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ASBT Inhibitors in PBC and PSC

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Abstract

Bile acids (BAs) have gained mainstream attention since the discovery of their key role as signalling molecules in health and disease. The apical sodium dependent transporter (ASBT) protein located in the terminal ileum plays an important physiological role in the enterohepatic circulation of BAs and therefore essential for the bile acid (BA) homeostasis. Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), the two most common cholestatic liver diseases are characterised by altered BA flow and bile acid composition which contribute to disease progression and symptom (pruritus) development. Therefore, changing the circulating BA pool in patients with PBC and PSC may have therapeutic implications. To this end, pharmacological inhibition of ASBT is fast emerging as an interesting target. In this chapter, we discuss recent evidence for potential therapeutic use of ASBT inhibitors (ASBTi) to treat PBC and PSC patients.

Introduction

Primary biliary cholangitis (PBC, previously referred as primary biliary cirrhosis) is a chronic intrahepatic non-suppurative destructive cholangitis with a prevalence of 1 in 1000 women over the age of 40 years. Primary sclerosing cholangitis (PSC) is a chronic progressive cholangitis of intra- and extrahepatic bile ducts that predominantly affects men and has strong association with inflammatory bowel disease (IBD). In untreated patients these two chronic cholangiopathies lead to progressive loss of bile ducts, biliary fibrosis and eventually cholestatic liver cirrhosis and associated complications of portal hypertension and increased risk of malignancy. The aetiology and pathogenesis of PBC and PSC are poorly understood but cumulative evidence point to complex interactions between environment, genetics and immune-mediated liver injury. Clinical features of PBC and PSC vary greatly between patients and there are well-recognised disease-associated symptoms, concurrent autoimmune diseases and complications. Currently available disease modifying and symptom-based treatment of PBC and PSC patients is unsatisfactory. For instance, ursodeoxycholic acid (UDCA), the current standard of care for PBC fails to improve liver biochemistry in ~40% of patients (UDCA non-responders) who are at higher risks of disease progression and poor quality of life [1]. In PSC, although UDCA improves serum liver tests it has no proven benefit on survival and therefore currently not recommended for routine treatment [2]. In addition, UDCA has limited therapeutic benefit in treating pruritus and fatigue, the two most common symptoms associated with PBC and PSC. Due to these limitations of UDCA there is an unmet need for new and effective treatment for these complex diseases.

By definition, PBC and PSC are characterised by impaired bile flow and altered bile acid (BA) composition which are important pathogenic factors for initiation and development of disease

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and symptoms. The latter is influenced by the extent of bile flow defect (cholestasis) and accumulation of toxic hydrophobic BAs (that cause liver cell damage) [1]. Therefore modulation of bile flow and BA composition is emerging as an important therapeutic strategy treating PBC and PSC.

ASBT

In health, BAs [cholic acid (CA) and chenodeoxycholic acid (CDCA)] are produced in the liver, secreted in the intestine and following their role in fat digestion most BAs (>95%) are re-claimed in the terminal ileum and returned to the liver via portal vein [3]. This efficient process of recycling of BAs is referred as enterohepatic circulation (EHC) which maintains the balance between hepatic synthesis and intestinal loss of BAs. The physiological importance of EHC has been well known for many decades [4] and the key proteins and sodium-dependent transporters involved in EHC have been characterised. It is now known that a number of specialized membrane transporters expressed on the apical (brush border) and basolateral membranes of the hepatocyte and ileal enterocytes mediate EHC of BAs (an in-depth review in [5]). Of these, the apical sodium dependent transporter (ASBT; also called as ileal bile acid transported, IBAT; gene symbol *SLC10A2*) located on the apical membrane of ileal enterocytes was first cloned in humans in 1995[6] and shown to function as a major gatekeeper for the intestinal compartment of EHC of BAs [7-9] (**Figure 1**). Indeed, by regulating the rate of biliary BA secretion it is considered a major determinant of BA pool size in the human body and is an essential regulator of lipid and cholesterol homeostasis [10]. Interestingly, ASBT mediated transport is not equal among BA species; conjugated (more hydrophilic) BAs are transported more efficiently than

unconjugated forms. Also, the affinity of ASBT is higher for dihydroxy BAs such as CDCA than for trihydroxy BAs such as CA, taurocholic acid (TCA) and glycocholic acid (GCA) [8].

