Recent Advances in Alcoholic Liver Disease
I. Role of intestinal permeability and endotoxemia in alcoholic liver disease

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Rao, R. K., A. Seth, and P. Sheth. Recent Advances in Alcoholic Liver Disease. I. Role of intestinal permeability and endotoxemia in alcoholic liver disease. Am J Physiol Gastrointest Liver Physiol 286: G881–G884, 2004; 10.1152/ajpgi.00006.2004.—A significant body of evidence indicates that endotoxiaemia and endotoxin-mediated hepatocellular damage play a crucial role in the pathogenesis of alcoholic liver disease. A close correlation between endotoxiaemia and the severity of alcohol-induced liver injury is supported by a number of clinical and experimental studies. Elevated intestinal permeability appears to be the major factor involved in the mechanism of alcoholic endotoxiaemia and the pathogenesis of alcoholic liver disease. Ethanol and its metabolic derivatives, acetaldehyde in particular, alter intra-cellular signal-transduction pathways leading to the disruption of epithelial tight junctions and an increase in paracellular permeability to macromolecules. Studies addressing the mechanisms of such epithelial disruption and the protective factors that prevent ethanol and acetaldehyde-mediated disruption of epithelial tight junctions are critically important in the investigations toward the search of preventative and therapeutic strategies for alcoholic liver disease.

acetaldehyde; PTP1B; phosphorylation; ethanol

THE PROGRESS IN INVESTIGATIONS on the mechanisms involved in the pathogenesis of alcoholic liver disease (ALD) has demonstrated that ALD is a result of complex pathophysiological events involving various types of cells, such as neutrophils, endothelial cells, Kupffer cells, and hepatocytes, and a variety of injurious factors such as endotoxins, oxidative stress, cytokines, and proteases. A striking observation is that endotoxiaemia and endotoxin-mediated alteration of liver cell functions play a crucial role in the pathogenesis of ALD. The mechanism of endotoxiaemia has been a subject of investigation for decades. Evidence suggests that there are three possible mechanisms involved in the alcohol-induced endotoxiaemia: 1) dysfunctional Kupffer cells with reduced ability to detoxify endotoxins, 2) bacterial overgrowth in the gut leading to excessive generation of endotoxins, and 3) disruption of intestinal barrier function and increase in permeability to endotoxins and bacteria. The third possibility has recently gained increasing attention. Alcohol consumption appears to increase intestinal permeability to macromolecules as evidenced by both clinical and experimental studies. Therefore, the action of ethanol at the level of gastrointestinal mucosa appears to be the first site of injury that leads to the development of a complex cascade of cellular responses resulting in hepatocellular injury.

ENDOTOXEMIA IN ALD

Endotoxins are LPSs derived from the cell wall of gram-negative bacteria. They are highly immunogenic and induce production of proinflammatory cytokines such as interleukin-1 and TNF-α. Bacteria inhabiting the lumen of colon and terminal ileum are the sources of endotoxins. Endotoxins normally penetrate the gut epithelium only in trace amounts; however, the absorption can be elevated under pathophysiological conditions. The evidence for the role of endotoxin-induced liver injury in ALD is provided by a number of studies. First, plasma endotoxin levels are higher in patients with ALD compared with those in normal subjects (7, 9, 19, 27) and patients with nonalcoholic cirrhosis (8). Second, alcohol-induced hepatitis in rats is associated with increased levels of plasma endotoxin (15, 16). Third, administration of antibiotics to rats reduces the growth of gram-negative bacteria in the intestinal lumen and prevents ethanol-induced endotoxiaemia and liver injury (1). A significant body of evidence indicates that endotoxin plays a crucial role in hepatocellular damage by activating Kupffer cells to secrete cytokines and affecting hepatic sinusoids to increase vascular permeability.

Plasma endotoxin levels in patients with alcoholic cirrhosis are several-fold greater than those in patients with nonalcoholic cirrhosis (8) and healthy subjects (7, 9, 19, 27). Plasma endotoxin levels in patients with ALD correlate well with the levels of TNF receptors and TNF-α (9). Additionally, a recent study showed a link between plasma endotoxin levels and the severity of liver disturbance in patients with alcoholic hepatitis (7). One patient with severe hepatitis showed a markedly high plasma endotoxin level. Endotoxiaemia also increased with the progress of the disease to the terminal stage. On the other hand, in most survivors, plasma endotoxin levels decreased during the recovery phase. Similarly, the levels of LPS-binding protein were found to be greater in patients with ALD compared with those in normal subjects. The plasma endotoxin levels were also increased in rats by chronic (17) or acute (15, 16, 26) administration of ethanol, which was associated with liver injury. Lactobacillus-mediated reduction in liver pathology score was associated with a significant decrease in alcohol-mediated endotoxiaemia (17).

