Pleiotropic functions of bile acids mediated by the farnesoid X receptor

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Abstract

In addition to their well-established role in the digestion and absorption of dietary lipids, bile acids (BAs) are recognized as signalling molecules in a wide range of metabolic processes. Bile acids regulate their own metabolism and enterohepatic circulation by activating the farnesoid X receptor (FXR). BAs have been shown to affect lipid metabolism, to decrease levels of circulating triglycerides, improve hyperglycemia and insulin signalling, directly act on the arterial wall and protect hepatocytes against cholestatic liver injury. Given that BAs are an integrated part of the complex metabolic network regulated by FXR, acting as a major underlying pathway, specific therapeutic targeting of this nuclear receptor represents an attractive therapeutic approach for a wide range of disorders. During a phase II clinical trial, the administration of a semisynthetic BA derivative 6-ethyl-chenodeoxycholic acid (6-ECDCA) to patients with diabetes, non-alcoholic fatty liver disease (NAFLD) and primary biliary cirrhosis (PBC), led to encouraging results, despite side effects being observed in pre-clinical studies. Chemical manipulations of the side chain and the steroid nucleus of BAs could lead to the discovery of novel semisynthetic BA derivatives that are more specific and selective FXR activators. (Acta gastroenterol. belg., **2012**, 75, **389-398**).

Key words : metabolism, diabetes, atherosclerosis, cholestasis, liver.

Introduction

In addition to their well-established role in the digestion and absorption of dietary lipids, bile acids (BAs) are recognized as signalling molecules in a wide range of metabolic processes, such as lipid, glucose and energy homeostasis. The BA mediated activation of the nuclear farnesoid X receptor (FXR) is a major underlying pathway for these effects (1). As a functional protein in the vasculature, FXR has been shown to counteract pro-inflammatory and pro-atherogenic responses in cardiovascular disease (2). Furthermore, by regulating BA synthesis, transport and detoxification as well as inflammation and fibrosis, FXR plays a pivotal role in hepatocyte protection during cholestasis. Alterations in BA signalling may contribute to the changes in lipid and glucose metabolism that are linked to some of the most prevalent diseases of western societies, such as type II diabetes, cardiovascular disease and non-alcoholic fatty liver disease (NAFLD). Most of these conditions are treatable today, but not (yet) curable. Unfortunately, combined drug treatment for the individual metabolic risk factor is often needed. The strategy of using a synergistic drug combination may reduce polypharmacy, which can reduce medical costs and improve patient compliance. However, alternative therapeutic strategies involve targeting all or multiple risk factors with single therapies. Given that FXR modulates each of these pathways, therapeutic targeting of this nuclear receptor *via* specific and potent agonists opens new perspectives for the treatment of numerous metabolic disorders (3).

The structure of the farnesoid X receptor

The farnesoid X receptor (FXR, NR1H4) is one of the 48 ligand-activated nuclear transcription factor proteins that detect the intracellular presence of specific hydrophobic molecules and respond by modulating the expression of target genes. FXR is classified as a nuclear BA receptor, since the BAs, at physiological concentrations, have been found to be the most potent endogenous ligands for this receptor (4). Two FXR genes (FXRα and FXRβ) have been identified in mammals. FXRβ is a separate gene in rodents, but it is a pseudogene in humans and does not function as a bile acid receptor. The FXRα gene (located on chromosome 12q23.1) encodes four FXR isoforms (FXRα 1-4), which are the result of the differential use of two promoters and an alternative splicing between two different sites in exon 5 (5). The four isoforms are expressed in a tissue-dependent manner. FXR is mainly expressed in hepatocytes, biliary epithelium, small bowel enterocytes, renal tubular cells and adrenal glands. Low expression levels have been reported in adipocytes, beta pancreatic cells, cardiac muscle, vascular endothelial and smooth muscle cells, lymphocytes and monocytes, whereas it has not been detected in the brain and skeletal muscle (6).

FXR protein has a typical nuclear receptor (NR) structure composed of modular domains (Fig. 1) (7). A highly conserved DNA-binding domain (DBD) in the N-terminal region, which contains two zinc finger motifs, interacts with specific DNA sequences (FXR response element, FXRE) in the promoter of its target genes. The C-terminal region is a moderately conserved ligand binding domain (LBD) that binds to natural or pharmaceutical ligands. The Hinge region is a structural

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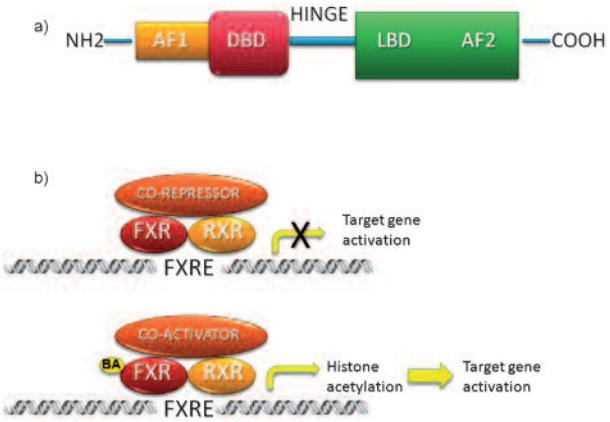

