Therapeutic targeting of bile acids

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Bile acids (BAs) are steroid-derived detergent molecules that are excreted by the liver and are responsible for fat emulsification in the small intestine, aiding fat digestion and absorption. There are several BA species that are differentiated structurally by their hydroxylation and conjugation status. BAs have been used as medicinals for several centuries, and the known properties of BAs as a shunt pathway for lipids led to the use of BA sequestrants in the treatment of hyperlipidemia. Relative to the deleterious hydrophobic BAs, the use of ursodeoxycholic acid (UDCA) led to their extensive use as the drug of choice in cholestatic liver diseases, though recent advances suggest that it may be replaced in the future by farnesoid X receptor (FXR) agonists or fibroblast growth factor 19 (FGF-19) analogs, both of which have the capacity to reduce the hepatocyte synthesis of BAs. The intraluminal effects of relatively high concentrations of di-α-hydroxy BA [though not at lower concentrations, which may be antisecretory (27)] result in diarrhea, and, conversely, neutralization of the effects of such BAs results in relief of BA diarrhea. Recent research has ushered in other therapeutic opportunities such as interaction with the microbiome and effects on energy expenditure, fat metabolism, and glycemic control. This review addresses the biochemical, pathophysiological, and mechanistic concepts associated with the use of BAs in therapeutics.

Synthesis of Bile Acids from Cholesterol

Chenodeoxycholic acid (CDCA) and cholic acid (CA) are the primary BAs that are synthesized in the liver from cholesterol (Fig. 1). In microsomes, cholesterol is converted by 7α-hydroxylase (CYP7A1) to 7α-hydroxycholesterol. Through a series of steps involving NADPH, coenzyme A (CoA), chenodeoxycholyl-CoA and cholyl-CoA are formed; formation of cholyl-CoA requires an additional hydroxyl group, catalyzed by the enzyme 12α-hydroxylase. Finally, these molecules are conjugated with the amino acids glycine or taurine in peroxisomes. The ratio of the glycine to the taurine conjugates is normally 3:1. In humans, an alternative BA synthetic pathway also exists, producing less than 10% of the total BAs. In this pathway, the mitochondrial sterol 27-hydroxylase (CYP27A1) generates 27-hydroxycholesterol and 3β-hydroxy-5-cholestenonic acid from cholesterol and the intermediates feed into a pathway leading to the formation of CDCA (30).

Chemical Structures of Bile Salts

The chemistry of BAs is characterized by one (lithocholic acid, LCA), two (CDCA; and deoxycholic acid, DCA), or three (cholic acid, CA) hydroxyl groups in α configuration. Another BA (which constitutes <5% of the BA pool) is UDCA, in which there is a hydroxy group in an α configuration at the third carbon atom and a hydroxy group in the β configuration at the seventh carbon atom (Fig. 2). The hydroxy groups are located on the hydrophilic side of the BA molecule (Fig. 1). Deconjugation (by bile salt hydrolase, which is commonly produced by the normal microbiota) and dehydroxylation of primary BAs in the colon result in the production of LCA and DCA (Fig. 2).

The chemistry of BAs determines their biological effects. For example, two hydroxy groups [3,7 or 3,12] in α configuration are associated with the increased fluid secretion by CDCA and DCA, respectively, in the colon. In contrast, UDCA (with 3α, 7β configuration) does not induce fluid secretion, and it reduces secretion caused by activators of adenyl cyclase, such as forskolin, (28).

Another example illustrating the structural specificity of actions of BAs is the induction of apoptosis in colonic epithelial cells (5) and hepatocytes (55). Thus, whereas CDCA, LCA, and DCA induce apoptosis, UDCA prevents apoptosis.

Enterohepatic Circulation of Bile Acids

BAs are excreted from the liver as conjugates to the amino acids taurine or glycine, and they are concentrated in the gall bladder before being released into the duodenum, typically in response to ingestion of a meal or concurrent with an interdigestive migrating motor complex in the neighboring stomach and proximal small intestine (48). BAs remain ionized in the duodenum. Ionization increases their solubility, allowing a

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sufficiently high concentration to achieve spontaneous formation of micelles, whereby the polar BAs surround fat molecules and can present the hydrophobic fat molecules (which are insoluble in the aqueous phase) to the brush border membrane of the small intestine for digestion and absorption.

