Binge Drinking Disturbs Hepatic Microcirculation after Transplantation: Prevention with Free Radical Scavengers

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ABSTRACT

Disturbances in hepatic microcirculation increase graft injury and failure; therefore, this study evaluates the effects of ethanol on microcirculation after liver transplantation. Donor rats were given one dose of ethanol (5 g/kg) by gavage 20 h before explantation, and grafts were stored in University of Wisconsin solution for 24 h before implantation. Acute ethanol treatment decreased 7-day survival of grafts from about 90 to 30%, increased transaminase release nearly 4-fold, and decreased bile production by 60%. Moreover, portal pressure increased significantly and liver surface oxygen tension decreased about 50%, indicating that ethanol disturbs hepatic microcirculation. Pimonidazole, a 2-nitroimidazole hypoxia marker, was given i.v. to recipients 30 min after implantation, and grafts were harvested 1 h later. Ethanol increased hepatic pimonidazole binding about 3-fold, indicating that ethanol led to hypoxia in fatty grafts. Ethanol also significantly increased free radicals in bile. Catechin (30 mg/kg i.v. upon reperfusion), a free radical scavenger, and Carolina Rinse solution, which contains several agents that inhibit free radical formation, minimized disturbances in microcirculation and prevented pimonidazole adduct formation significantly. These treatments also blunted increases in transaminase release and improved survival of fatty grafts. Destruction of Kupffer cells with GdCl3 (20 mg/kg i.v. 24 h before explantation) or inhibition of formation of leukotrienes with MK-886 (50 μM in University of Wisconsin or rinse solution) also minimized hypoxia and improved survival after transplantation. Taken together, these results demonstrate that ethanol disturbs hepatic microcirculation, leading to graft hypoxia after transplantation, most likely by activating Kupffer cells and increasing free radical production.

Organ donors are often victims of accidents involving either chronic or acute consumption of ethanol. For example, in 1993, more than 40,000 fatalities due to traffic accidents occurred in the United States; approximately 44% of them were alcohol-associated (Anonymous, 1994). Moreover, in a study at the University of Pittsburgh in 1991, 48% of donors had a blood ethanol level between 4 and 40 mg/dl (Hassanein et al., 1991). Therefore, ethanol consumption is common in organ donors. It is well known that ethanol exposure causes fatty infiltration, inflammation, degeneration, and necrosis in the liver, and hepatic lipids are elevated after exposure to even a single, inebriating dose of ethanol (Ylikahri et al., 1972). Previous studies have shown that both acute and chronic ethanol treatments significantly increase primary nonfunction after liver transplantation in rats (Gao et al., 1995; Zhong et al., 1996). Moreover, grafts from victims with elevated blood alcohol levels have high rates of primary graft failure (Hassanein et al., 1991). Therefore, the mechanism(s) of alcohol-induced fatty graft failure must be understood if the pool of usable donor organs is to be expanded.

Previous studies have shown that leukocyte margination and platelet adhesion are increased and blood flow is reduced after liver transplantation (Takei et al., 1996). Moreover, disturbances in hepatic microcirculation increase graft injury and failure (Marzi et al., 1990). A recent study has shown that Kupffer cells (KCs), the major source of vasoactive and chemotactic mediators such as thromboxanes, leukotrienes, platelet-activating factor, and tumor necrosis factor-α in the liver, are activated by acute ethanol treatment (Enomoto et al., 1998). Moreover, ethanol increases oxidative stress, which activates nuclear factor-κB and phospholipase A2, thus increasing production of vasoactive and chemotactic cytokines, platelet-activating factor, and eicosanoids. Therefore, it is possible that ethanol disturbs hepatic microcirculation, leading to hypoxia and graft injury.

Organ donation is often associated with accidents that...
frequently involve binge drinking. Acute ethanol treatment mimics binge drinking and provides an inexpensive and convenient model. Accordingly, the purpose of this study was to evaluate the effects of ethanol exposure on microcirculation and hypoxia after liver transplantation following acute treatment with one large dose of ethanol to mimic binge drinking.