Fig. 1. — Molecular biology of FXR. a) Protein structure of FXR. An N-terminal region contains highly conserved DNA-binding domain (DBD) and ligand-independent AF1 transactivation domain. A C-terminal region contains moderately conserved ligand binding domain (LBD) and ligand-dependent AF2 transactivation domain. A hinge region connects the DBD and LBD. b) FXR forms a heterodimer with retinoid X receptor (RXR) that binds to FXR-response element (FXRE), a DNA sequence in the promoter of its target genes. In the absence of ligand, the FXR heterodimer is associated in complex with co-repressor, leading to transcriptional repression. Following ligand binding to the LBD of FXR, the heterodimer undergoes conformational changes, leading to the release of co-repressor and the recruitment of co-activator proteins. Co-activator proteins possess a histone-acetyltransferase activity that allows the transcription of target gene.

domain modulating the receptor activity after phosphorylation of key amino acids. A ligand-independent transactivation AF-1 domain and a ligand-dependent transactivation AF-2 domain, which mediate interactions with co-regulatory proteins after receptor activation, are located in the N- and C-terminal regions, respectively. FXR is, in a similar manner to other NRs, an obligate partner of the retinoid X receptor (RXR). The FXR/RXR heterodimer usually binds (but not exclusively) to the DNA sequences composed of two inverted repeats separated by one nucleotide (IR1) and can be activated by ligands for both receptors (BAs and / or 9-*cis*-retinoic acid). Ligand binding induces conformational changes of the FXR protein causing the release of co-repressor proteins and the recruitment of co-activator proteins in an AF-2 dependent manner, which promote chromatin remodelling and activation of transcription machinery on the target gene (7).

FXR agonists

BAs and their conjugates are both endogenous ligands for FXR. The primary hydrophobic BA, chenodeoxycholic acid (CDCA), is the most potent natural activator of human FXR (EC~10 M) (8). Secondary BAs : deoxycholic (DCA) and lithocholic acid (LCA), which are the products of gut metabolism of primary BAs, can also activate FXR, but to a much lesser extent than CDCA. Primary cholic acid (CA) is a weak FXR agonist, whereas hydrophilic ursodeoxycholic acid (UDCA) cannot activate FXR (9). Therefore, the manipulation of the gut microbiome may change the bile salt pool and bring about associated changes in FXR mediated transcriptional pathways (10). In addition, BAs can activate other nuclear receptors, including the pregnane X receptor (PXR), the constitutive androstan receptor (CAR), the vitamin D receptor (VDR) and the plasma membrane G protein-coupled receptor, TGR5, and thus, modulate a number of different cell signalling pathways (11). Since BAs can activate multiple signalling pathways, a number of specific synthetic (GW4064 and fexaramine) and a semi-synthetic (6E-CDCA) agonists have been developed in order to dissect FXR-specific signalling (12). The potent non-bile acid derivative FXR ligand, GW4064, has been used in a number of animal studies, but its limited bioavailability has prevented its evaluation in clinical trials. 6E-CDCA (EC~0.1 M) has about 100-fold higher agonistic activity than the most potent natural ligand, CDCA (13). Thus far, 6E-CDCA has been evaluated in clinical trials for liver diseases, such as primary biliary cirrhosis, NASH and type II diabetes mellitus (14,15).

FXR regulates bile acid metabolism

BAs are amphipatic, detergent-like molecules, synthesized in the liver from cholesterol. Due to their physicochemical properties, which allow them to facilitate the intestinal absorption of lipophilic nutrients, BAs may be cytotoxic molecules in higher concentrations. BA cytotoxicity increases linearly with hydrophobic index in the following order : UDCA < CA < CDCA < DCA < LCA. Therefore, the size and composition of the BA pool need to be tightly controlled. The primary role of FXR is the regulation of intracellular BA levels in the liver (16). This is achieved by regulation of the expression of genes involved in BA synthesis, transport and detoxification (Fig. 2).