The apical Na\(^+\)/H\(^+\)-dependent bile salt transporter (ASBT) [also called ileal BA transporter (IBAT) or SLC10A2 (solute carrier family 10, member 2)] is responsible for the active reuptake of BAs in the terminal ileum to enter the portal circulation, reabsorbing ~95% of BAs and transporting them to the liver. BAs are agonists of the FXR, the nuclear receptors found in epithelial cells including ileal enterocytes and hepatocytes. The level of enterocyte BA leads to the production of the endocrine factor, FGF-19, which is released from ileal enterocytes into the portal circulation and activates FGF receptor 4 (FGF-R4) on the hepatocyte membrane in a process that involves interaction with klotho. This activation downregulates CYP7A1, inhibiting BA synthesis. This enterohepatic circulation of BAs occurs about five times per day.

The 5% BA pool that is not absorbed in the ileum undergoes deconjugation and dehydroxylation by colonic bacteria BAs to produce secondary BAs, DCA, and LCA (22). Disorders of the enterohepatic circulation result in BA malabsorption.

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### Table: Primary vs. Secondary Bile Acids

<table>
<thead>
<tr>
<th>Primary BA</th>
<th>Secondary BA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholic acid (CA)</td>
<td>Deoxycholic acid (DCA)</td>
</tr>
<tr>
<td>Chenodeoxycholic acid (CDCA)</td>
<td>Ursodeoxycholic acid (UDCA), Lithocholic acid (LCA)</td>
</tr>
</tbody>
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### Diagram: Bile Acid Structures

- **Bile Acid**
  - CDCA: \(\alpha\)-OH
  - CA, DCA: \(\alpha\)-OH
  - LCA, UDCA: H

- **Conjugation Sites**
  - CDCA: 3αOH, 7αOH
  - CA, DCA: 12αOH
  - LCA, UDCA: 12αOH

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Fig. 1. Synthesis of primary bile acids from cholesterol in hepatic microsomes. The main primary bile acids, cholic acid and chenodeoxycholic acid, are synthesized in hepatocyte microsomes via classic or alternative pathways; the bile acids are then conjugated with glycine or taurine in liver peroxisomes to produce the amphipathic structure that contains both hydrophobic (lipid-soluble) and polar (hydrophilic) regions. The cholesterol portion of a bile acid is hydrophobic and the amino acid conjugate is polar and hydrophilic.
Bile Acid Malabsorption

There are three classes of BA malabsorption. Type 1, also called ileal dysfunction and impaired reabsorption, is found in Crohn’s disease. Type 2, also called idiopathic BA diarrhea, produces increased fecal BAs, watery diarrhea, and response to BA sequestrants in the absence of ileal or other obvious gastrointestinal disease. Type 3 is found in other gastrointestinal disorders that affect absorption such as small intestinal bacterial overgrowth, celiac disease, or chronic pancreatitis (19). Recent studies have proposed mechanisms to explain idiopathic BA diarrhea: first, defective feedback inhibition of hepatic synthesis caused by reduced production of FGF-19 by ileal enterocytes (53); second, genetic variations reducing the levels of hepatic proteins involved in feedback regulation of BA synthesis (klothoB and FGFR4 genes) (11, 56); third, a gain-of-function variation in the gene that results in upregulation of the membrane bound BA receptor, TGR5 or G protein-coupled BA receptor 1 (GPBAR1) (12).

Current and Potential Therapeutic Applications of Bile Acid Modulation

Bile acid sequestration for hyperlipidemia. BAs provide a natural excretory pathway for hepatic cholesterol. BA sequestrants (e.g., cholestyramine, colestipol, colesvelelam) bind negatively charged BAs and bile salts in the small intestine and interrupt the enterohepatic circulation of BAs, leading to increased conversion of cholesterol into bile within the liver. These effects result in decreased hepatocyte cholesterol content, promoting an increase in LDL receptors and increasing the clearance of LDL from the circulation (24). BA sequestrants that lower LDL cholesterol result in up to 10% increase in HDL (“good”) cholesterol; unfortunately, they also increase serum triglyceride levels. Thus BA sequestrants are most useful in patients with high LDL cholesterol levels as the major lipid abnormality. BA sequestrants can be combined with statins or niacin to achieve greater reduction in LDL cholesterol, and they tend to be used alone as initial therapy with elevated LDL cholesterol in patients at low risk, or in those intolerant of statins. There are problems with BA sequestrants, including poor compliance with treatment with the resins, interference with the absorption of concomitant medications, aggravation of severe constipation, and contraindication in patients with possible bowel or total biliary obstruction (24).

