Bile Acid Transporters: Role in Health and Disease

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Bile Acid Uptake Transporters as Targets for Therapy

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Keywords

Sodium taurocholate cotransporting polypeptide · Apical sodium-dependent bile acid transporter · Bile acid signaling · Hepatitis B virus · Cholestasis

Abstract

Background: Bile acids are potent signaling molecules that regulate glucose, lipid and energy homeostasis predominantly via the bile acid receptors farnesoid X receptor (FXR) and transmembrane G protein-coupled receptor 5 (TGR5). The sodium taurocholate cotransporting polypeptide (NTCP) and the apical sodium dependent bile acid transporter (ASBT) ensure an effective circulation of (conjugated) bile acids. The modulation of these transport proteins affects bile acid localization, dynamics and signaling. The NTCP-specific pharmacological inhibitor myrcludex B inhibits its hepatic uptake of conjugated bile acids. Multiple ASBT-inhibitors are already in clinical trials to inhibit intestinal bile acid uptake. Here, we discuss current insights into the consequences of targeting bile acid uptake transporters on systemic and intestinal bile acid dynamics and discuss the possible therapeutic applications that evolve as a result.

Introduction

Bile acid transport into the liver and intestine is maintained by transport proteins, present at the apical membrane of ileocytes and basolateral membrane of hepatocytes. In this review, we focus on hepatic bile acid uptake via sodium taurocholate cotransporting polypeptide (NTCP) and intestinal bile acid uptake via apical sodium-dependent bile acid transporter (ASBT) and the (possible) benefits of pharmacologically targeting these transporters in various pathophysiological conditions. First, the discovery of NTCP as the key uptake receptor for hepatitis B and D virus (HBV/HDV) pointed to novel applications of NTCP targeting in virology. Second, physiological and pharmacological downregulation of the hepatic and intestinal bile acid uptake machinery during cholestasis might provide hepatoprotection. Third, the modulation of bile acid transport is expected to alter bile acid dynamics, which in turn will affect the activation of the main bile acid sensors farnesoid X receptor (FXR) and transmembrane G protein-coupled receptor 5 (TGR5), with multiple consequences on metabolism of bile acids, cholesterol, lipids and glucose.

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Bile Acid Formation and the Enterohepatic Circulation

Bile excretion plays a pivotal role in the elimination of endogenous and exogenous (toxic) compounds, such as bilirubin, heavy metals and drug metabolites. Bile mainly contains phospholipids, cholesterol and bile acids combined in micelles [1]. Bile acids are amphipathic molecules, characterized by the ability to form an interface between lipids and water. In the small intestine, bile acids improve dietary lipid digestion and transport across enterocytes.

Synthesis of bile acids starts in the pericentral hepatocytes via the rate-limiting conversion of cholesterol into 7α-hydroxycholesterol by the hepatic microsomal enzyme cholesterol 7α-hydroxylase (CYP7A1). After a complex biosynthetic pathway involving multiple enzymes, the main primary bile acids chenodeoxycholic acid (CDCA) and cholic acid (CA) are formed (~80% of the human bile acid pool) [2]. Specifically in mice, CDCA is converted to muricholic acid. In the last step of biosynthesis, bile acids undergo conjugation with glycine (predominantly in humans) or with taurine (predominantly in rodents). Secreted bile acids are predominantly in their conjugated form, that is, in the negatively charged state, which prevents passive membrane-diffusion. Secondary bile acids are formed from these primary bile acids by bacterial modification in the distal intestine [3]. Bile acids are efficiently retained within the enterohepatic circulation (only 3–5% is lost via feces), due to reabsorption in the terminal ileum by the apical sodium-dependent bile acid transporter (*SLC10A2*/ASBT) and excretion into portal blood by the heterodimeric organic solute transporter α/β (*SLC51A/B*/OSTα/β) and other basolateral extrusion pathways in ileocytes [4, 5]. In physiological situations, fecal bile acid loss is compensated for by de novo synthesis from cholesterol in the liver, and thereby boosts cholesterol elimination from the body [6]. Bile acids are eventually recycled from portal blood at the hepatic basolateral membrane by 2 transport systems: the sodium-dependent taurocholate cotransporting polypeptide (*SLC10A1*/NTCP) and members of the sodium-independent organic anion transporting polypeptide (*SLCO*/OATP) transport family [7, 8]. In humans, the physiological bile acid concentration ranges from <5 μM (fasting) to 3–7 μM (postprandial) in systemic blood. In portal blood, the postprandial bile acid peak is more evident (from 4–27 to 22–55 μM) [9, 10]. Thus, the hepatic uptake machinery efficiently limits the escape of bile acids to the general circulation, with a first-pass extraction fraction ranging from 50 to 90% depending on the bile acid structure [11].