Primary BAs are synthesized in a multistep process. The majority of primary BAs are formed *via* the so-called classical pathway and only about 6 % *via* the alternative pathway. Currently, there are two FXR-dependent mechanisms for the inhibition of CYP7A1, the rate-limiting enzyme in the classical BA biosynthetic pathway that defines the size of BA pool (17). In the liver, FXR induces expression of the atypical NR, small heterodimer partner (SHP/NR0B2) that lacks a DNA binding domain and is a common transcriptional repressor of nuclear receptors (18). SHP forms non-functional heterodimers with DNA binding activators, including nuclear receptors, and inhibits their transcriptional activity. The interaction of SHP with liver-related homolog-1 (LRH-1/NR5A2 or human α-fetoprotein transcription factor) results in the inhibition of CYP7A1 transcription (19). In addition to CYP7A1, SHP inhibits

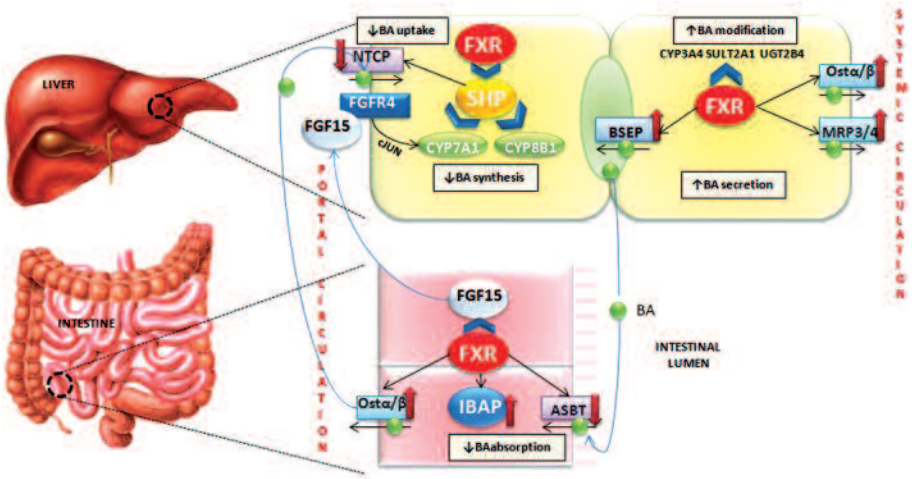

Fig. 2. — Regulation of bile acid homeostasis by hepatic and intestinal FXR.
Under physiological conditions, bile acids activate FXR in the liver. Activated FXR induces SHP, which inhibits the transcription of CYP7A1 and CYP8B1 gene. In the intestine, activated FXR induces FGF15/19, an intestinal hormone that induces FGFR4 on the hepatocytes and via cJUN inhibits CYP7A1. FXR not only suppresses synthesis of bile acids, but also regulates its enterohepatic circulation. In the liver, FXR induces biliary bile acid excretion by BSEP and MRP2 transporters, located at the apical membrane of hepatocytes. Under cholestatic conditions, FXR induces hepatocyte basolateral transporters Ostα/β and MRP3/4, providing an alternative excretion route for bile acids into the systemic circulation. In the ileum, FXR decreases reabsorption of conjugated bile acids via ASBT, while inducing IBAP and Ostα/β promotes enterohepatic bile acid circulation. Phase I (CYP3A4) and phase II (SULT2A1 and UGT2B4) of bile acid detoxification are also positively regulated by FXR, rendering bile acids more hydrophilic and less toxic.

CYP8B1, an enzyme of the acidic pathway that controls the hydrophobicity of the BA pool by controlling the ratio of CA to CDCA. This is achieved through the SHP-mediated repression of hepatocyte nuclear factor-4α (HNF4α/NR2A1) (20). By inducing the expression of SHP, BAs inhibit the transcription of other genes *via* FXR, such as sterol-regulatory-element-binding-protein-1c (SREBP1c), in order to decrease *de novo* lipogenesis.

CYP7A1 gene regulation has an important impact on liver and terminal ileum metabolism. FXR induces the expression of mouse ileal fibroblast growth factor 15 (FGF15, the rodent ortholog of human FGF19). This peptide hormone is transported to the liver *via* portal blood and activates the hepatocyte FGFR4 receptor, a widely distributed receptor linked to tyrosine kinase activity that represses CYP7A1 through a c-Jun n-terminal kinase (JNK)-dependent pathway (21). The human ortholog, FGF19 has recently been shown to be expressed in both the intestine and the liver, in contrast to the ileal specific expression of mouse FGF15 (22). In addition to its effect on the regulation of BA synthesis, FGF-15/19 has been shown to reduce body weight in animal models and improve insulin signalling, leading to associated effects on metabolic syndrome (23). Thus, FGF-15/19 and its receptor, FGFR4, are potential targets for pharmacological manipulation in metabolic disorders.

Before its secretion into the bile, BAs are activated by acetyl-CoA and conjugated with taurine or glycine. This process is catalyzed by the BA CoA synthase (BACS) and BA-CoA :amino acid N-acetyltransferase (BAAT) enzymes that are positively regulated by FXR (24). Conjugated BAs are actively secreted into the bile *via* two ABC transporters : the bile salt export pump (BSEP, ABCB11) and the multidrug resistance-associated protein 2 (MRP2, ABCC2). Both of these receptors are directly induced by FXR at the transcriptional level in order to maintain the orthograde biliary BA output and prevent the accumulation of BAs in the liver and consequent liver injury (25,26). The human phosphatidylcholine export pump (MDR3/2, ABCB4) is also positively regulated by FXR. MDR3 is essential for the maintenance of the BA / lipid ratio in bile in order to prevent bile duct injury by non-micellar bound BAs. Mutations in the BSEP and MDR3 proteins are responsible for progressive familial intrahepatic cholestasis (PFIC) type 2 and 3 (27,28). When orthograde biliary BA output is reduced, retrograde BA secretion *via* basolateral Ostα/Ostβ or MRP3/4 represents an alternative elimination route through the urinary tract. This efflux system is directly transactivated by FXR under cholestatic conditions (29).