Ursodeoxycholic acid: from traditional oriental medicine to treatment of cholestatic liver diseases. Bile from 44 different animals (both invertebrates and vertebrates, including human bile) has been used for centuries for a host of maladies in traditional Chinese medicine beginning in the Zhou dynasty (~1046-256 BCE) (54). The principal chemicals in animal bile are steroidal detergent-like molecules, bile salts, bile pigment (bilirubin and its glucuronides), membrane lipids, vitamins A, D, E, and K, and melatonin. Bile was claimed to improve liver function, dissolve gallstones, inhibit bacterial and viral multiplication, and promote cardiac chronotropism (regularity of heart rhythm) and was used as an anti-inflammatory, antipyretic, antioxidant, sedative, anticonvulsant, antiinflammatory, anticoagulent, antidiabetic, and antispasmodic. Pig, wild boar, and human bile diluted with alcohol were shown to form an artificial skin for burns and wounds one thousand years ago in the Tang dynasty (618–907 CE).

In Japan, UDCA was brought to market in the 1950s by Tokyo Tanabe as a liver tonic, based on the legendary therapeutic effects of bear bile. It was packaged as 10 mg tablets. The recommended dose likely had no pharmacodynamic effects (23).

UDCA is the drug of first choice in the treatment of cholestatic liver diseases, the most common being primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) (26). PBC is characterized by interlobular duct destruction, female preponderance (10 F:1 M), autoimmune with antimitochondrial antibodies in 95% of patients, and several known T lymphocyte targets. In contrast, PSC is characterized by scarring and irregularity of the caliber of intra- and extrahepatic ducts, is more common in males (2 M:1 F), and is associated with ulcerative colitis and unknown T lymphocyte targets (26, 51). UDCA counteracts the cholestatic components in PBC and PSC and exhibits anti-inflammatory and immunomodulatory properties.

The beneficial effects of treatment with UDCA include 1) protection against cytotoxicity due to more toxic BAs; 2) stimulation of hepatobiliary secretion with increased hydrophilicity index of the circulating BA pool, antioxidant activity (due in part to an enhancement in glutathione levels); 3) cytoprotection against BAs and cytokine-induced injury; 4) immunosuppressive properties by interfering with B and T cell functions; 5) inhibition of liver cell apoptosis through targeting of mitochondrial function and integrity, reduction of endoplasmic reticulum stress, and interactions with survival signals in the cAMP, Akt, nuclear factor kappa-B (NF-κB), mitogen-activated protein kinases (MAPK), and phosphatidylinositol 3-kinase (PI3K) signaling pathways (39, 41).

In PBC, normalization of biochemical values occurs within 2 years in 20% of patients and in an additional 15% of patients after 5 years. Although long-term, high-dose UDCA therapy is associated with improvement in serum liver tests in PSC, it unfortunately does not improve survival and appears to be associated with higher rates of serious adverse events such as mortality, need for liver transplantation, and development of esophageal or gastric varices (31, 32). In fact, meta-analyses show that UDCA does not significantly impact the mortality or need for transplantation in either PBC or PSC (20, 51).

Bile acid “push or pull” for bowel dysfunctions in irritable bowel syndrome. BAs have been used as therapy for gallstones and cholestatic liver disease since the early 1970s. CDCA was associated with development of diarrhea. In gallstone patients with chronic constipation, CDCA increased stool frequency and loosened stool consistency compared with placebo (6). Some BAs are essentially physiological laxatives. On the other hand, UDCA was associated with no diarrhea. These clinical observations were consistent with the physiological studies performed in animal models and in vitro (13, 28). Fluid secretion occurs through a number of mechanisms, including stimulation of intracellular messengers such as cAMP and calcium, which results in activation of chloride secretion through the cystic fibrosis transmembrane regulator (2, 3, 38). Apart from inducing colonic secretion, BAs induce high-amplitude propagated contractions of the colon (4). These effects are, at least partly, mediated through TGR5, which has been identified in enteric cholinergic and nitricergic neurons and...
Bile Acids and the Microbiome

BA pool size is a function of microbial metabolism of BAs in the intestines. The host and microbiome regulate the BA pool size. Thus there are differences between germ-free and conventional mammals; there is higher synthesis of BAs in germ-free than in conventional mice; in the germ-free animals, the primary BA, tauro-β-muricholic acid, inhibits FXR, reducing FGF-15 (the mouse homolog of FGF-19) in the portal circulation and, thereby, enhancing hepatocyte BA synthesis (for review, see Ref. 43).

The host produces a conjugated hydrophilic BA pool, maintained by selective antagonism of FXR in the intestine and liver. The presence of intestinal microbial flora results in deconjugation and dehydroxylation of the primary BAs (tauro-liver. The presence of intestinal microbial flora results in deconjugation and dehydroxylation of the primary BAs (tauro-

Effects on Metabolism Through FXR and TGR5 Bile Acid Receptors

Enteronococrine cells in the epithelial layer of the gut are involved in chemosensing; thus there is a rapid increase in circulating glucagon-like peptide (GLP)-1 and GLP-2 (which are produced by intestinal L cells in the ileocolonic mucosa) after a meal, suggesting that humoral signals from the proximal bowel activate L-cell secretion before direct nutrient exposure in the distal intestine. The factors mediating this rapid activation of release of GLP-1 and GLP-2 are glucose-stimulated insulinoenic peptide (GIP) released from enteronococrine K cells in the duodenum, and neural reflexes involving gastrin-releasing peptide (also called bombesin).