ASBT Inhibition

In recent years, ASBT has gained more attention as a specific drug target to inhibit EHC and alter the circulating pool of BAs. There are specific effects of pharmacological inhibition of ASBT which have therapeutic potential. First, inhibition of intestinal reabsorption of BAs leads to increased BA load in the colon (**Figure** 1) and causes BA-induced diarrhoea. The latter effect is being utilised to treat constipation [11]. Second, decreased return of BAs to liver results in increased hepatic BA synthesis as a result of negative feedback regulation (inactivation of hepatic FXR)[12]. This lowers serum cholesterol level (due to increased conversion of cholesterol into BAs) which is an additional benefit in lipid metabolism and metabolic disorders and supports ASBT inhibitors as novel hypolipidaemic dugs [13]. Third, ASBT inhibition may have anti-diabetic action mediated through BA-TGR5 axis. BAs in the colon activate TGR5 (highly expressed in colon) resulting in stimulation of expression and secretion of the incretin GLP-1, a hormone that lowers plasma glucose [14]. Finally, yet untested hypothesis suggests increased load of BAs in the colon may significantly impact the gut microbiome with potential secondary effects on cholestatic diseases [15].

At the turn of the century, at least five classes of chemically divergent specific ASBT inhibitors (ASBTi) were developed (**Table 1)** mostly for the treatment of hypercholesterolemia. The main ASBTi that have entered phase 2 trials are summarised in **Table 2**. In the following sections we

review recent evidence to support therapeutic potential of ASBTi as novel therapy for PBC and PSC patients.

Experimental evidence

There are conflicting data on intestinal absorption of BAs during cholestasis. An adaptive regulation leading to downregulation of intestinal ASBT has been shown in both animal and human studies [16,17]. In contrast, increased absorption of BAs has been reported in PBC, thus contributing to cholestasis in this condition [18]. Nevertheless, ASBT inhibition is an attractive therapeutic option in cholestatic conditions based on the hypothesis that interrupting the EHC of BAs may also reduce the circulating BA pool and hepatic levels of potentially cytotoxic BAs.

Early reports from non-cholestatic animal studies demonstrated that SC-435 (an ASBTi) leads to increased faecal BA (and diarrhoea) and reduced FXR stimulation, lower FGF19 synthesis, and consequently enhanced BA synthesis, expanding the BA pool and lowering plasma cholesterol[12,19,20]. More recently, effects of ASBTi in animal models of cholestasis have been reported. Miethke *et al.* treated mdr2$^{-/-}$ mice (an established animal model for chronic cholestasis with some features of sclerosing cholangitis) with SC-435 for 14 days. They observed 8-fold increase in faecal BA excretion associated with 65%, 98.9% and 98.8% decrease in hepatic, serum and biliary concentrations of BAs, respectively. The anti-cholestatic and anti-inflammatory effects of SC-435 was evidenced by decreased markers of liver injury; plasma ALT and bilirubin concentrations decreased by 86% and 93%, respectively and serum ALP by 55%. They also observed an improvement in liver histology of sclerosing cholangitis with decreased fibrosis and favourable alteration in the biliary phosphatidylcholine/BAs ratio

indicating decreased bile toxicity. In addition, the livers from SC-435 treated mice showed reduction in the proinflammatory Ly6C$^+$ and increase in anti-inflammatory Ly6C$^-$ subset of monocytes accompanied by reduced levels of F4/80$^+$ CD11b$^+$ Kupffer cells and Gr1$^+$ CD11b$^+$ neutrophils. Taken together, the results from this study demonstrated the potential of ASBTi in halting progression of murine sclerosing cholangitis during the early phase of disease process [21].

Baghdasaryan *et al.* also examined the effects of a specific intestinal ASBTi (A4250, Albireo pharma, Sweden) in eight week old Mdr$^{-/-}$ (Abcb4$^{-/-}$) mice (model of cholestatic liver injury and sclerosing cholangitis). After four weeks of treatment with A4250 they observed reduced serum ALT, ALP and BA levels, decreased hepatic expression of proinflammatory (*Tnf-a, Vcam1, Mcp-1*) and pro-fibrogenic (*Col1a1,Col1a2*) genes and bile duct proliferation. Furthermore, A4250 was shown to significantly reduce bile flow and biliary BA output with preserved HCO3$^-$ and biliary phospholipid (PL) secretion resulting in an increased HCO3$^-$/BA and PL/BA ratio [22].