The indirect activation of FXR in the distal ileum, *via* SHP and LRH-1, downregulates the expression of the apical sodium-dependent bile salt transporter (ASBT) at the enterocyte brush border membrane, which decreases the reabsorption of conjugated BAs (30). By inducing both the ileal bile acid-binding protein (IBAP), which promotes the movement of BAs from the apical to the basolateral membrane of enterocytes, and the basolateral efflux system, Ostα/Ostβ, FXR induces secretion of BAs into the portal circulation. As in the intestine, the FXR-regulated active transport system for BAs exists in the

cholangiocytes and proximal renal tubular cells (31). Hepatic uptake of BAs is mediated by a sodium-dependent bile acid transporter, NTCP (SLC10A1), and a family of multi-specific organic anion transporters (OATPs ; SLC21A). Both of these transporters are down-regulated indirectly, *via* SHP, with a consequent decrease in hepatocyte BA uptake (32).

In addition to the regulation of their own synthesis and transport, BAs, by activating FXR, induce the enzymes involved in BA detoxification. BAs are detergent molecules, inherently cytotoxic for hepatocytes, and their accumulation in the liver is associated with cholestasis-induced hepatic injury. The ability of FXR to induce BA biotransformation and elimination is more evident under pathological conditions (e.g. cholestasis). Phase I of biotransformation – hydroxylation (mediated by CYP3A4) and phase II – conjugation with sulfate (mediated by dehydroepiandrosterone sulfotransferase 2A1, SULT2A1) and glucuronidate (mediated by UDP-glucuronosyltransferase 2B4, UGT2B4) are positively regulated by FXR (33-35). These reactions render BAs more hydrophilic and less toxic, allowing urinary excretion of BAs, which can become the favored elimination route for BAs accumulating under cholestatic conditions.

The role of FXR in cholesterol metabolism and atherosclerosis

Activation of FXR, by downregulating CYP7A1, reduces the conversion of cholesterol into BAs, which represents the main pathway for cholesterol elimination in the body. This was confirmed by the observation that the long-term administration of CDCA in humans with gallstone disease produced a slight increase in LDL cholesterol levels (36). Opposing this effect of FXR activation on cholesterol homeostasis is the effect of FXR activation in ileal enterocytes where it leads to decreased bile salt absorption and thus increased loss of bile salts by intestinal excretion. Furthermore, *in vitro* studies performed in human hepatocyte cell lines demonstrated that CDCA increases LDL receptor gene expression and activity (37).

Another unfavourable effect in subjects treated with CDCA is a decline in high density lipoprotein (HDL) levels, reported in a different set of clinical studies (38). FXR activation reduced the expression of apolipoprotein A1, the primary constituent of HDL, which defines its size and shape. This observation raises concerns regarding the therapeutic value of FXR agonists. However, recent data showed that cardioprotective HDL activity was determined by the capacity to transport cholesterol from peripheral tissues to the liver, not by the HDL level *per se* (39,40). Therefore, there is a need to establish whether FXR activation can modify HDL composition and hence the capability of HDL to remove cholesterol from peripheral cells, by activating the lecithin-cholesterol acyl transferase enzyme and delivering the resulting cholesteryl ester to the liver. Another FXR target is paraoxonase-1 (Pon-1) whose function is the inactivation of proatherogenic lipids produced by oxidative modification of LDL. FXR represses Pon-1 activity through the FGF15/19 – FGFR4 – JNK pathway (41). On the other hand, FXR activation seems to contribute to reverse cholesterol transport, a process that results in the delivery of cholesterol from peripheral tissues to the liver for biliary disposal and consequent intestinal elimination. This effect occurs due to the regulation of the expression of phospholipid transfer protein (PLTP), which is responsible for the transfer of phospholipids and cholesterol from LDL to HDL, as well as the expression of the scavenger receptor, B1 (SR-B1), which is involved in the uptake of HDL by the hepatocytes (42).

