induced increase in albumin leakage at 30 min in both the dextrin-fed and ethanol-fed groups.

Changes in intensity of DHR fluorescence in the venular walls. Figure 6 shows the changes in the fluorescence intensity of the H$_2$O$_2$-sensitive probe DHR in the ethanol-fed group during the reperfusion period. Within 10 min, areas of fluorescence intensity were observed along the venule, then gradually increased until the end of the observation period. Although DHR activity was largely confined to the vascular endothelium, an area of fluorescence intensity corresponding to mast cells and emigrated leukocytes was also observed.
in the interstitium. Figure 7A compares the time course of changes in DHR fluorescence ratio to the baseline intensity on the venular walls after the I/R period in the dextrin-fed and ethanol-fed groups. There were no significant changes in DHR intensity during the observation period in the sham-operated groups. In the I/R groups, the intensity of DHR fluorescence was significantly increased just after reperfusion in the ethanol-fed group and beginning at 10 min after reperfusion in the dextrin-fed group. In the ethanol-fed group, these values were dramatically increased at 20 min after reperfusion, and the values of DHR intensity were significantly greater compared with those in the dextrin-fed group at 20 and 30 min after reperfusion. However, the further increase in DHR at 20–30 min was not observed in the dextrin-fed group.

Fig. 6. Fluorographic representation of dihydrorhodamine 123 (DHR) oxidation in the rat mesentery after superfusion of DHR (top left, transillumination image; bottom left, baseline fluorescent image) and 10 (top right) and 30 (bottom right) min after reperfusion in the ethanol-fed rat. White spots, indicating the sites of DHR oxidation, are observed along the venular endothelium and in the interstitium.
As shown in Fig. 7B, treatment with SOD significantly attenuated the I/R-induced increase in DHR fluorescence ratio on the venular wall at 30 min after reperfusion in the ethanol-fed group but not in the dextrin-fed group, whereas NAC treatment significantly inhibited the I/R-induced increase of DHR fluorescence ratio in both groups. The increased DHR fluorescence ratio induced by I/R injury was significantly aggravated by the treatment with DMA in the dextrin-fed group but not in the ethanol-fed group.

Mast cell degranulation along microvessels. Figure 8 shows the ratio of degranulated mast cells in the area along the microvessels 30 min after the reperfusion period and the effects of SOD, NAC, and DMA treatment. In the sham-operated groups, the mast cell degranulation ratio was 21.0 ± 3.0% in the dextrin-fed group (n = 6) and 23.0 ± 4.0% in the ethanol-fed group (n = 6). It was significantly increased in I/R animals in both the dextrin-fed and ethanol-fed groups, and there was no significant difference in these values between the two groups. Both SOD and NAC treatment significantly attenuated the I/R-induced increase of mast cell degranulation in both groups. DMA treatment, however, did not alter the I/R-induced increase of mast cell degranulation in either the dextrin-fed or ethanol-fed group.

DISCUSSION

One of the major objectives of this study was to compare the responsiveness of microvessels to I/R between normal dextrin-fed rats and chronic ethanol-fed rats by simultaneous measurement of leukocyte adherence and albumin leakage in postcapillary venules. The results obtained in this model indicate that ische-
parameters was significantly increased in the ethanol-fed rats compared with the dextrin-fed animals at 20–30 min after reperfusion. These observations suggest that there are two different stages in the process of microvascular disturbances by I/R and that only the later stage appears to be aggravated by chronic ethanol treatment.

We used the H$_2$O$_2$-sensitive fluorescent probe DHR, which has been successfully used to measure intracellular H$_2$O$_2$ levels in cultured endothelial cells and in other cell types, in an effort to monitor the oxidative stress elicited by I/R (6, 38). DHR has biological properties that make it an ideal agent for detecting secondary H$_2$O$_2$-dependent reactions in living tissue by fluorescence videomicroscopy. Royall and Ichihara (39) recently showed evidence that oxidation of DHR is not due to H$_2$O$_2$ alone but appears to occur by reactions involving reactive oxygen species. We have shown that oxidative stress in microvasculature subjected to I/R in vivo is more enhanced in ethanol-fed animals at 20–30 min after reperfusion. The remarkable difference in DHR oxidation in postcapillary venules between dextrin-fed and ethanol-fed animals in the later stage indicates that a greater oxidative stress occurred in the ethanol-fed rats.

Another important observation in the present study is that the antioxidant SOD effectively attenuated the microcirculatory disturbances induced by I/R at 30 min, particularly in the ethanol-fed group but partially in the dextrin-fed group. The difference in microvascular injury between the two groups was not observed after SOD treatment except after DHR, suggesting that, at least in the later stage, oxygen-derived free radicals may play a significant role as a causative mediator in ethanol-induced microcirculatory disturbances.

