How bile acids confer gut mucosal protection against bacteria

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Bile is a complex mixture of organic and inorganic molecules that is stored in the gallbladder and released into the proximal small intestine when a meal is eaten. Bile is both an excretory secretion, to eliminate cholesterol, bilirubin, and waste products, and a digestive secretion, to promote lipid absorption. The dominant organic constituents of bile are conjugated bile acids (also termed bile salts), glycine or taurine N-acyl amidated derivatives of bile acids that are formed from cholesterol in the liver cell. Bile acids are wedge-shaped, water soluble, amphipathic molecules with a hydrophobic side and a hydrophilic side. Adsorption of bile acid anions to lipid bilayers of dietary membrane lipids or fatty acids (derived by pancreatic lipase acting on triglyceride) increases the curvature of the bilayers, ultimately converting them to mixed micelles (1, 2). Such micellar solubilization of polar lipids greatly increases their rate of diffusion to the epithelial surface of the small intestine, and micellar solubilization is essential for the efficient absorption of lipids. Although most lipid absorption occurs in the proximal small intestine, conjugated bile acids themselves are not absorbed together with the solubilized lipids in the jejunum but pass to the distal small intestine, where they are efficiently absorbed by an active transport system present in the epithelium of the terminal ileum. Efficient intestinal reclamation of bile acids leads to the accumulation of a recycling pool of conjugated bile acids that circulates one or more times with each meal (3). Enterohepatic cycling of bile acids provides large quantities of bile acids for digestion.

The remarkable ability of bile acids to solubilize polar lipids during digestion has generally been considered the sole function of conjugated bile acids in the small intestine. Work during the past decade (see below) has suggested that luminal conjugated bile acids in conditions of bile acid deficiency in the intestine abolished bacterial overgrowth and reduced bacterial translocation to intestinal lymph nodes (6, 8). Unfortunately, in vitro studies (11) suggest that the antimicrobial effect of conjugated bile acids is quite weak when compared with that of unconjugated bile acids and cast doubt on the validity of the effect being mediated solely by conjugated bile acids. A possible explanation for this paradox is that the antimicrobial effect of administered conjugated bile acids may be mediated by fatty acids (partly present as soaps) that are associated with the conjugated bile acids in mixed micelles in the proximal small intestine. Long-chain fatty acids have been known for many decades to have potent antimicrobial effects (12, 13). Nonetheless, irrespective of the mechanism, these experiments provided strong evidence for a second physiological function of conjugated bile acids in the proximal small intestine: to prevent bacterial overgrowth by their antimicrobial activity.

Inagaki et al. (4) present compelling evidence, based on studies in mice, that the antibacterial effect of conjugated bile acids in the distal small intestine is mediated by a cellular pathway involving the nuclear receptor farnesoid X receptor (FXR), an orphan receptor that is activated by conjugated bile acids. Activation of FXR by conjugated bile acids induced the expression of genes whose products prevent bacterial overgrowth and promote epithelial integrity. The authors first determined that intestinal FXR mRNA levels were three times higher in the ileal epithelium, where bile acids are absorbed, than in the epithelium of the proximal small intestine. To test which genes are regulated by FXR in the ileum, they administered GW4064, a potent FXR agonist developed by Glaxo Wellcome (14). Using microarray analysis of ileal RNA, several genes with potential functions in mucosal defense were found to be up-regulated by GW4064, including inducible nitric oxide synthetase (iNOS), whose enzymatic product, nitric oxide, is well known to possess direct antimicrobial activity.