Materials and Methods

Animals and Liver Transplantation. Inbred female Lewis rats (200–230 g) were used in liver transplantation experiments to prevent immunological interference. Donor rats were treated with ethanol (5 g/kg body weight, dissolved in normal saline at a concentration of 20%) or an equal volume of saline vehicle by gavage 20 h before explantation. Previous studies showed that this dose of ethanol dramatically increased hepatic triglyceride content (Ylikahri et al., 1972) and decreased graft survival after transplantation (Zhong et al., 1996). This dose of ethanol resulted in a peak blood ethanol concentration of about 370 mg/dl in about 2 h, which declined to undetectable levels in 8 to 10 h (Wendell and Thurman, 1979). No respiratory suppression was observed in animals receiving this treatment; therefore, it provides a convenient model mimicking fatty grafts caused by binge drinking. Gadolinium chloride (GdCl₃; 20 mg/kg) dissolved in acidic saline (pH 3.0–3.5) was given to some donors i.v. 24 h before surgery to destroy KCs (Hardonk et al., 1992). Rats were anesthetized with Metofane (Schering-Plough Animal Health, Union, NJ) by inhalation in a breathing cone; animals usually woke up within 30 min after surgery. Liver transplantation was performed using a technique described elsewhere (Zimmermann et al., 1979). Briefly, heparin (200 IU) in 0.5 ml of lactated Ringer’s solution was injected into the subhepatic vena cava, and a 4-mm-long stent prepared from polyethylene tubing (PE 50) was inserted into the common bile duct and secured with a 6–0 suture. Livers were flushed in situ with 5 ml of lactated Ringer’s solution (0–4°C) or Carolina Rinse solution, which contains several compounds that inhibit free radical formation. These components include desferrioxamine (1.0 mM), which chelates iron, thus inhibiting the Fenton reaction; allopurinol (1.0 mM), which inhibits superoxide formation by xanthine oxidase and tryps radicals; and glutathione (3.0 mM), which inactivates radicals (Gao et al., 1991). In some experiments, MK-886 (50 μM) or cethinic (400 μM), a flavonoid that scavenges free radicals and singlet oxygen (Slater, 1981), was added to lactated Ringer’s solution. For implantation, the liver of the recipient was removed after clamping the suprahepatic vena cava, portal vein, and subhepatic vena cava, and implanted by connecting the suprahepatic vena cava with a running suture, then inserting cuffs into the appropriate vessels and securing them with a 6–0 silk suture. The bile duct was anastomosed with an intraluminal stent. Implantation surgery required less than 50 min; during this time the portal vein was clamped for 18 to 20 min. Catechin was injected (30 mg/kg i.v.) into some recipients upon opening of vascular clamps. Survival was monitored for 7 days after surgery. All animals in this study received humane care in compliance with institutional guidelines as outlined in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. All experiments were performed in accordance with protocols approved by the Animal Use Committee of the University of North Carolina.

Measurement of Serum Aspartate Aminotransferase (AST) and Bile Production. Blood samples were collected from the inferior vena cava for the first 3 h after implantation to assess AST release. Serum was obtained by centrifugation and stored at –20°C, and AST activity was determined using a commercially available analytical kit from Sigma Chemical Co. (St. Louis, MO). The common bile duct was cannulated with polyethylene tubing (PE 50, Clay Adams Brand, Becton-Dickinson, Sparks, MD) in some experiments, and bile was collected for 3 h after organ implantation. Rates of bile production were calculated from time of bile collection, the volume of bile, and the liver wet weight.

Assessment of Arterial Blood Pressure, Microcirculation, and Hypoxia. After implantation of the liver, the right carotid artery was cannulated with polyethylene tubing (PE 50), and blood pressure was measured using a low pressure analyzer (LPA-200, Digi-Med, Louisville, KY).

To assess hepatic microcirculation after transplantation, a needle connected to a polyethylene tube (PE 90) was inserted into the portal vein. Portal pressure was measured by changes in the height of a water column 3 h after implantation. Oxygen tension on the liver surface, another indicator of hepatic microcirculation (Adachi et al., 1995), was measured with a Teflon-shielded, Clark-type oxygen electrode, which was placed gently on the liver surface with the aid of a micromanipulator at the times indicated in the figures. Oxygen tension was measured at three to five points on the liver surface at several times, and averages were calculated. Metofane was always removed at least 15 min before measuring surface oxygen tension (i.e., the rat was breathing air when surface oxygen tension was measured). Liver surface oxygen tension measured this way was not significantly different from the oxygen tension found in animals that were anesthetized with pentobarbital (50 mg/kg i.p.) but were not exposed to inhaled anesthesia. Therefore, measurement of liver surface oxygen tension was not influenced by anesthesia.

