REVIEW

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Bile Acid Diarrhea: Prevalence, Pathogenesis, and Therapy

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Bile acid diarrhea (BAD) is usually seen in patients with ileal Crohn's disease or ileal resection. However, 25% to 50% of patients with functional diarrhea or diarrhea-predominant irritable bowel syndrome (IBS-D) also have evidence of BAD. It is estimated that 1% of the population may have BAD. The causes of BAD include a deficiency in fibroblast growth factor 19 (FGF-19), a hormone produced in enterocytes that regulates hepatic bile acid (BA) synthesis. Other potential causes include genetic variations that affect the proteins involved in BA enterohepatic circulation and synthesis or in the TGR5 receptor that mediates the actions of BA in colonic secretion and motility. BAs enhance mucosal permeability, induce water and electrolyte secretion, and accelerate colonic transit partly by stimulating propulsive high-amplitude colonic contractions. There is an increased proportion of primary BAs in the stool of patients with IBS-D, and some changes in the fecal microbiome have been described. There are several methods of diagnosing BAD, such as ^{75}selenium homotaurocholic acid test retention, serum C4, FGF-19, and fecal BA measurement; presently, therapeutic trials with BA sequestrants are most commonly used for diagnosis. Management involves the use of BA sequestrants including cholestyramine, colestipol, and colesevelam. FXR agonists such as obeticholic acid constitute a promising new approach to treating BAD. **(Gut Liver 2015;9:332-339)**

Key Words: Malabsorption; FGF-19; FXR; C4; CYP7A1; Klotho β

INTRODUCTION: BILE ACIDS, FAT ABSORPTION, AND THE ENTEROHEPATIC CIRCULATION

Bile acids (BAs) are detergent molecules[1] that are synthesized in the liver and are responsible for solubilization of fatty acids and monoglycerides (the lipolysis products of triglycerides), facilitating digestion and lipid absorption in the small intestine. The different BA molecules are differentiated by hydroxylation and conjugation. Chenodeoxycholic acid (CDCA) and cholic acid (CA) are primary BAs synthesized from cholesterol and conjugated with taurine or glycine in the liver; in the colon, bacteria deconjugate and dehydroxylate the BAs to form, respectively, lithocholic acid and deoxycholic acid (DCA).[2]

Taurine or glycine conjugation of the BAs permits the ionization of BAs which increases their solubility and their impermeability to cell membranes, allowing BAs to reach the critical micellar concentration for spontaneous formation of micelles. In the micelles, the polar BAs surround the insoluble, hydrophobic fatty acids and monoglycerides and present the hydrophobic fat molecules to the enterocyte brush border membrane of the small intestine for digestion and absorption.

The apical Na^+-dependent bile salt transporter (ASBT) (also called ileal BA transporter or SLC10A2 [solute carrier family 10, member two]) is responsible for the active reuptake of BAs in the terminal ileum. This reabsorbs approximately 95% of BAs in the terminal ileum and results in a functional enterohepatic circulation of BA,[3] transporting the BAs back to the liver. Several molecular mechanisms are involved in the enterohepatic circulation: farnesoid X receptor (FXR) is expressed in ileal enterocytes and hepatocytes; BAs are agonists of the FXR; sensing of the enterocyte BA pool by FXR affects the liver by way of the endocrine factor, fibroblast growth factor 19 (FGF-19); FGF-19 is released from enterocytes into the portal circulation and activates FGF receptor 4 (FGF-R4) in hepatocytes in a process that involves interaction with klothoβ on the hepatocyte membrane, resulting in downregulation of cholesterol 7α-hydroxylase (CYP7A1) and therefore inhibition of the BA synthesis. Cholerheic or BA diarrhea is thought to result predominantly from the interruption of the enterohepatic circulation.[3]

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CLASSIFICATION OF BILE ACID MALABSORPTION/ DIARRHEAS

The causes of BA diarrhea (BAD) are based on the original classification of BA malabsorption (BAM):

Type 1: Ileal dysfunction and impaired reabsorption, e.g., Crohn's disease

Type 2: Primary, or idiopathic, BAD produces a similar picture of increased fecal BAs, watery diarrhea, and response to BA sequestrants in the absence of ileal or other obvious gastrointestinal disease

Type 3: Other gastrointestinal disorders which affect absorption, such as small intestinal bacterial overgrowth, celiac disease, or chronic pancreatitis

A fourth category of BAD may result from excessive hepatic BA synthesis; for example, the oral hypoglycemic drug, metformin, is associated with increased hepatic BA synthesis.[4-6]

WHAT'S NEW IN UNDERSTANDING THE ETIOPATHOGENESIS OF IDIOPATHIC BILE ACID DIARRHEA?

