Bile-Acid-Induced Intestinal Dysfunction: Implications to Protracted Infantile Diarrhea and Malnutrition

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The pharmacologic effect of exogenous bile acids as cathartic agents is well known. Early formulations used as purgatives took advantage of the laxative effect of bile acids through administration of bile enemas or orally administered desiccated ox bile. Multiple studies have suggested that endogenous bile acids may also, in certain situations, induce diarrhea. The mechanism of the cathartic effect is better understood today; it appears that alterations in colonic motility as well as electrolyte and water transport are effected. In addition, it is known that bile acids are potent detergents and are capable of inducing membrane damage. In children with protracted diarrhea (with varying degrees of malnutrition), bile acids may serve to exacerbate or perpetuate the diarrhea and malabsorption and hence contribute to the vicious cycle (i.e., maintain a “sick bowel”) and lead to continuing malnutrition.

EFFECTS OF BILE ACIDS ON THE INTESTINE

Bile acids have multiple effects on intestinal function—under specific conditions, they are capable of inducing changes in ion transport, motor activity, mucosal morphology, and mucosal permeability. If diarrhea is looked on as a failure of colonic salvage, it is easy to understand the role of bile acids in effecting water transport (1–4). In studies performed using the perfused rat colon as a model, Forth et al. showed that bile acids were able to block sodium transport (5). Subsequent studies by Mehkjian et al. have demonstrated that bile acids alter fluid and electrolyte movement in human jejunum, ileum, and colon when each segment is perfused with varying concentrations of specific bile acids (3,6). It has been shown that dihydroxy bile acids induce net fluid secretion at high concentrations, whereas at lower concentrations, they are able to block absorption of fluid and electrolytes. This effect is specific for the dihydroxy bile acids, since perfusion with trihydroxy bile acids such as cholic acid does not induce these changes (3).

Structure–function studies have shown that in order to cause net secretion, bile acids must (a) be di-α-hydroxylated (e.g., chenodeoxycholic acid or deoxy-
cholic acid) (Fig. 1); conjugation lessens but does not abolish the effect; (b) be present in elevated concentrations (i.e., approximately 1.5 mM or greater); and (c) be present in the aqueous phase (i.e., the supernatant obtained after centrifugation at 100,000 g for 3 hr); in addition, (d) an appropriate pH (i.e., 7.5-8.0) must exist; the alkaline media will keep dihydroxy bile acids in solution (1,3,4,6-13). There is a strong correlation between fecal pH and the percent fecal dihydroxy bile acids in the aqueous supernatant (12). Several studies have related the degree of diarrhea to bile acid loss; there is a good correlation between fecal water and dihydroxy bile acid excretion as measured either in the fecal homogenate or in the aqueous supernatant phase (7).

All of the conditions required for bile acids to mediate diarrhea are present following short-length ileal resection. In situations in which more extensive ileal resection has occurred, less bile acid is present in solution; however, fatty acid derivatives may then serve to mediate a cathartic effect on the colonic mucosa and produce secretion (14-16).

FIG. 1. The effect on fluid absorption of rabbit colon perfusion with equimolar (5 mM) concentrations of nine different bile acids compared with perfusion with control buffer solution. This study demonstrated that marked secretion of water occurred only during perfusion with di-α-hydroxy bile acid (deoxycholic acid, DCA; chenodeoxycholic acid, CDCA; and 7α-OH, 12α-OH = 7α-OH, 12α-OH-5β-cholanoic acid). No significant reduction of fluid absorption was observed with any other bile acid (CA, cholic acid; UDCA, ursodeoxycholic acid; CON, buffer). (From Chadwick et al., ref. 9, with permission.)
MECHANISMS OF BILE-ACID-INDUCED SECRETION

Bile acids (especially when deconjugated) are potent surface-active anionic detergents and are capable of penetrating membrane lipid bilayers. At high concentrations, bile acids solubilize membrane phospholipids and release membrane-bound proteins, thereby altering membrane structure, function, and permeability. Fluid secretion induced by dihydroxy bile acids has been well studied in the hamster jejunum and in the canine colon as well as in the human large and small bowel (9,17,18).