In summary, use of intestinal ASBTi in animal models of sclerosing cholangitis demonstrates that interruption of EHC improves cholestatic liver and bile duct injury, suggesting that ASBTi may represent a novel and promising treatment for cholangiopathies.

Clinical evidence

The three main ASBTi currently being investigated in early phase trials are: A4250, GSK2330672 and Lopixibat chloride (LUM001) (**Table 2**). In non-cholestatic population ASBTi have been shown to be effective in changing circulating BA levels. For instance, in a

randomised, double blind, placebo controlled study of 24 healthy subjects one week treatment with A4250 was found to be safe, well tolerated and produce significant decrease in plasma total BAs and FGF19 levels and increase plasma C4 (a marker of hepatic BA synthesis) and faecal BAs. The main adverse events were abdominal discomfort, nausea and mild diarrhoea which were dose-dependent[23]. GSK2330672 has been developed as specific, non-absorbable, highly potent human ASBTi [24] and has been investigated in double-blind, randomized trials of patients with type2 diabetes mellitus (T2DM) [25]. It was shown to reduce circulating BAs, fasting plasma glucose and cholesterol (LDL, non-HDL and total cholesterol) levels and increase C4. Diarrhoea was the most common adverse event associated with GSK2330672 which appeared to be dose-independent.

Pruritus, a common and debilitating symptom associated with PBC and PSC is specifically being targeted with novel ASBTi. The rationale for this is cholestatic pruritus is linked to circulating BAs and reducing their levels may improve the symptom. The results of CLARITY study investigating use of oral Lopixibat chloride (LUM001) in PBC patients with pruritus was presented at the International Liver Congress (EASL 2016)[26]. In this double blind, randomised placebo controlled trial PBC patients on stable doses of UDCA (or intolerant to UDCA) with baseline pruritus score >4 for each of two consecutive weeks in the screening period were randomised to daily Lopixibat 10 mg, 20 mg or placebo. The 13-week treatment period comprised dose-escalation (3–4 weeks) and stable-dosing periods (9–10 weeks). The primary endpoint was change in adult ItchRO weekly sum score at week 13 or early termination. The results from 66 enrolled patients (61 completed the study) showed significant decrease in ItchRO

score from baseline in the within group comparison (26% Lopixibat, $p < 0.0001$ and 23% placebo, $p < 0.0001$) but no significant difference between group comparison (Lopixibat vs. placebo, $p = 0.47$). The changes in serum ALP from baseline were not significant for either group but reduction in mean serum BA levels (-14.23 vs.10.05) and increase in C4 levels (13.49 vs. -2.21) were greater for Lopixibat group compared to placebo. GSK2330672 is also being investigated as a potential treatment for pruritus and a phase 2 randomised, double blind, placebo controlled cross-over trial in PBC patients with pruritus has recently been completed and the results are awaited[27].

Limitations

There are few potential concerns with the use of ASBTi. Increased spill over of BAs in the colon is known to cause diarrhoea, which is an adverse event and may limit the regular use of the drugs. Long-term BA malabsorption resulting from ASBTi may produce fat soluble vitamin deficiencies, hyperoxaluria, urolithiasis and increased incidence of pigment and cholesterol gallstones[28]. More importantly, increased BAs in the colon may also have malignant potential for colorectal cancer (CRC) development and this risk needs careful evaluation. In a recent study, ASBT-deficient ($Slc10a2^{-/-}$) mice showed a 54% increase in aberrant crypt foci (earliest histological marker of colon neoplasia) and 70% and 59% increase in colon tumour number and size, respectively[29]. Additionally, gut microbial dysbiosis has been linked to CRC [30,31] and ASBTi induced increased BA load in the colon may adversely affect the gut microbial diversity and increase the risk of CRC. However, there are no data to support or refute these hypothesis in the short-term human studies reported so far. Therefore, long-term clinical studies would be needed to fully understand the potential concerns of CRC with ASBTi.