Recent literature has identified several novel potential mechanisms in the development of idiopathic BAD (Fig. 1).[7]

1. Defective feedback inhibition of bile acid biosynthesis by FGF-19

FGF-19 produced in the ileum in response to BA absorption regulates hepatic BA synthesis.[8] In a landmark article, Walters *et al*.[9] reported lower serum FGF-19 in patients with BAM and an inverse relationship between FGF-19 and serum C4 (a surrogate of the rate of hepatic BA synthesis). These results were replicated by others.[10,11]

2. Genetic mutations in the apical sodium-bile acid transporter

Genetic mutations in the apical sodium-bile acid transporter (ASBT) are extremely rare.[12,13] In addition, defective BA uptake into ileal mucosal biopsies was excluded by Bajor *et al*.[14]

3. Accelerated small bowel transit bypassing active bile acid transport in the ileum

Accelerated small bowel transit bypassing active BA transport in the ileum has been hypothesized as a cause of BAM in idiopathic[15] and postradiation cases.[16,17] While this is theoretically possible, it seems unlikely given the ASBT's affinity for BA, and it is unclear whether the accelerated small bowel transit is a cause or an effect of the BAM.

4. Genetic variations in the proteins involved in feedback regulation of bile acid synthesis, specifically *KlothoB* gene and *fibroblast growth factor 4* gene

The role of these genetic variants is based on significant associations of SNP rs17618244 in the *KlothoB* (KLB) gene with colonic transit in diarrhea-predominant irritable bowel syn-

