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Bile Acids in Cholestasis and its Treatment

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ABSTRACT

Bile acids (BA) are key molecules in generating bile flow, which is an essential function of the liver. In the last decades there have been great advances in the understanding of the role of a number of specific transport proteins present at the sinusoidal and canalicular membrane domains of hepatocytes and cholangiocytes in generating and maintaining bile flow. Also, a clearer understanding on how BA regulate their own synthesis and the expression and/or function of transporters has been reached. This new knowledge has helped to better delineate the pathophysiology of cholestasis and the adaptive responses of hepatocytes to cholestatic liver injury as well as of the mechanisms of injury of biliary epithelia. In this context, therapeutic approaches including the use of new hydrophilic BA such as the conjugation-resistant nor- ursodeoxycholic acid, nuclear receptor (FXR, PPAR-alpha) agonists, FGF19 analogues, inhibitors of the apical sodium-depend bile acid transporter (ASBT) and modulators of the inflammatory cascade triggered by BA are being studied as novel treatments of cholestasis. In the present review we summarize recent experimental and clinical data on the role of BA in cholestasis and its treatment.

Key words. Bile acids. Bile flow. Cholestasis. Liver diseases. Inflammation. Cell injury. Signaling. Innate immunity. Neutrophils.

INTRODUCTION

Bile formation by the liver is a crucial physiological function as serves as an excretory route for endo- and xenobiotics and allows the digestion and absorption of lipids from the intestinal lumen.[1,2] Secretion of bile occurs as a consequence of an osmotic filtration process in which the secretion of osmotically active components from hepatocytes to canalicular space determines the passing of water through intercellular tight junctions that are impermeable to bigger size molecules.[2] Among these active solutes secreted in the canalicular pole are bile acids (BA), glutathione, and various organic anions, as well as bicarbonate, proteins, organic cations and lipids.[2,3] BA are the main solutes in bile and are considered the major osmotic driving force in its production.[3] Canalicular excretion of BA occurs after a vectorial transport process including three phases: first, a highly efficient uptake from portal blood in the hepatic sinusoid; second, intracellular transport in the hepatocyte (that can be accompanied by chemical modifications such as conjugation with glucuronic acid or aminoacids, hydroxylation, sulfation, etc.) and, finally, excretion to biliary canaliculi.[4] Upon secretion into the intestine BA undergo an enterohepatic circulation that is mediated by a highly efficient recapture (95% of secreted BA) mechanism in the terminal ileum that in turn secrete BA into mesenteric blood that return to the liver via portal vein for reuptake at the sinusoidal pole of hepatocytes. In the last two decades, considerable advances have been made in the understanding of the mechanisms at play in the generation and regulation of BA flux in the enterohepatic circulation.[5,6] Specifically, the elucidation of the molecular features of hepatocytic transport proteins as well as of the pathobiology of biliary epithelia and the role of several nuclear receptors in regulating the expression of key transporters and enzymes, have provided a more detailed knowledge of the regulation of hepatobiliary transport in physiological and pathological conditions such as cholestasis.[2,7] Also, the role of BA as main drivers of bile flow as well as its importance in determining cell injury and death in both hepatocytes and cholangiocytes as well as recent information on how BA may trigger an inflammatory response in the setting of cholestasis had led to a focused research on BA-mediated liver injury and to the

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design of new BA-based therapeutic strategies that are currently under study with promising results.[8,9] In the present review, we summarize recent advances on the role of BA in cholestasis and provide a synopsis on how this knowledge is being translated into potentially effective therapies for cholestatic liver injury.