The exact mechanism of diarrhea production remains speculative and is probably multifactorial; several postulated pathophysiologic mechanisms (or alterations of mechanisms normally occurring in the colon) may be operant. Active transport of sodium and chloride in the colon, dependent on energy from membrane-bound ATPase, is coupled with osmotic absorption of water.

One theory suggests that bile acids will increase the activity of adenylate cyclase, leading to cyclic AMP generation and an increased cellular secretion of electrolytes and water (19,20). Recent studies by Camilleri et al. suggest that bile acids alter mucus secretion (Fig. 2), abolishing the cytoprotective effect, and therefore lead to damage of mucosal cells; this in turn causes an alteration in permeability and a predominance of "crypt-like" (secretory) cells (9,10,21,22,22a) (Figs. 3, 4). As an alternative/additive effect, bile acids alter colonic motility (increase myoelectric activity), leading to decreased dehydroxylation and an increased amount of cathartic compounds such as chenodeoxycholic acid in solution (23,24). It is also possible that bile acids decrease ATP generation in the mucosa by their known detergent effect on membrane-bound enzymes such as ATPase (25). In addition to their effect in isolation on colonic mucosa, recent preliminary studies suggest that bile acids may interact with adenylate cyclase in the induction of fluid secretion by enterotoxin and prostaglandin E1 (26).

INTERRUPTION OF THE ENTEROHEPATIC CIRCULATION

Bile acids are normally restricted to the anatomic components of the enterohepatic circulation (i.e., the liver, biliary tract, intestine, and portal venous system) (27). This is made possible by efficient ileal reabsorption as well as rapid hepatic extraction of these molecules from the portal venous blood. Interruption of the enterohepatic circulation, as may occur in several clinical situations (ileal resection, inflammation, or bypass), will result in spillage of excess quantities of bile acid into the colon. It is possible that colonic salvage, i.e., passive absorption of bile acids, occurs to a limited degree; however, the majority remains in the lumen. The unabsorbed bile acids lead to specific clinical symptoms and disturbances of bile acid metabolism (Fig. 5). The resultant syndrome, which has been called "cholerheic enteropathy" by Hofmann (28), is associated with diarrhea as well as a tendency toward cholesterol gallstone disease, renal stone formation, maldigestion, and steatorrhea (2).
PROTRACTED INFANTILE DIARRHEA

Whether or not bile acids contribute to the spectrum of disease termed protracted infantile diarrhea is not known. However, several studies have suggested that alterations in bile acid metabolism, either primary or secondary, may result in excess spillage of bile acids into the colon.

Primary Bile Acid Malabsorption

The precise mechanism involved in ileal active transport of bile acids is undefined; however, it has been suggested that the “transport system” or “ileal receptor” may be absent or malfunction without apparent ileal disease. Therefore, a rare inborn error may be one of the “idiopathic forms” of protracted infantile

FIG. 2. Effect of 5.0 mM sodium chenodeoxycholate (CDC) on net fluid transport and output of protein-bound hexose (mucus secretion) and DNA; influence of concomitant administration of parenteral atropine and carbachol. (From Camilleri et al., ref. 22, with permission.)
diarrhea, i.e., primary bile acid malabsorption. This condition may serve as a prototype for disturbances of bile acid metabolism in acquired (i.e., secondary) bile acid malabsorption.

