Preventing Gut Leakiness by Oats Supplementation
Ameliorates Alcohol-Induced Liver Damage in Rats

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ABSTRACT

Only 30% of alcoholics develop liver disease (ALD) suggesting that additional factors are needed. Endotoxin is one such factor, but its etiology is unclear. Since the gut is the source of endotoxin, we sought to determine whether an increase in intestinal permeability (leaky gut) is required for alcohol-induced endotoxemia and liver injury and whether the gut leakiness is preventable. For 10 weeks, rats received by gavage increasing alcohol doses (to 8 g/kg/day) and either oats (10 g/kg) or chow b.i.d. Intestinal permeability was then assessed by urinary excretion of lactulose and mannitol. Liver injury was evaluated histologically, biochemically (liver fat content), and by serum aminotransferase. Alcohol caused gut leakiness that was associated with both endotoxemia and liver injury. Oats prevented these changes. We conclude that chronic gavage of alcohol in rats is a simple experimental model that mimics key aspects of ALD, including endotoxemia and liver injury, and can be useful to study possible mechanisms of endotoxemia in ALD. Since preventing the gut leakiness by oats also prevented the endotoxemia and ameliorated liver damage in rat, our results suggest that alcohol-induced gut leakiness 1) may cause alcohol-induced endotoxemia and liver injury and 2) may be the critical cofactor in the 30% of alcoholics who develop ALD. Further studies are needed to determine whether ALD in humans can be prevented by preventing alcohol-induced gut leakiness, studies that should lead to the development of useful therapeutic agents for the prevention of ALD.

Clinically significant liver damage leading to cirrhosis and liver failure is a common and serious complication of excessive alcohol consumption (Burbige et al., 1984; Grant et al., 1988). It occurs only in a subset (~30%) of alcoholics suggesting that “heavy drinking” (ethanol consumption) is necessary but not by itself sufficient to cause alcohol-induced liver disease (ALD) and that other contributing factors must be involved. Although hepatitis C infection and hemochromatosis trait have been suggested, neither is present in the majority of patients with ALD.

A more plausible cofactor is endotoxemia with recent clinical and experimental observations strongly supporting this idea (Bode et al., 1987; Nolan, 1988). In this view, alcohol-induced liver damage is triggered by a hepatic necro-inflammatory cascade, which is initiated by circulating, gut-derived endotoxin. First, high levels of endotoxin have been reported in the serum of alcoholics with liver disease (Bigatello et al., 1987; Lumsden et al., 1988; Tachiyama et al., 1988; Fukui et al., 1991; Schafer et al., 1995) and alcohol-fed animals (Nanji et al., 1993). Second, there is a correlation in animal models between endotoxin levels and the severity of alcohol-induced liver damage (Nanji et al., 1994). Third, treatments such as antibiotics or lactobacillus that lower the blood endotoxin levels in alcohol-fed rats are associated with significantly less severe liver damage (Nanji et al., 1994; Adachi et al., 1995). Fourth, it is known that circulating endotoxins have a synergistic effect with alcohol in causing the activation of hepatic macrophages and the over-production of tissue-damaging cytokines and oxidants (prime features of the hepatic necro-inflammatory cascade) (Bhagwandeen et al., 1987; Shibayama et al., 1991; Hansen et al., 1994; Nanji et al., 1995; Enomoto et al., 1998).

Less well established is the cause of endotoxemia in patients with ALD. Abnormally high endotoxin levels in blood could result from 1) increased production of endotoxin by abnormal gut flora, 2) increased permeation of endotoxin from gut lumen into the portal circulation through the intestinal wall (gut leakiness), 3) shunting of blood away from the liver (as seen in advanced liver damage with portal hypertension), or 4) defective Kupffer cell function since these cells are normally responsible for removing endotoxin.