Hepatic Basolateral Uptake Systems

NTCP is a family member of the solute carrier 10 family and is present on the basolateral membrane of hepatocytes. NTCP adopts a dimeric or even higher order quaternary structure, in which the individual subunits form functional units [12]. Recently, the crystal structure of ASBT was solved revealing a structure with 9 transmembrane domains, with an exoplasmic N terminus and cytoplasmic C terminus [13], and a similar conformation might be expected for NTCP. NTCP-mediated uptake of taurocholate was demonstrated with published K_m values that vary from 5 to 84 μM for human NTCP and 8–61 μM for rodent NTCP [14]. Besides bile acids, NTCP is a transporter of steroidal hormones and a variety of drugs [15, 16]. In the liver, NTCP is distributed equally along all liver lobules, but uptake of conjugated bile acids occurs predominantly in periportal cells (zone 1), as these are exposed to the highest concentrations of bile acids [17]. In humans, Ho et al. [18] described ethnicity-dependent polymorphisms that are associated with decreased transport function in vitro. However, to date, only one individual with NTCP deficiency was described, featuring a single homozygous mutation (p.R252H), phenotypically characterized by high plasma conjugated bile acid levels without any signs of liver injury or pruritus [19]. Similarly, a subset of *Slc10a1*/NTCP knockout mice displays strongly elevated conjugated bile acids levels in plasma [20], confirming a primary role for NTCP in hepatic clearance of conjugated bile acids. In addition, several adult NTCP knockout mice showed physiological bile acid levels, indicating that an NTCP-independent uptake of conjugated bile acids must exist.

The presence of additional Na^+-dependent membrane transporters has been suggested, in particular, bile acid transport by microsomal epoxide hydrolase [21], but little experimental evidence supports this notion [22]. Hepatic bile acid uptake can also be mediated by (one or more members of) the Na^+-independent OATP transporter family. All OATPs are 12 transmembrane domain glycoproteins with broad substrate preference, such as (un)conjugated bile acids, bilirubin and numerous drugs [23]. The most abundant hepatic OATP subfamilies are OATP1A and OATP1B, and are designated Oatp1a1, Oatp1a4 and Oatp1b2 in rodents [8]. It is difficult to estimate the role of each single OATP-isoform in vivo, as there is large substrate overlap and rodent Oatps have no direct human orthologue [24]. In humans, 2-gene biallelic human OATP1B1 and OATP1B3 deficiency is known as the Rotor syndrome, character-