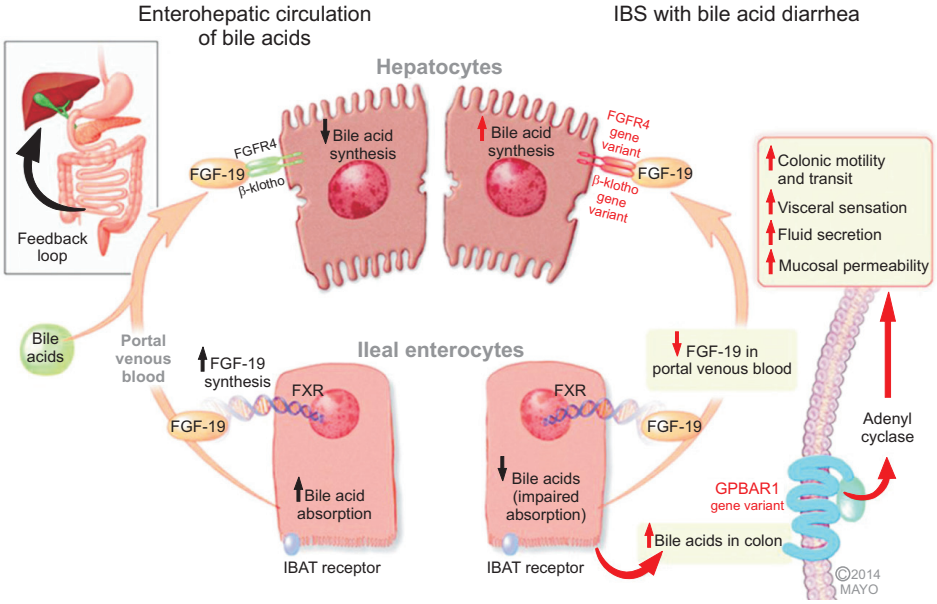

Fig. 1. Mechanisms of bile acid (BA)-related bowel dysfunction in irritable bowel syndrome with diarrhea (IBS-D) or idiopathic BA diarrhea (Adapted from Camilleri M. J Physiol 2014;592(Pt 14):2967-2980).[7] Enterohepatic circulation of bile acids: Ileal enterocytes absorb bile acid through a receptor-mediated process (ileal bile acid transporter [IBAT]). Intracellular bile acids activate the farnesoid-γ receptor to increase fibroblast growth factor 19 (FGF-19) synthesis. FGF-19 in the portal circulation downregulates hepatocyte bile acid synthesis. Disorders of FGF-19 synthesis by ileal enterocytes or genetic variations of FGFR4 or β-klotho lead to excess bile acid concentration in the colon, resulting in activation of the G protein-coupled bile acid receptor 1 (GPBAR1, or TGR5) with enteroendocrine cell stimulation (e.g., release of 5-hydroxytryptamine) and stimulation of colonic motility with acceleration of colonic transit, activation of visceral sensation and fluid secretion (through increased intracellular cAMP, increased mucosal permeability or chloride ion secretion). Genetic variation in GPBAR1 (*TGR5*) is associated with increased colonic transit in IBS-D.

drome (IBS-D).[18] Pharmacogenetic studies of the influence of KLB (rs17618244) on the dose-response effects of administered chenodeoxycholate on the emptying rate of the ascending colon[11] suggest that KLB variation may influence colonic response to BAM, and exome DNA sequencing studies showed KLB (rs1015450, downstream) association with fecal BAs and FGFR4 (rs1966265, nonsynonymous) with colonic transit.[19]

5. Upregulation of the membrane bound bile acid receptor, TGR5 or GPBAR1

TGR5, or GPBAR1, is a member of the G protein-coupled receptor superfamily that functions as a cell surface receptor for BA,[20] including colonic epithelial cells,[21] regulating basal and cholinergic-induced secretion in rat colon[22] and colonic transit.[23] We have recently shown that genetic variation in *GPBAR1* predisposes to quantitative changes in colonic transit and BA excretion.[24]

CELLULAR MECHANISMS OF BILE ACID DIARRHEA

BA chemistry determines effects on colonic mucosa; in general, the surface active properties that lead to increased colonic mucosal permeability and electrolyte and water secretion are associated with two hydroxyl groups at the 3,7 (CDCA) or 3,12 (DCA) positions in the α-configuration. BAs regulate many cell types in the gut wall and beyond by activating nuclear and plasma membrane receptors. Of these, the G protein-coupled receptor, TGR5, has emerged as a key mediator of the nongenomic actions of BAs. TGR5 is a cell-surface receptor that couples to Gαs, formation of cAMP, activation of protein kinase A and extracellular signal-regulated kinases, and inhibition of inflammatory signaling pathways.[25]

The mechanisms of diarrhea include increased mucosal permeability;[26] water secretion through activation of CFTR via adenylate cyclase[27,28] and inhibition of apical Cl/OH exchange;[29] lubrication by increased mucus secretion (a direct effect on goblet cells);[30,31] and acceleration of colonic motility, likely via TGR5 stimulation of myenteric ganglionic neurons.[23] BAs induce colonic high amplitude propagated contractions.[32]

PREVALENCE OF BILE ACID DIARRHEA

Type 1 BAD is caused by ileal disease or resection, typically due to Crohn's disease or radiation ileitis. The classical papers of Hofmann and Poley[33-35] described the association of ileal disease of <100 cm length with diarrhea; when the extent of involvement was over 100 cm, there was associated steatorrhea as a result of BA deficiency.

Type 2 BAD is currently considered diarrhea without morphological abnormalities. Several studies have documented BAM in one-third to one-half of patients with chronic diarrhea or IBS-D, as summarized in a systematic review.[36] Overall, the systematic review found that BAM was reported in 32% of patients with symptoms consistent with IBS-D, and there was a dose-response relationship to treatment with BA binders, based on severity of BAM assessed by ^{75}selenium homotaurocholic acid test (^{75}SeHCAT) retention at 7 days. Similar results were found in recent studies of patients presenting to an outpatient gastroenterology clinic in the United Kingdom[37] and in a prospective research study at Mayo Clinic of local patients with IBS-D.[10,38] In fact, the IBS-D patients had evidence of increased fecal BA excretion and increased hepatic BA synthesis.