Abnormal intestinal flora as the main cause of endotoxemia seems unlikely, since it is not commonly present in

ABBREVIATIONS: ALD, alcoholic liver disease; AST, aminotransferase; L/M, lactulose/mannitol; BAL, blood alcohol levels.
alcoholics and because liver damage can occur in rats having normal intestinal flora. Shunting of the blood away from the liver may be an important factor for endotoxemia in patients with advanced liver disease, but clearly this cannot be a factor for the initiation of liver damage where there is no portal hypertension. Abnormal intestinal permeability (leaky gut) is a possible candidate cofactor for liver damage and may be a major cause of endotoxemia in liver disease (Hill et al., 1997). Our group has shown that intestinal permeability is increased in alcoholics with liver disease but not in alcoholics without liver disease (Keshavarzian et al., 1999), suggesting that alcohol-induced gut leakiness is a necessary cofactor for ALD. To show that the leaky gut causes endotoxemia and liver damage, one should demonstrate that preventing gut barrier leakiness prevents liver damage in alcoholics.

Although there is no technique for restoring normal gut barrier function in humans, investigators have successfully restored gut barrier function in animal models. For example, oats supplementation attenuated gut leakiness induced by methotrexate injections in rat (Mao et al., 1996). It is not known, however, whether alcohol-induced gut leakiness in rats can also be restored by oats and whether this prevents alcohol-induced liver damage.

Accordingly, current aims were to 1) establish an animal model of chronic alcohol-induced liver injury that is associated with gut leakiness and endotoxemia, and 2) use this model to determine whether oats prevent the alcohol-related increase in intestinal permeability and attenuate endotoxemia and liver damage.

Materials and Methods

Animal Subjects

Male Sprague-Dawley rats (Zivic-Miller Laboratories, Zelienople, PA; n = 36, 250–300 g, initial body weight) were acclimated for 6 to 7 days, at 22 ± 1°C with a 12:12-h dark-light cycle. During acclimation, rats were given water and standard laboratory food (rat chow) ad libitum. During the experiment, alcohol or dextrose and other treatments were administered intragastrically by gavage twice daily. A 12-gauge needle was used (Popper & Sons, New Hyde Park, NY). Alcohol-fed rats received alcohol gavage (4 ml) twice daily starting with an initial dose of 2 g/kg/day. This dose was progressively increased during weeks 1 and 2 to a maintenance dose of 8 g/kg/day (solutions maximally containing 50–60% alcohol) that was continued for 8 more weeks. Control rats received an isocaloric amount of dextrose, also by gavage. Rats also received intragastric feedings of a slurry of either powdered rat chow or oats (10 g/kg; Quaker Oats, Barrington, IL). All rats also had regular rat chow available (ad libitum) throughout the 10-week period. Rats were weighed daily. Four treatment groups were studied: 1) alcohol/chow (n = 10); 2) alcohol/oats (n = 10); 3) dextrose/chow (n = 5); and 4) dextrose/oats (n = 5). Gut permeability was measured (as described below) at baseline and at the end of 10 weeks. Blood samples were taken for blood alcohol levels 1 h after gavage, 3 weeks after initiation of alcohol (i.e., after the final ethanol dose of 8 g/kg/day had been administered for at least 5 days). At 10 weeks, the animals were humanely killed by CO₂ inhalation, followed immediately by cardiac puncture (for blood collection), and laparotomy for the collection of intestinal and liver tissues.