Pimonidazole, a 2-nitroimidazole compound, is reductively activated at low oxygen concentrations and binds to cell molecules that possess free thiol groups (Raleigh and Koch, 1990; Arteel et al., 1995). Pimonidazole adducts accumulate in vivo in intact, awake animals and measure tissue hypoxia directly at the cellular level (Arteel et al., 1995; Durand and Raleigh, 1998). To evaluate hypoxia in grafts of awake animals, pimonidazole hydrochloride was dissolved in normal saline at a concentration of 120 mg/ml and injected into the tail vein (120 mg/kg) 30 min after implantation. Grafts were perfused 1 h later to remove blood, and pimonidazole binding was determined in liver homogenates using a competitive enzyme-linked immunosorbent assay (ELISA) procedure as described elsewhere (Arteel et al., 1995). Protein levels in tissue homogenates were measured with the bicinchoninic acid assay using a commercially available kit.

Protein-bound pimonidazole in liver sections was also determined immunohistochemically. Paraffin blocks of formalin-fixed liver tissue were sectioned at 6 μm and pimonidazole adducts were detected with a biotin-streptavidin-peroxidase indirect immunostaining method (Arteel et al., 1995). Sections were hydrated and treated briefly with 0.01% protease (pronase E) and exposed to mouse monoclonal anti-pimonidazole IgG₁ antibody (3.44.6.7.) in PBS-Tween for 2 h at 37°C. Rat-adsorbed horse anti-mouse antibody was then applied to the sections for 30 min. Once the antibody-biotin-peroxidase complex was formed, 3,3’-diaminobenzidine chromogen was added as the peroxidase substrate. After the immunostaining procedure was completed, a counterstain of hematoxylin was applied, followed by mounting with crystal mount solution. Some liver sections were also stained with osmium and examined microscopically for fatty infiltration (Luna, 1968).

Detection of Free Radical Adducts. To assess free radical formation by liver grafts, the spin trapping reagent α-(4-pyridyl 1-oxide)-N-tert-butyl nitronate (4-POBN, 1 g/kg body weight) was dissolved in 0.5 ml of normal saline and injected slowly into the tail vein after opening the vascular clamps. A cannula (PE 50) was placed in the common bile duct, and bile was collected for 1 h after implantation into 50 μl of 30 mM dipyridyl on ice to prevent ex vivo free radical formation. Samples were stored on dry ice until analysis. Bile
samples were thawed, placed in a quartz electron spin resonance (ESR) cell, and scanned repeatedly until the interfering ascorbate semiquinone signal disappeared in approximately 1 h. Free radical adducts were detected with a Bruker ESP 106 ESR spectrometer (Bruker, Billerica, MA). Instrument conditions were as follows: 20-mW microwave power, 1.0-G modulation amplitude, and 80-G scan range. Spectral data were stored on an IBM-compatible computer and were analyzed for ESR hyperfine coupling constants by computer simulation (Duling, 1994).

Assay for Hepatic Triglycerides. To assess triglyceride content in the grafts, liver tissue was homogenized in an equal volume of normal saline and extracted with a mixture of chloroform and methanol (2:1) as described elsewhere (Brodie et al., 1961). Zeolite (Sigma) was added to remove phospholipids in the extract. The resulting extract was dried under nitrogen and dissolved in 2 ml of Plasmanate (Bayer, West Haven, CT), and triglycerides were determined enzymatically (Bucolo and David, 1973). Triglyceride content was standardized with DNA content in the liver tissue to rule out any possible influences of weight changes due to fat accumulation. DNA content in the liver tissue was measured using the bisbenzimidazole method as described elsewhere (Labarca and Paigen, 1980).

Statistical Analysis. All groups were compared by using the $\chi^2$ or ANOVA plus Student-Newman-Keuls posthoc tests as appropriate, and differences were considered significant at the $p < .05$ level.