In accordance with previous observations, activation of FXR has both anti and pro-atherosclerotic properties (Fig. 3). In addition to its systemic influence on dyslipidemia and hyperglycemia, FXR may also directly act at the level of the arterial wall. Even though the vascular wall is not typically involved in BA metabolism, FXR has been found to be expressed in the vascular smooth muscle and endothelial cells (43). FXR activation may have a beneficial effect on vascular tone, vascular inflammation and neointimal proliferation. Activation of FXR regulates vascular tone by repressing the potent vasoconstrictive peptide, endothelin-1 (ET-1), and by inducing the production of the vasodilating agent, nitric oxide (NO) (44,45). Furthermore, FXR activation increases the production of physiological vaso-relaxant agent, hydrogen sulphide (H_2S), by vascular smooth muscle cells, which reduces the portal pressure and attenuates the endothelial dysfunction of isolated and perfused cirrhotic rat livers (46).

Activation of FXR by the synthetic ligands : 6-ECDCA and GW4064, inhibits inflammatory responses and the migration of vascular smooth muscle cells by inhibiting interleukin-1β-induced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), by reducing NF- B activation in a SHP-dependent manner. FXR ligands also reduce the migration of both rat and human aortic vascular smooth muscle cells induced by platelet-derived growth factor-β (PDGF-β) (47). However, CDCA, DCA and LCA treatment induces the expression of adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1) and E-selectin, whereas GW4064 only increases adhesion molecule expression at high concentrations. This induction of adhesion molecules promotes adhesion of monocytes to endothelial cells, a crucial step in the initiation of the atherosclerosis process (48).

A complete picture of the role of FXR activation in atherosclerosis is not yet clear. Translating the findings from rodent models to humans is not straightforward because the cholesterol is mostly transported by HDL and the data with regard to atherosclerosis are often contradictory. Development of tissue-specific and gene-selective FXR agonists would provide the avoidance of

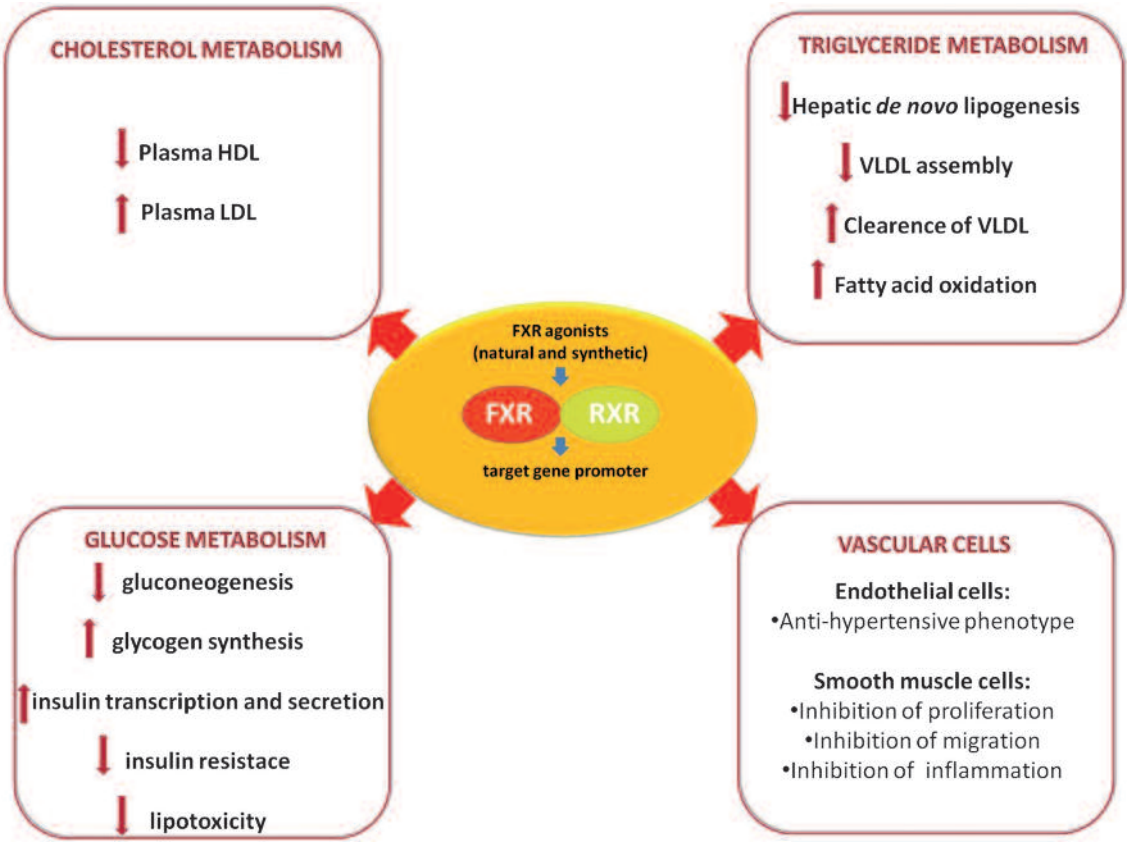

Fig. 3. — Pleiotropic effects of FXR activation.
The activation of FXR/RXR heterodimer by bile acids or synthetic ligands, regulates the expression of genes involved in cholesterol, triglyceride and glucose metabolism. FXR activation leads to the decrease in glucose and triglyceride plasma levels, improved insulin secretion and peripheral insulin sensitivity. FXR activation is associated with decreased HDL and increased LDL cholesterol levels, two undesirable effects in the patients with cardiovascular risk. However, FXR activation in the arterial wall contributes to vasodilatation and anti-atherogenic phenotype, which may counteract the dyslipidemic effect.

the side effects that are potentially detrimental in patients with cardiovascular risk.