The authors then performed bile duct ligation to determine whether such up-regulation was associated with suppression of bacterial overgrowth in vivo. As anticipated, bile duct ligation in WT mice caused an >10-fold increase in aerobic bacteria and a doubling of anaerobic bacteria in ileal and cecal contents. It also caused bacterial invasion of the intestinal mucosa and increased aerobic bacterial translocation to mesenteric lymph nodes. These notable effects of bile duct ligation were abolished by oral administration of GW4064 in WT mice but not in mice genetically deficient in FXR (developed by Frank Gonzalez and his colleagues at the National Institutes of Health; ref. 14). Thus, the bacterial overgrowth and bacterial translocation that had been attributed to a conjugated bile acid deficiency in older studies were corrected by GW4064 despite conjugated bile acid levels in the small intestine being negligible because of bile duct ligation. Based on these results, the authors propose that conjugated bile acids activate FXR and that such activation, in turn, induces the expression of gene products that promote antimicrobial defense and epithelial integrity in the distal small intestine.

Missing in the experimental design of this paper was an examination of the effects of conjugated bile acid administration. If bile acids affect microbial levels in the distal small intestine through only FXR-dependent mechanisms, one would expect that their administration, like that of GW4064, would have no effect in

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FXR-deficient mice. Practically, such experiments may be confounded by severe toxicity, and even death, because administration of conjugated bile acids to bile duct-ligated mice results in bile acid accumulation in the hepatocyte with consequent induction of apoptosis/necrosis (15). The paper also did not examine proximal intestinal content, so it is not known whether GW4064 influenced the presumed bacterial overgrowth in the duodenum and jejunum.

It must be further noted that although the identification of FXR-regulated genes by microarray analysis suggests their involvement in antibacterial intestinal defense, such data are merely correlating and not necessarily causal. Indeed, mice lacking iNOS are not known to have bacterial overgrowth in the small intestine (16), even though they are far more susceptible to infections by enteric bacterial pathogens (17). Thus, up-regulation of iNOS may not be solely responsible for the observed beneficial effects of FXR activation. The particular microarray used in the work covered only ~50% of all murine genes, and only a single time point was examined, leaving open the possibility that other FXR target genes are expressed in the small intestine and responsible for the effects of GW4064. Finally, FXR may mediate antimicrobial functions independent of changes in gene expression as probed by microarray analysis.

Bile acid concentrations fall to low, submicellar levels in the terminal ileum because of active bile acid absorption (18), and fatty acids are unlikely to be present as fatty acid absorption occurs in the proximal small intestine. Reflux of bacterial-rich cecal content across the ileocecal valve results in continuous bacterial seeding of the terminal ileum. A defense mechanism that inhibits luminal bacterial growth based on the carrier-mediated entry of conjugated bile acids into the ileal enterocyte should be more robust than that other FXR target genes are present in very low concentrations in the ileal lumen. If this reasoning is correct, conjugated bile acids, probably together with fatty acids, inhibit bacterial growth in the proximal small intestine directly by their pharmacological properties and in the distal small intestine indirectly by their signaling properties. This concept is illustrated in Fig. 1.

Multiple factors contribute to the low bacterial content of the human small intestine (19). These factors include gastrin acidity that kills ingested organisms, IgA that is secreted in bile and by the intestinal epithelium, antimicrobial peptides (e.g., defensins) that are secreted by Paneth and other epithelial cells, and propulsive intestinal motility. Long-chain fatty acids solubilized in conjugated bile acid-mixed micelles also may play a role in the proximal small intestine as discussed. Inagaki et al. (4) describe the secretion in the distal small intestine of as-yet-unidentified microbial agents evoked by conjugated bile acids activating FXR in the ileal enterocyte. These different mechanisms are likely to act synergistically in controlling microbial levels in the small intestine.

In addition to their surfactant properties that render them powerful solubilizers and bacteriostatic agents, bile acids are now known to signal a variety of systems in the liver and intestine by interaction with multiple nuclear receptors (20). This seminal paper from the laboratory of Steven Kliewer describes an important signaling function for conjugated bile acids in the distal small intestine. It also confirms the power of genetic techniques and the availability of a nuclear receptor agonist to elucidate unexpected and previously undescribed physiological functions in vertebrates. Future studies should elucidate the relative roles of conjugated bile acids and/or fatty acids in promoting antibacterial defenses in the small intestine and define which gene products resulting from bile acid-induced FXR activation promote such defenses in distal intestinal content.