MECHANISMS IN ENDOCRINOLOGY

FXR signalling: a novel target in metabolic diseases

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Abstract

During the last decades, it has become clear that the gastrointestinal tract plays a pivotal role in the regulation of glucose homeostasis. More than 40 hormones originate from the gastrointestinal tract and several of these impact glucose metabolism and appetite regulation. An astonishing example of the gut's integrative role in glucose metabolism originates from investigations into bile acid biology. From primary animal studies, it has become clear that bile acids should no longer be labelled as simple detergents necessary for lipid digestion and absorption but should also be recognised as metabolic regulators implicated in lipid, glucose and energy metabolism. The nuclear farnesoid X receptor (FXR) is a part of an exquisite bile acid-sensing system that among other things ensures the optimal size of the bile acid pool. In addition, intestinal and hepatic FXR also impact the regulation of several metabolic processes such as glucose and lipid metabolism. Accordingly, natural and synthetic FXR agonists and certain FXR-regulated factors (i.e. fibroblast growth factor 19 (FGF19)) are increasingly being evaluated as treatments for metabolic diseases such as type 2 diabetes and non-alcoholic fatty liver disease (and its inflammatory version, non-alcoholic steatohepatitis). Interestingly, decreased FXR activation also benefits glucose metabolism. This can be obtained by reducing bile acid absorption using bile acid sequestering agents (approved for the treatment of type 2 diabetes) or inhibitors of intestinal bile acid transporters, that is the apical sodium-dependent bile acid transporter (ASBT). This article discusses recent clinical trials that provide insights about the role of FXR-FGF19-targeted therapy for the treatment of metabolic diseases.

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Introduction

Today, bile acids are acknowledged as metabolic integrators and in recent years, several clinical and (patho) physiological studies have explored this paradigm from a

human perspective. It has been established that bile acids stimulate the secretion of the gut-derived glucagon-like peptide 1 (GLP-1), an incretin hormone with profound

Invited Author's profile

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glucose-lowering and satiety-promoting capabilities (1, 2). The mechanism behind this phenomenon is believed, in part, to be bile acid-induced activation of the bile acid receptor, Takeda G protein receptor 5 (TGR5), which is located on the basolateral side of intestinal GLP-1-secreting L cells (3). Moreover, bile acids activate the nuclear farnesoid X receptor (FXR) in the intestine and liver, whereby hepatic bile acid synthesis from cholesterol is repressed (4, 5). In recent years, it has been established that FXR is also implicated in metabolic control, possibly via FXR-derived actions on lipid and glucose metabolism, and possibly via alterations in bile acid metabolism (6). Several FXR-related effects are mediated partly through the hormone fibroblast growth factor 19 (FGF19), which is secreted from the intestine upon postprandial bile acid stimulation. FGF19 actions in the liver lead to reduced *de novo* bile acid synthesis via the FGF-receptor 4 (FGFR4) and the co-receptor beta-Klotho, which synergise with the small heterodimer protein (7). Intriguing findings in rodents have demonstrated that FGF19 displays insulin-like actions and that the FXR-FGF-19 axis may constitute a therapeutic target in patients with type 2 diabetes and non-alcoholic fatty liver disease (and its inflammatory version, non-alcoholic steatohepatitis) (8). Indeed, FXR signalling is centrally positioned in the so-called gut-liver axis; a reciprocal interaction that takes place between the gut and its microbiota and bile acids on the one hand, and the liver on the other. Effectively, bile acids produced in the liver regulate microbiota composition and intestinal barrier function, and gut products regulate bile acid synthesis and glucose and lipid metabolism in the liver (see Chávez-Talavera *et al.* (9) for a detailed review of the role of FXR in lipid metabolism). There is growing evidence that the gut-liver axis disruption leads to the progression of most forms of chronic liver diseases (10). The main features of a disrupted gut-liver axis are shared by fatty liver disease, including an altered intestinal microbiota, gut barrier damage and ensuing increased permeability, and changes in luminal levels of bile acids (10).

Since the US approval of the bile acid sequestrant colesevelam for the treatment of type 2 diabetes, the modulation of bile acid receptor activation with subsequent implications for glucose metabolism has been a busy pursuit in many research groups around the world (11). Bile acid sequestrants bind bile acids in the intestinal lumen, diverting them from the enterohepatic circulation. The resulting depletion of the bile acid pool is accompanied by a compensatory increase in hepatic low-density lipoprotein (LDL) receptor expression, which causes a reduction in plasma LDL cholesterol

concentrations. With regard to the glucose-lowering properties (i.e. reduction in haemoglobin A1c (HbA1c) of ~0.5% (12)), the underlying mechanisms remain speculative; however, reduced FXR activation (due to intraluminal trapping of intestinal bile acids) alongside increased TGR5-mediated GLP-1 release seems to play a role (13, 14). Presently, several 'bile acid programmes' targeting metabolic diseases such as type 2 diabetes and fatty liver disease are ongoing, but also other liver diseases including primary biliary cholangitis, primary sclerosing cholangitis, and portal hypertension constitute targets for bile acid-based pharmacotherapy (15).

In the present paper, the role of FXR-FGF19 signalling for metabolic regulation will be outlined, and the apparent paradox that both activation and deactivation of FXR may benefit glycaemic regulation and metabolism will be discussed in the context of recent clinical trials of drugs targeting FXR in metabolic diseases.

