Bile acids in regulation of inflammation and immunity: friend or foe?

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ABSTRACT

Apart from their pivotal role in dietary lipid absorption and cholesterol homeostasis, bile acids (BAs) are increasingly recognised as important signalling molecules in the regulation of systemic endocrine functions. As such BAs are natural ligands for several nuclear hormone receptors and G-protein-coupled receptors. Through activating various signalling pathways, BAs not only regulate their own synthesis, enterohepatic recirculation and metabolism, but also immune homeostasis. This makes BAs attractive therapeutic agents for managing metabolic and inflammatory liver disorders. Recent experimental and clinical evidence indicates that BAs exert beneficial effects in cholestatic and metabolically driven inflammatory diseases. This review elucidates how different BAs function as pathogenetic factors and potential therapeutic agents for inflammation-driven liver diseases, focusing on their role in regulation of inflammation and immunity.

Introduction

The first documentation of bile dates back to 1550 B.C. in Ebers Papyrus, when it was described as a useful remedy and agent to purge (1). Bile acids (BAs) are principal components of the bile, which further contains bilirubin, cholesterol, phospholipids, proteins (*e.g.* albumin and immunoglobins), water and electrolytes (2). Due to their detergent chemical features which makes BAs to attack cell membranes, and their capability to stimulate the secretion of cytokines and chemokines, BAs were traditionally categorised as tissue-damaging and proinflammatory molecules (5, 6). Accumulation of BAs in various (particularly cholestatic) liver diseases is considered a major driver of hepatic inflammation, fibrogenesis and carcinogenesis. Moreover, systemic accumulation of BAs may also damage extrahepatic organs and tissues such as the kidneys (*e.g.* cholemic nephropathy) (3, 4). On the other hand, BA-based therapies have so far mainly focused on hydrophilic, less toxic BAs such as ursodeoxycholic acid (UDCA). Due to its cytoprotective, antiapoptotic, immunomodulatory and choleretic effects, UDCA has over the past decades been therapeutically used in a range of cholestatic and metabolic liver diseases (6, 7).

More recently it has become apparent, that BAs are ligands for several nuclear hormone receptors including farnesoid X receptor (FXR; also known as NR1H4) and G-protein-coupled receptors, such as TGR5 (also known as GPBAR1, M-BAR and BG37) (8-11). BAs act as enterohepatic hormones when they undergo enterohepatic circulation in the hepatobiliary and gastrointestinal (GI) tract. Through activating various signalling pathways, BAs regulate their own synthesis, transport and metabolism, and also immune homeostasis. This makes them attractive therapeutic agents in managing metabolic and liver disorders (9-11). Recent data suggest, that via activation of BA receptors, BAs exert beneficial effects in many inflammation-driven diseases (12-19).

This review summarises current knowledge concerning the role of BAs in the regulation of inflammation and immunity from preclinical to clinical studies, thereby focusing on different BA-modulated signalling pathways and BA-based therapies relevant in the modulation of inflammation and immunity.

Bile acids in a nutshell

BAs are a group of water-soluble, amphipathic molecules biochemically derived from cholesterol. Their synthesis is a complex and multienzyme-regulated process which mainly occurs in hepatocytes (20). Before secretion from hepatocytes, primary BAs are conjugated with glycine or taurine, converting them from weak acids to strong acids

that are impermeable to cell membranes and thus accumulate in the bile and the intestinal environment (20). Once conjugated, primary BAs (*e.g.* cholic acid and chenodeoxycholic acid (CDCA)) reach the gut, where they are microbially transformed into secondary BAs (*e.g.* deoxycholic acid (DCA) and lithocholic acid), which are reabsorbed in the distal intestine and subsequently return to the liver. Each molecule undergoes multiple of these enterohepatic recirculations before finally being excreted in the feces (20). Maintenance of BA homeostasis where BAs exert their pleiotropic physiological functions, such as stimulation of bile flow, facilitation of intestinal absorption of cholesterol, fat-soluble vitamins and lipids, and antimicrobial and metabolic effects is tightly regulated by BA transporters and BA sensing nuclear receptors at the molecular level (Fig. 1) (21, 22).