BILE ACID TRANSPORT, BILE ACID-INDUCED TOXICITY AND HEPATOCELLULAR ADAPTIVE RESPONSES IN CHOLESTASIS

The vectorial transport of BA by hepatocytes involves several transport proteins and enzymes including the sinusoidal transporters sodium taurocholate co-transporting polypeptide (NTCP/SLC10A1), members of the anion Transporting Polypeptides (OATPs/SLCO) family, conjugation enzymes and the ATP-dependent efflux pump BSEP (bile salt export pump [also know as ABCB11]).[4,7] These proteins allow to a rapid transition of BA from blood to bile and maintain a low intracellular BA concentration (estimated in the micromolar range). This is crucial to maintain hepatocyte integrity as BA are both signaling and detergent molecules that, at higher concentration (≥ 50 μM or mM), may cause apoptosis, activate proinflammatory genes, and eventually induce cellular necrosis.[10-12] This inherent cytotoxicity of BA plays a role in liver damage in cholestatic conditions where bile secretion is impaired and BA accumulate inside hepatocytes and, in the case of cholangiopathies, leak into the surrounding tissue due to injury of bile ducts.[10] Of note, in the cholestatic setting changes in the expression of hepatobiliary transporters occur that may represent a compensatory response aiming to limit the accumulation of potentially toxic biliary constituents. These changes include down-regulation of BA uptake, down-regulation of BA synthesis and upregulation of BA excretion through increased BSEP or other transporters able to provide alternative excretory routes.[13,14] These adaptive responses are mediated by the activation of several nuclear receptors such as FXR, PXR, CAR and SHP as well as by enterohormones such as FGF-19, which is produced in the ileum and also in hepatocytes (in humans).[7,14] FXR is a major player as is a dedicated BA receptor that influences a myriad of pathways both in hepatocytes and in other resident cells such as Kupffer, endothelial and hepatic stellate cells.[15] In hepatocytes in particular, upon upregulation of SHP, FXR mediates a downregulation of NTCP and of CYP7A1, a key enzyme in BA synthesis. FXR also directly up-regulates BSEP, thus promoting BA excretion.[7] In humans, FGF-19 may also be a player down-regulating CYP7a1.[16] Finally, alternative excretory transport proteins located at the basolateral membrane of hepatocytes (i.e. OSTα,β, MRP3, and MRP4) that are expressed at low levels in physiological conditions and become up-regulated during cholestasis.[7] Thus, if BA secretion is impaired adaptive responses may limit BA accumulation inside hepatocytes thus preventing hepatocellular damage. If these responses are insufficient cell damage and death may occur either by apoptosis or necrosis.[12] Of note, it has been also shown that in cholestatic hepatocytes BA can trigger hepatocyte-specific inflammatory response that involves increased expression of cytokines such as Ccl2, Cxcl2, and IL-8 that in turn can contribute to neutrophil recruitment and augment local inflammation.[11,17] This response is partially dependent of activation of toll like receptor-9 presumably by BA-induced mitochondrial damage and the release of mitochondrial DNA.[17] In addition to the local inflammation promoted by BA in other scenarios such as in cholangiopathies or bile duct diseases, mechanical obstruction lead to increased biliary pressure and the occurrence of biliary infarcts and the leak of BA and other biliary constituents into surrounding tissue that may activate proliferative reactions and hepatic fibrogenesis leading to disease progression and ultimately to cirrhosis.[10]

BA AND CHOLANGIOCYTES IN CHOLESTASIS

Advances in the pathobiology of biliary epithelia have also been significant in the last two decades.[18,19] Cholangiocytes, the epithelial cells lining the intra- and extrahepatic biliary tree, are heterogeneous polarized cells that contain a significant amount of transport proteins that allow the secretion of large amounts of bicarbonate (via the Cl-/HCO3- exchanger (anion exchanger 2 [AE2]), water (through aquaporin-1 [AQP-1]) and chloride (through the low conductance cystic fibrosis transmembrane conductance regulator [CFTR]) that enrich canalicular bile and contribute to regulate biliary pH, which is important for activation of pancreatic enzymes and the absorption of lipophilic organic compounds. Cholangiocytes also express BA transporters (the apical sodium-dependent bile acid transporter [ASBT] is present in the apical membrane and a truncated form of the same transporter [referred to as t-Asbt] is located at basolateral membrane of cholangiocytes) that allow for reabsorption of conjugated BA. Also, passive absorption of protonated unconjugated BA can occur through passive absorption. The re-uptake of BA in cholangiocytes followed by re-secretion into the blood of peribiliary plexuses is referred as the "cholehepatic shunt pathway", which leads to BA return to hepatocytes for re-secretion into bile augmenting its choleretic action.[8] Finally, some *in vitro* e *in vivo* evidence suggest that biliary BA concentration and composition may eventually regulate some cholangiocyte functions by activating differing signaling pathways and (i.e. calcium protein kinase C [PKC],

phosphoinositide 3-kinase [PI3K], mitogen-activated protein [MAP] kinase and extracellular signal-regulated protein kinase [ERK] among others) thus inducing changes in cholangiocyte secretion, proliferation and survival. It has been also shown that cholangiocyte proliferation is critically dependent of the BA receptor TGR5, which is located in the cholangiocytes cilia.[18]