Intestinal Permeability

Intestinal permeability in rat was assessed after an 8-h fast as previously described by us for humans (Keshavarzian et al., 1994) except that the animals received intragastric administration of 0.5 ml of a solution containing lactulose (L) 100 mg/kg body weight, mannitol (M) 6 mg/kg body weight, and sucrose 200 mg/kg body weight. Rats were housed individually in metabolic cages, and urine was collected for the first 5 h. To promote urine output, each rat also received 10 ml of a Ringer-lactate solution subcutaneously, just prior to sugar administration. Only rats with adequate urinary output (2.4 ml or more) were included in the analysis. Urinary sugar levels were measured by gas chromatography as we previously described (Keshavarzian et al., 1994). Briefly, urine volumes were determined, and aliquots of each sample were centrifuged at 10,000g for 5 min. Aliquots (200 μl) of supernatant were mixed with an internal standard solution containing inositol and phenylmethylglucoside. The mixture was evaporated to dryness, and the residue was taken up in 200 μl of pyridine containing 25 mg/ml hydroxylamine. Aliquots (100 μl) of the supernatant (containing the oxime derivatives of reducing sugars) were transferred to a vial containing 100 μl of trimethylsilylmidazole. After 30 min of incubation at 70°C, 2 μl of the supernatant were analyzed by gas chromatography. An increase in the urinary L/M ratio was used as an index of increased gut permeability (Hollander, 1992).

Serum Endotoxin

A commercial kit was used to assay endotoxic levels in blood [Bacterial Endotoxins Test in The United States Pharmacopeia (1995)]. Briefly, blood was collected, then centrifuged at 400g for 15 min at 4°C, and the serum obtained was stored at −70°C in endotoxin-free vials (Sigma, St. Louis, MO). Measurements were done within 1 month of collection. Serum was diluted 1:10 in pyrogen-free water and heated to 75°C for 30 min to remove any potential endotoxin inhibitors. The samples were then incubated at 37°C for 30 min with a Limulus amebocyte lysate (Kinetic-QCL; BioWhittaker, Walkersville, MD). The reaction was stopped with 35% acetic acid. Rat sera were assayed against a standard curve of endotoxin concentrations of 0.1, 0.05, and 0.01 EU/ml. Microplates containing sera or standard were then read spectrophotometrically at 405 nm.

Blood Alcohol Levels (BAL)

Serum ethanol was measured with an alcohol dehydrogenase kit (Sigma) that was used according to the protocol supplied by the manufacturer.

Liver Injury

The following markers were measured at week 10.

Serum Liver Enzymes. Serum aspartate aminotransferase (AST) was measured using a commercial assay kit (Sigma).

Liver Histology. Liver tissues were fixed in formalin (10%) and embedded in paraffin. Tissue sections were cut at 4 μm and stained with H&E. Two gastrointestinal pathologists (S.J. and S.Y.), each unaware of the group assignments of the samples, examined three sections of each liver tissue for macrovesicular steatosis, microvesicular steatosis, inflammation, and necrosis. The presence or absence of these abnormalities and their severity was tallied to yield a score between 0 and 4 as described below. A histological liver damage index was calculated by adding the scores for these four parameters. Thus, a maximum liver damage score was 16.

The severity of macrovesicular steatosis was scored as 0 (no hepatocytes), 1 (less than 25% hepatocytes), 2 (26–50%), 3 (51–75%), and 4 (greater than 75% hepatocytes) containing small droplets of fat. The percentage of hepatocytes demonstrating large droplets of fat was used to assess the degree of macrovesicular steatosis and was also rated 0 to 4 (according to increasing degree) similar to microvesicular steatosis. The severity of the inflammation was scored as 0 to 4 [none (0), minimal (1), mild (2), moderate (3), and severe (4)] and was based on the degree of portal and lobular inflammation and evidence of piecemeal and spotty necrosis. The degree of necrosis and necrotic hepatocytes was scored as 0 to 4 (none, minimal, mild, moderate, and severe, respectively).
Liver Fat Content. The total liver fat content was measured gravimetrically as previously described (Folch et al., 1957). Briefly, lipids were extracted from 1 g of liver tissue by homogenizing tissues in 2:1 chloroform-methanol (v/v) and then filtering the homogenates. The filtrate containing the tissue lipids accompanied by nonlipid substances was freed from nonlipids by adding 5-fold its volume of water to the filtrate. The organic phases were poured into tared tubes, dried (evaporation of solvent), and weighed.

Intestinal Injury

Sections of small and large bowel were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections were cut at 4 μm and stained with H&E. A pathologist (S.J.) unaware of the treatment groups evaluated the intestinal mucosa for the following parameters: epithelial cell injury/loss, mucin (goblet cell) loss, mucosal edema, and the degree of inflammatory cells within the lumina propria and in the epithelial layer (intraepithelial lymphocytes).