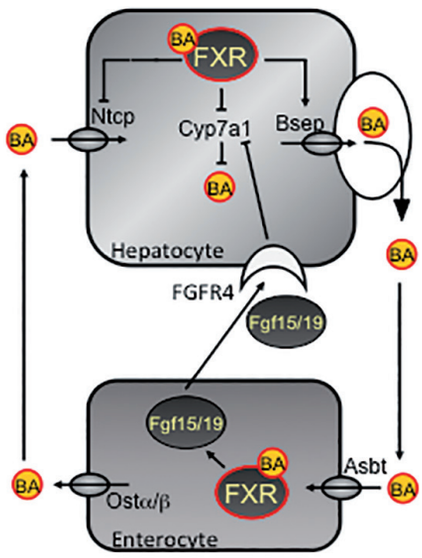

Fig. 1. Maintenance of BA homeostasis is tightly regulated by BA transporters and BA sensing nuclear receptor FXR at the molecular level. Approximately 95% of BAs are reabsorbed by enterocytes of the ileum through Asbt. Binding between BAs and FXR activate FGF15/19 transcription which is in turn subsequently secreted into portal circulation. In the ileum, BAs alternatively exit enterocytes through Ostα/β and then are taken up by Ntcp in liver. In hepatocytes, BAs bind to FXR which block transcription of Cyp7a1, a rate-limiting enzyme in the conversion of cholesterol to primary BAs, thus reducing BA synthesis. In addition, FGF15/19 binds to surface receptor FGFR4 in hepatocytes to slow down BAs synthesis also through suppressing Cyp7a1. Bile salts are released from the liver through Bsep.
BAs: bile acids; Asbt: apical sodium dependent bile acid transporter; FXR: farnesoid X receptor; FGF15/19: fibroblast growth factor 15/19; Ostα/β: organic solute transporter α/β; FGFR4: fibroblast growth factor receptor 4; Ntcp: Na^+-taurocholate cotransporting polypeptide; Bsep: bile salt export pump.

Bile acids as cause of hepatocyte and bile duct injury

Bile is a sophisticated combination of organic and inorganic compounds with BAs being major components. BAs form mixed micelles with phospholipids and cholesterol when they are excreted into the lumen of the bile ducts, which is essential for promoting biliary elimination and reducing detergent activity of monomeric bile acids, thus preventing toxicity of high biliary bile acid concentrations to cholangiocytes (2, 4). Disturbances of normal hepatobiliary transport, altered bile composition and retention of bile flow may result in accumulation of potentially toxic BAs in hepatocytes and/or the formation of "toxic bile" which has increased detergent activities damaging plasma membranes and the capability to stimulate cell death pathways and to induce oxidative stress (2, 8, 9). The Mdr2/abcb4 mouse is a well-established animal model to study the effect of "toxic bile". Mdr2/abcb4 mice lack the canaliluclar phospholid export pump and can, therefore, not excrete phosphatidylcholine into bile, which results in BA toxicity due to increased concentration of free non-micellar bound BAs which disrupt cell membranes and cell junctions.

Cytotoxicity of pathophysiological concentrations of BAs leads to non-specific detergent effects and receptor-mediated signalling. Hydrophobic BAs, such as glycochenodeoxycholic acids (GCDCA) and taurochenodeoxycholic acids (TCDCA), which predominate in cholestatic humans and rodents, are suspected to induce cell apoptosis by directly acting as strong detergents on cell membranes (5, 7). However, serum BA levels in cholestatic patients are insufficient to produce a significant detergent effect; therefore, hepatocyte injury from BAs ultimately inducing cell death by either necrosis or apoptosis appears to result from other cellular mechanisms. Recent data suggest that pathological concentrations of BAs induce apoptosis by activating death receptors in a Fas and TRAIL dependent fashion, including recruitment of Fas-associated death domain (FADD), activation of Caspase-8 and cytoplasmic Bid, as well as downstream effector caspases, such as Bax and Bak which in turn transduce death stimuli to mitochondria (22, 23).