The suspicion that defective bile acid transport was responsible for the initiation of certain cases of the protracted infantile diarrhea syndrome followed the observation of a beneficial response to cholestyramine administration in two patients with intractable diarrhea (29). Subsequent investigation of these patients documented elevated concentrations of bile acids in stool homogenate, marked fecal loss of labeled bile acids, marked contraction of cholic acid pool size with an increased fractional turnover rate, and a decreased concentration of intra-
FIG. 4. Light microscopic appearance of rabbit colonic mucosa after perfusion (4 hr) with ursodeoxycholic acid (A) or chenodeoxycholic acid for 2 (B) or 4 (C) hr. In contrast to lack of damage after ursodeoxycholic acid, perfusion with chenodeoxycholic acid led to a loss of the normal lobulated appearance, loss of epithelial cells and crypt cells, and mucus depletion. With a longer period of perfusion (4 hr), there was heavy infiltration of the lamina propria with neutrophils and the appearance of surface fibrin. (From Chadwick et al., ref. 9, with permission.)
luminal bile acids (29-31). The concentration of bile acids in fasting serum was markedly depressed, and there was no postprandial rise as might be expected in individuals with an intact ileal absorptive mechanism (32,33). Recent studies have demonstrated reduced uptake of taurocholate by ileal tissue obtained from these patients via peroral intestinal biopsy (34). There are other reported cases of apparent primary bile acid malabsorption in children, all of whom presented with intractable diarrhea and increased fecal bile acid concentrations (35-37).

**Postenteritis (Acquired/Secondary) Bile Acid Malabsorption**

It is well known that there is disturbed fat absorption following infectious enteritis, although the mechanisms may not be as simple as initially suggested. Jonas et al. studied 10 patients who, during recovery from acute gastroenteritis, had persistent failure to thrive despite apparently adequate nutritional intake (38). All had significant steatorrhea, deficiency of intraluminal bile acids, impaired fat incorporation into the micellar phase, and increased fecal bile acid excretion. The authors concluded that there is a transient ileal dysfunction with an associated excess bile acid excretion, which may be responsible for impaired fat digestion or absorption and persisting diarrhea following acute gastroenteritis. There was, however, no indication as to whether or not small bowel bacterial overgrowth (see below) was present in these patients, a factor that could have altered the intraluminal milieu and contributed to the maldigestion.
ALTERATIONS IN BILE ACID METABOLISM IN THE CONTAMINATED SMALL BOWEL SYNDROME

Certain bacteria, such as obligate anaerobes, are capable of 7α-dehydroxylation of bile acids (e.g., conversion of cholic acid to deoxycholic acid), thereby generating dihydroxy bile acids, which are potential secretagogues. In addition, deconjugation of bile acids catalyzed by bacterial cholylamidases liberates unconjugated compounds, which are less efficient in promoting micellar solubilization. Unconjugated bile acids are poorly soluble and either precipitate intraluminally or, more commonly, are absorbed by passive nonionic diffusion, thereby short circuiting the enterohepatic circulation. As mentioned, unconjugated bile acids are capable of causing direct damage to intestinal mucosa leading to malabsorption of nutrients. Dawson and Isselbacher initially suggested that the steatorrhea seen in patients with the stagnant loop syndrome was caused by deconjugation of bile acids with dihydroxy bile acid inhibition of fatty acid esterification (39). In rats with experimentally created blind pouches, jejunal transport of glucose and tyrosine is impaired unless concomitant bile duct ligation was carried out (40). Factors, other than the direct effects of bile acids, such as bacterial-elaborated enzymes (proteases) or toxins also mediate injury (41-43).

A mild secondary proliferation of metabolically active colonic bacteria in the upper segments of the small intestine (jejunal colonization) is a frequent finding in infantile diarrheal disease and childhood malnutrition in tropical ecosystems (44-48). The consequences of mild degrees of bacterial contamination and possible biotransformation of bile acids are not known; however, they most likely will be of clinical significance. Ament et al. described patchy mucosal lesions in the jejunum of patients with intestinal stasis and bacterial overgrowth (49). These investigators suspected that the changes were caused by unconjugated dihydroxy bile acids formed by intraluminal bacterial deconjugation (and 7α-dehydroxylation). There is much experimental evidence to support their suspicion. In laboratory animals, mucosal damage caused by unconjugated dihydroxy bile acids occurs in a concentration-dependent manner; the relative effects are paralleled by their ability to lyse erythrocytes (as a model of membrane damage) (Fig. 6) (50). Perfusion with low doses (1.0 mM) of deoxycholic acid and chenodeoxycholic acid caused villus tip alterations and shedding of cellular debris (51). Other studies have produced similar effects but required concentrations up to 4.0 mM. There were no obvious lesions produced in human volunteers after infusion of 1 to 2 mM deoxycholic acid but there was definite evidence of fat malabsorption (52). However, it is not known whether these studies, performed in perfused segments with pure bile acid solutions, are applicable to the in vivo clinical situation in which a mixture of various bile acids and lipids is in contact with the intestinal mucosa. Keeling et al. have found that the toxic effects of deoxycholic acid were abolished when a mixed micelle solution (i.e., taurocholate and oleic acid) was coin fused (53).