Bile acids and triglyceride metabolism

Very important data obtained nearly four decades ago showed the significant role of BAs in triglyceride metabolism regulation. The administration of CDCA reduced plasma triglyceride levels, both in individuals suffering from the gallstone disease and in those with familial hypertriglyceridemia (49,50).

The molecular mechanism underlying the hypotriglyceridemic effect of CDCA has been linked to FXR activation. Indeed, FXR activation regulates triglyceride metabolism at different levels. FXR is involved in the control of hepatic *de novo* lipogenesis, one of the fatty acid sources used for the assembly of VLDL. Both BAs and synthetic FXR agonists, *via* SHP, repress the transcription of sterol regulatory element-binding protein 1c (SREBP-1c), a key regulator of several genes involved in fatty acids and triglyceride synthesis, including fatty acid synthase (FAS) (51). Furthermore, the expression of microsomal triglyceride transfer protein (MTP), which facilitates the transfer of triglycerides, cholesterol esters, and phospholipids to newly synthesized apoB, is downregulated by FXR. Serum triglyceride levels reflect the balance between production and clearance of triglyceride-rich lipoproteins, such as VLDL and chylomicrons. Lipoprotein lipase (LPL) is a key enzyme involved in the lipolysis of these particles. Apolipoprotein (apo) C-III is an inhibitor of LPL activity, whereas apoC-II and apoA-V are LPL activators. FXR activation induces apoC-II expression and human apoA-V promoter activity in liver cells. Conversely, the FXR/RXR heterodimer represses the expression in the liver of apoC-III, a major constituent of VLDL that inhibits lipoprotein lipase (52,53).

FXR facilitates the clearance of VLDL by inducing the expression of the VLDL receptor, a protein that plays a major role in the metabolism of postprandial lipoproteins by enhancing LPL-mediated triglyceride hydrolysis (54). VLDL is highly homologous to the LDL receptor, but is present in trace amounts in the liver. However, this receptor is highly expressed in skeletal muscle, heart, adipose tissue and macrophages. FXR also increases the expression of syndecan-1, a transmembrane

proteoglycan that binds the remnant particles before their transfer to receptors. In human primary hepatocytes, FXR ligands induce the expression of peroxisome proliferator-activated receptor α (PPARα) and its target gene, pyruvate dehydrogenase kinase-4 (PDK-4), in order to promote fatty acid oxidation (55).

Collectively, these findings support the concept by which FXR activation decreases the plasma triglyceride levels by suppressing hepatic lipogenesis and triglyceride secretion and by increasing the clearance of triglyceride-rich lipoproteins from the blood (Fig. 3). These observations suggest that FXR activation may have a beneficial effect in patients with hypertriglyceridemia and NAFLD, two common features of the metabolic syndrome.

Bile acids and glucose homeostasis

Over a number of years, a growing body of evidence has shown that BA metabolism is altered in patients with type II diabetes, and that modification of the BA pool can improve glycemic control in such patients. This raises the question as to whether the changes in BA homeostasis are either a cause or a consequence of the metabolic disturbances observed in the course of diabetes and whether the regulation of BA homeostasis may improve diabetes-associated metabolic complications (56).

FXR null mice develop the signs of insulin resistance (57). Activation of FXR by agonists or hepatic overexpression of constitutively active FXR by adenovirus-mediated gene transfer, have been shown to reduce the blood glucose levels in murine models of obesity and diabetes (58). These results indicated that BAs are involved in the regulation of glucose metabolism by FXR-mediated pathways (Fig. 3).

The liver controls blood glucose levels by modulating gluconeogenesis, glycogen synthesis and glycolysis. Via FXR activation, BA have been shown to decrease the hepatic glucose production as a consequence of down regulation of the gluconeogenic program (59). CDCA treatment of human hepatoma cell lines decreased expression of the genes involved in gluconeogenesis, including phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase) and 1,6-bisphosphatase (60). Another recent study has shown that intestinal hormone, FGF15/19, an FXR target, suppressed postprandial hepatic gluconeogenesis (61). In addition to the regulation of hepatic glucose production, FXR also improves regulation of hepatic glycogen synthesis, one of the principal pathways in glucose homeostasis (62).