The degree of epithelial cell injury/loss was graded as follows: 0 (none), 1 (focal superficial epithelial cell injury), 2 (1–2 glands lost with or without mild superficial ulceration), 3 (3 glands lost with or without moderate areas of ulceration), or 4 (4 or more glands lost with or without severe mucosal ulceration). The degree of cellular mucin depletion was graded as 0 (none), 1 (focal), 2 (1–2 glands lost), 3 (3–4 glands), or 4 (greater than 5 glands lost). The degree of lamina propria edema was assessed by 0 (none), 1 (focal), 2 (superficial), 3 (superficial to mild), and 4 (diffuse edema). The inflammatory infiltrate was scored qualitatively as 0 (no increased inflammation), 1 (mild), 2 (moderate), 3 (severe and focal), and 4 (severe and diffuse).

Statistical Analysis

The normal value for intestinal permeability was defined as mean ± 2 S.D. in control rats. Small bowel leakiness was defined as an elevated L/M ratio (greater than 2 S.D. above the mean for controls). For parametric analysis (in which a continuous variable was measured and the data were normally distributed), a Student's t test was used when two groups were compared and analysis of variance followed by Tukey's post hoc test when more than two groups were compared. For nonparametric analysis, the Mann-Whitney U test was used when two groups were compared and Kruskal-Wallis followed by Dunn's post hoc test when more than two groups were compared. The data are presented using box plots. The median is denoted by the central horizontal line of the box (note that when the median is close to or equal to the 25th or 75th percentile, then this line is not visually detectable). The bottom, top, and midline of the box show the 25, 75, and 50% (median) rankings, respectively. The box height gives the interquartile range. The lower and upper whiskers give the 10 and 90% rankings. Outliers are denoted by circles.

Information on Veterinary Care

The study was approved by Institutional Animal Care and Use Committee of the Hines Veterans Affairs Hospital where the animals were housed. All animals received humane care according to the criteria outlined by the National Academy of Sciences (1985). Veterinary care was provided by a licensed veterinarian in our American Association for Laboratory Animal Care approved facility.

Results

Body Weight and BAL. Alcohol feeding did not lead to any significant weight loss and there were no significant differences in final body weight between the control (492 ± 2.6 g) and ethanol- (489 ± 3.2 g) fed groups. Our ability to maintain rats at normal weight and prevent malnutrition is probably because all animals received adequate calories for daily nutritional needs by gavage of oats or chow. At week 3, BAL 1 h after ethanol administration by gavage were similar in the oat-fed groups (382 ± 167 mg/100 ml) and the Chow-fed groups (398 ± 70 mg/100 ml). This indicates that the administration of oats did not affect alcohol absorption and that any observed effects of oats on liver damage were not likely to be due to the effects of oats on alcohol absorption and/or the level of exposure of the liver or gut to alcohol.

Intestinal Permeability. Eight of ten rats receiving dextrose produced adequate urine volume (>2.4 ml within 5 h) and were used for analysis. There were no significant differences in L/M ratio between dextrose-fed rats that received oats (n = 4) and those that received chow (n = 4). Accordingly, data from these two groups were pooled for statistical analyses into one merged “control” group. For L/M ratios in controls, the mean was 0.16; the median was 0.15. We defined gut leakiness as an L/M ratio greater than 0.46 because this value equals the mean ± 2 S.D. of values for controls.

Nine of ten rats receiving alcohol + chow had adequate urine production. Chronic daily alcohol gavage caused a significant increase in gut leakiness score (mean = 0.67; median = 0.47). The urinary L/M ratio in alcohol-fed rats at 10 weeks was significantly higher (p = 0.002) than in dextrose-fed rats (Fig. 1). Six of the nine alcohol/chow-fed rats (66%) had leaky guts (L/M > 0.46).