Schneider and Viteri studied the luminal events responsible for decreased fat
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FIG. 6. The effects of deoxycholate or taurocholate on erythrocyte stabilization and lysis curves (percentage of hemolysis after 5 min). Deoxycholate, between 0.125 and 0.5 mM, stabilized the erythrocytes, but at concentrations above 0.5 mM, hemolysis increased until, at 1.0 mM, near complete lysis was seen. Taurocholate, up to at least 16.0 mM, stabilized the membrane. (From Gullickson et al., ref. 50, with permission.)

absorption and decreased capacity of duodenal contents to achieve micellar solubilization of lipids in 18 children with severe protein–calorie malnutrition (54,55). The authors found that previously postulated factors such as low pancreatic lipase activity and dilution played a minor role. However, they did find an increased concentration of free bile acids in all patients. Micellar lipid decreased as the ratio of free bile acids to conjugated bile acids increased. They postulated that a decrease in conjugated bile acids and an increase in free bile acids (presumably as a result of bacterial overgrowth) play an important role in decreasing micellar lipid formation and in causing decreased lipid absorption in infants with protein–calorie malnutrition. In addition, the possible ameliorative effect of the mixed micelle is absent under these conditions.

Another contributing factor is the ability of deconjugated bile salts to inhibit intestinal transport processes (40,51,56,57). Gracey et al. have shown that deconjugated bile acids inhibit monosaccharide transport both in vitro and in vivo (58–61). Several investigators have found deconjugated bile salts in the small intestine of infants with protracted diarrhea and secondary monosaccharide intolerance (62,63). Challacombe studied infants with protracted diarrhea but was unable to consistently find deconjugated bile acids in duodenal samples (64). These, however, may represent false negatives since multiple samples taken from various levels of proximal small intestine may be needed to document the presence of unconjugated bile salts.

Teichberg et al. examined jejunal macromolecular absorption in protein–calorie-malnourished rats following jejunal exposure to 0.5 mM dihydroxy bile acids (65). They found that the combined stresses (infusion of deconjugated
bile acid and malnutrition) led to alterations more severe than found with either perturbation alone (Table 1). Infusion of deoxycholic acid induced an increased penetration of a macromolecular tracer (horseradish peroxidase) across the jejunal epithelium and demonstrable ultrastructural damage (Fig. 7) (66). The authors therefore postulate an additional mechanism whereby the malnourished child with jejunal colonization is at an increased risk for the acquisition of foreign antigens or toxins. This effect may contribute to the observations of Rothman et al., who have shown an increased intestinal uptake of antigen in malnourished rats (67).

Therefore, it is possible that bacterial overgrowth, even of a mild degree, as may be seen in protein–calorie malnutrition, leads to the proliferation of obligate anaerobes, which will catalyze deconjugation and 7α-dehydroxylation of bile acids; the resultant derivatives are toxic to the intestinal mucosa and inhibit transport function.

DIAGNOSIS OF BILE ACID MALABSORPTION

Various methods have been utilized for the quantitative and qualitative analysis of bile acids in various body fluids and for detection of interruption of the enterohepatic circulation (14,68). However, these tests are difficult to perform and are not readily available; therefore, they are of little applicability to clinical practice. Reliance on relatively simple measures or reliable screening tests may be of help in detecting patients with bile-acid-mediated diarrhea and in assessing the role of bile acids in protraction of infantile diarrhea.