Bile acid metabolism and enterohepatic circulation

The rate-limiting enzyme in the classical pathway of bile acid synthesis from cholesterol in the liver is 7- α -hydroxylase (cytochrome P450 7A1, CYP7A1). The primary bile acids are cholic acid and chenodeoxycholic acid, which are conjugated with taurine and glycine and are excreted into bile and stored in the gallbladder (16). Postprandial gallbladder contraction delivers bile acids into the small intestine facilitating digestion and absorption of fat. Most of the conjugated bile acids (~95%) are absorbed in the terminal ileum by active transporters and transported via the portal circulation to the liver and recycled (enterohepatic circulation). The unabsorbed (~5%) cholic acid and chenodeoxycholic acid reaching the colon are deconjugated by bacterial bile salt hydrolases and 7 α -dehydroxylated by bacteria to secondary bile acids, predominantly deoxycholic acid and lithocholic acid. These microbiota-mediated processes increase the hydrophobicity of the bile acids, which support the passive absorption across the colonic epithelium and return to the liver for reuse in the enterohepatic circulation (16). An intermediary product in the synthesis of bile acids, 7- α -hydroxy-4-cholesten-3-one (C4), is present in blood plasma and its plasma concentration has been shown to reflect bile acid synthesis and the enzymatic activity of CYP7A1 (17, 18).

Bile acid receptors – FXR and TGR5

The primary bile acid receptor, FXR, is highly expressed in the intestine and the liver (Fig. 1). It is a natural receptor for bile acids and most potently activated by chenodeoxycholic acid. FXR is a major regulator of human bile acid metabolism via diverse effects on bile acid transport proteins and synthesis of bile acids as a result of intestinal and hepatic FXR activation. Being a nuclear receptor, FXR is activated in ileal enterocytes or hepatocytes when bile acids are absorbed. Intestinal FXR stimulation leads to synthesis and secretion of FGF19, which binds to the hepatic receptor complex FGFR4/beta-Klotho. FGF19 inhibits bile acid synthesis through the inhibition of CYP7A1, but FGF19 also has the ability to activate FGFR4 and to induce hepatocyte proliferation (19). Hepatic FXR activation also leads to the induction of small heterodimer protein, which inhibits the transcription of CYP7A1. In recent years, it has become clear that FXR and small heterodimer protein are central for metabolic homeostasis regulating glucose levels and hepatic fat content, two functions which are finely regulated to maintain a beneficial metabolic state (5, 16, 20).

The TGR5 receptor is located on cholangiocytes, the epithelial surface of the gallbladder and intestinal cells, including the GLP-1-producing enteroendocrine L cells. TGR5 is also found on smooth muscle cells, neural cells, brown adipose tissue, immune cells including dendritic cells and macrophages. TGR5 is most potently activated by

lithocholic acid, a relatively unimportant bile acid. Besides inducing GLP-1-secreting actions (21, 22, 23), several animal models have reported bile acid-mediated TGR5 activation to suppress hepatic macrophage activation, induce gallbladder relaxation and refilling and to promote intestinal motility (11, 24, 25).

The 'FXR conundrum' – activation vs deactivation

As mentioned, bile acid-sequestering therapy reduces FXR activation leading to improved glycaemic control in patients with type 2 diabetes. Such indirect deactivation of FXR signalling represents an example of gut–liver axis disruption, launched by hampering the enterohepatic circulation of bile acids. It has turned out, however, that FXR agonism also exerts beneficial effects on metabolism and glycaemic regulation, which has been demonstrated in both animal and clinical studies (see subsequently). The reason for this discrepancy is far from clear but highlights the enormous complexity of FXR signalling. For example, a recent mouse study demonstrated that the selective inhibition of intestinal FXR attenuated hepatic gluconeogenesis (26), but FXR has also been shown to be required for the stimulatory effects of glucagon on fasting-induced hepatic gluconeogenesis (27). In addition, FXR deficiency increased GLP-1 plasma concentrations (26), supporting an important study by Trabelsi *et al.*, which demonstrated that FXR activation in L cells inhibits

Figure 1

A schematic representation of the enterohepatic circulation of bile acids and the current FXR/FGF19-targeting drug classes under clinical investigation. ASBT, apical sodium-dependent bile acid transporter; CYP7A1, cholesterol 7- α -hydroxylase; FGF19, fibroblast growth factor 19; FGFR4, fibroblast growth factor receptor 4; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide 1; TGR5, Takeda G protein receptor 5.

intracellular glycolytic pathways leading to decreased production and secretion of GLP-1 (14). Conversely, hepatic FXR deficiency in the mouse has been shown to increase gluconeogenesis thus worsening glucose intolerance and insulin resistance (28, 29). Accordingly, both hepatic overexpression of FXR and FXR activation by the FXR synthetic agonist, GW4064, restored glucose intolerance and insulin resistance in mice (30). In rats, the FXR agonist, obeticholic acid, reversed insulin resistance, protected against weight gain and improved lipid abnormalities (31). In fact, several animal studies have shown that the FXR activation represses gluconeogenesis and increases hepatic glycogen synthesis (26, 28, 30, 32), perhaps with the contribution from FXR-mediated increase in FGF19 (33). This apparent 'FXR paradox' could indicate that the role of FXR during the pathogenesis of metabolic dysfunctions might differ between the liver and the intestine (34, 35).