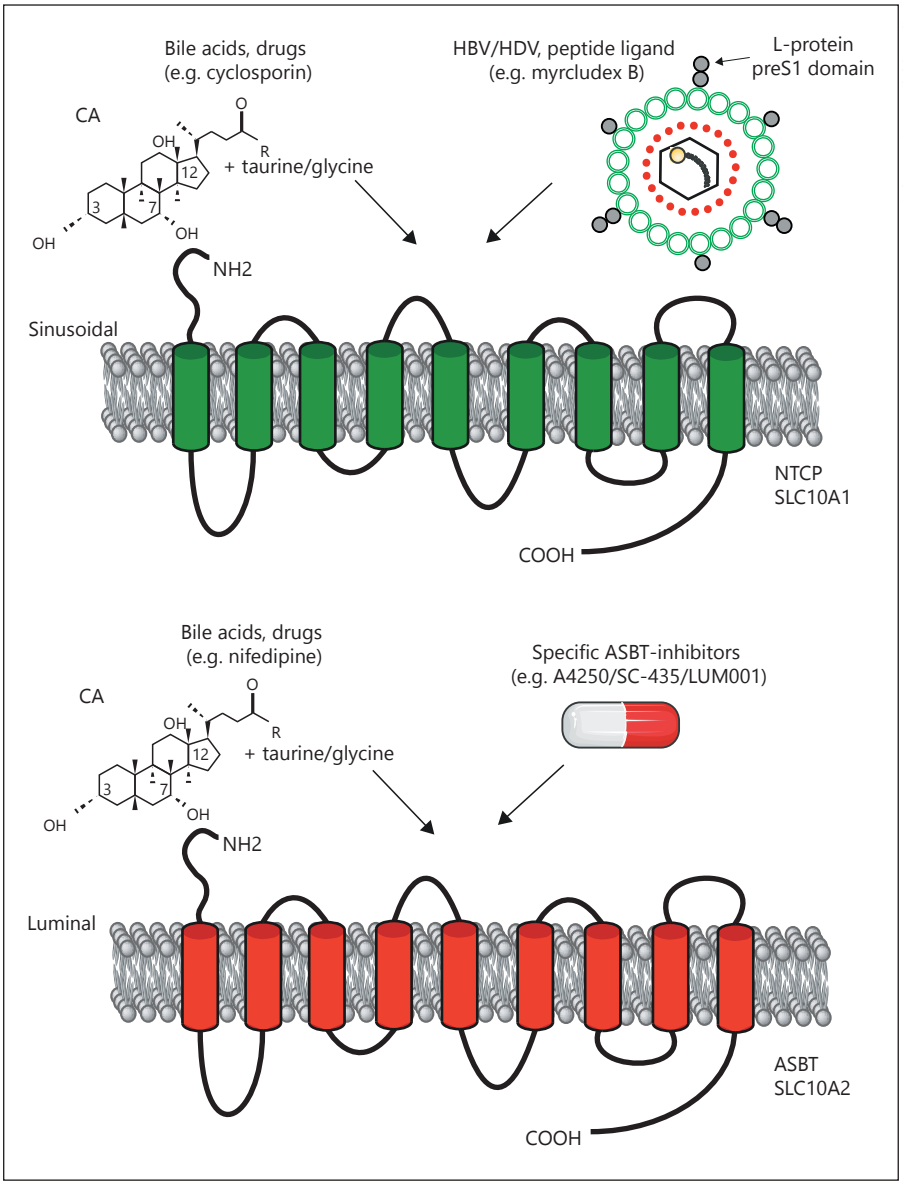

Fig. 1. NTCP and ASBT: topology and ligands. Both NTCP (upper panel) and ASBT (lower panel) from the SLC10 family contain 9 transmembrane spanning domains. Conjugated bile acids are natural substrates for these transporters, and recently the preS1 domain of HBV was found to specifically bind to NTCP. Also, the HBV-derived lipopeptide myrcludex B strongly binds to and inhibits NTCP in vitro and in vivo. Many ASBT-specific inhibitors (e.g. A4250) were developed over the past decade to increase fecal bile acid excretion.

ized by high conjugated plasma bilirubin levels [25]. Evidence for human OATP polymorphisms affecting endogenous bile acid uptake is sparse. Interestingly, mice lacking all Oatp1a/1b-family members (*Slco1a/1b* knockout mice) display 13-fold elevated levels of unconjugated bile acid in blood [26], whereas conjugated bile acid levels remained mostly unchanged, and similar results were found in knockout mice lacking only *Slco1b2* [27]. The role of the rodent Oatp1a-isoforms in bile acid transport is less obvious; nevertheless, taurocholate uptake was reduced in primary hepatocytes isolated from Oatp1a4-null mice, and to slighter extent in Oatp1a1-null mice [28]. Other studies suggest that Oatp1a1 and Oatp1a4 preferably transport secondary unconjugated bile acids, thereby altering intestinal bile acid metabolism [29, 30].

NTCP as Receptor of HBV/HDV

Beside the role of NTCP as major transporter for conjugated bile acids, NTCP was recently found to be the main receptor for HBV and HDV viral particles [31], and the specific NTCP inhibitor myrcludex B is currently being tested in phase II trials as an HBV/HDV entry inhibitor [32, 33] (fig. 1). Myrcludex B is a synthetic lipopep-

tide based on the preS1 domain of the HBV envelop protein targeting NTCP, and effectively inhibits HBV entry in vitro and in vivo [34, 35]. Pharmacokinetic studies with myrcludex B show rapid hepatic accumulation (within minutes) [20] where it has a half-life of ~12 h. Liver-specific binding is also observed in non-HBV susceptible animals (dogs, rats and mice) [36] and NTCP specificity was recently confirmed using NTCP knockout mice [20]. HBV entry inhibition is clinically important in the treatment of HBV/HDV infection, where it could be applied in combination with other anti-viral drugs, such as interferon or tenefovir. An interim report of a phase Ib/IIa clinical trial showed a reduction of HDV RNA levels towards undetectable levels upon 12 or 24 weeks of treatment especially using a combination of myrcludex B and interferon, although HBsAg did not show any reduction with this treatment duration [32]. Myrcludex B treatment resulted in elevated plasma bile acids levels, which was well tolerated and the drug showed no adverse effects.