Bile acids may initiate or perpetuate diarrhea in children in which the clinical criteria described above are met and in whom fecal pH values are consistently in the alkaline range. After careful evaluation to rule out known causes of protracted diarrhea, duodenal intubation (to determine the presence of unconjugated dihydroxy bile acids or bacterial overgrowth) and biopsy may provide useful data. An initial simple therapeutic trial would then be to attempt to bring about a decrease in fecal frequency and fecal weight through the administration

| Table 1. Percentage of villi with cytochemically demonstrable horseradish peroxidase penetration* |
|---------------------------------|-----------------|-----------------|-----------------|
| Protein–energy malnourished     | Well nourished  | *P (PEM vs. WN) |
| Bile salt-free                  | 5.4             | 7.8             | NS              |
| 0.5 mM Taurocholate             | 4.2             | 2.9             | NS              |
| 0.5 mM Cholate                  | 12.0            | 6.0             | NS              |
| 0.5 mM Deoxycholate             | 67.0*           | 53.3*           | <0.01           |

* Epon thick sections were studied unstained by phase microscopy, and villi were scored for HRP penetration into the lamina propria.

* P < 0.01 vs. bile salt-free, taurocholate, and cholate.

Modified from Teichberg et al. (65), with permission.
FIG. 7. Cytochemical demonstration of horseradish peroxidase (HRP) penetration. Phase-contrast light micrographs (×600–700) of villi from jejunal preparations perfused with an isotonic NaCl-glucose solution containing 0.5 g/100 ml of HRP, with or without bile salts. A: Control (bile acid-free); dense reaction product for HRP is confined to the brush border. B: 0.5 mM deoxycholic acid; HRP is seen on brush border between epithelial cells and in the lamina propria (arrows). C: 5.0 mM deoxycholic acid; HRP is seen on the brush border, between epithelial cells, diffusely staining many of the cells and in the lamina propria (arrow). (From Fagundes-Neto et al., ref. 66, with permission.)

of cholestyramine. Cholestyramine is a resin composed of particles of polystyrene-containing positively charged groups that nonspecifically bind anions (69,70). It is, therefore, capable of exchanging chloride anions for bile acids, since the latter undergo tighter electrostatic and hydrophobic bonding. This high affinity for dihydroxy bile acids will, of course, remove these compounds from suspension
and prevent them from mediating secretion. It must be understood, however, that the resin may bind numerous compounds; therefore, the effect is not specific.

Another screening test that has been used is the bile acid meal study. This simple procedure involves measurement (by radioimmunoassay or enzymatic assay) of the concentration of bile acids achieved in peripheral serum following a meal; this represents a spillover of portal venous bile acids that had been absorbed by ileal active transport (32,33,71). The magnitude of daily fecal bile acid excretion can be measured via several methods in the 24- to 72-hr collection (31,72). Tracer studies using isotope-labeled bile acids may give information regarding the fate of enterohpatically circulating bile acids. Tests such as this are used extensively in adult patients and may achieve wider clinical utility when stable-isotope-labeled tracers are readily available (73). Similarly, with the availability of stable isotopes, the cholyglycine breath test may achieve wider utility, especially in the diagnosis of bacterial overgrowth. This test depends on the fact that bacterial cholyglycine hydrolase is required to deconjugate bile acids; therefore, exposure of cholyglycine to intestinal flora will lead to a release of the glycine fraction. The absorbed glycine is partially metabolized to CO$_2$; the appearance rate of CO$_2$ in breath will be proportional to the rate of deconjugation. This test has been widely used in the diagnosis of bile acid malabsorption in adults (74). The recent experimental validation and commercial availability of $\gamma$-ray-emitting radioisotopes of bile acid derivatives (such as $^{75}$Se-selenium taurohomocholic acid, SeHCAT) provides investigators and clinicians with a rapid, reliable method for determination of bile acid loss in feces (75,76). The SeHCAT is absorbed almost exclusively in the ileum and is excreted at the same rate as taurocholate; the isotope can be readily measured using external whole-body counting techniques.

MANAGEMENT

Evidence obtained during evaluation of patients with diarrhea may confirm bile-acid-induced diarrhea. Therefore, there may be a period of time during nutritional rehabilitation of the infant with protracted diarrhea in which administration of bile-acid-sequestering agents may be of benefit. This, however, is a secondary goal of therapy, and the diagnosis of specific causes of protracted infantile diarrhea and nutritional rehabilitation are of key importance.