Two clinical studies with obeticholic acid have provided valuable insights into the effects of FXR activation in humans. In the FLINT study, 72 weeks of treatment with obeticholic acid was superior to placebo in improving several pathological aspects of non-alcoholic steatohepatitis including steatosis, fibrosis and inflammation (36). In another study, 6 weeks of treatment with obeticholic acid demonstrated improved placebo-corrected insulin sensitivity in patients with non-alcoholic fatty liver disease and type 2 diabetes together with a reduction in markers of liver fibrosis (37). However, these two studies have provided conflicting results regarding the effect on glycaemic regulation (see subsequently).

Direct FXR antagonism (as opposed to indirect FXR deactivation induced by bile acid sequestering therapy) might be obtained with ursodeoxycholic acid, a natural bile acid used for hepatobiliary disorders including primary biliary cholangitis and cholestatic diseases (16). Ursodeoxycholic acid has been studied intensely in patients with non-alcoholic fatty liver disease (38) and ursodeoxycholic acid-stimulated bile acid synthesis has been known for years (39). Intriguingly, a recent study in patients with non-alcoholic fatty liver disease demonstrated that ursodeoxycholic acid increased the bile acid formation by reducing FGF19, resulting in CYP7A1 induction (mirrored by elevated C4), indicating clear FXR antagonistic properties (40). In two high-dose trials, ursodeoxycholic acid (~2000 mg once daily) demonstrated increased insulin sensitivity in patients with non-alcoholic steatohepatitis and obesity, respectively (41, 42). Also, in a small Japanese study, 16 patients with type 2 diabetes (HbA1c ~7.2%) were randomised to treatment

with 12 weeks of ursodeoxycholic acid (900 mg once daily) followed by 12 weeks sitagliptin (50 mg once daily) or vice versa (43). Ursodeoxycholic acid-induced small decreases in weight and HbA1c when administered before sitagliptin, and HbA1c (but not weight) also decreased when ursodeoxycholic was administered after sitagliptin. Notably, ursodeoxycholic increased early phase GLP-1 secretion (evaluated with a liquid high-fat meal test), indicating bile acid-dependent activation of TGR5 and/or deactivation of FXR (14).

Thus, aside from indirect FXR deactivation induced with bile acid-sequestering therapy (with proven efficacy in type 2 diabetes), direct FXR antagonism may equally impose benefits. Conversely, preclinical and clinical evidence suggest that FXR activation may also be efficacious in improving insulin sensitivity and metabolic diseases such as fatty liver disease. This leaves us with the apparent paradox that both inhibition of FXR signalling and FXR activation may be of therapeutic value in metabolic disease.

Indirect FXR deactivation – insights from clinical studies

The mechanism explaining the beneficial effect of bile acid sequestrants on glycaemic regulation remains to be established (44). As opposed to animal studies, the majority of human studies have not been able to show marked effects on hepatic glucose handling (45), whereas increased splanchnic glucose utilisation may play a role (34). Also, bile acid sequestrants have been suggested to reduce intestinal glucose absorption (46). Our group has shown, in patients with type 2 diabetes, that 2 weeks of treatment with bile acid sequestrants elicited placebo-corrected reductions in plasma glucose concentrations, reduced FGF19 secretion, increased lipogenesis and a shift towards a more hydrophilic bile acid pool, indicating FXR antagonistic signalling (13). Although TGR5 may be activated even by sequesterant-bound bile acids (47), we have been unable to show acute effects of bile acid sequestrants on GLP-1 secretion, which may be in agreement with a basolateral localisation of TGR5 on GLP-1-producing L cells (2, 13, 22).

Inhibition of intestinal bile acid uptake via the specific inhibitor of the apical sodium-dependent bile acid transporter (ASBT) constitutes another way to indirectly reduce intracellular FXR activation – as well as potentially increasing intestinal bile acid-induced TGR5 activation leading to increased GLP-1 secretion

(48, 49). In a randomised, placebo-controlled crossover phase 2 study in patients with type 2 diabetes ($n=15$), 7 days of treatment (add-on to metformin) with the ASBT inhibitor, GSK672 (titrated to 90 mg twice daily), caused a baseline-corrected reduction in weighted mean 24-h plasma glucose of 1.93 mmol/L (95% CI: 0.82–3.04) compared with placebo (50). In a second parallel-group study ($n=75$) in patients with type 2 diabetes, 14 days of treatment with GSK672 10–90 mg twice daily (add-on to metformin) elicited placebo-corrected reductions from baseline in fasting plasma glucose of 1.21 mmol/L (95% CI: 0.28–2.14) and weighted mean 24-h plasma glucose of 1.33 mmol/L (95% CI: 0.35–2.30) (50). The observed glucose-lowering impact of GSK672 was similar to the effects seen after treatment with sitagliptin (third arm). GSK672 also reduced LDL cholesterol (up to a maximum of ~40%) and apolipoprotein concentrations. The most common adverse event associated with GSK672 was diarrhoea (22–100%). Of note, both studies demonstrated reduced insulin concentrations following treatment with GSK672, which might point to an insulin-independent glucose-lowering mechanism of this drug. Interestingly, this finding stands opposite to a previous study in patients with chronic constipation demonstrating that ASBT inhibition elicits increased plasma GLP-1 and reduced plasma glucose concentrations, an effect most likely mediated by increased bile acid-dependent activation of TGR5 in the terminal ileum and colon (48, 49, 51). However, more recent clinical trials with volixibat, a highly potent and selective ASBT inhibitor, did not demonstrate effects on plasma GLP-1 concentrations in patients with type 2 diabetes or non-alcoholic steatohepatitis. Of note, volixibat reduced plasma glucose and insulin to the same extent as GSK672 (52, 53).