Results

Survival after Liver Transplantation. After implantation of grafts, survival rates were 86% in control rats but only 29% in the alcohol-treated group. Catechin, a free radical scavenger, and Carolina Rinse solution restored rates to 60 to 63%. Destruction of KCs with GdCl$_3$ elevated survival rates significantly to 70% (Fig. 1). Leukotrienes, which are synthesized from arachidonic acid via 5-lipoxygenase, are potent chemoattractants (LTB$_4$) and smooth muscle constrictors (LTC$_4$, LTD$_4$, and LTE$_4$) (Anggard, 1985). MK-886, which inhibits activation of 5-lipoxygenase, thus blocking leukotriene synthesis (Gillard et al., 1989), also improved survival of grafts exposed to ethanol to 67%. (Fig. 1).

AST Release and Bile Production. Serum AST levels were 34 U/l in untreated controls without transplantation. One single dose of ethanol (5 g/kg i.g.) did not significantly change this value. However, AST levels increased gradually to about 650 U/l over 3 h after implantation in controls. In contrast, recipients receiving grafts from ethanol-treated rats under similar conditions had AST levels of 2432 U/l. Treatment with the radical scavenger catechin or destruction of KCs with GdCl$_3$ blunted the elevation of serum enzymes (i.e., values only rose about 40% as high; ethanol + catechin was not significantly different from ethanol + GdCl$_3$) and minimized injury to ethanol-exposed grafts immediately after implantation (Fig. 2, top).

In control rats not receiving liver transplantation, bile flow rates were around 92 $\mu$g/liver/h, and acute ethanol treatment did not significantly change this value. After transplantation, bile was produced at rates of about 27 $\mu$g/liver/h in control rats but was reduced about 60% in grafts from alcohol-treated rats. Catechin increased rates to 41 $\mu$g/liver/h whereas GdCl$_3$ treatment before transplantation returned bile production to control values (25 $\mu$g/liver/h; Fig. 2, bottom). Ethanol + catechin was significantly different from ethanol + GdCl$_3$.

Effects of Ethanol, Catechin, and Gadolinium Chloride on Hepatic Microcirculation. The mean arterial blood pressure was about 90 mm Hg in controls 2 h after transplantation. Ethanol did not alter this value. To evaluate hepatic microcirculation, portal pressure and liver surface oxygen tension were measured. Without liver transplantation, portal pressure was 7.5 ± 0.1 cm of H$_2$O in controls ($n = 4$), and ethanol did not significantly alter this value. Three hours after implantation, portal pressure was around 7.2 ± 0.2 cm of H$_2$O in controls ($n = 6$) and was increased to 9.1 ± 0.4 by acute alcohol treatment ($n = 10$, $p < .05$ by ANOVA and Student-Newman-Keuls posthoc test; data not shown). Catechin minimized this increase to 7.6 ± 0.5 cm of H$_2$O ($n = 5$, $p < .05$ compared with the ethanol group but $p > .05$ compared with the controls by ANOVA plus Student-Newman-Keuls posthoc test). GdCl$_3$ also blunted the increases to 7.6 ± 0.1 cm of H$_2$O ($n = 4$, $p < .05$ compared with the ethanol group but $p > .05$ compared with the controls by ANOVA plus Student-Newman-Keuls posthoc test).

Hepatic surface oxygen tension was around 82 $\mu$M in controls without transplantation, a value not altered signifi-
significantly by acute ethanol treatment (data not shown). Fifteen minutes after implantation, hepatic surface oxygen tension was around 80 \text{mM} in the control group, and values decreased gradually to about 68 \text{mM} over 3 h (Fig. 3). In contrast, surface oxygen tension reached only about 31 \text{mM} in the alcohol-treated group, reflecting surface hypoxia due to disturbances in hepatic microcirculation. Catechin minimized the decrease of oxygen tension caused by alcohol treatment significantly, whereas GdCl3 reversed the decrease almost completely.

Graft hypoxia was also evaluated in awake animals after implantation by using a tissue hypoxia marker, pimonidazole. Previous studies showed that pimonidazole detects hypoxia in the liver (Arteel et al., 1995). Figure 4 depicts representative images of liver grafts in which pimonidazole adducts were detected immunohistochemically. In livers from controls, pimonidazole adducts were minimal and accumulated primarily in pericentral regions (Fig. 4, top left); this is probably due to the natural low oxygen tension in these regions. Treatment with ethanol increased the relative area of pimonidazole dramatically with binding extending from the pericentral regions toward periportal areas (Fig. 4, top right), reflecting much more extensive hypoxia in fatty grafts. The area of pimonidazole-labeled cells in grafts from ethanol-treated rats was decreased significantly after treatment with catechin or Carolina Rinse solution (Fig. 4, middle). Destruction of KCs with GdCl3 or inhibition of leukotriene synthesis with MK-886 also blunted increases in pimonidazole binding caused by ethanol (Fig. 4, bottom).