Other therapies

In addition to ASBTi, other attractive therapeutic agents for cholangiopathies include FXR agonists, FGF19 analogues, *nor*UDCA, PPAR and TGR5 agonists[32]. The mechanism(s) of action of these drugs differs from that of ASBTi with primary effect on inhibiting hepatic BA synthesis and alter the load or composition of hepatic BA pool.

As summarised in **Table 3** the effects of these drugs on BA synthesis, serum BA and FGF19 levels are contradictory and it is ironic that they are all being pursued with a common goal of improving cholestasis. For instance, ASBTi improve cholestasis (at least in animal models) but inhibit FGF19 production in contrast to FXR agonists which also improve cholestasis but stimulate the synthesis of FGF19. It is noteworthy that although FGF19 down-regulates BA synthesis it induces hepatocyte proliferation and prolonged exposure in animal studies has been shown to cause hepatocellular carcinoma [33]. This potential tumorigenic action of FGF19 is of a concern but circumvented by recent development of engineered FGF19 variants without mitogenic actions[34]. Pre-clinical studies suggest FGF19 analogues have potential in the treatment of PBC and PSC. It is equally intriguing that PBC patients treated with FXR agonist (obeticholic acid) that effectively inhibits BA synthesis developed pruritus as an adverse event[35] but ASBTi which cause rebound increase in BA synthesis are being developed to treat pruritus.

In summary, the field of BA based therapeutics in cholestasis is evolving and drugs with paradoxical and diverse BA-mediated effects are being rapidly developed. In future, it is possible

that various combination therapies targeting BA homeostasis is the best way to effectively treat

PBC and PSC[28].

Conclusion

In the quest for novel therapies in PBC and PSC ASBT inhibitors are emerging as important therapeutic options. They block the uptake of BAs in the terminal ileum, increase their excretion in the faeces and decrease the amount of BAs returning to the liver via enterohepatic circulation. Experimental and early clinical studies confirm ASBT inhibitors are effective in reducing total BA levels with compensatory increase in hepatic BA synthesis. Reductions in total and LDL cholesterol are additional advantages which can help treat metabolic diseases. A variety of ASBT inhibitor compounds are emerging and their utility is now being explored across PBC and PSC as well as NASH, with a view to both reducing cholestatic and liver injury and also addressing pruritus management. BA induced diarrhoea is the main adverse event associated with ASBT inhibitors. Future long-term studies will need to explore the impact of these drugs on potential risk of colon cancer development.

Acknowledgements

Disclosure Statement

Figure 1

ASBT inhibitors selectively block the BA transporter ASBT located on the apical side of ileal enterocytes and inhibit BA re-uptake. As a result, BAs spill over into the colon and are lost via faeces. This reduces the amount of BAs returning to liver via portal circulation.
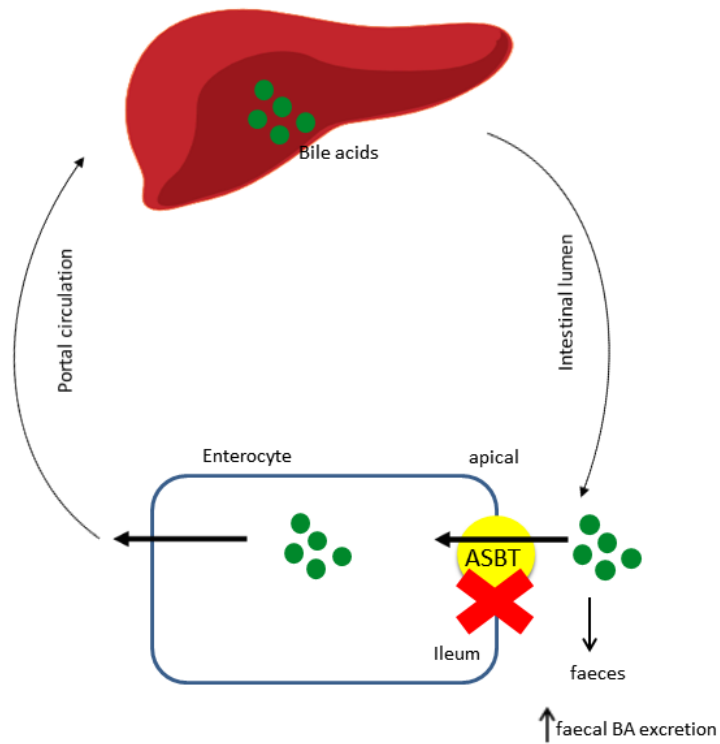
Table 1