Taken together, most data indicate that bile acid sequestrants and ASBT inhibitors work primarily at the enterohepatic level where they may influence the absorption kinetics and hepatic utilisation of glucose via reduced FXR activation. Additionally, TGR5 activation, which increases intestinal motility and GLP-1 production, may play a role.

FXR agonism – insights from clinical studies

Obeticholic acid is a synthetic derivative of chenodeoxycholic acid and a potent and specific FXR agonist (54). As mentioned, the two available studies examining the effect of obeticholic acid on glycaemic control have reported conflicting results in terms of gluco-

metabolic effects. The randomised, placebo-controlled proof-of-concept, phase 2 trial by Mudaliar *et al.* examined the effect of treatment with 25 mg ($n=20$) or 50 mg ($n=21$) obeticholic acid once daily compared to placebo ($n=23$) in patients with type 2 diabetes and non-alcoholic fatty liver disease (37). The hyperinsulinaemic-euglycaemic clamp method was used to assess insulin sensitivity before and after a 6-week treatment period (no additional glycaemic outcomes were reported). The study reported approximately 25% increase in glucose infusion rate in the two obeticholic acid treatment groups, which point to improved insulin sensitivity. However, the absolute increase in glucose infusion rates was small, and there were some baseline imbalance regarding fasting glucose levels, HbA1c and entry level glucose infusion rates, which suggest that the finding of obeticholic acid-induced increase in insulin sensitivity be interpreted with caution. The other study (FLINT) was a randomised, placebo-controlled phase 2 trial of obeticholic acid including 283 patients with non-alcoholic steatohepatitis (without cirrhosis). The study was stopped early when it showed a clear benefit with improved steatosis, lobular inflammation and fibrosis scores on repeat biopsy (36). The primary endpoint was a minimum two-point histological improvement in non-alcoholic fatty liver disease activity score without worsening of fibrosis. After 72 weeks, 50 (45%) of 110 patients receiving 25 mg obeticholic acid daily met the primary endpoint compared to 23 (21%) of 109 in the placebo group ($P < 0.001$). 50% of the 83 patients had type 2 diabetes but no placebo-corrected improvements in HbA1c or basal plasma glucose concentrations following 72 weeks of treatment with 25 mg obeticholic acid once daily were demonstrated. In fact, a statistically significant placebo-corrected deterioration of insulin sensitivity as measured by the homeostatic model assessment of insulin resistance (HOMA-IR) was evident in patients treated with obeticholic acid. Thus, the FLINT study could not corroborate Mudaliar *et al.*'s findings regarding insulin sensitivity. A subsequent *post-hoc* analysis examined the combined effects of weight loss and obeticholic treatment (i.e. additive reduction of alanine aminotransferase and liver histology) on various glycaemic parameters (HbA1c, plasma glucose and HOMA-IR) (55). Two hundred patients were included in the analysis (102 treated with obeticholic acid and 98 treated with placebo) and had similar baseline characteristics compared with the complete study population. Surprisingly, the *post-hoc* analysis demonstrated that the favourable effects of weight loss on alkaline phosphatase, lipids and blood glucose seen in placebo-treated patients were absent or reversed on

obeticholic acid treatment. The subsequent phase 3 trial (REGENERATE) has randomised ~2400 patients (including ~2100 patients with stage 2–3 liver fibrosis) to placebo, 10 mg or 25 mg obeticholic acid daily (56). The study is on-going with patients expected to have follow-up for at least 4 years to evaluate the long-term clinical benefits of treatment but an 18-month interim analysis has been published recently (57). The primary endpoint was defined as improvement of fibrosis by at least one stage with no worsening of non-alcoholic steatohepatitis (histological assessment) or resolution of non-alcoholic steatohepatitis (histological assessment) with no worsening of fibrosis. One thousand nine hundred and sixty-eight patients with stage 1–3 fibrosis were enrolled and received at least one dose of study treatment; 931 patients (539 [58%] females) with biopsy-proven stage 2–3 fibrosis were included in the primary analysis. One of these outcomes (fibrosis improvement without worsening of non-alcoholic steatohepatitis) was achieved in 71 (23%) of the 308 patients in the 25 mg group compared with 37 (12%) of the 311 patients in the placebo group ($P=0.0002$). The other outcome (resolution of non-alcoholic steatohepatitis with no worsening of fibrosis) was achieved in 36 (12%) of 308 patients in the 25 mg group vs 25 (8%) of 311 patients in the placebo group, which was not statistically significant ($P=0.18$). Obeticholic acid treatment had a beneficial effect on other markers of hepatocellular injury such as alanine and aspartate aminotransferase. Also, gamma-glutamyl transferase concentration in plasma, a suggested marker of metabolic and cardiovascular risk (58), declined rapidly and was generally stable at month 3. The most common adverse events of obeticholic acid treatment were increase (~20%) in LDL cholesterol (a class effect of FXR agonists), which was suggested to be transient and reversible by month 6 in those who initiated statins (66 (10%) in the placebo group, 155 (24%) in the obeticholic acid 10 mg group, and 159 (24%) in the 25 mg obeticholic acid group), and dose-dependent mild-to-moderate pruritus (123 (19%) in the placebo group, 183 (28%) in the 10 mg obeticholic acid group, and 336 (51%) in the 25 mg obeticholic acid group). High-density lipoprotein (HDL) cholesterol and triglycerides decreased dose-dependently through the month 18. In terms of hepatobiliary events, more patients in the 25 mg obeticholic group developed gallstones or cholecystitis (3%) compared to placebo (<1%) and 10 mg obeticholic acid (1%). Perhaps, this may be due to increased saturation of cholesterol in the gallbladder and bile acid hydrophobicity, but may also arise via FXR-stimulated FGF19, which is implicated in gallbladder refilling (59).