FXR deficiency leads to impaired glucose tolerance and insulin resistance. Animal models of diabetes showed decreased levels of FXR expression, an effect that could be reversed by the administration of insulin (63). FXR deficiency in mice is associated with glucose intolerance, and insulin resistance in adipose tissue and skeletal muscle, which was reflected by reduced peripheral glucose disposal (64,65). Since FXR is not expressed in muscle cells and very low levels are detectable in white adipose tissue, it is conceivable that the molecular mechanisms behind the peripheral insulin sensitizing effect of FXR could be indirect. The phenomenon of 'lipotoxicity' is described in the absence of the FXR, based upon ectopic lipid deposition in insulin target tissues. FXR deficiency is characterized by increased circulating free fatty acid (FFA) levels and elevated intramuscular trygliceride and FFA contents. Tissue-specific overexpression of lipoprotein lipase in skeletal muscle has been shown to specifically induce muscle insulin resistance. Furthermore, the excess circulating FFAs exerts a number of deleterious effects on mitochondrial function and insulin signalling (66). A similar mechanism operates in beta pancreatic cells, which causes a reduction in insulin secretion (67). Hence, the activation of FXR decreases the levels of triglycerides and FFA may reduce the 'lipotoxicity' phenomenon and restore insulin secretion and sensitivity. Moreover, maintenance of insulin sensitivity at the level of the adipocytes is essential for preventing the inappropriate release of fatty acids, hepatic triglyceride accumulation and lipotoxic injury to hepatocytes, recognized as non-alcoholic steatohepatitis (NASH) (68).

Pancreatic β cells express FXR mRNA and protein. 6-ECDCA significantly induces insulin transcription and secretion by murine β-TC6 cells and human pancreatic islets. FXR knockdown in β-TC6 cells abrogates these effects induced by 6-ECDCA, implying that insulin transcription and secretion are FXR dependent processes (69).

BAs may also affect glucose homeostasis in an FXR-independent fashion. Modulation of the BA pool by administration of BA sequestrants in patients with type II diabetes, improved glycemic control and decreased plasma haemoglobin A1c levels (70). Administration of BA sequestrants, in addition to having a lipid lowering effect, has been shown to increase the glucagon-like 1 peptide (GLP-1) from intestinal L-cells through the activation of membrane BA receptor, TGR5 (71). Tauro-UDCA improves insulin resistance by attenuating endoplasmic reticulum stress, which contributes to the development and progression of a number of diseases, including neurodegenerative disorders, diabetes, obesity, cancer and cardiovascular disease (72,73).

Given that BAs and their derivatives are amphiphilic steroids, BAs have been recognized as transport promoters with significant potential to facilitate the absorption of different therapeutic agents. Bile salts increase the permeability of membranes, both to low molecular weight compounds as well as to macromolecules, such as insulin (74). Moreover, BAs have also been shown to potentiate the action of hypoglycaemic and hypolipidemic agents (75-77).

Therefore, the potential use of BAs in the regulation of glucose homeostasis could be achieved by two main approaches : as hypoglycaemic agents (*via* FXR-dependent and independent pathways) and as absorption-

enhancing agents (78). The modification of the BA pool size or its composition (by BA substitution or intestinal sequestration), therapeutic manipulation of the intestinal flora microenvironment by probiotics (with secondary effects on the BA pool composition) and manipulation of BA receptors, such as FXR, represent attractive and promising strategies in the development of new anti-diabetic agents.

FXR and cholestasis

Cholestasis is a pathological condition characterized by impairment or cessation of the bile flow with consequent liver damage (necrosis, fibrosis and cirrhosis). Irrespective of the aetiology of cholestasis, the accumulation of toxic hydrophobic BAs in the liver is thought to play a key role in cholestasis-associated liver damage. Thus, reductions in hepatic BA overload and the hydrophobic index of the BA pool are recognized as therapeutic goals for the management of cholestasis. The use of UDCA, a polar BA that reduces the hydrophobicity and toxicity of the BA pool, is the only pharmacological intervention recognized for the management of cholestasis (79). The discovery of FXRs new function, as the master regulator of BA homeostasis, makes this receptor an attractive pharmacological target for the management of cholestatic liver disease (Fig. 3). As mentioned above, the activation of FXR reduces the BA pool size by downregulating CYP7A1 and CYP8B1 expression, decreasing hepatocyte BA uptake *via* NTCP and increasing biliary BA excretion *via* BSEP (BA-dependent bile flow) and MRP2 (BA-independent bile flow) (32). In addition to stimulating the orthograde, biliary excretory route, FXR activates the alternative, retrograde BA overflow in systemic circulation *via* OSTα/β and MRP3 transporters, as well as in phases I and II of detoxification, which stimulate renal BA excretion under cholestatic conditions (80). Therefore, when intrahepatic and systemic BA levels rise, FXR orchestrates an adaptive response in order to counteract potential liver injury. Systemic FXR activation by specific ligands : 6-ECDCA and GW4064, has been shown to reduce liver damage in animal models of cholestasis induced by bile-duct ligation (BDL), α-naphtylisothiocyanate (ANIT) and ethinyl estradiol (81,82).