BAs are also known to induce ligand-independent activation of Fas in hepatocytes, followed by caspase-8 activation (24). This mechanism is suggested to be associated with BA-induced oxidative stress, which is induced by different pathways including activation of NADPH oxidase and BAs directly targeting mitochondria, thereby increasing mitochondrial permeability transaction (MPT) and release of reactive oxygen species (ROS) (24-26). Moreover, BAs may also induce endoplasmic reticulum (ER) stress in hepatocytes which largely depends on their hydrophobicity features (27).

Accumulation of BAs in various (particularly cholestatic) liver diseases is a major driver of hepatic inflammation, fibrogenesis and carcinogenesis. Systemic BA accumulation may also damage extrahepatic organs and tissues such as kidney (*e.g.* cholemic nephropathy). A recent study showed that BAs act as inflammagens to directly activate early growth response factor-1 (Erg-1) dependent and independent signalling networks in hepatocytes that stimulate production of proinflammatory mediators, including cytokines, chemokines, adhesion molecules, and other proteins that influence immune cell levels and function in a well-established animal model (28).

BAs modulate innate and adaptive immunity: from homeostasis to inflammation

BAs induce liver injury and inflammation mediated through neutrophils

Neutrophils are the most abundant phagocytes of the innate immune system that first arrive at inflamed foci. There they change their phenotype, become activated and release cytotoxic molecules (*e.g.* ROS, defensins, lacto-

ferrin, cathelicidins and chemokines) to attract more neutrophils (29). Under pathologic condition, such as cholestasis when excessive hydrophobic BAs accumulate in the liver, neutrophils aggravate acute liver injury after bile duct ligation (BDL) by CD18-dependent extravasation from sinusoids and ROS formation (30, 31). Recent studies revealed advanced mechanistic insights on how BAs mediate neutrophils to cause inflammation and liver injury in cholestasis. In a BDL mouse model it was demonstrated that hydrophobic BAs such as CDCA and DCA can induce hepatocellular expression of the adhesion molecule ICAM-1 and the neutrophil chemoattractant CxCL1 through activation of Erk1/2and Egr-1 (32). Neutrophil cytotoxicity occurs when they leave hepatic sinusoids and adhere to parenchymal cells via ICAM-1/Mac-1interaction. Additionally, neutrophils via NADPH oxidase generate superoxide radicals, which subsequently react to hydrogen peroxide. ROS produced by neutrophils may directly diffuse into hepatocytes and may thus contribute to increased intracellular oxidative stress, probably leading to hepatocyte death. ROS released by neutrophils have been further shown to contribute to liver sinusoidal endothelial cell damage in cholestasis (28). Some BAs, such as lithocholic acid (LCA), which is elevated in cholestasis, are suggested to stimulate neutrophils and thus to increase formation of oxygen radicals in this pathological state (33). In accordance with observations from experimental studies, it has been shown that neutrophils from cholestatic patients are "pre-primed", a state when they are "prepared for action" and respond to stimulation in an aggressive hyper-reactive fashion by releasing more toxic products (31).

BAs hamper phagocytosis of Kupffer cells and monocytes and enhance inflammatory monocyte recruitment

Residing within sinusoids in liver, Kupffer cells (KCs) are tissue-resident macrophages that scavenge apoptotic cells and pathogens, and produce pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-α in response to inflammation. In animal model for obstructive cholestasis, BAs reversibly impair phagocytosis activity of KCs. This suppressive effect of BAs on KCs is associated with their hydrophobic features (34). Decreased clearance of bacteria by KCs might explain why cholestatic syndromes are often complicated by gut-derived microbiota translocation which can further lead to sepsis.

As in KCs, phagocytosis capability of monocytes is also hampered by BAs in animal models and cholestatic patients (34, 35). Cholestasis induces trafficking of $Ly6C^{high}$ monocytes to the liver because they are attracted by CCL2 released from activated KCs. Interestingly, BA retention was associated with an activation of cerebral endothelium that recruits TNF-α producing monocytes into the brain. Enhanced TNF-α release within the brain may contribute to the development of cholestasis-associated sickness behavior, including fatigue (36).