The normal endotoxin levels in human subjects (7, 9, 19) and rats (11, 13, 15) are maintained at a very low level due to the intestinal barrier function and Kupffer cell-mediated detoxification of endotoxins in liver. Endotoxiaemia is defined as a condition with plasma endotoxin levels >2.5 endotoxin units (EU)/ml (18). However, the comparison of plasma endotoxin levels has been difficult due to several factors. Although in general, one nanogram of endotoxin is considered equivalent to 12 EU, different types of LPSs show different degrees of toxic activity. The different procedures of sample preparation and different assay methods used to measure endotoxin can affect the plasma endotoxin values. The endotoxin levels in normal subjects are reported to be 0.3–10.4 pg/ml (7, 9, 19), whereas

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in all studies, endotoxin levels in patients with ALD were five- to eightfold greater than the corresponding values in normal subjects. Similarly in rats, normal levels of plasma endotoxin varied from undetectable to 13 pg/ml (11, 13, 15), whereas alcohol administration increased the plasma endotoxin by six- to eightfold. Studies by Lambert et al. (13) suggested that the endotoxin levels at specific locations such as liver and portal blood might be more important than its level in systemic plasma.

It is well established that the development of alcohol-induced liver injury is more rapid in women than in men, and alcoholic hepatitis progresses more rapidly in women (22). A similar gender difference in sensitivity to alcoholic liver injury has been established in rats, because plasma endotoxin levels in alcohol-fed female rats were greater than those in alcohol-fed male rats (16). Furthermore, rats administered with antibiotics, polymyxin B, and neomycin showed virtually no growth of gram-negative bacteria in the intestinal lumen, decreased plasma endotoxin levels by nearly 75%, and a complete prevention of alcohol-induced increases in plasma aspartate aminotransferase levels (1). Therefore, the role of endotoxemia in ALD is supported by both the clinical and experimental studies.

The mechanism by which alcohol induces endotoxemia is not clear; however, several possibilities have been proposed (Fig. 1). One possible mechanism is diminished phagocytosis of endotoxin by Kupffer cells. Chronic administration of ethanol in rats decreases phagocytic function of certain populations of Kupffer cells (24). Furthermore, acute alcohol administration lowers the phagocytic activities of recruited polymorphonuclear cells and Kupffer cells more rapidly in female rats than in male rats (25), which correlates well with the higher sensitivity of female rats to develop alcoholic liver damage. Therefore, it is possible that reduced phagocytic activities of Kupffer cells may reduce the clearance of endotoxins from the circulation and contribute to endotoxemia. However, this hypothesis is complicated to some extent by the fact that endotoxin-mediated Kupffer cell function is essential for the development of alcoholic liver damage. Therefore, it seems paradoxical that Kupffer cells can be responsible for liver damage, whereas they are simultaneously responsible for detoxifying the injurious endotoxins.

Clinical and experimental studies showed bacterial overgrowth in the small intestine by ethanol administration (5). Bacterial overgrowth certainly increases the volume of the source of endotoxin, which may contribute to the alcoholic endotoxemia. However, the role of bacterial overgrowth in endotoxemia is undermined by the barrier function of the gastrointestinal epithelium, which prevents the diffusion of endotoxins from the gut lumen into the interstitial tissue. Therefore, the alteration of gastrointestinal epithelial barrier function and an increased intestinal permeability to endotoxins appear to be involved in the mechanism of alcohol-induced endotoxemia.

**INTESTINAL PERMEABILITY IN ALD**

Disruption of the gastrointestinal barrier function and the diffusion of luminal toxins and pathogens into the systemic circulation are central to the pathogenesis of a number of diseases. Studies using permeability markers such as polyethylene-glycol, chromium-EDTA, mannitol/lactulose, or sucrose showed that the gastrointestinal permeability to macromolecules is significantly greater in alcoholics compared with that in normal subjects (10, 12). Alcohol administration increases gastrointestinal permeability in both normal subjects and in patients with ALD (12). Although in some patients, the intestinal permeability is reduced to normal levels by 1–2 wk of sobriety, in many patients, the abnormality in gastrointestinal permeability persists even after 2 wk of sobriety (12).