In addition to cholestyramine, which, of course, has achieved the widest usage, other bile-acid-binding resins have been used. This is partially because cholestyramine is unpalatable and is associated with a high rate of complications (constipation, exacerbation of steatorrhea, etc.) (77,78). Sali et al. have studied the bile-acid-binding properties of antacids and have shown that administration of 15 ml of an aluminum hydroxide-containing preparation was associated with a decrease in bile-acid-mediated diarrhea in adults (79). Commonly available antacids (containing trivalent cations) such as Aludrox®, Amphogel®, Gelusil®,
and Maalox® are theoretical alternatives to cholestyramine administration (80,81).

Agents capable of decreasing intestinal motility such as codeine and loperamide have been used on a trial basis in bile-acid-mediated diarrhea (82–84). These drugs may have a theoretical advantage in that they may increase the ability of the colon to absorb water by suppressing motility, or they may act by stimulating net uptake of water and electrolytes; they have been shown to decrease net colonic water loss in chenodeoxycholic acid-induced diarrhea in experimental animals (82,84). Propranolol, an inhibitor of adenylate cyclase, was postulated to be effective in abolishing deoxycholic acid-induced secretion in colonic mucosa (85); however, subsequent studies have failed to prove the efficacy of this drug. Measures to inhibit colonic mucous secretion (and possible depletion) or to increase mucosal resistance (i.e., enhanced cytoprotection) may also be useful in bile-acid-mediated diarrhea. There are, however, no clinical trials of any of these drugs, and the exact role in altering the course of bile acid diarrhea remains to be defined.

SUMMATION

Protracted diarrhea and associated nutrient malabsorption occur in infants in at least two settings: (a) prolonged recovery from an acute episode of enteritis, possibly complicated by inadequate nutrient intake, and (b) less extensive disease occurring in a malnourished infant. Bile acids may, hypothetically, play a key role in perpetuating the cycle of diarrhea—malabsorption—malnutrition and persistent mucosal injury. The following interrelationships (Fig. 8) are proposed. Mucosal injury induced by infectious enteritis or by preceding or ongoing malnutrition is associated with several pathophysiologic alterations in addition to diffuse impairment of nutrient absorption. Factors that promote bacterial overgrowth (altered motility, decreased gastric acid secretion, altered immunity, etc.) may occur and allow the appearance, in the proximal small intestine, of deconjugated dihydroxy bile acids. These compounds are able to alter transport of nutrients such as monosaccharides as well as cause further damage to the mucosa. This may lead to marked alterations in mucosal permeability and allow increased uptake of macromolecules (foreign antigens or toxins). The exacerbation of mucosal injury further impairs the infant’s ability to absorb oral nutrients.

If the mucosal injury is diffuse, ileal dysfunction may occur; impaired bile acid active transport by the ileum will lead to the spillage of excess quantities of bile acids into the colon, where they will induce a net secretion and further diarrhea. Mucosal injury is associated with impaired cholecystokinin (CCK) release; therefore, the gallbladder will empty sluggishly and incompletely, so that the bile acid pool size is effectively reduced. This, in combination with deconjugation/dehydroxylation of bile acids and bile acid malabsorption, leads
to decreased intraluminal bile acid levels (to below the critical micellar concentration) and, therefore, steatorrhea. Potential hepatobiliary complications of these induced alterations are increased absorption through the damaged mucosa of hepatotoxic substances (such as lithocholic acid or E. coli endotoxin) and gallstones resulting from the combined effect of gallbladder stasis, decreased bile acid pool size, and sepsis. The resultant cholestasis may further impair bile acid metabolism.

In the face of such complex disturbances in pathophysiology, the management of these infants is difficult. Attention must be given to prompt nutritional rehabilitation, correction of the bacterial overgrowth, and dampening of the effect of bile acid on colonic mucosa. Aggressive, appropriate nutritional management of infants with acute enteritis may prevent initiation of the downhill spiral.

REFERENCES