BAs selectively expand myeloid dendritic cells in liver

The liver is constantly exposed to gut-derived pathogen associated molecular patterns (PAMPs), which are transferred via the portal blood. Under steady-state conditions, hepatic dendritic cells (DCs) remain in an immature state expressing low levels of MHC class II molecules and low or undetectable levels of costimulatory molecules such as CD40, CD80 and CD86, making them weak T cell stimulators (37). Liver DCs are known to play an important role in inducing hepatic tolerance by priming functional $CD4^+CD25^+$ Tregs against harmless antigens (37). However, BA-induced inflammation can convert liver DCs from a tolerogenic to an activating hyper-reactive phenotype, resulting in a selective expansion of hepatic myeloid DCs as demonstrated by a mouse model of obstructive cholestasis (38). Hepatic accumulation of BAs convert liver DCs to an immunogenic myeloid phenotype with enhanced ability to prime allogeneic and syngeneic T lymphocytes and to secrete pro-inflammatory cytokines under inflammatory stimulation (38-41).

BAs and T lymphocytes

Hepatic T lymphocytes show remarkable heterogeneity regarding their diverse immunological profiles since they are able to perform multiple pro-inflammatory and anti-inflammatory functions in liver diseases. Under steady condition, hepatic immunological tolerance in controlling effector T cell activity is induced by liver antigen presenting cells (APCs), such as liver sinusoidal endothelial cells, KCs and DCs, through their priming of Tregs (42). However, during cholestasis, BAs seem to transform liver APCs from a tolerogenic to an immunogenic phenotype and also directly alter hepatic T cell immunity (41-44).

In a model of biliary obstruction, upregulation of intrahepatic PD-1 expression results in dysfunction of liver bulk T cells (43). Further, acute inflammation during obstructive cholestasis is associated with hepatic Th17 cell infiltration (43). Secretion of IL-6 and IL-1β by biliary epithelial cells in response to a high concentration of BAs also contributes to induction of Th17 cells, which is accompanied by an increased number of neutrophils in cholestasis (43).

Since BAs up-regulate MIP-2 and other cytokines in hepatocytes through Egr-1, they are pivotal for Th17 infiltration and response (44). Additionally, BAs up-regulate IL-23 in hepatocytes through AKT and JNK activation, which greatly contributes to Th17 expansion and promotes the production of IL-17A. In turn, IL-17A synergistically enhances production of MIP-2 and IL-23 by hepatocytes in response to BAs. Enhanced production of IL-23 leads to the formation of a positive feedback loop, which further elicits inflammation during cholestasis (44).

Bile acid-activated receptors (FXR and TGR5) and control of inflammation and immunity

BAs can act as signalling molecules with hormonal actions mediated through activation of dedicated BA receptors such as the nuclear BA receptor farnesoid X receptor (FXR/ NR1H4) (Fig. 1) and the membrane-bound BA receptor TGR5 (also called GPBAR1 or M-BAR/BG37) (45). Besides FXR

and TGR5, BAs are able to activate other nuclear receptors (NRs) such as pregnane X receptor and vitamin D receptor. Ligand-activated NRs such as FXR control a broad range of metabolic processes including hepatic BA transport and metabolism, lipid and glucose metabolism, drug disposition, hepatic regeneration, inflammation, fibrosis, cell differentiation and tumour formation (45). Moreover, FXR mediates anti-inflammatory and immunomodulatory actions and controls intestinal permeability. FXR has anti-inflammatory properties in the liver and intestine mainly by interacting with Nuclear Factor Kappa Light-Chain Enhancer of Activated B Cells (NF-κB) signalling (46). FXR agonists might, therefore, represent useful agents to lower inflammation in cells with high FXR expression such as hepatocytes and prevent or delay inflammation-driven liver diseases. Recently, FXR has also been implicated in activation of hepatic natural killer T cells and hepatic accumulation of myeloid-derived suppressor cells, counteracting immune-mediated liver injury in rodents (47).

Outside the liver, BA-dependent FXR activation also controls bacterial overgrowth and maintains mucosal integrity in the small intestine under physiological conditions by inducing the transcription of multiple genes involved in intestinal mucosal defense against microbes (48). These FXR effects in the gut could explain how luminal bile acids reduce bacterial overgrowth, bacterial translocation and endotoxaemia in cirrhotic rats in addition to their detergent and direct bacteriostatic properties (48). Therefore, FXR agonists could be clinically relevant to prevent gut-derived complications in patients with liver cirrhosis. Conversely, gut microbiota metabolises BAs to secondary BAs, which in turn modulates BA signalling.