It has been estimated that 1% of the population of Western countries suffers from BAD.[39]

INTERACTIONS OF THE MICROBIOME OF THE COLON AND BILE ACIDS

The colonic microbiome is responsible for the dehydroxylation of cholic and chenodeoxycholic acids to the secondary BAs, deoxycholic and lithocholic acids. Gut microbiota also regulate expression of fibroblast growth factor 15 in the mouse ileum and cholesterol CYP7A1 in the liver by FXR-dependent mechanisms.[40] The microbiome influences the generation of BAs and other organic acids in the murine colon.[41] In humans, BA pool size and composition appear to be major regulators of microbiome structure, which, in turn, appears to be an important regulator of BA pool size and composition.[42] Ongoing research seeks to unravel the contributions of the microbiome and BA composition to diverse conditions including colorectal cancer,[43] inflammatory bowel disease,[44] and irritable bowel syndrome.[45]

CHANGES IN THE PROFILE OF FECAL BILE ACIDS IN IRRITABLE BOWEL SYNDROME

Several studies have now reported the profile of fecal BAs in patients with IBS-D. Duboc *et al*.[45] reported that the percentage of fecal primary BA was significantly higher in IBS-D patients than in healthy controls, and it was significantly correlated with stool consistency and frequency. They also reported a significant increase of *Escherichia coli* and a significant decrease of *leptum* and *bifidobacterium* in IBS-D patients. Shin *et al*.[38] confirmed that fecal levels of primary BAs (cholic and chenodeoxycholic [CDCA] acids) were higher in 31 subjects with IBS-D, compared with 30 healthy controls, and also reported that the proportions of fecal secretory BAs (chenodeoxycholic and deoxycholic [DCA] acids) were lower in 30 IBS-C patients compared with controls. An extension study of the latter cohort involving 64 patients with IBS-D confirmed the differences in the proportions of primary and secondary BAs in feces of patients with IBS-D. In addition, the phenotypes of patients with IBS-D and increased total fecal excretion of >2,337 μmol per 48 hours differed from that of IBS-D patients with normal fecal BA excretion, including higher body mass index, increased fecal fat excretion, higher

proportion of primary BAs (CA and CDCA) in stool, and a trend to faster colonic transit.[46]

DIAGNOSIS OF BILE ACID MALABSORPTION

Table 1 summarizes the diagnostic tests for BAM and their pros and cons.[47]

1. Direct measurements of bile acids

^{14}C-glycocholate breath and stool test, ^{75}selenium homotaurocholic acid test (SeHCAT), 7α-hydroxy-4-cholesten-3-one (C4), and fecal BAs are direct measurements of BAs or surrogates for the rate of hepatic synthesis of BAs, which is proportional to BAM.

The ^{14}C-glycocholate (^{14}C-BA) breath and stool test is based on the principles[48] that bacterial overgrowth in the small intestine enzymatically degrades the ^{14}C-BA, releasing ^{14}C-glycine which is absorbed into the portal circulation, is rapidly metabolized in the liver, and is exhaled into the breath as an early peak (typically <60 minutes from ingestion) of $^{14}CO_2$. If ^{14}C-BA is not reabsorbed in the terminal ileum and enters the large intestine, the ^{14}C-BA is deconjugated by colonic bacteria and, if not absorbed by passive diffusion in the colon, is excreted in stool. This test is no longer widely utilized since development of less complex tests that have no radiation exposure.

The ^{75}SeHCAT utilizes a synthetic ^{75}selenium homotaurocholic BA that is resistant to bacterial degradation[49] and passive diffusion.[50] Like a natural BA, it is either actively absorbed in the terminal ileum or excreted into stool, and unaltered by its passage through the colon. The test involves the patient ingesting a capsule containing ^{75}SeHCAT; retention of the isotope in the body at 7 days is measured noninvasively by whole body gamma counter and expressed as a percentage of administered dose. BA may undergo five enterohepatic circulations per day with ~5% loss in the stool with each circulation; retention rates of 5%, 10%, and 15% are used to estimate the relative severity of BAM.