Approximately 56% of the patients in the REGENERATE trial had type 2 diabetes and, consequently, received antidiabetic medication (not specified in the study). In patients with type 2 diabetes, a transient increase in glucose (from ~7 to ~8 mmol/L) and HbA1c (from ~7 to 7.4%) was observed upon the initiation of obeticholic acid compared to placebo treatment. However, the increases were short-lived and returned to baseline at month 6. In patients without type 2 diabetes, mean plasma glucose concentrations and HbA1c were slightly increased compared to placebo and stayed at this level during all 18 months. However, these changes were small and not likely to be clinically relevant. Thus, based on evidence from the FLINT and REGENERATE trials, obeticholic acid (i.e. potent FXR agonism) does not seem to impact on glycaemic homeostasis in non-alcoholic steatohepatitis patients with or without type 2 diabetes. However, obeticholic acid resulted in a dose-dependent decrease in bodyweight of approximately 2%. Although weight loss is important for this patient population, a weight loss of this size is not expected to influence histological parameters of non-alcoholic steatohepatitis (60). Importantly, the combination of obeticholic acid and statin for curbing obeticholic acid-induced increase in LDL cholesterol has been investigated in a randomised, double-blind, placebo-controlled phase 2 study (CONTROL) in patients with non-alcoholic steatohepatitis ($n=84$) (61). Patients were assigned (1:1:1:1) to placebo or 5 mg, 10 mg or 25 mg obeticholic acid once daily during a 16-week double-blind phase. Once daily atorvastatin treatment (10 mg) was initiated from week 4 with subsequent titration. By week 8, LDL cholesterol concentrations fell below baseline in all treatment groups with no clinical benefit of higher doses. Importantly, the combination was generally safe and well tolerated.

Several other FXR agonists are being evaluated for the treatment of non-alcoholic steatohepatitis (Table 1). As opposed to obeticholic acid, the majority of these newer FXR agonists are non-steroidal (i.e. PX-104/GS-9672/cilofexor and tropifexor), which largely means non-bile acid-type, and lack enterohepatic circulation. Thus, some of these agents are suggested to primarily agonise intestinal FXR, whereas obeticholic acid agonises FXR more systemically (62, 63). PX-104, a synthetic and non-steroidal high-affinity FXR agonist, was studied in an open-label, proof of concept phase 2a trial in 12 non-diabetic patients with non-alcoholic fatty liver disease (64). Liver enzymes (alanine aminotransferase and gamma-glutamyl transferase) and insulin sensitivity (estimated by clamp-like index (CLIX)) improved slightly

Table 1 List of completed and ongoing clinical trials investigating FXR agonists, ASBT inhibitors and FGF19 analogues for the treatment of metabolic diseases and liver diseases.

Name	Company	Drug class	Indication	Status
FXR agonists				
INT-747/obeticholic acid	Intercept pharmaceuticals	FXR agonist (steroid scaffold)	NASH, compensated fibrosis, PBC (approved), PSC	Rejected by FDA (NASH)
PX-102	Gilead (Phenex pharmaceuticals)	FXR agonist	NASH	Phase 1
LJN-452/triopifexor	Novartis	FXR agonist	NASH and PBC	Phase 2
PX-104	Phenex pharmaceuticals	FXR agonist	NAFLD	Phase 2 (discontinued)
GS-9674/cilofexor	Gilead (Phenex pharmaceuticals)	FXR agonist	NASH, PBC, PCS	Phase 2/3
INT-767	Intercept pharmaceuticals	FXR/TGR5 agonist	Liver fibrosis	Phase 1
LMB-763/nidufexor	Novartis	FXR agonist	NASH, diabetic nephropathy	Phase 2
EDP-305	Enanta	FXR agonist (steroid scaffold)	NASH and PBC	Phase 2
EYP001	Eryo pharmaceuticals	FXR agonist	NASH	Phase 2
MET409	Metacrine	FXR agonist	NASH, IBD	Phase 1
TERN-101	Terns pharmaceuticals	FXR agonist	NASH	Phase 2
ASBT inhibitors				
Elobixibat (A3309)	Albireo	ASBT inhibitor	Constipation/NAFLD/NASH	Phase 2
Odevixibat (A4250)	Albireo	ASBT inhibitor	PFIC, Alagille syndrome, PBC, cholestasis	Phase 2/3
Maralixibat (SHP625/LUM001/lopixibat)	Mirum	ASBT inhibitor	PFIC, Alagille syndrome, PBC syndrome, PSC, PBC	Phase 3
Linerixibat (GSK2330672)/GSK-672	GSK	ASBT inhibitor	PBC, T2D, cholestasis	Phase 2/3
Volixibat (SHP626/LUM002)	Mirum	ASBT inhibitor	NASH, T2D	Phase 2
FGF19 analogues/other				
NGM-282/alderfermin	NGM biopharmaceuticals	FGFR4 agonist/FGF19 variant	T2D, PBC, NASH, cirrhosis	Phase 2
NGM-313/MK-3655	NGM biopharmaceuticals	Beta-Klotho/FGFR1 c receptor agonist	Obesity, insulin resistance, NAFLD/NASH	Phase 1