On the other hand, the increase in leukocyte adherence preceded the increase in DHR fluorescence, with the former becoming significant just after reperfusion and the latter at 10 min after I/R (Figs. 2A and 7A). These findings suggest that accumulated and activated leukocytes provoke the oxidative stress in microvascular walls subjected to I/R. Although it was first proposed that xanthine oxidase-derived oxidants mediate the microvascular injury associated with reperfusion of the ischemic intestine in 1981 (11), recent studies have suggested that leukocyte NADPH oxidase-derived oxygen radicals also play a role in the process of microvascular injury (10). In the present study, albumin leakage was significantly attenuated by SOD at 10 min, whereas leukocyte adherence was attenuated by SOD after 20 min. These findings support the involvement of xanthine oxidase-derived active oxygens in the early phase and granulocyte-derived oxygen metabolites in the later phase. However, the leukocyte recruitment in the early phase may at least in part be mediated by oxygen radical-independent mechanisms, because SOD treatment did not significantly inhibit leukocyte recruitment immediately after the reperfusion period in either the dextrin-fed or the ethanol-fed animals.

Another important observation in this study was the significant mast cell degranulation elicited by I/R and the ability of antioxidants to prevent this degranulation response, whereas there was no significant difference between the percentage of mast cells degranulated in the dextrin-fed and ethanol-fed animals. Kubes et al. (14) have proposed that inhibition of nitric oxide synthesis allows accumulation of superoxide, which in turn causes mast cell degranulation. They proposed that mast cell products, including platelet-activating factor, released in response to superoxide exposure promote leukocyte adhesion in postcapillary venules. Thus the early burst of mast cells may significantly contribute to the greater oxidant stress in microvasculature at a later time.

In the present study, microvascular disturbances elicited by I/R were significantly aggravated in chronic ethanol feeding. Greater lipoperoxidation or increased malondialdehyde production after reperfusion has been reported in the liver of chronic ethanol-treated rats (9, 46). We have previously demonstrated that chronic ethanol consumption aggravates endotoxin-induced leukocyte adhesion, which may result in hepatic microcirculatory disturbances and leukocyte adhesion to the sinusoidal wall and may be associated with increased tumor necrosis factor-α levels (35). Altered microvascular responses induced by chronic ethanol feeding, such as an increased contractile response to endothelin-1 (3) and decreased hepatic sinusoidal perfusion following hemorrhagic shock (2) or impaired dilation of cerebral resistance arterioles (26), have been observed. The microvascular damage due to alcohol abuse may include various changes in vascular endothelial cells or smooth muscle cells, such as morphometric changes (25, 44), changes in the number of receptors (24, 26), changes in signal transduction (1) or energy metabolism, and alteration of redox states (8, 20). However, regular alcohol consumption produces cardioprotective effects (27, 30–32), suggesting that different responses and sensitivities to chronic alcohol feeding in the different organs may exist.

Promotion of generation of oxygen free radical species after chronic ethanol consumption may be closely associated with impairment of defense systems against oxidative stress in the microvasculature. Our results clearly demonstrated that perturbations of glutathione content by two different agents influence susceptibility to I/R-induced injury in vivo. First, NAC, a permeable precursor for γ-glutamylcysteine synthase, is known to increase glutathione content by stimulating glutathione synthesis (28, 37). Albumin leakage and increased DHR, which are thought to be related to H$_2$O$_2$ production after reperfusion in particular, were drastically inhibited by NAC in both the dextrin-fed and ethanol-fed animals. These findings are consistent with glutathione peroxidase catalyzing GSH-dependent reduction of H$_2$O$_2$, which is a major pathway of H$_2$O$_2$ metabolism in many cells (34). Therefore, the decrease of GSH levels may largely contribute to oxygen radical-induced microvascular injury in the later stage, especially in ethanol-fed animals. Chronic ethanol intake
has been reported to induce a decrease in glutathione content, whereas malondialdehyde, a lipid peroxidation product, was found to be increased in rat peripheral nerves (4) and in the erythrocytes of alcoholic patients (23). Fernandez-Checa et al. (7) demonstrated that the GSH transport system from cytosol into mitochondria is impaired in chronically ethanol-fed rats, resulting in the development of increased susceptibility to oxidant stress. Second, DMA is known to interact with glutathione by direct conjugation and via glutathione-S-transferase, thus reducing GSH level without thione -ity to oxidant stress. Second, DMA is known to interact with glutathione by direct conjugation and via glutathione-S-transferase, thus reducing GSH level without thione -ity to oxidant stress.

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