Serum 7 α-hydroxy-4-cholesten-3-one (C4) measures BA synthesis, 90% of which is regulated by the rate-limiting enzyme, cholesterol CYP7A1. C4 is a downstream product of CY-

Table 1. Diagnostic Tests for Bile Acid Malabsorption and Their Pros and Cons

BAM diagnosis	Advantages	Disadvantages
Therapeutic trial with BA sequestrant	Clinically applicable, widely used	Not definitive diagnosis of BAM; nonspecific amelioration of diarrhea due to other causes; poor compliance with some BA sequestrants
^{14}C glycocholate	May identify small bowel bacterial overgrowth	Radiation exposure, β emission, long $t_{1/2}$
		Varying normal values
		Positive breath excretion at 2–4 hr does not differentiate BAM from small bowel bacterial overgrowth
		Laborious test method (stool collection)
^{75}SeHCAT	γ Emission, short $t_{1/2}$, with decreased radiation to extra-abdominal organs	Not available in United States
	Well-defined normal values; level of isotope retention predicts response to BA sequestrant	Radiation exposure
	Simple test method: 2 patient visits	
Serum C4	No radiation	Fasting sample, diurnal variation
	Normal values reported in adults	Requires further validation
	Not dependent on age, gender or cholesterol	False-positive in liver disease, treatment with statins and altered circadian rhythm
	Simple blood test: 1 patient visit	
Serum FGF-19	No radiation; commercial ELISA assay	Moderate sensitivity and specificity; requires further validation
Fecal BA	No radiation	Variable daily fecal BA excretion, requires at least 48 hr sample collection
	Measures total and individual BAs	Cumbersome method (stool collection)
Urine 2-propanol and acetamide	No radiation; urine sample	Special technology required: Field Asymmetric Ion Mobility Spectrometer; requires replication and validation

Updated from Vijayvargiya P, et al. Clin Gastroenterol Hepatol 2013;11:1232-1239.[47]
BAM, bile acid malabsorption; BA, bile acid; ^{75}SeHCAT, ^{75}selenium homotaurocholic acid test; FGF-19, fibroblast growth factor 19; ELISA, enzyme-linked immunosorbent assay.

P7A1. Serum C4 is a simple blood test, but it requires standardized specimen collection time because of diurnal variability.[51] Accurate method for measurement uses liquid chromatography-tandem mass spectrometry.[52] The clinical performance of the C4 assay demonstrated a sensitivity of 90%, specificity of 79%, negative predictive value of 98%, and positive predictive value of 74% when compared to the ^{75}SeHCAT test. The high negative predictive value makes the assay attractive as a screening test to rule out BAM.[53] C4 was unrelated to age, gender or serum cholesterol when analyzed against potential covariates.[53] When compared to elevated 48-hour fecal BA excretion, elevated serum C4 did not identify phenotype differences (such as increased fecal fat and colonic transit) among patients with IBS-D, other than documenting the increased fecal BAs among those with elevated serum C4, defined as >47.1 ng/mL.[46] In summary, serum C4 test is applicable to a majority of patients, but requires further clinical validation including responsiveness to BA sequestrants therapy or FXR agonists in patients with BAM.

Fecal measurements to quantify total and individual fecal BAs are technically cumbersome and not widely available.[10,38,46] These Mayo Clinic studies showed that IBS-D is associated with higher serum C4, higher total fecal BA, and increased secretory BAs (e.g., CDCA, DCA). In addition, high fecal BA excretion was associated with a more significant IBS-D phenotype, characterized by higher fecal fat and a trend toward accelerated colonic transit.[46] Indeed, fecal BA excretion and colonic transit were validated as biomarkers that identified mechanisms that could be targets of treatment in patients with IBS-D.[54] Excretion of >2,337 μmol per 48 hours (upper limit of normal) is used as an index of BAM.[46,54]

An enzymatic 3α-steroid dehydrogenase assay indirectly measures fecal BA. 3α-Steroid dehydrogenase is used to oxidize deconjugated BAs and produces NADH, which is then measured biochemically. This method requires proper stereotactic alignment of enzyme and substrate and with a variety of conjugations (sulfonation, glucuronidation) of BAs while they are in the small intestine. This method would lack precision if it was used to measure concentrations of BAs in small bowel fluid or ileostomy effluent. In addition, because it does not assess BAs with hydroxyl groups in the β-configuration, it tends to underestimate total BAs.