It is not clear whether permeability is caused by an abnormality in the gastroduodenal barrier function or the intestinal barrier function. Keshavarzian and co-workers (12) showed that acute ethanol intake increases gastroduodenal permeability without affecting the intestinal permeability, whereas chronic alcohol abuse in alcoholics shows no effect on gastroduodenal permeability while increasing the intestinal permeability. The location of barrier dysfunction is important in understanding its role in ALD because the large intestine is the main source of bacterial endotoxin. A recent study by Keshavarzian et al. (10) compared the gastrointestinal permeability changes with liver disease in alcoholic subjects. Both gastroduodenal permeability and intestinal permeability were significantly higher in alcoholics with symptoms of liver injury than those in alcoholics without liver damage or normal subjects or patients with nonalcoholic liver disease. This clearly ties the gastrointestinal permeability with the liver damage in alcoholics. However, it is not clear whether the permeability changes were caused by the liver damage or whether it is the cause of liver damage. The current information indicates that the gastrointestinal barrier function provides resistance to alcohol-induced liver damage until the secondary factors promote the disruption of barrier function and increase endotoxin absorption.

A correlation between intestinal permeability and alcoholic liver damage was also demonstrated in experimental models of ALD using rats and mice. A number of studies using animal models (6, 11, 13, 15, 23) demonstrated that chronic or acute administration of ethanol increases gastrointestinal permeability to macromolecular markers, such as mannitol, lactulose, polyethylene-glycol, and dextran as well as endotoxin, the LPS. At present, there is no animal model that can mimic symptoms similar to those seen in patients with ALD. However, the most recent studies show that ethanol-induced gastrointestinal permeability is associated with elevated plasma endotoxin levels and liver injury (6, 11, 15), indicating that a correlation among intestinal permeability, endotoxemia, and liver injury can be demonstrated in animal models as seen in patients with ALD. Therefore, the results of these experimental studies are relevant to our understanding of the mechanism of the pathogenesis of ALD. It is not clear at present whether

![Fig. 1. Schematic representation of different mechanisms involved in ethanol-induced endotoxemia in alcoholic liver disease.](http://ajpgi.physiology.org/)

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changes in both gastroduodenal and intestinal permeability are important in causing alcoholic liver injury. In a carefully developed rat model of ALD, Mathurin et al. (15) showed that liver injury induced by chronic ethanol administration is associated with the elevated intestinal permeability, whereas it had no influence on gastroduodenal permeability.

The mechanism of ethanol-induced intestinal permeability is not clear. None of the studies have reported any gross morphological changes in the intestinal epithelium. A few studies showed that the tight junctions of gastrointestinal epithelium are disrupted in ethanol-fed rats (23), suggesting the disruption of epithelial barrier function as a possible mechanism involved in the ethanol-induced increased in intestinal permeability.

**DISRUPTION OF EPITHELIAL BARRIER FUNCTION BY ETHANOL**

The barrier function of intestinal epithelium is provided by tight junctions, the highly specialized junctional complexes located at the apical end of epithelial cells. Tight junctions form the barrier to the diffusion of allergens, toxins, and pathogens from the intestinal lumen into the interstitial tissue. The disruption of tight junctions increases intestinal permeability to injurious factors, which result in mucosal inflammation. Tight junctions are organized by interactions among a wide variety of proteins localized specifically at the tight junctions (2). Occludin, claudins, and junction adhesion molecules are the major transmembrane proteins that interact with intracellular plaque proteins such as zona occludens (ZO)-1, -2, and -3, which in turn interact with the actin cytoskeleton to anchor occludin and other transmembrane proteins at the apical end of the lateral membrane. In the intestine, disruption of tight junctions may lead to increased permeability to allergens, toxins, and pathogens, which appears to be a common mechanism involved in the pathogenesis of a number of gastrointestinal diseases, such as inflammatory bowel disease, celiac disease, and ALD.

Most recent studies have addressed the possible effect of ethanol on paracellular permeability and disruption of epithelial tight junctions in a cell culture model of intestinal epithelium, the Caco-2 cell monolayer (3, 14, 20). Paracellular permeability has been evaluated by measuring transepithelial electrical resistance (TER) and unidirectional flux of extracellular markers such as inulin and mannitol. Ethanol at 5% concentration produced an ∼10% transient decrease in TER, but acetaldehyde (0.1–0.6 mM), the metabolic product of ethanol, potently reduced TER by 70% and increased paracellular permeability to inulin by nearly fourfold (3, 20). The weak effect of ethanol may be attributed to the very low level of expression of alcohol dehydrogenase in Caco-2 cells. A study by Ma et al. (14) indicated that ethanol at 5–10% concentration reduced TER of Caco-2 cell monolayers by 30–50% and increased mannitol permeability twofold by a myosin light chain kinase-dependent mechanism. Such a mechanism may exist in the gastroduodenal region, where alcohol levels can be expected to be high. However, there is no evidence for such high levels of alcohol being maintained in the small and large intestine. Increased permeability in Caco-2 cell monolayers by 2.5–15% ethanol was shown in another recent study (4); however, this permeability was caused by loss of cell viability.