Whereas immunohistochemistry detects predominantly protein-bound adducts, quantitation of pimonidazole binding with ELISA detects both protein and nonprotein adducts (e.g., GSH adducts). The level of pimonidazole binding in control liver grafts was 272 pmol/mg protein; one dose of ethanol increased binding more than 3-fold (Fig. 5). Catechin or Carolina Rinse solution diminished binding to about 240 pmol/mg protein, whereas destruction of KCs (GdCl3) or inhibition of leukotriene synthesis with MK-886 also blunted the increase in binding due to ethanol significantly (Fig. 5).

**Fat Content in Liver Grafts after Binge Drinking.** Disturbances in hepatic microcirculation could be a result of fat accumulation in the liver; therefore, liver sections were examined microscopically with osmium staining. Fat droplets were barely detectable in parenchymal cells in grafts from control rats (Fig. 6, top left). However, microvesicular and macrovesicular fat droplets (black staining) were numerous in parenchymal cells from livers of rats treated acutely with ethanol (Fig. 6, top right). The extent of fatty infiltration and its lobular distribution in grafts from rats treated with ethanol receiving catechin (Fig. 6, middle left), Carolina
Fig. 4. Representative images of pimonidazole binding in liver grafts after transplantation. Conditions as in Fig. 1. Pimonidazole (120 mg/kg i.v.) was injected 30 min after implantation. Grafts were perfused with Krebs-Henseleit buffer (pH 7.4) 1 h later to remove blood and fixed with 1% paraformaldehyde; then sections were stained immunohistochemically (Arteel et al., 1995). Representative images are shown: top left, control; top right, ethanol; middle left, ethanol + catechin; middle right, ethanol + Carolina Rinse; bottom left, ethanol + GdCl₃; bottom right, ethanol + MK-886.
Solution before implantation. Values are means ± S.E.M. (ANOVA; n = 4–5 in each group). *p < .05 compared with control; †p < .05 compared with the ethanol group.

Rinse (Fig. 6, middle right), GdCl₃ (Fig. 6, bottom left), or MK-886 (Fig. 6, bottom right) were similar to those of grafts from rats receiving ethanol alone. Hepatic triglyceride content determined enzymatically was about 0.8 mg/mg DNA in the control group, whereas values were elevated 2.8-fold by ethanol treatment. GdCl₃ did not significantly alter triglyceride content in the liver under these conditions (Fig. 7), confirming the histological findings.

**Free Radical Production after Liver Transplantation.** Because oxidative stress could lead to microcirculatory disturbances (Ikai et al., 1994), free radical production after liver transplantation was evaluated using the spin trapping reagent 4-POBN and ESR. A six-line ESR spectrum due to a 4-POBN/pentyl radical adduct or a closely related species. Pentyl free radicals would be formed in vivo upon the β-scission of arachidonic or linoleic acid-derived alkoxy radicals (Kadisaka et al., 1998). Catechin blunted free radical adduct formation with values reaching only about 20% of those observed in grafts from ethanol-treated rats (2.0 ± 0.6 arbitrary units, Fig. 8). When fatty grafts were rinsed with Carolina Rinse solution before implantation, free radical adduct formation was reduced by about 50% (5.0 ± 1.5 arbitrary units). Destruction of KCs with GdCl₃ also reduced free radical production significantly (3.0 ± 0.6 arbitrary units, Fig. 8).

**Discussion**

**Binge Drinking Disturbs Microcirculation in Fatty Grafts after Transplantation.** Hepatic microcirculation is pivotal for graft survival; however, cold storage/reperfusion causes critical injury to sinusoidal lining cells (Marzi et al., 1989). Disturbance of hepatic microcirculation increases graft injury and failure after transplantation (Marzi et al., 1990), whereas rinsing grafts with warm lactated Ringer’s to improve microcirculation increases survival (Takei et al., 1991a). Previous studies have shown that the velocity of polymorphonuclear neutrophils decreases and leukocyte adhesion increases after liver transplantation (Marzi et al., 1990). Moreover, platelet adhesion is increased and blood flow is reduced (Takei et al., 1991b). It is known that infusion of ethanol disturbs microcirculation, which leads to liver injury (Hijioka et al., 1991). Therefore, it is possible that ethanol increases graft injury by disturbing the microcirculation, which leads to graft hypoxia and injury after transplantation. Indeed, liver surface oxygen tension decreased dramatically and portal pressure increased in fatty grafts from ethanol-treated rats (Fig. 3), confirming that ethanol disturbs the microcirculation.