In addition to FXR, BAs exert anti-inflammatory effects via activation of TGR5. More specifically, TGR5 activation by BAs inhibits pro-inflammatory cytokine release and reduces phagocytic activity in rabbit alveolar macrophages, human monocytic leukaemia cells and isolated rat KCs (49, 50). Using an *in vitro* model of human monocyte-derived DCs (MDDCs), it has been demonstrated that TGR5 activation by BAs can induce differentiation of IL-12 hypo-producing MDDCs through TGR5-cAMP pathway. This indicates that the TGR5 pathway may be a novel therapeutic target for Th1 dominant chronic inflammatory disorders, such as Crohn's disease and psoriasis (51). Notably, polymorphisms of TGR5 have been linked to primary scerlosing cholangitis (PSC) and ulcerative colitis. By induction of FXR and TGR5 activation BAs improve clinical scores and prolong survival via reducing inflammatory immune cell infiltration to the central nervous system and blocking activation of myeloid cells in mouse model of experimental autoimmune encephalitis (18, 19). Similar to FXR, TGR5 can now be targeted pharmacologically by highly potent agonists, some of which are dual FXR and TGR5 ligands.

Among pharmacological FXR agonists, most data are so far available for obeticholic acid (OCA; also known as 6-ethyl-chenodeoxycholic acid), which has been clinically tested in primary biliary cholangitis (PBC), non-alcoholic steatohepatitis (NASH) and portal hypertension (53, 54). As such OCA improved biochemical and immunological parameters of cholestasis in PBC patients not responding to (or not tolerating) UDCA. In line with the results obtained from combination therapy with UDCA in non-responders, OCA monotherapy also achieved a significant reduction of cholestasis in untreated PBC patients (53, 54). Dose-dependent pruritus was the most common adverse event in patients receiving higher doses of OCA. OCA is currently tested in PSC in a US trial; targeting concomitant inflammatory bowel disease in PSC by FXR may also be an attractive concept (53, 54).

A pilot study with OCA therapy in type 2 diabetics with non-alcoholic fatty liver disease (NAFLD) showed improvement of liver function and, hepatic and peripheral insulin sensitivity (54). A larger placebo-controlled study (FLINT trial) with 283 NASH patients randomised to either OCA or placebo for 72 weeks showed improvement of liver histology including a significant reduction in fibrosis by OCA compared to placebo. In contrast to the pilot study, insulin sensitivity (assessed by HOMA index) deteriorated compared to placebo. Pruritus, a side effect already seen in studies with PBC patients, was also seen in those with NASH, although to a lesser degree (54). Moreover, patients treated with OCA showed an increase of LDL and a decrease of HDL cholesterol; long-term follow-up data are needed to further evaluate the impact of FXR ligands on cardiovascular risk in NAFLD/NASH (54).

OCA has been shown to reduce portal pressure in preclinical models and patients with liver cirrhosis and may also play a key role in maintaining gut integrity (48). Loss of FXR signalling has been linked to inflammation-driven hepatic carcinogenesis, while FXR ligands protect from development of hepatocellular carcinoma (HCC) in preclinical mouse models (55). One of the concerns of FXR therapy may be stimulation of tumour development via FGF15/19; a recently developed FGF-19 mimetic apparently lacks these potentially carcinogenic properties (55).

In summary, the broad immunometabolic actions of BA-activated receptors hold considerable promise for the treatment of a wide range of metabolic and cholestatic liver diseases, perhaps even including complications of end-stage liver disease such as portal hypertension and HCC.

UDCA as therapy and its immunoregulatory effects

BAs have been considered as toxic molecules driving progression of liver diseases, due to their ability to induce tissue damage and inflammation, which has been closely linked to their hydrophobicity (5). Therefore, BA-based therapies have so far traditionally focused on hydrophilic, less toxic BAs, such as UDCA. UDCA has been used therapeutically in a range of cholestatic and metabolic liver diseases over the past decades.