**ROLE OF ACETALDEHYDE IN DISRUPTION OF INTESTINAL EPITHELIAL TIGHT JUNCTIONS**

Mounting evidence indicates that ethanol is oxidized to acetaldehyde in the gastrointestinal tract and suggests that acetaldehyde may contribute to the pathogenesis of alcohol-related diseases (21). In addition to mucosal alcohol dehydrogenases, intestinal bacteria seem to play a significant role in the oxidation of ethanol to acetaldehyde (21). The capacity of colonic mucosa and microbes to oxidize acetaldehyde to acetate is low compared with that in other tissues, suggesting a greater ability of colon to accumulate acetaldehyde. High levels of acetaldehyde appear to be accumulated in the colonic mucosa and lumen in alcoholics. Due to its volatility, it is difficult to measure the acetaldehyde level in tissues without underestimating it. Studies by Salaspuro (21) demonstrated that the intracolonic acetaldehyde level in rats may reach as high as 3 mM. In cell culture studies, acetaldehyde at 0.1–0.6 mM concentrations disrupts tight junctions and increases paracellular permeability in Caco-2 cell monolayers (3, 20). Therefore, it is likely that intracolonic acetaldehyde in alcoholics may play an important role in disrupting the tight junctions and increasing the permeability to endotoxins.

Acetaldehyde disrupts the tight junction and increases paracellular permeability by inducing redistribution of tight junction proteins from the intercellular junctions (Fig. 2). This effect of acetaldehyde on tight junction and paracellular permeability is mediated by a tyrosine kinase-dependent mechanism (3). Acetaldehyde does not alter the overall tyrosine kinase activity, but it effectively inhibits protein tyrosine phosphatase activity in Caco-2 cells. Acetaldehyde induces dramatic inhibition of protein tyrosine phosphatase-1B and partial inhibition of protein tyrosine phosphatase-1C and -1D activities. Acetaldehyde-induced disruption of the tight junction and inhibition of protein tyrosine phosphatase activities is associated with the increase in tyrosine phosphorylation of a wide spectrum of proteins, including ZO-1, a tight junction protein, and β-catenin, an adherens junction protein (3). Recent studies demonstrated that acetaldehyde dissociates protein tyrosine phosphatase-1B from the E-cadherin/β-catenin complex and induces tyrosine phosphorylation of E-cadherin and β-catenin. Furthermore, acetaldehyde interacts directly with protein tyrosine phosphatase-1B to form a phosphatase-inactive acetaldehyde-protein tyrosine phosphatase-1B adduct (R. K. Rao, unpublished data). Therefore, acetaldehyde can modify intra-
cellular signal-transduction pathways to destabilize the tight junction protein complex leading to increased permeability to endotoxins. Generation and accumulation of acetaldehyde in the intestinal lumen may play a crucial role in the onset of a cascade of cellular responses that ultimately lead to endotoxemia and liver injury.

FUTURE DIRECTIONS

The observation that acetaldehyde increases the paracellular permeability suggests that endotoxin absorption may be prevented by inhibiting alcohol dehydrogenase activity. However, the organs will still have to deal with ethanol, which is directly responsible for injury in many tissues, including gastrointestinal epithelium. Therefore, the most beneficial approach to deal with the increased permeability is to prevent acetaldehyde-induced disruption of intestinal barrier function. This can be achieved by 1) accelerating the clearance of acetaldehyde, i.e., by activation or over expression of aldehyde dehydrogenase; and 2) by intervening with biochemical mechanisms involved in the acetaldehyde-induced increase in permeability. Therefore, the factors that prevent alcohol- and acetaldehyde-induced intestinal permeability are essential to provide a basis for the design of therapeutic regimens for ALD. Recent studies have provided some information regarding this issue. Alcohol-induced intestinal permeability and liver damage could be reduced by diets supplemented with oat (11) or zinc (13). Therefore, future studies to understand the mechanisms involved in alcohol-induced intestinal permeability and the mechanisms associated with the protective factors that ameliorate alcohol-induced intestinal permeability are critically important in designing the preventive and therapeutic strategies for ALD.

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