Cholangiocyte injury is a key phenomenon in certain cholestatic diseases and therefore aspects related to cholangiocyte responses to injury are also of importance to the understanding of cholestasis pathophysiology and treatment.[20] When injured, cholangiocytes respond acquiring a neuroendocrine phenotype and, in response to a myriad of stimuli, proliferate leading to bile duct hyperplasia, which is a common histological hallmark of cholestatic diseases.[18] Injury of biliary cells can be immune-mediated, toxically-induced or related to mechanical factors (i.e. biliary obstruction). In all these settings, direct cytotoxicity of BA could play a role as increased luminal BA can damage cholangiocytes membrane, induce autophagy and promote cellular senescence, which is associated to secretion of pro-inflammatory and pro-fibrotic signals.[18,21] Bicarbonate secretion and the existence of an intact cholangiocyte glycocalyx have been hypothesized to form a "bicarbonate umbrella" that prevents protonation of biliary BA and cellular damage by bile acid monomers.[22,23]

NEW CONCEPTS IN TREATING CHOLESTASIS WITH BA-BASED THERAPIES

Based in the information summarized above, new strategies are been exploited as therapeutic strategies for cholestatic diseases.[8,22,24] These approaches include:

- To limit BA accumulation by reducing bile acid pool size.
- To improve hepatoprotection and induce choleresis thus limiting cholangiocyte damage.
- To modulate BA-induced inflammation.

Hepatocellular BA accumulation could be prevented in several ways. FXR agonists, that evoke the above mentioned adaptive responses (reduction of BA import and synthesis, and increase of BA export) in hepatocytes, have been shown to be beneficial in animal models of cholestasis. Recently, this knowledge was succesfully translated into the clinic after the first-in-class FXR agonist obeticholic acid was shown to exercise positive effects in primary biliary cholangitis (PBC), a prototypic cholestatic disease.[25] These results determined the drug FDA approval and new efforts by the pharmaceutical indutry in developing new FXR agonists. Although beneficial, obeticholic acid has some disturbing adverse effects, such as pruritus, that may be reduce with by new agents.[26]

The use of FGF19 analogues and ASBT[27] inhibitors are additional strategies to limit intracellular BA by down regulating BA synthesis and preventing recapture of BA in the ileum thus reducing BA pool.[8,24] These approaches are being applied and trials are ongoing with promising results.[28,29]

The hepatoprotective properties of hydrophilic BA has been exloited with ursodeoxycholic acid (UDCA), a natural BA that has been proven to be an effective therapy for PBC.[22,30] UDCA has multiple mechanisms of action including stimulation of bile flow (through post-transcriptional actions leading to increased insertion of transporters sucha as BSEP into the canalicular membrane), stimulation of biliary bicarbonate secretion in hepatocytes and cholangiocytes as well as antiapoptotic and anti-inflammatory actions [reviewed in[22]]. A modified molecule of UDCA, Nor-UDCA, which is a side-chain-shortened derivative resistant to amidation, which increases its cholehepatic shunting, has been shown beneficial effects in animal models[31] and is entering clinical testing with positive preliminary results in primary sclerosing cholangitis.[32]

Finally, modulation of the inflammatory responses is another potential target in cholestasis.[11] In this regard, it has been recently shown that BA can act as damage signals and activate the multimolecular protein complex inflammasomne in Kupffer cells[33] amplifying local inflammation upon injury. At the same time, FXR serves as a negative regulator of the NLRP3 inflammasome and therefore this pathway can also be targeted by FXR agonists.

CONCLUDING REMARKS

A deeper understanding of BA synthesis and transport regulation, the role of BA as signaling molecules in health and disease and the mechanisms underlying BA toxicity in hepatocytes and cholangiocytes has allowed designing new therapeutic approaches for cholestatic diseases. Exploiting FXR-related mechanisms is starting to show positive results and the outcome of ongoing clinical trials investigating new FXR agonists are eagerly awaited. Additionally, other therapeutic approaches targeting the pathways described in this review are also entering clinical testing and, if proven effective, they will open the exciting possibility of drug combinations aiming to modulate different BA-related pathways. Agents acting at this level may be significantly improve the outcome of patients with cholestatic diseases and offer them an expanded therapeutic armamentarium that not long ago was restricted to UDCA or a watch-and-wait approach.

CONFLICT OF INTEREST

The authors declare not to have conflict of interests related to this scientific work.

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