BAs are also signaling molecules (Fig. 3) that induce gut hormone secretion from enteronococrine cells, such as secretion of GLP-1 through activation of the TGR5, resulting in part of the metabolic “bonus” related to the effects of BAs. BAs have been implicated in metabolic regulation, through FXR-mediated regulation of energy substrate mobilization and storage in the liver. It is intriguing that, in addition to BAs themselves inducing these metabolic effects, the BA sequestrants colesevelam and colestimate have similar metabolic effects. Thus colesevelam lowers glucose and lipid levels in Type 2 diabetes, and colestimate increases secretion of GLP-1 in patients with Type 2 diabetes (reviewed in Ref. 18). The mechanisms mediating the glucose-lowering effect of BA sequestrants remain a matter of controversy (46). Nevertheless, FXR is thought to play a pivotal role in the improved metabolism and weight loss after bariatric surgery, particularly sleeve gastrectomy (44), which is associated with elevated plasma BA levels (35, 47). The beneficial effects of bariatric surgery include metabolic actions via FXR (inhibition of glycolysis and lipogenesis) and TGR5 (increased energy expenditure and improved glucose metabolism) and GLP-1-induced stimulation of insulin secretion and retardation of gastric emptying, contributing to the inhibition of appetite (reviewed in Ref. 29).

A poorly absorbed FXR agonist termed fexaramine has also been developed that selectively activates intestinal FXR (16). In mice, this FXR agonist potently induces FGF-15, which
induces the transdifferentiation of white adipose tissue to brown adipose tissue. The browning of the adipose tissue results in substantial metabolic improvements in mice fed an otherwise obesity-inducing diet.

Dual activation of the BA nuclear receptors, FXR and TGR5 (with INT-767), protects mice against atherosclerosis (33), consistent with the fact that BA agonists of these two receptors have the potential to alter several aspects of glucose and fat metabolism (Fig. 3).

In summary, BAs are involved in the regulation of energy and glucose and lipid metabolism via FXR- and TGR5-mediated signaling pathways.

Applications of an FXR Agonist, Obeticholic Acid, in Human Gastrointestinal and Liver Diseases

Obeticholic acid (or 6-ethyl CDCA) is an FXR agonist that is ~100 times more potent than naturally occurring BAs (58). When administered to patients with BA diarrhea, the frequency and consistency of bowel movements are reduced (52). Obeticholic acid increased median fasting FGF-19 and significantly reduced hepatocyte synthesis (fasting C4) of BAs, thereby improving stool frequency and consistency (52).

Efficacy of obeticholic acid was tested in PBC and inadequate response to UDCA on alkaline phosphatase in a randomized, controlled trial and open extension (21): biochemical parameters were improved, but the patients developed worse pruritus through an unclear mechanism.

Effects of obeticholic acid in nonalcoholic steatohepatitis showed improvement of several parameters (serum alanine aminotransferase, γ-glutamyl transpeptidase, alkaline phosphatase) relative to baseline, including improvements in fibrosis, hepatocellular ballooning, steatosis, and lobular inflation (36). The mechanism is thought to involve FXR activation, decreasing hepatic lipogenesis by downregulation of the transcription factor SREBP (sucrose responsive element binding protein)1c and increasing SIRT (sirtuin)1.

Moreover, a phase 2, double-blind, placebo-controlled, proof-of-concept study tested the effects of obeticholic acid in patients with both Type 2 diabetes mellitus and nonalcoholic fatty liver disease with increased insulin sensitivity and reduced markers of liver inflammation and fibrosis (34).

Problems encountered with obeticholic acid are association with increased concentrations of total serum cholesterol and LDL cholesterol and decreased concentrations of HDL cholesterol. These changes developed within 12 wk of the start of treatment, decreased in magnitude during treatment, and were not sustained upon treatment discontinuation (36).

Conclusion

BAs may find a role in clinical therapeutics for a variety of diseases. The clearest therapeutic effects are in restoring normal bowel functions and among liver diseases for cholestatic or nonalcoholic fatty liver disease. There may be a potential therapeutic role in treatment of metabolic diseases including hyperlipidemia and Type 2 diabetes mellitus. Many of these effects involve a role of FXR and TGR5 modulation in these diverse diseases. Future research will consolidate the roles of BAs and their agonists and antagonists or sequestrants for these diverse indications.

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