Inhibition of Bile Acid Uptake to Ameliorate Cholestatic Liver Injury

Cholestatic liver damage occurs when bile flow is impeded, leading to the accumulation of toxic bile acids within hepatocytes and causing liver damage, inflammation and fibrosis. Current therapies in cholestatic liver disease are recently reviewed by Beuers et al. [37]. Nuclear receptors (NRs), bile acid transporters and hepatic enzymes play a key role in orchestrating bile acid metabolism to protect against the accumulation of toxic bile acids [38]. Classical intrahepatic bile acid sensing by FXR [39–41] leads to the recruitment of the atypical NR that has only a ligand-binding domain, called short heterodimer partner (SHP) [42, 43], which represses bile acid biosynthesis via the downregulation of CYP7A1. Hepatic FXR activation by (semi-)synthetic FXR agonists has been successful in cholestatic animal models [44, 45] in order to induce hepatic bile acid efflux and reduce bile acid uptake and such therapies are now being tested in phase II and III trials in PBC and primary sclerosing cholangitis [46]. In addition to FXR-dependent transcriptional repression of the bile acid uptake machinery, posttranscriptional regulation of the plasma membrane expression and function of NTCP is expected to be relevant during cholestasis, as reviewed elsewhere [47]. So far, the effectiveness of (further) inhibition of basolateral hepatic bile acid uptake in the context of cholestasis was only studied using OATP1A1 knockout mice, which were not protected against hepatic injury after bile-duct ligation [48]. The role of additional (pharmacological) NTCP-inhibition during cholestasis is not reported yet. The finding that myrcludex B is well tolerated, even at dosages that inhibit NTCP and lead to increased bile acid levels in plasma, makes this strategy likely to be tested in vivo.

When we focus on the modulation of intestinal bile acid transport, most studies investigated intestinal bile acid sequestrants (e.g. cholestyramine and colesevelam) as first-line agents in the treatment of cholestatic pruritis. Colesevelam proved to be effective in reducing plasma bile acid levels by ~50%, but failed to reduce pruritis [49]. Currently, clinical studies are evaluating ASBT inhibitors as a novel pharmacological treatment for cholestasis. The rational of ASBT inhibition is based on an increase of fecal bile acid elimination and preventing bile acid return to the liver, thereby potentially reducing the bile acid pool by 80%. The ASBT inhibitor A4250, previously used in a clinical phase I study [50], showed improvement of cholestatic liver injury in multi-drug resistance P-glycoprotein 2-deficient mice [51]. In a similar study, 2 weeks of treatment with SC-435, a different ASBT inhibiting small molecule, demonstrated reduced bile acid pool size and attenuation of cholestasis in the same mouse model [52]. At present, human studies are being performed, but the outcome of these promising studies is not published yet (https://clinicaltrials.gov/ct2/show/NCT02061540).

Targeting FXR tissue-specifically during cholestasis attracted considerable attention since the discovery of fibroblast growth factor 15/19 (FGF15/19). This hormone, when released from the gut, binds to the tyrosine kinase receptor FGF receptor 4/β-Klotho on hepatocytes, which activates the jun N-terminal kinase 1/2 signaling pathway [53]. Intestinal FXR activation [54] and the FGF19 mimetic M70 [55, 56] dampen cholestatic liver injury by strongly reducing hepatic bile acid synthesis and the circulating bile acid pool. So, mouse studies clearly show hepatoprotection through (gut-specific) FGF15/19 signaling, primarily by reducing bile acid pools, sharing its mode of action with ASBT-inhibition. NGM282 (the engineered variant of human FGF19) is now tested in a phase II trial in PBC patients unresponsive to ursodeoxycholic acid treatment [57].

Bile Acid Dynamics in Relation to the Metabolic State

Besides the role of bile acids in cholesterol and lipid metabolism, bile acids are important signaling molecules regulating glucose metabolism, inflammation and energy