FXR, farnesoid X receptor; IBD, inflammatory bowel disease; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cholangitis; PCS, primary sclerosing cholangitis; PFIC, progressive familial intrahepatic cholestasis; T2D, type 2 diabetes; TGR5, Takeda G protein receptor 5.

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in seven out of eight patients after 4 weeks of treatment, whereas hepatic steatosis (assessed by magnetic resonance spectroscopy) was unaffected. The area under the curves (AUC) of serum glucose, insulin or HOMA-IR did not change, but the AUC of C-peptide measured by OGTT was significantly decreased from baseline to end of the treatment (specific values not reported). No changes in aspartate aminotransferase, alkaline phosphatase or bilirubin concentrations were observed. PX-104 had no effect on serum LDL cholesterol, HDL cholesterol, triglycerides or other of the measured circulating lipids and lipoproteins. There were no serious adverse events, but two patients experienced short intervals of cardiac arrhythmia (isolated polymorphic premature ventricular contractions with a singular ventricular triplet in one patient without symptoms). A relationship between study medication and ventricular extrasystoles could not be ruled out leading to the termination of the study after 12 patients without replacement of previous drop-outs (further drug development was abandoned). Cilofexor/GS-9674, an 'intestinally restricted' FXR agonist (62), was tested in a randomised, double-blind, placebo-controlled phase 2 trial in patients with non-cirrhotic non-alcoholic steatohepatitis, diagnosed by MRI-proton density fat fraction (MRI-PDFF) $\geq 8\%$ and liver stiffness ≥ 2.5 kPa by magnetic resonance elastography or historical liver biopsy. One hundred and forty patients were randomised to receive cilofexor 100 mg ($n=56$), 30 mg ($n=56$), or placebo ($n=28$) orally once daily for 24 weeks (65). The randomisation was stratified by type 2 diabetes status (55% in total). At week 24, patients receiving 100 mg cilofexor had a median relative decrease in MRI-PDFF of 22.7%, compared with an increase of 1.9% in those receiving placebo. The 30 mg-group had a negligible decrease in MRI-PDFF. Serum gamma-glutamyl transferase, C4, and primary bile acids decreased in both treatment groups. Notably, no significant changes were seen in LDL cholesterol, HDL cholesterol or triglycerides. In terms of glycaemic regulation, no clear benefit of cilofexor on serum glucose, insulin, HOMA-IR or HbA1c was demonstrated compared with placebo. However, in patients achieving at least 30% reduction in MRI-PDFF (vs $<30\%$ reduction), a consistent reduction in HOMA-IR (-19% vs 9%), insulin (-23% vs 4%), HbA1c (1.3% vs 4.1%), and body weight (-2.2% vs -0.4%) was demonstrated, indicating improved insulin resistance. Cilofexor was generally well tolerated, but moderate to severe pruritus was more common in patients receiving 100 mg cilofexor (14%) than in those receiving 30 mg cilofexor (4%) and placebo (4%). Similar results have been reported for tropifexor, another non-steroidal

FXR agonist. An interim analysis reported data on a phase 2 study (FLIGHT-FXR) assessing several doses of tropifexor for safety, tolerability, and efficacy in patients with non-alcoholic steatohepatitis (66). At 12 weeks, a significant decrease in hepatic steatosis of at least 5% assessed by MRI-PDFF was observed in 33.3% of patients treated with 90 μg , 27.8% treated with 60 μg , and 14.6% treated with placebo. Furthermore, a dose-response decrease in gamma-glutamyl transferase concentrations was observed as well as increase in FGF19. Adverse events and pruritus were reported to be comparable between 90 μg tropifexor arm and placebo. However, a mild dose-related increase in LDL cholesterol and decrease in HDL cholesterol were observed in the 60 and 90 μg arms. Data on glycaemic regulation were not reported.