Nine of ten rats receiving alcohol + oats produced adequate urine. Oat supplementation prevented alcohol-induced gut leakiness, and none of these alcohol/oats-fed rats had a leaky gut (mean = 0.10; median = 0.11). Indeed, alcohol-fed rats that received oats had an L/M ratio that was not significantly different from the dextrose-fed rats (Fig. 1).

Intestinal Histology. Alcohol caused mild and mostly focal histological changes in the small bowel (Fig. 2). There was no significant mucosal injury or loss of mucosal lining. However, half the alcohol-fed rats developed focal and mild (score = 1) mucosal injury with abnormal epithelial cells without gross breach of the mucosal lining. There was no

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**Figure 1.** The effect of chronic alcohol administration (10 weeks) on L/M ratio in alcohol/chow- and alcohol/oat-fed rats. In the box plots, median is denoted by the center horizontal line. The bottom and top of the box, respectively, show the 25 and 75% rankings and therefore the interquartile range. The 10 and 90% rankings are denoted by the lower and upper whiskers. Outliers are denoted by circles. Chronic alcohol administration significantly increased the L/M ratio compared with controls (*p < 0.05). Oat supplementation significantly lowered the L/M ratio compared with alcohol/chow (+p < 0.05). There was no statistically significant difference between the group receiving alcohol + oats versus the group receiving dextrose + chow (control).
were significantly lower than in alcohol/chow-fed rats had elevated serum endotoxin levels, with a mean of 1.89 pg/ml. Serum endotoxin levels in controls were 0.43 pg/ml, none having detectable endotoxin levels in their serum, but chronic alcohol consumption caused gut leakiness that is associated with both endotoxemia and liver injury and that oats supplementation ameliorates all of these changes. Several points bear further discussion.

**Liver Injury.** Chronic daily alcohol gavage caused liver injury by all three of our outcome measures. Alcohol-fed rats developed steatohepatitis with macrovesicular and microvesicular steatosis associated with increased inflammatory cells, spotty necrosis, and Councilman bodies (Fig. 4). When these changes were quantitated using histological liver damage index scores, there was a significant increase in the scores of alcohol/chow-fed rats (median = 6) compared with controls (median = 3) (Fig. 5). This indicates that daily alcohol consumption caused a definite but mild steatohepatitis. Steatosis was corroborated by biochemical measurement of fat (total fat content of the liver). Fat content of alcohol/chow-fed rats (mean = 46 mg/g of tissue; median = 30) was significantly higher than that of controls (mean = 20; median = 16) (Fig. 6). Serum AST levels, an index of liver cell injury, were significantly elevated in alcohol/chow-fed rats (mean = 161 IU/liter; median = 160) as compared with controls (mean = 88; median = 88) (Fig. 7) corroborating the findings for steatohepatitis.

Oat supplementation ameliorated the deleterious effects of chronic alcohol on the liver. It prevented spotty necrosis and liver cell death, and it markedly decreased steatosis (Fig. 4). This was reflected in significantly lower scores for liver histology (median = 3) (Fig. 5), fat content (mean = 25; median = 19) (Fig. 6), and AST (mean = 116; median = 110) (Fig. 7) compared with alcohol + chow rats. There was no significant difference in histological score or fat content between alcohol + oats rats and control rats. However, AST levels were still significantly higher in alcohol + oats rats than in controls.

**Discussion.** We found that chronic administration of alcohol to rats induces gut leakiness that is associated with both endotoxemia and liver injury and that oats supplementation ameliorates all of these changes. Several points bear further discussion.

**Endotoxin Levels.** All control and alcohol-fed rats had detectable endotoxin levels in their serum, but chronic alcohol exposure caused significant endotoxemia. The mean serum endotoxin levels in controls were 0.43 pg/ml, none having levels higher than 0.74 pg/ml. The mean endotoxin level in the alcohol/chow-fed group was 1.89, which is over 4-fold higher than the mean for the control group, and this increase in the alcohol/chow-fed group was 1.89, which is over 4-fold higher than the mean for the control group. Oat supplementation significantly lowered the endotoxin levels compared with alcohol/chow (+p < 0.05). There was no statistically significant difference between the group receiving alcohol + oats versus the group receiving dextrose + chow (control).

