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Dissolution of lipids from mucus: A possible mechanism for prompt disruption of gut barrier function by alcohol

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HIGHLIGHTS

- Alcohol causes prompt increase in gut permeability.
- Alcohol causes prompt decrease in mucosal surface hydrophobicity.
- Changes above are related to dissolution of lipids from mucus by alcohol.

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ABSTRACT

Acute and/or chronic alcohol ingestion has been shown to exacerbate the morbidity and mortality rate associated with acute mechanical and/or thermal trauma. While alcohol ingestion can affect many organs and systems, clinical and preclinical studies indicate that alcohol ingestion can cause a 'leaky gut' syndrome which in turn contributes to infection and systemic organ dysfunction. This study investigated the acute effect of alcohol on gut barrier function. Using an in vivo isolated gut sac model of naïve male rats, each individual gut sac was injected with different concentrations (0, 5, 10, 20, and 40%, v/v) of alcohol. After different times of alcohol exposure, each isolated gut segment was harvested and intestinal permeability and mucosal surface hydrophobicity (a physiologic marker of mucus barrier function) were measured as well as luminal DNA, mucus, protein and free fatty acids. The results showed that alcohol caused dose-dependent and time-dependent increases in gut permeability and decreases in mucosal surface hydrophobicity, with significant changes to be observed 5 min after treatment with 10% alcohol. In addition, it is further found that these changes in permeability and hydrophobicity are more closely associated with increased intestinal luminal free fatty acids levels but not protein or DNA levels. These results suggest that alcohol may cause loss of gut barrier function by extracting and dissolving lipids from the mucus with a resultant decrease in mucosal surface hydrophobicity, which is a critical component of gut barrier function.

1. Introduction

Alcohol use is endemic worldwide and contributes to morbidity and mortality. According to the World Health Organization (WHO), it is estimated that about two billion people consume alcoholic beverages worldwide and that 76.3 million of these people have diagnosable alcohol-use disorders (Ghosh et al., 2012). Furthermore, in 2004, 3.8% of all global deaths and 9.0% of male deaths in the United States were attributable to alcohol (Rehm et al., 2009). Not only has alcohol use been associated with damage to multiple organs (Esper et al., 2006; Guidot and Hart, 2005; Lieber, 1995; Moss and Burnham, 2003), nearly 38% of alcohol-related deaths involve intentional and unintentional injury (Rehm et al., 2009). In fact, studies show that approximately 50% of male burn patients as well as trauma patients have positive blood alcohol concentrations at the time of admission to the hospital (Rivara et al., 1993; Scalfani et al., 2007). Because acute and/or chronic alcohol ingestion appears to exacerbate the morbidity and mortality of patients with mechanical or thermal trauma, a number of clinical and preclinical animal studies have investigated this relationship (Bird and Kovacs, 2008; Dinda et al., 1996; Kaur, 2002; Li et al., 2011). One area that has received special attention is the relationship between enteral alcohol ingestion and loss of gut barrier function, where

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studies have documented that an alcohol-induced increased absorption of endotoxin due to a "leaky gut" plays a critical role in alcoholic hepatitis and cirrhosis (Bjarnason et al., 1984; Keshavarzian et al., 1999; Purohit et al., 2008). Additionally, the adverse effects of an impaired gut barrier have been recognized in several non-cirrhotic patient populations including ICU patients and patients sustaining major burn or mechanical trauma (Cherpitel, 1997, 2007; Rivara et al., 1993). Because of the relationship between intestinal dysfunction and the development of the systemic sepsis (SIRS) and multiple organ dysfunction syndromes (MODS), we and others have focused investigative attention on the mechanisms leading to gut injury and gut-induced MODS (Deitch, 2001; Deitch et al., 2006; Leaphart and Tepas, 2007; Nieuwenhuijzen and Goris, 1999). Most recently, these studies have high-lighted the important role that the mucus layer plays in normal gut barrier function and how its loss can result in increased gut permeability (Qin et al., 2008, 2011; Sharpe et al., 2010). Thus, the object of this study was to begin to characterize the effects of enteral alcohol on normal small bowel function with a special focus on its physiologic effects on the mucus layer and the associated changes in intestinal permeability.

2. Materials and methods

2.1. Animals

Specific pathogen-free male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 300–350 g (about 10–12 weeks old) were housed under barrier-sustained conditions and kept at 25 °C with 12-h light/dark cycles. The rats had free access to water and chow (Teklad 22/5 Rodent Diet W-8640, Harlan Teklad, Madison, Wis). All rats were maintained and all experiments were conducted in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of Rutgers, etc.