Most of the methods for the assessment of microcirculation, such as in vivo microscopy or detection of hemoglobin absorbance on the liver surface, are conducted in anesthetized animals, which could influence microcirculation. To avoid this potential problem, pimonidazole, a 2-nitromidazole compound, was used in this study to assess graft hypoxia in vivo after transplantation in the absence of anesthesia. This compound is reductively activated at low oxygen concentrations and binds to cellular macromolecules (Durand and Raleigh, 1998). It has several distinct advantages. It accumulates in vivo in intact, awake animals and measures tissue hypoxia directly at the cellular level, unlike other methods. Pimonidazole binding was increased massively after reperfusion in ethanol-treated grafts (Figs. 4 and 5), indicating that significant tissue hypoxia was associated with increased graft injury (Fig. 2, top), inhibited liver function (Fig. 2, bottom), and decreased survival (Fig. 1). Importantly, the use of free radical scavengers, destruction of KCs, and inhibition of biosynthesis of leukotrienes with MK-886, which improved graft microcirculation (Figs. 3 and 4), significantly decreased injury (Fig. 2), and reduced graft failure (Fig. 1). Taken together, these data are consistent with the hypothesis that ethanol causes graft injury by disturbing the hepatic microcirculation, leading to hypoxia.

**Fat Accumulation Is Not Responsible for Graft Hypoxia.** How ethanol disturbs graft microcirculation leading to
Fig. 6. Fatty infiltration in liver grafts. Conditions as in Fig. 1. After implantation (1.5 h), grafts were rinsed with normal saline followed by infusion of 10 ml of 4% paraformaldehyde. Fixed tissue was stained with osmium (Luna, 1968), and sections were counterstained with eosin. Typical images (original magnification, 200×) of at least four sections in each group. Top left, control; top right, ethanol-treated group; middle left, ethanol + catechin; middle right, ethanol + Carolina Rinse; bottom left, ethanol + GdCl$_3$; bottom right, ethanol + MK-886.
hypoxia after transplantation remains unknown. One attractive hypothesis is that it is due to fatty infiltration caused by ethanol. It is well known that alcohol induces fatty liver: even one single large dose of alcohol significantly increases hepatic lipid content (Ylikahri et al., 1972). Severely steatotic grafts transplant poorly, often necessitating replacement surgery resulting in high mortality (Todo et al., 1989); therefore, biopsies are performed at many transplant units, and livers with highly elevated fat infiltration are often not utilized. Fat droplets in parenchymal cells could theoretically cause obstruction of the hepatic microcirculation mechanically, and rupture of parenchymal cells in a liver with severe steatosis could result in release of fat globules into the hepatic microcirculation (Todo et al., 1989). However, livers from rats fed ethanol-containing high-fat or low-fat diets chronically have similar cell injury after hypoxia/reoxygenation, although only the former exhibit fatty infiltration (Zhong et al., 1998). In this study, one large dose of ethanol caused massive fat accumulation in hepatocytes (Fig. 6); however, free radical scavengers, removal of KCs, and inhibition of leukotriene biosynthesis all dramatically improved hepatic microcirculation reflected by higher liver surface oxygen tension, lower pimonidazole binding, lower portal pressure, and higher bile flow [Figs. 2 (bottom), 3, and 4] without affecting hepatic fat content (Fig. 6). Therefore, it is concluded that accumulation of fat droplets in parenchymal cells is not responsible for disturbances in hepatic microcirculation in the binge drinking model studied here.

Fig. 7. Effects of acute ethanol and gadolinium chloride treatment on hepatic triglyceride content. Conditions as in Fig. 1. Liver homogenates were extracted with a mixture of chloroform and methanol (2:1, v/v) (Brodie et al., 1961), and hepatic triglycerides were determined by enzymatic methods. Data are expressed per unit DNA to rule out any possible effect of liver weight changes due to fat accumulation. Values are means ± S.E.M. (p < .05 by ANOVA; n = 4 in each group). *p < .05 for comparison with controls (Student-Newman-Keuls test).