**Liver Injury.** Chronic daily alcohol gavage caused liver injury by all three of our outcome measures. Alcohol-fed rats developed steatohepatitis with macrovesicular and microvesicular steatosis associated with increased inflammatory cells, spotty necrosis, and Councilman bodies (Fig. 4). When these changes were quantitated using histological liver damage index scores, there was a significant increase in the scores of alcohol/chow-fed rats (median = 6) compared with controls (median = 3) (Fig. 5). This indicates that daily alcohol consumption caused a definite but mild steatohepatitis. Steatosis was corroborated by biochemical measurement of fat (total fat content of the liver). Fat content of alcohol/chow-fed rats (mean = 46 mg/g of tissue; median = 30) was significantly higher than that of controls (mean = 20; median = 16) (Fig. 6). Serum AST levels, an index of liver cell injury, were significantly elevated in alcohol/chow-fed rats (mean = 161 IU/liter; median = 160) as compared with controls (mean = 88; median = 88) (Fig. 7) corroborating the findings for steatohepatitis.

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**Discussion.** We found that chronic administration of alcohol to rats induces gut leakiness that is associated with both endotoxemia and liver injury and that oats supplementation ameliorates all of these changes. Several points bear further discussion.
First, we conclude that chronic gavage of alcohol in rats is a simple experimental model that mimics key aspects of ALD in humans, including endotoxemia and liver injury, and can be used to explore mechanisms causing endotoxemia and liver damage in ALD. Our alcohol-fed rats developed Councilman bodies, spotty necrosis, and microsteatosis similar to histological findings in alcoholics with steatohepatitis. However, our model does not produce liver damage identical to that in ALD; damage is milder than in other animal models such as continuous alcohol infusion (Mathurin et al., 2000) and in ALD. However, we reported a similar gut leakiness in this model (Mathurin et al., 2000).

Second, the fact that alcohol-induced liver injury is associated with both endotoxemia and gut leakiness in our model and that preventing the gut leakiness by oats simultaneously ameliorates the endotoxemia and liver damage is consistent with our hypothesis that gut leakiness is a causal cofactor in alcohol-induced liver injury. Of course, to establish such a causal link between alcohol-induced gut leakiness and liver damage, reliable methods for assessing changes in intestinal permeability are essential. Investigators have used macromolecules that are neither metabolized nor actively transported across the intestinal epithelial barrier (Hollander, 1992) including sugars like lactulose and mannitol (Hollander, 1992). These sugars are not digested by the intestinal brush border and passively diffuse across the intestinal mucosal layer. This assay of epithelial permeability has been validated in various clinical and experimental settings and has yielded comparable results to those obtained using other markers for gut leakiness such as Cr-EDTA (Hollander, 1992). Equally important, leaky gut as defined by either high urinary L:M ratio or increased urinary Cr-EDTA is present in many patients with Crohn’s disease (Bjarnason et al., 1995; Macpherson and Maloy, 1999) where endotoxin from the gut lumen has been strongly implicated in its pathophysiology (Gardiner et al., 1995; Campieri and Gionchetti, 1999).

Fig. 4. Histological micrograph of liver specimen from normal controls (chow-fed rats, panel A), ethanol-fed rats (panel B) and rats fed ethanol + oats (panel C). Ethanol resulted in diffuse microvesicular fat (panel B), spotty necrosis with lymphocytic inflammatory cell infiltrate and a Councilman body (panel D, white arrow), spotty necrosis with inflammatory cell infiltrate (panel E), and Kupffer cell hyperplasia (panel D). Oats significantly decreased microvesicular fat and eliminated Councilman body and inflammatory cell infiltrate, with minimal steatosis and no necrosis (panel C). H&E, 400×.