2. Indirect measurements of bile acids

Serum FGF-19 is a useful screening test for BAD,[39] given the inverse relationship between C4 and FGF-19 originally described by Walters *et al*.[9] It has been validated in studies using ^{75}SeHCAT retention as the gold standard[55] and by comparisons with serum C4.[56] In the study of Pattni *et al*.[56] of 258 patients, sensitivity and specificity of FGF 19 at 145 pg/mL for detecting a C4 level >28 ng/mL were 58% and 79%, respectively, and for C4 >60 ng/mL (denoting high BA synthesis), the sensitivity and specificity of FGF-19 were 74% and 72%, respectively.[55] The attraction of this diagnostic method is the ease of the enzyme-linked immunosorbent assay and the measurements based on a morning, fasting serum sample. Further validation studies, including responsiveness to therapy of BAM, are eagerly awaited.

Urine 2-propanol and acetamide[57] are volatile organic compounds produced by the gut bacterial cleavage of BAs. This method uses an electronic nose (that mimics the biological olfactory system)[58] and a Field Asymmetric Ion Mobility Spectrometer that separates ionized molecules based on their different mobilities in a high electric field.[59] These volatile organic compounds were detected in urine of 23 patients with BAD (confirmed by ^{75}SeHCAT), in contrast to 42 patients with ulcerative colitis and 45 healthy controls. Further studies are awaited.

MANAGEMENT OF BILE ACID DIARRHEA

1. Intraluminal bile acid binders

Cholestyramine and colestipol are generally considered first-line treatment for BAD; however, poor palatability results in low patient compliance.[60] Several open label studies have recently demonstrated efficacy of these BA sequestrants in patients with IBS-D, especially those with evidence of BAM.[61,62] For example, colestipol treatment improved IBS symptoms (IBS severity scoring system 220±109 vs 277±106; $p<0.01$), and 15 of 27 patients also fulfilled criteria for treatment response (adequate relief ≥50% of weeks 5 to 8), suggesting benefit both in bowel symptoms and global symptoms.

Alternatives are being tested, even though there are no large clinical trials specifically for the indication of BAD. Thus, patients may prefer colesevelam at a dose of up to 1.875 g, twice a day. In a pharmacodynamics study of 24 unselected patients with IBS-D,[63] emptying of the ascending colon took an average of 4 hours longer in patients given colesevelam (1.875 g, twice a day) compared with placebo, treatment effect was significantly associated with baseline serum C4 levels, and colesevelam caused greater ease of stool passage and somewhat firmer stool consistency. In an unpublished open-label study (Camilleri 2014, unpublished) of the same dose of colesevelam in 12 IBS-D patients with elevated fecal BA excretion, we have also shown that colesevelam sequestered BAs and resulted in significantly firmer stool consistency.

2. Experimental agents inhibiting bile acid diarrhea by cellular mechanisms

FGF-19 production is stimulated by the FXR agonist, obeticholic acid,[64] which may potentially reverse the FGF-19 deficiency postulated in BAM that leads to excessive hepatocyte BA synthesis. This treatment has been associated with improved stool frequency and consistency in a preliminary study of patients with BAD.[65] Another FXR agonist, GW4064, attenuated Cl^- secretory responses to both Ca^{2+} and cAMP-dependent agonists, and may be efficacious in the treatment of BAD through

antisecretory actions on the colonic epithelium.[66]

CONCLUSION AND FUTURE DIRECTION

The pioneering work conducted 40 years ago by giants in this field (Drs. Alan Hofmann, Donald Small, Hans Fromm, and Vinton Chadwick) is finally going to have an impact beyond the patients with ileal resection or ileal Crohn's disease. BA diarrhea is finally appreciated as a significant cause of functional, otherwise unexplained, chronic diarrhea in about one-third of such patients. The availability of simple diagnostic stool tests (fecal BA excretion performed at the time of fecal fat measurement) and, even more applicable, serum or urine tests will enhance the ability of physicians to diagnose this eminently treatable disorder.

In the future, BA sequestration with tablet formulations that are associated with higher compliance or Farnesoid X receptor agonists will impact the care of patients and likely reduce overall healthcare costs by reducing the need for expensive tests like colonoscopy and biopsies or treatments like biologic agents in patients with Crohn's disease.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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