FXR agonist combination therapies for the treatment of fatty liver disease are increasingly being pursued, with one of many treatment concepts being combination with GLP-1 receptor agonists, which themselves constitute promising candidate agents in the field (67). Indeed, a recent mouse study demonstrated that the co-administration of obeticholic acid and the GLP-1 receptor agonist IP118 was superior to either monotherapies in terms of reducing hepatic liver enzymes, steatosis, inflammation and fibrosis. Moreover, in diet-induced obese mice, the combination reduced body weight to a greater degree (-25.5%) than IP118 alone (-12.5%) and further improved glucose tolerance and reduced hepatic fat content (68). This treatment concept was recently investigated in a phase 2, proof-of-concept, five-arm trial in non-alcoholic steatohepatitis (69). The trial evaluated combinations of semaglutide, a GLP-1-receptor agonist, with cilofexor and/or firsocostat, an acetyl-coA carboxylase inhibitor. All regimens were well tolerated, and exploratory efficacy endpoints assessing biomarkers of liver health at 24 weeks in *post-hoc* analyses showed statistically significant improvements in hepatic steatosis (measured by MRI-PDFF) and liver injury (measured by serum alanine aminotransferase) in the combination arms vs semaglutide alone.

Taken together, the 18-month interim analysis of the REGENERATE trial provides us with further insights into the metabolic effects of synthetic FXR agonism: the obvious downsides of obeticholic acid therapy are an increase in plasma LDL cholesterol, moderate pruritus, and transient increase in glycaemic parameters, as was also seen in the FLINT trial (36, 55). Indeed, it is worrisome that FXR agonist treatment may necessitate statin therapy for curbing cholesterol increases, especially in patients with a high cardiovascular risk, such patients

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with non-alcoholic steatohepatitis patients. The non-steroidal FXR agonists, however, seem to inflict only minor effects on lipid parameters and appear neutral regarding glucose-lowering activity. On the positive sides of obeticholic acid therapy are a decrease in body weight, serum triglycerides, and gamma-glutamyl transferase, as well as improvement in liver fibrosis and other markers of liver injury (alanine and aspartate aminotransferase), which have been reproduced in the trials with the newer (non-steroidal) FXR agonists. The lack of significant effects on serum lipids of non-steroidal FXR agonists as opposed to obeticholic acid is intriguing, and may reflect a different balance between intestinal contra hepatic/systemic FXR agonism (although other factors such as treatment duration, molecular structures, potencies, patient populations and lipid management may also be at play) (62, 63). According to Gege *et al.*, cilofexor, due to its specific tissue distribution and physicochemical properties, is an intestinally biased FXR agonist, which is still bioavailable but mostly lacks transcriptional activity in the liver (62). Tropifexor, on the other hand, seems less intestinally biased, and, subsequently, yields a mild dose-related increase in LDL cholesterol and a decrease in HDL cholesterol in the 60 and 90 µg arms. The ratio of intestinal- vs liver-specific FXR activation is a topic of intense discussion in the literature, especially concerning lipoprotein cholesterol changes and pruritogenic effects. At this point, clinical studies need to demonstrate how synthetic FXR agonists compare to steroidal ones in this regard. Thus, at first sight, obeticholic acid and other FXR agonists seem well tolerated with mild-to-moderate pruritus being the most common adverse event. However, post market reports of serious liver injury in patients with primary biliary cholangitis receiving obeticholic acid prompted a boxed warning by the Food and Drug Administration in 2018. Finally, the metabolic consequences of FXR agonists warrant caution, as indicated by the FLINT and REGENERATE trials, and, if ever approved for non-alcoholic steatohepatitis, long-term safety and efficacy must be assessed thoroughly, especially regarding cardiovascular risk and liver injury.

FGF19-based therapy in humans

FGF19, the downstream signalling hormone of FXR, has also been suggested for the treatment of metabolic disease (Table 1). However, the FGFR4-dependant mitogenic actions of FGF19 (activation of hepatic FGFR4) have precluded clinical development of FGF19 analogues until

FGF19 variants were generated which lacked the ability to induce hepatocyte proliferation but retained antidiabetic effects in obese mice (for details, see (19, 70)). The FGF19 variant NGM-282 (also known as aldafermin or M70) was tested in a randomised, double-blind, placebo-controlled phase 2 study in 82 patients with biopsy-confirmed non-alcoholic steatohepatitis (70). The patients received either 3 mg or 6 mg s.c. NGM-282 or placebo. The primary endpoint was the absolute change from baseline to week 12 in liver fat content (assessed by MRI-proton density fat fraction). 20/27 (74%) in the 3 mg group and 22/28 (79%) in the 6 mg group achieved at least a 5% reduction in absolute liver fat content from baseline, respectively, vs 2/27 (7%) in the placebo group. Overall, the safety profile was acceptable with most patients expediting grade 1 adverse events (injection site reactions, diarrhoea, abdominal pain, and nausea). As expected, plasma LDL cholesterol concentrations increased following the treatment with both NGM-282 doses, which is consistent with potent FGF19-induced inhibition of CYP7A1 (i.e. reduced cholesterol catabolism). A multicentre, open-label, phase 2 dose finding study was initiated to assess whether co-administration of a statin could manage the cholesterol increase seen in patients receiving treatment with NGM-282 (71). The study showed that the co-administration of rosuvastatin (titrated to 40 mg once daily) with NGM-282 (0.3 mg, 1 mg or 3 mg) from week 2 until end of treatment (week 12) resulted in rapid decline (below baseline) in plasma concentrations of total cholesterol and LDL cholesterol in all dose groups. Importantly, the magnitude and response rate in liver fat reduction were similar to the previously mentioned initial NGM-282 study (70), suggesting that the co-administration of rosuvastatin does not attenuate the efficacy of NGM-282 on steatosis reduction. A longer phase 2 study of 24 weeks (with serial liver biopsies) was conducted in 78 patients with biopsy-proven non-alcoholic steatohepatitis and liver fibrosis (72). The NGM-282 group (1 mg once daily) had a reduction of absolute liver fat content (primary endpoint) of 7.7% vs 2.7% in the placebo group (difference: 5.0%, 95% CI: 8.0–1.9, $P=0.002$). Fibrosis improvement (≥ 1 stage) with no worsening of non-alcoholic steatohepatitis was achieved in 38% of patients receiving NGM-282 vs 18% of patients receiving placebo ($P=0.10$). Resolution of non-alcoholic steatohepatitis was observed in 24% of the patients given NGM-282 vs 9% of patients given placebo ($P=0.20$). A *post-hoc* analysis revealed that concurrent achievement of both fibrosis improvement and resolution of non-alcoholic steatohepatitis was achieved by 24% in the NGM-282 group vs 0% in the placebo group ($P=0.015$).