Dried bile from black bear was documented as therapeutic agent more than a thousand years ago as a remedy for

cholestasis at the Tang dynasty in China (1). UDCA is a major primary BA and makes up 60% of the total BA pool in black bears (56). In humans, UDCA is regarded as a minor secondary BA as it is formed by 7β-epimerisation of CDCA by the intestinal microbiota. The portion of UDCA in the human total BA pool is less than 3% (57).

UDCA has been shown to have liver protective properties such as reduction of oxidative stress, inhibition of apoptosis, stimulation of bile flow, increased detoxification of cholephilic compounds and induction of the "biliary HCO_3 umbrella" to protect biliary epithelial cells against cytotoxicity of hydrophobic BAs. Currently, UDCA is recommended for treatment of various cholestatic disorders such as PBC (58-64).

Various immunoregulatory effects of UDCA have so far been observed in PSC patients. These include reduction of cytokine secretion by lymphocytes, production of immunoglobulins, downregulation of MHC class I molecule expression on hepatocytes, blocking of mast cell activation and inhibition of eosinophil activation and degranulation. The immunosuppressive effects of UDCA are partly mediated by activation of glucocorticoid receptor (GR) in a ligand-independent way and by NF-kB transcription via GR-p65 (65-69).

UDCA also exerts immunosuppressive effects in the lung. In an asthma mouse model, UDCA reduced airway inflammation via repressing interaction duration between DCs and T cells. Further, UDCA promotes bone marrow-derived DCs (BMDCs) to secrete IL-12, thus, suppressing the potential of BMDCs to prime for Th2-dependent eosinophilic airway inflammation. In addition, UDCA enhances migration of BMDCs, which limits interaction between BMDCs and T cells, and subsequently results in reduced cytokine production of T cells (70).

norUDCA as therapy and its immunoregulatory effects

24-norursodeoxycholic acid (norUDCA) is a side-chain shortened derivate of UDCA. Due to this structural modification, norUDCA is relatively resistant to amidation with taurine or glycine and has profoundly different pharmakokinetic and therapeutic properties. Instead of undergoing a full enterohepatic circulation norUDCA undergoes cholehepatic shunting, resulting in "ductular targeting" to inflamed bile ducts/ductules and hepatic enrichment (71-72). Cholehepatic shunting also leads to a bicarbonate-rich hypercholeresis which counteracts bile acid toxicity and reinforces the biliary "bicarbonate umbrella". As such, norUDCA (but not UDCA) reverses sclerosing cholangitis in the experimental Mdr2/Abcb4 knockout mouse (Mdr2/Abcb4-/-) cholangiopathy model for PSC while UDCA aggravates bile infarcts in cholestatic conditions with (complete or partial) biliary obstruction (73). Notably, neither norUDCA nor its parent compound UDCA have relevant affinities for dedicated bile acid receptors such as FXR or TGR5. However, norUDCA has potent anti-inflammatory properties in cholangiocytes and macrophages, inhibits NF-κB and mTOR signalling, alleviates ER stress and restores abnormal cell cycle regulation (74, 75, 77). norUDCA stimulates autophagy, which resulted in reduced alpha-1-antritrysin (a1AT) protein accumulation and attenuated liver injury in a mouse model of a1AT deficiency (76). Moreover, a recent study demonstrated beneficial effects of norUDCA (but not UDCA) on granuloma size and hepatic fibrosis in a mouse model of *Schistosoma mansoni* infection; the latter is the world-leading cause of hepatic fibrosis and portal hypertension (78). The anti-inflammatory properties of norUDCA were attributed to MHC class II protein expression on DCs and macrophages, and reduced proliferation of T-lymphocytes and pro-fibrogenic Th2 cytokines (IL-13 and IL-4) (78). These properties may also contribute to anti-inflammatory and anti-fibrotic effects of norUDCA (78). Based on these preclinical data, norUDCA is currently being tested in clinical trials for PSC and NASH.

Summary and future perspectives

BAs mediate various metabolic, anti-inflammatory and immunomodulatory effects, which makes them an attractive new therapeutic strategy in managing metabolic and inflammatory liver disorders. Moreover, BAs may also act as prognostic biomarkers in cholestatic, metabolic and inflammatory disorders (*e.g.* sepsis). The emerging diagnostic, prognostic and therapeutic potential of BAs for extrahepatic disorders deserves further studies.

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