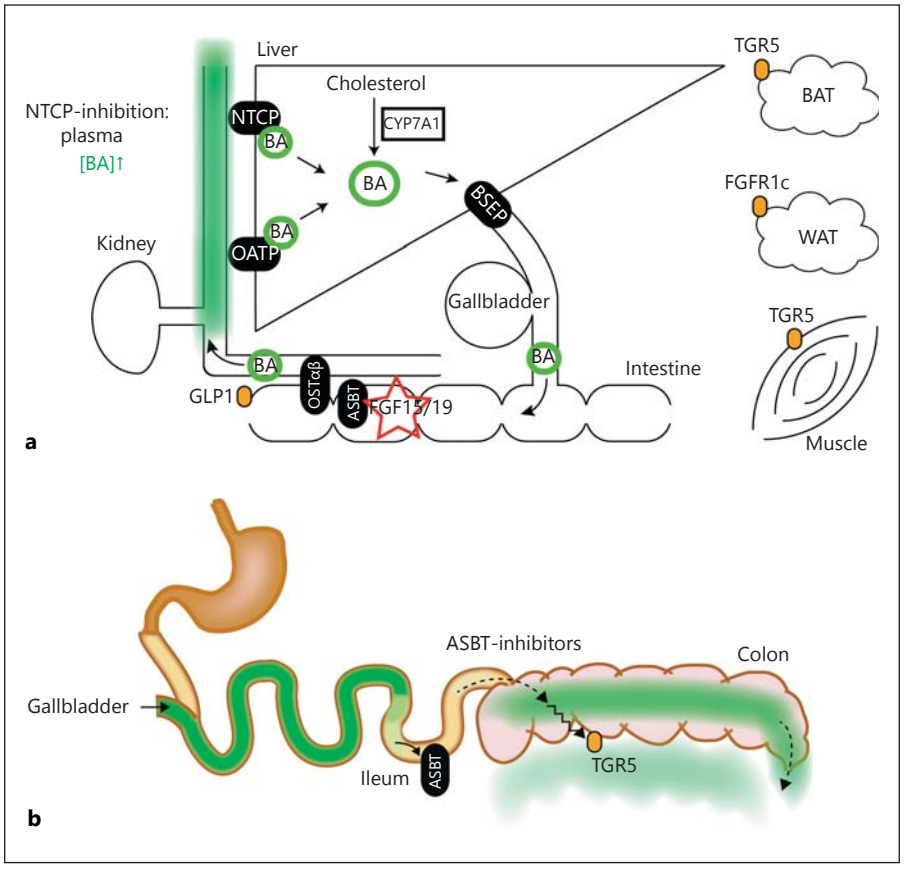

Fig. 2. Enterohepatic circulation of bile acids. **a** Overview of the distinct hepatic and intestinal bile acid transporters. The key transporters are BSEP, ASBT, OSTα/β, OATP and NTCP (schematically depicted). Upon intracellular bile acid sensing, FXR/SHP and FGF15/19 become activated and regulate bile acid synthesis (i.e. predominantly CYP7A1). Furthermore, spillover of bile acids into the systemic circulation might also activate the TGR5 receptor, present on the basolateral side in various tissues, such as BAT, muscle and in the colon. **b** Pharmacological inhibition of ileal bile acid uptake (by bile acid binding resins or ASBT inhibitors) induces the presence of bile acids in the colon and increases fecal bile acid loss. The latter contributes to cholesterol catabolism. Increased GLP1 release in the distal intestine improves systemic glucose handling and is likely induced by (passive) the translocation of bile acids leading to basolateral stimulation of TGR5.

expenditure. Detection of bile acids is mostly mediated via the receptors FXR and TGR5, and not exclusively in the liver and intestine [58, 59]. Targeting bile acid signaling is, therefore, appealing to treat metabolic diseases such as diabetes and atherosclerosis. In the following sections, we discuss the (possible) beneficial metabolic effects of modulation of bile acid dynamics by the inhibition of ASBT and NTCP transport activity as this is expected to alter the FXR/TGR5 activity.

Inhibition of intestinal bile acid uptake specifically via ASBT is recognized for its low-density lipoprotein (LDL)-cholesterol lowering effect [60]. Preclinical studies further showed that ASBT-inhibition reduces hepatic triglyceride and cholesterol accumulation in high fat diet-fed mice [61, 62]. Similarly, bile acid–binding resins, including cholestyramine and colesevelam, have been shown to reduce serum total sterols including LDL-cholesterol values. This effect is mainly due to increase in cholesterol catabolism to replenish bile acids lost via the feces. However, their use for this application is limited, mainly because compliance remains a challenge due to the gastrointestinal side effects.