Fig. 5. The effect of chronic alcohol administration (10 weeks) on liver histology scores in alcohol/chow- and alcohol/oat-fed rats. Data are presented in the box plot. Chronic alcohol administration significantly increased histology scores compared with controls (*p < 0.05). Oat supplementation significantly lowered histology scores compared with alcohol/chow (+p < 0.05). There was no statistically significant difference between the group receiving alcohol + oats versus the group receiving dextrose + chow (control).

Fig. 6. The effect of chronic alcohol administration (10 weeks) on total liver fat levels in alcohol/chow- and alcohol/oat-fed rats. Data are presented in the box plot. Chronic alcohol administration significantly increased total fat levels compared with controls (*p < 0.05). Oat supplementation significantly lowered total fat levels compared with alcohol/chow (+p < 0.05). There was no statistically significant difference between the group receiving alcohol + oats versus the group receiving dextrose + chow (control).
Additionally, in the current study, alcohol administration significantly increased AST levels compared with controls (*p < 0.05). Oat supplementation significantly lowered AST levels compared with alcohol/chow (+p < 0.05). Serum endotoxin levels in alcohol- and oat-fed rats were still significantly higher than in controls.

Similarly, we reported elevated L/M in alcoholics with liver disease (Keshavarzian et al., 1999).

We chose urinary L/M as a marker of gut leakiness because it is a reliable and widely accepted method, and it is more sensitive than either variable alone (Hollander, 1992). Using a ratio eliminates confounding factors that affect sugar absorption and clearance such as gastric emptying, intestinal transit, renal function, and urinary output.

Another question is whether using permeation to small molecules, such as lactulose or mannitol, reflects permeation of larger molecules, such as endotoxin or its fragments. As stated above, several studies demonstrated that the L/M ratio is a reliable marker for general gut leakiness (increased paracellular permeability) in different diseases (Bjarnason, 1995). This is not surprising since the rate of passive permeation of molecules across the intestinal barrier is not solely dependent on molecular weight; other factors such as molecular diameter and hydrophobicity are also important (Lloyd, 1998). Furthermore, we demonstrated for rats continuously infused with alcohol that increased L/M predicts increased absorption of orally administered endotoxin into the circulation (Mathurin et al., 2000). Additionally, in the current study, there was a significant correlation between L/M and endotoxemia (r² = 0.54, p < 0.001). These data strongly suggest that an elevated L/M is a reasonable marker of elevated gut leakiness to endotoxin.

Our current rat data support our previous human data (Keshavarzian et al., 1999) that gut leakiness only occurs in alcoholics with liver disease, and the idea that the main cause of endotoxemia seen in alcoholics with liver disease is gut leakiness. Previous reports have demonstrated the importance of endotoxemia as a cofactor in alcohol-induced liver damage in both humans and animals (Bhagwandeen et al., 1987; Shibayama et al., 1991; Hansen et al., 1994; Nanji et al., 1994, 1995; Adachi et al., 1995; Enomoto et al., 1998). It is now well established that alcohol and endotoxin have a synergistic deleterious effect on the liver (Bhagwandeen et al., 1987; Shibayama et al., 1991; Hansen et al., 1994; Nanji et al., 1995; Enomoto et al., 1998). What is still unclear is the cause of endotoxemia in ALD. The source of serum endotoxin is the gut. The serum endotoxin level reflects the difference between the rate of endotoxin entering the circulation from the gut and the rate of endotoxin cleared by Kupffer cells in the liver. Thus, abnormalities in either process can contribute to changes in endotoxin levels. However, Kupffer cell dysfunction does not seem to be the initiating factor for endotoxemia. Although Kupffer cell dysfunction can be present in ALD, it is a consequence and not a cause of advanced liver disease (Enomoto et al., 1998). Thus, if endotoxemia is a required cofactor for ALD, then Kupffer cell dysfunction cannot be the causative factor for endotoxemia. However, it might exacerbate endotoxemia after liver disease is already established. Endotoxemia could alternatively be due to increased transport of endotoxin from the gut into the circulation due to either increased endotoxin production by intestinal flora or disruption of the intestinal barrier. Indeed, bacterial overgrowth has been reported in alcoholic cirrhosis (Morencos et al., 1995), but this occurs in only 5% of alcoholics without ascites and in 30% of more advanced liver disease with ascites, suggesting that bacterial overgrowth may be a result of advanced liver disease rather than a contributing cause of ALD. Furthermore, regardless of the severity of liver disease, a majority of patients with ALD do not have bacterial overgrowth. Thus, bacterial overgrowth is not a likely cause of endotoxemia in a majority of patients with ALD. Therefore, gut leakiness is the most plausible explanation for endotoxemia in ALD, and our findings support this notion.