Fig. 8. ESR spectrum of free radical adducts after transplantation. Conditions as in Fig. 1. The spin trapping reagent 4-POBN (1 g/kg body weight) was dissolved in 0.5 ml of normal saline and injected slowly into the tail vein upon opening of vascular clamps. Bile was collected into 50 μl of 30 mM dipyridyl on ice to prevent ex vivo free radical formation for 1 h after injection of 4-POBN. Free radical adducts in bile were detected with a Bruker ESP 106 EPR spectrometer. Typical spectra (n = 4–5 in each group). A, control; B, ethanol; C, ethanol + catechin; D, ethanol + Carolina Rinse; E, ethanol + GdCl3.

Oxidative Stress Plays an Important Role in Ethanol-Induced Disturbances in Hepatic Microcirculation after Transplantation. An important finding of this study is that catechin, a free radical scavenger, and Carolina Rinse significantly improved hepatic microcirculation and minimized ethanol-induced hypoxia after transplantation (Figs. 3–5). These results suggest that ethanol disturbs hepatic microcirculation, most likely by increasing oxidative stress (Fig. 9). Indeed, free radical production increased 2-fold in grafts from ethanol-treated rats, an effect that was blocked by catechin and Carolina Rinse (Fig. 8). Reactive oxygen species could cause damage to endothelial cells, thus disturbing the microcirculation. Alternatively, previous studies have shown that reactive oxygen species induce vasoconstriction in isolated perfused rat livers (Ikai et al., 1994), and ischemia/reperfusion stimulates secretion of vasoactive and chemotactic mediators (Fig. 9) such as platelet-activating factor (Nishiyama et al., 1993) and leukotrienes (Clavien et al., 1993), thus causing vasoconstriction, leukocyte plugging, and platelet adhesion (Fig. 9). Oxidative stress activates nuclear factor-κB, a transcription factor responsible for the expression of proinflammatory cytokines and cell adhesion molecules (Lin et al., 1995). In addition, reactive oxygen species activate phospholipase A2 (Goldman et al., 1997), which increases production of lipid-derived vasoactive and chemotactic mediators such as eicosanoids and platelet-activating factor (Fig. 9). All of these pathophysiological alterations caused by oxidative stress could contribute to disturbances of graft microcirculation and hypoxia. Indeed, MK-886, which inhibits activation of 5-lipooxygenase thus blocking biosynthesis of leukotrienes (Gillard et al., 1989; Fig. 9), significantly mini-
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as platelet-activating factor, leukotrienes, thromboxanes, and toxic cytokines in the liver (Decker, 1990; Fig. 9). Indeed, destruction of KCs with GdCl₃ largely blocked free radical production and significantly minimized microcirculatory disturbances caused by ethanol in this study (Figs. 4 and 8). Thus, it is concluded that acute ethanol treatment (“binge drinking”) causes graft hypoxia after transplantation most likely by activating KCs and increasing formation of free radicals. Reactive oxygen species activate production of chemotactic and vasoactive mediators, which leads to microcirculatory disturbances and hypoxia. GdCl₃ destroys KCs, catechol scavenges free radicals, and MK-886 inhibits synthesis of leukotrienes, thereby minimizing ethanol-induced graft hypoxia, liver injury, and mortality (Fig. 9).

Fig. 9. Diagram depicting the working hypothesis by which binge drinking causes hypoxia in fatty grafts after transplantation. It is proposed that acute ethanol treatment (“binge drinking”) causes graft hypoxia after transplantation by the following mechanisms: ethanol activates KCs and increases formation of free radicals via NADPH oxidase and xanthine oxidase (XO). Reactive oxygen species stimulate production of chemotactic and vasoactive mediators such as eicosanoids, platelet-activating factor, and cytokines, which causes vasoconstriction and increases white blood cell and platelet adhesion, leading to microcirculatory disturbances and hypoxia. GdCl₃ destroys KC, catechol scavenges free radicals, and MK-886 inhibits synthesis of leukotrienes, thereby minimizing ethanol-induced graft hypoxia, liver injury, and mortality. SC, stellate cell; EC, endothelial cell; EtOH, ethanol; O₂⁻, superoxide radicals; Xan, xanthine; HK, hypoxanthine; PLA₂, phospholipase A₂.

References


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