The protective effects of FXR activation are ascribed to reduced BA synthesis and hepatocellular uptake, as well as induced biliary BA and phospholipid excretion into the bile. Stimulation of reduced transporter function may be beneficial for cholestasis conditions where transporter defects are the causative factors (e.g. oestrogen-induced cholestasis, and hereditary cholestatic diseases, such as progressive familial intrahepatic cholestasis, sepsis-associated cholestasis and intrahepatic cholestasis of pregnancy) (83). However, most of the clinically relevant cholestatic disorders are the consequence of bile duct obstruction (e.g. large bile duct obstruction by stones or tumours, small bile duct obstruction, as observed in primary sclerosing cholangitis (PSC), or bile duct loss (i.e. vanishing bile duct syndromes, such as late stage PBC). Therefore, the induction of the canalicular transport systems by hepatic FXR activation may be detrimental. Stimulation of bile flow with hydrophilic ursodeoxycholic acid (UDCA) in a mouse model of sclerosing cholangitis and in bile duct ligated mice increased liver injury, aggravated bile infarcts and induced hepatocyte necroses. Increased liver injury is caused by increased biliary pressure due to UDCA's choleretic activity leading to rupture of cholangioles (84). During cholestasis, the enterohepatic circulation of BAs is perturbed, due to a significant decrease of BAs in the intestinal lumen, while, at the same time, accumulating in the liver. The absence of BAs in the intestine during obstructive extrahepatic cholestasis leads to intestinal bacterial overgrowth and translocation across the intestinal mucosal barrier, which can result in systemic infection (85). Modica *et al.* (86) have shown that selective activation of intestinal FXR protects against obstructive extrahepatic cholestasis, both in the liver by reducing the size and hydrophobic index of the total BA pool and in the intestine by preserving the intestinal mucosa integrity associated with a reduction of bacterial translocation and subsequent inflammation. Furthermore, selective activation of intestinal FXR, in a mouse model with constitutively active intestinal FXR, was sufficient to protect against the inherited form of cholestasis, such as PFIC3 (progressive familial intrahepatic cholestasis type 3, induced by mutation of MDR2 transporters) (86). It has been recently shown that the livers of cholestatic patients were able to ectopically express FGF19, thus the increase in FGF19 expression represented an adaptive hepatic response in order to protect against the progression of cholestasis (22). Therefore, it would be of great interest to study the mechanisms of hepatoprotection by intestinal FXR and its downstream target, FGF15/19 activation and their role in expression of BA transporters in enterohepatic and non-enterohepatic tissues during cholestasis.

Because long-term cholestasis leads to the development of biliary cirrhosis, direct inhibition of fibrosis may also be an attractive strategy. FXR appears to antagonize hepatic inflammatory processes by antagonizing the nuclear factor kappa B pathway (87). Activation of FXR by 6-ECDCA reduces liver fibrosis in a rodent model of bile duct obstruction, hepatic stellate cells trans-differentiation *via* SHP and the peroxisome proliferator-activated receptor γ (PPARγ) (88). Since FXR was barely detectable in human HSC and myofibroblasts, there has been concern about these findings (89). However, during phase II studies, administration of 6-ECDCA in patients with primary biliary cirrhosis (PBC) with incomplete response to UDCA, showed significant improvement in biochemical cholestasis parameters, which suggested that 6-ECDCA may be a potential first-line agent for the treatment of PBC (14). The major side effect was pruritus, which was found to be dose related. Other cholestatic diseases, such as primary sclerosing

cholangitis and cystic fibrosis, would be attractive candidates for a future study. However, the potent choleric effects of FXR agonists suggest that caution should be exersized in patients who may have an obstructive component to their cholestatic liver disease (90).

Conclusion and prospects for future research

During the past decade, the discovery of FXR as a key regulator of BA metabolism has increased understanding of the molecular basis behind BA homeostasis and enabled a link to be made between BAs and other signalling molecules that regulate metabolism. Modulation of FXR activity may be exploited for the pharmacological management of the all aspects of metabolic syndrome, as well as for liver disorders, such as NAFLD, PBC and cholestatic liver disease.

Since the activation of FXR may have potentially undesirable effects, the identification of tissue or gene-selective FXR modulators may enhance specificity and reduce the side effects of this therapeutic approach. At this point, the lack of clinical data impedes the final judgement of FXR as a pharmacological target in humans. However, it is very encouraging that 6-ECDCA improved insulin signalling and reduced triglyceride levels in patients with diabetes mellitus and NAFLD (15), as well as improving biochemical cholestatic parameters in patients with PBC (14). Chemical manipulations of the side chain and the steroid nucleus of BAs may lead to the discovery of novel semisynthetic BA derivatives that could be more specific and selective FXR activators. Furthermore, the accumulating knowledge in BA signalling and FXR function, derived from *in vitro* experiments and animal models of different metabolic conditions, represents the essential basis for translation of these findings to safe clinical use.

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