2.2. Experimental procedure

To reduce the number of animals as well as the variation among animals, the small intestine after flushing was divided into several segments, with each receiving a different treatment. Below is the detailed description of the procedure.

After anesthesia with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and laparotomy, the luminal content of the small intestine was flushed out through three cuts, each about three-fourths of the gut circumference and effort was made to avoid cutting visible blood vessels: first cut at about 5 cm down the ligament of treitz, the second cut about 50 cm distal from the first cut, and the third cut at about 5 cm above the end of the ileum. The gut was gently flushed with 60 ml warm saline to remove the luminal contents. Flushing was done to reduce any potential confounding effects caused by differences in the amount as well as composition (digestive proteases, bile salts, etc.) of the luminal contents between the intestinal segments to be tested and to facilitate the assay of various luminal parameters.

After gently milking out the flushing solution, the small intestine was sequentially divided into different segments with a length of up to 15 cm for each, isolated by ligation. For the time course study, each segment was injected with 20% alcohol at a volume of 0.1 ml/cm and harvested at different time points. For the dose response study, the different segments were injected with 0–40% alcohol that encompassed the alcohol content of commonly used alcoholic beverages from beer and wine to spirits like brandy, whiskey and vodka. By the end of the incubation period, the blood vessels to the segment were ligated and the segment was harvested. The luminal contents were collected and stored at −80 °C. A 4 cm piece of the segment was cut off and used to measure gut mucosal hydrophobicity. Another 6 cm piece was taken to measure gut permeability. The luminal contents were subsequently assayed for DNA, protein, free fatty acids, and mucus levels.

To reduce the potential variation caused by the location of the different segments, the segments corresponding to the different treatment were rotated among the different animals as demonstrated in Table 1 for the dose–response study.

2.3. Measurement of intestinal mucosal permeability

Intestinal permeability was measured using the everted gut sac method and the fluorescent tracer, fluorescein isothiocyanate dextran (MW 4000 Da; FD4) as described in our previous study (Qin et al., 2008). Briefly, the intestinal segment was everted using a thin metal rod. One end of the segment was secured with 4–0 silk to the grooved tip of a 1-ml plastic syringe containing 0.5 ml modified Krebs–Henseleit bicarbonate buffer (KHBB, pH 7.4). A ligation was made 4 cm away from the tip and the everted gut sac was suspended in a 100-ml beaker containing 80 ml of KHBB with added FD4 (20 μg/ml). The solution in the beaker was maintained at 37 °C temperature in a water bath, and a gas mixture containing 95% O_2 and 5% CO_2 was bubbled continuously. A 1.0-ml sample was taken from the beaker before placing the everted gut sac to determine the initial external (mucosal surface) FD4 concentration. The everted gut sac was distended gently by injecting the 0.5 ml of KHBB and incubated for 30 min in the KHBB solution containing FD4. After that, the fluid on the serosal side was aspirated into the syringe and put into a centrifuge tube. The samples were centrifuged for 10 min at 1000 × *g*. Two hundred microliters of the supernatant were put into the wells of a microplate and fluorescence was measured by a PerkinElmer LS-50 fluorescence spectrophotometer (Palo Alto, CA) at an excitation wavelength of 492 (slit width, 10 nm) and an emission wavelength of 515 nm (slit width, 10 nm). Permeability was expressed as the mucosal-to-serosal clearance of FD4 calculated using the following equations:

$$M = ([\mathrm{FD4}]_{\mathrm{serosal}}) \times 0.5$$

$$F = \frac{\mathrm{M}}{30\ \mathrm{min}}$$

$$C = \frac{(F/[\mathrm{FD4}]_{\mathrm{mucosal}})}{A}$$

where M is the mass (in ng) of FD4 in the gut sac at the end of the 30-min incubation period, $[\mathrm{FD4}]_{\mathrm{serosal}}$ is the FD4 concentration in the serosal fluid aspirated from the sac at the end of the 30 min incubation period, F is the flux of FD4 (in ng/min) across the mucosa, $[\mathrm{FD4}]_{\mathrm{mucosal}}$ is the FD4 concentration measured in the beaker at the beginning of the 30 min incubation period, $A = \Pi$ LD which is the calculated area (in cm^2) of the mucosal surface, and C is the clearance of FD4 (in nl min^{-1} cm^{-2}) across the mucosa.

Table 1
Treatment of the different segments from upper jejunum to middle ileum of the different animals in the dose–response study.

	Gut segments (upper jejunum to middle ileum)				
	1	2	3	4	5
Rat #1	0	5%	10%	20%	40%
Rat #2	40%	0	5%	10%	20%
Rat #3	20%	40%	0	5%	10%
Rat #4	10%	20%	40%	0	5%
Rat #5	5%	10%	20%	40%	0