Interestingly, fewer patients in the REGENERATE phase 3 trial (4% in the obeticholic group vs 0% in the placebo group) achieved this combined endpoint as compared with NGM-282. This efficacy may be attributed to much higher peak plasma NGM-282 concentrations (≥ 10 ng/mL) indicating greater exposure compared with that achieved by FXR agonists, which elevate endogenous FGF19 to peak concentrations of 0.3–2 ng/mL (57, 72, 73).

In terms of glycaemic efficacy, NGM-282 did not induce changes in HbA1c, insulin, glucose or HOMA-IR compared with placebo. Placebo-corrected changes in weight and BMI were also not affected by NGM-282 treatment. Accordingly, in a recent multicentre, randomised, double-blind, placebo-controlled study, 28 days of NGM282/aldafermin failed to relieve hyperglycaemia in patients with type 2 diabetes ($n = 81$) as evidenced by the lack of significant change in plasma glucose or HbA1c (74). Thus, contrary to expectations from the field, FGF19 analogues lack the glucose-lowering activity compellingly demonstrated in animal models when tested in patients with non-alcoholic steatohepatitis or type 2 diabetes. However, NGM-282 has proven highly effective in patients with non-alcoholic steatohepatitis, significantly reducing liver fat content.

Conclusions

In recent years, several animal and human studies have indicated that FXR and bile acid signalling impact on energy, glucose, and lipid metabolism. However, the initial optimism regarding clinical effectiveness towards type 2 diabetes has worn off to some degree as recent clinical trials have been unable to demonstrate significant gluco-metabolic benefit of FXR agonists. Accordingly, although bile acid composition and metabolism in type 2 diabetes is altered, these changes are small and probably of lesser importance seen in light of the massive metabolic changes that arise as a consequence of obesity, fatty liver disease and insulin resistance (6, 10). The lacking optimism with regard to utilising FXR as a treatment target in type 2 diabetes may also reflect the low efficacy of bile acid sequestrant treatment (reduction in HbA1c ~0.5%), despite the fact that these agents lower LDL cholesterol and cardiovascular risk (12, 75, 76). In addition, bile acid sequestrant treatment is cumbersome and has limiting gastrointestinal side effects, and should only be considered in selected patients (77).

The ASBT inhibitors may represent a new strategy to indirectly inhibit FXR signalling, but so far only small

clinical studies have been conducted demonstrating some benefit in terms of glycaemic regulation. The intriguing concept of direct FXR antagonism has never been examined in large clinical studies in diabetes. However, a specific FXR antagonist, HS218, has been shown to suppress gluconeogenesis in mouse primary hepatocytes, and improve glucose homeostasis in mice with type 2 diabetes. This effect was achieved by inhibiting the FXR-induced increase in the promoter activity of the key gluconeogenic gene peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1-alpha), leading to the repression of PGC-1-alpha and inhibition of peroxisome proliferator-activated receptor alpha (PPAR-alpha) (78).

While agents with FXR antagonistic properties have moved slightly out of the limelight, FXR agonists are continuously gaining dominance in the targeting of FXR. As noted, however, although FXR plays a role in metabolic physiology and disease, spanning from obesity to fatty liver disease and type 2 diabetes, current trials remind us of the need for a careful re-examination of previous conclusions drawn from animal studies regarding the role for FXR in glucose regulation and that the clinical development of FXR agonists (and FGF19 analogues) should focus instead on liver diseases. Indeed, activation of the FXR-FGF19 signalling pathway for the treatment of fatty liver disease constitutes a promising treatment concept, suggesting that this is where the future of FXR agonists (and FGF19 analogues) lies. Here, numerous FXR agonists combat against a multitude of candidate agents striving to be the first approved drug for the treatment of non-alcoholic fatty liver disease, one of the last uncharted territories in the pharma landscape – and a projected US \$15 billion market (62, 79). This achievement, however, is a difficult task as recently demonstrated by the Food and Drug Administration's rejection of obeticholic acid for fibrosis associated with non-alcoholic steatohepatitis (80). Time will tell whether the non-steroidal FXR agonists (i.e. cilofexor or tropifexor), alone or in combination with other agents, will gain more success, that is confirmative phase 3 trials, than their predecessors.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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