Bile acid–binding resins also have a beneficial effect on glucose handling. For example, the bile-acid sequestrant Colestilan induces glucagon-like peptide 1 (GLP1) release from the colon [63]. GLP1 increases insulin secretion and improves insulin sensitivity. Similarly, colesevelam improved glucose homeostasis. This effect seems mostly mediated by TGR5 activation [63, 64], and partially through the inhibition of FXR signaling [65]. Bile acid–induced GLP1 release occurs upon the activation of TGR5 at the basolateral (blood) side of L-cells [66, 67]. Nevertheless, ASBT inhibitors and bile acid–binding resins both stimulate the release of enterohepatic hormones, including GLP-1, and inhibit the uptake of bile acid into the circulation. In our view, the most direct explanation for this apparent discrepancy is that TGR5-mediated effects occur after (passive) bile acid translocation to the basolateral side of the colonocyte (fig. 2). ASBT-inhibition and bile-acid sequestration result in the increased presence of bile acid in the colon. Bile acid sequenstrants do not covalently bind bile acids, and some diffusion is still likely. The secondary bile acids lithocholic and deoxycholic acid, (and their corresponding taurine and gly-

cine conjugates) are most potent activators of TGR5 in vitro, so local elevation of these bile acid species could stimulate GLP-1 release into the circulation. At present, it is unclear whether elevated bile acid levels in the systemic circulation also would stimulate GLP1-secretion. Thomas et al. [68] demonstrated that the positive effects on glucose homeostasis of the CA-derived TGR5 agonist INT-777 were also mediated by intestinal TGR5. However, INT-777 was provided orally in this study, so TGR5 might have been activated from either the systemic circulation or by local diffusion across the colonic epithelium.

In general, little is known about the influence of endogenous circulating bile acids on metabolic processes, and whether inhibition of hepatic bile acid (re)uptake boosts energy expenditure and/or lipid metabolism. The activation of TGR5 by bile acids is linked to increased energy expenditure in brown adipose tissue (BAT) and muscle [69]. We postulate that such effects are mimicked by (transiently) increased bile acids levels that would occur with NTCP inhibition. This process could also play a role in the beneficial metabolic consequences of bariatric surgery. Bariatric procedures might induce bile-acid signaling by increased circulating bile acid levels, as shown in several studies [70, 71]. Previously, ileal interposition surgery in mice showed strongly elevated plasma bile acid levels [72], without changes in hepatic *Ntcp* mRNA. Beneficial effects of vertical sleeve gastrectomy (VSG) on body weight and glucose tolerance are dependent on both FXR [73] and TGR5 [74], suggesting that bile acid signaling is indeed relevant. Interestingly, mRNA-seq in VSG mice showed significant downregulation of hepatic *Ntcp* and *Oatp1b2*, possibly explaining the increase in bile acid levels in plasma [75].

What happens to the liver when NTCP is inhibited and/or bile acid levels in the systemic circulation increase? TGR5 is not detected in hepatocytes, but is expressed in sinusoidal endothelial cells where its activation has a hepatoprotective role by inducing nitric oxide synthase in a cAMP-dependent manner [76]. Furthermore, TGR5 activation in Kupffer cells decreases the release of proinflammatory cytokines IL-6 and TNF-α [77, 78] and (more generally) dampens macrophage-mediated inflammation by inhibiting the NFκB-pathway [79]. A high-fat diet caused more liver steatosis in male TGR5-knockout mice [80], suggesting that TGR5 activation prevents non-alcoholic fatty liver disease. INT-777 reduces liver fatty acid and triglyceride content as well as plasma triglycerides [68]. A beautiful study using glucocorticoid receptor–deficient mice pointed to a contribution of NTCP-governed bile acid dynamics to metabolism [81]. These mice showed impaired hepatic bile acid uptake by the downregulation of NTCP, which reduces dietary fat absorption and increases BAT mitochondrial uncoupling. Reduced bile acid uptake via NTCP could also dampen hepatic FXR activation. The (metabolic) consequences of this action are currently not clear, although some first insights were obtained using a mouse model, where human hepatocytes repopulated the liver of urokinase plasminogen activator/severe combined immunodeficiency mice. NTCP inhibition using myrcludex B resulted in increased CYP7A1 expression, suggesting reduced FXR activity [82]. Chronic HBV infection in these humanized mice had a similar effect. Further studies are required to assess the (metabolic) consequences of NTCP inhibition, as effects on lipid, glucose or energy metabolism were not investigated in this study.

In summary, targeting bile acid uptake transporters, NTCP and ASBT, has exciting implications for the fields of virology, cholestasis and metabolism of glucose, lipid and energy, although therapeutic efficacy and long-term (side-)effects of altering the bile acid dynamics needs to be further elucidated.

Disclosure Statement

The authors have nothing to disclose.

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