Although it is possible that alcohol-induced leakiness is a consequence rather than a cause of liver injury, we deem this unlikely. First, the degree of liver injury in our model is mild, and it is unlikely that this mild injury causes systemic changes that lead to organ failures such as gut leakiness. Second, we showed (Keshavarzian et al., 1999) that liver disease in man in the absence of alcoholism is not associated with gut leakiness. A time course study might indeed further confirm our conclusion.

Alcohol can induce gut leakiness by either gross structural changes or more subtle changes in functional aspects of the cytoarchitecture of cells and their cytoskeletons. Several studies (Burbige et al., 1984) have demonstrated that alcohol can cause histological changes in intestinal mucosa. These changes are usually focal and subtle. Indeed, in the present study, alcohol caused mild but detectable histological changes in intestinal mucosa. But these histological changes did not seem to be the main cause of gut leakiness since similar changes were noted in alcohol + oats-fed rats who had normal L/M ratios.

Another possible mechanism is a change in the cytoskeleton that regulates paracellular permeability. Using monolayers of intestinal cells, we demonstrated that alcohol-induced leakiness is associated with disruption of both actin and microtubule cytoskeletons (Banan et al., 1998, 1999). These changes were secondary to oxidation and nitration of actin and tubulin. Further studies are needed to determine whether alcohol-induced gut leakiness in humans is also due to cytoskeletal changes.

Our findings that oats supplementation prevents gut leakiness in alcohol-fed rats is consistent with previous reports that oats restore normal intestinal barrier in methotrexate-treated rats (Mao et al., 1996). These reports suggest that the
beneficial effects of oat supplementation on intestinal barrier are not limited to a specific injurious agent. This is important because there are many illnesses in which abnormal intestinal permeability occurs, including inflammatory bowel disease (Strobel et al., 1984; Hollander, 1992) and nonsteroidal anti-inflammatory drug-induced gastrointestinal damage (Wallace, 1997; Sigthorsson et al., 1998). Further studies are needed to determine whether oats are beneficial in such conditions.

The mechanism by which oats decreases endotoxin levels and blunts the deleterious effects of ethanol is not established. Oats may prevent alcohol absorption. This possibility was excluded, however, because blood alcohol levels were identical in chow- and oat-fed rats. Hence, the beneficial effect of oats on intestinal barrier appears to be due to prevention and/or reversal of the effects of alcohol on barrier integrity. Oats may also decrease serum endotoxin levels by lowering endotoxin production through its effect on bacterial flora or by absorbing endotoxin in the lumen. Although this mechanism cannot be excluded and may be important for prevention of liver damage, it would not explain oats-improved improvement of barrier function.

We currently believe that the beneficial effect of oats on alcohol-induced liver injury is due to oats-mediated preservation of gut barrier integrity and prevention of endotoxemia. Nevertheless, oats may directly benefit the liver. Studies are needed to further evaluate the mechanism of the effects of oats on intestinal and hepatic functions and to determine whether oats has a beneficial effect on gut barrier in humans, and whether oral supplementation of oats or similar substances are possible therapeutic options in the treatment and prevention of alcoholic liver disease.

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References


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