Different classes of ASBT inhibitors (ASBTi)

Class	Compound	Comments	Reference
Dimeric bile acid analogues	PB3, S0960,		[36-38]
Benzothiazepine derivatives	2164U90	competitive inhibitor of murine ASBT	[39,40]
	264W94	~500-fold more potent inhibitor than 2164U90	[41]
Benzothiepine derivatives	SC-435	• potent and non-absorbed • increases faecal BA excretion, decreases total and LDL-cholesterol plasma levels and enhances expression of the hepatic LDL receptor	[19,20,42]
Naphthol derivatives	S8921	mixed competitive and non-competitive ASBT inhibitor	[43,44]
4-oxo-1-phenyl-1,4,-dihydroquinoline derivatives	/		[45]

Table 2

ASBT inhibitors in clinical development

Compound	Sponsor	Clinical trial number	Phase	Disease
A4250	Albireo	NCT02360852	2	Cholestatic pruritus
		NCT02630875	2	Paediatric cholestasis
GSK2330672	GlaxoSmithKline	NCT01416324	1	Healthy subjects
		NCT01929863	2	T2DM
		NCT02202161	2	T2DM
LUM001 (SHP-625 or Lopixibat)	Shire	NCT01904058	2	PBC
		NCT02061540	2	PSC
		NCT01903460 NCT02057692 NCT02117713	2	Alagille Syndrome
		NCT02057718	2	PFIC
Volixibat (SHP-626)	Shire	NCT02787304	2	NASH

Abbreviations: ASBT, apical sodium-dependent bile acid transporter; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; PFIC, progressive familial intrahepatic cholestasis ;T2DM, type 2 diabetes mellitus

Table 3

An overview of potential future therapies in cholestatic diseases

	ASBT inhibitors	FXR agonists	FGF19 analogues	*nor* UDCA	PPAR agonists	TGR5 agonists
Examples	Lopixibat chloride (LUM-001), GSK2330672	INT-747 (Obeticholic acid, OCA), INT-767, GW4064, GSK2324, PX-102, MFA-1, Way362450, Fexaramine, LJN452, GS-9674	NGM282, M70	-	Fenofibrate, bezafibrate, GFT505 MBX-8025	INT-777 XL475
Target disease(s)	PBC, cholestatic pruritus	PBC, PSC, NASH,	PBC, PSC, NASH	PSC, NASH	PBC, PSC, NASH	PSC, NASH
Phase of development	II	III (OCA in PBC)	II	II	II III (bezafibrate in PBC)	Preclinical
mechanism(s) of action	↓ intestinal BA reabsorption	↓ hepatic BA synthesis	↓ hepatic BA synthesis	'cholehepatic shunting', induces a bicarbonate-rich hypercholeresis	↓ hepatic BA synthesis, anti-inflammatory	↑bile flow, anti-inflammatory, ↑energy expenditure, ↑ GLP-1, ↑ insulin sensitivity
C4 levels¶	↑	↓	↓	NA¥	NA	NA
Serum BA levels	↓	↓	↓	NA	↓	NA

Serum FGF19 levels	↓	↑	↑	NA	NA	NA
Adverse events (AEs)	diarrhoea, abdominal pain	pruritus (obeticholic acid)	lower GI symptoms, diarrhoea	No serious AEs	Hepatotoxicity (fibrates)	NA
References	[26]	[35]	[46]	[47]	[48,49]	[49]

Abbreviations: ASBT, apical sodium dependent BA transporter; BA, bile acid; GLP-1, glucagon-like-peptide-1; FGF, fibroblast growth factor; FXR, farnesoid X receptor; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cholangitis; PPAR, peroxisome proliferator-activated receptor; PSC, primary sclerosing cholangitis; TGR, transmembrane G protein-coupled receptor.

NA, no available data

¶ C4(7a-hydroxy-4-cholesten-3-one) is an intermediate in the hepatic BA synthesis pathway. Serum C4 levels act as surrogate marker for CYP7A1 enzyme activity.

¥ *nor*UDCA has no relevant affinity to FXR, therefore unlikely to change hepatic BA synthesis.

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