The Farnesoid X Receptor
A Molecular Link Between Bile Acid and Lipid and Glucose Metabolism

Thierry Claudel, Bart Staels, Folkert Kuipers

Abstract—Bile acids are the end products of cholesterol metabolism. They are synthesized in the liver and secreted via bile into the intestine, where they aid in the absorption of fat-soluble vitamins and dietary fat. Subsequently, bile acids return to the liver to complete their enterohepatic circulation. The Farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily and has emerged as a key player in the control of multiple metabolic pathways. On its activation by bile acids, FXR regulates bile acid synthesis, conjugation, and transport, as well as various aspects of lipid and glucose metabolism. This review summarizes recent advances in deciphering the role of FXR in the context of hepatic lipid and glucose homeostasis and discusses the potential of FXR as a pharmacological target for therapeutic applications. (Arterioscler Thromb Vasc Biol. 2005;25:2020-2031.)

Key Words: bile acid ■ FXR ■ glucose metabolism ■ lipid ■ nuclear receptor

As a direct consequence of the obesity epidemic,1–3 the prevalence of the metabolic syndrome is increasing at an alarming rate.4 Almost 25% of the adult US population is currently affected, and the situation is even worse in people older than 60 years.5 The metabolic syndrome has been defined by the National Cholesterol Education Program as a cluster of at least 3 of 5 criteria: insulin resistance and glucose intolerance, abdominal obesity, hypertension, low high-density lipoprotein (HDL) cholesterol, and hypertriglyceridemia.6 Given the fact that the metabolic perturbation associated with the metabolic syndrome predisposes to cardiovascular diseases and stroke,7 novel and more specific therapeutic strategies are urgently needed. Certain ligand-activated nuclear receptors provide promising new targets for this purpose. In this review, we discuss the biology of the Farnesoid X receptor (FXR, NR1H4), recently identified as a modulator of lipid and glucose homeostasis.

Nuclear receptors are transcription factors that, on ligand binding by specific molecules and cofactor recruitment, regulate the expression of specific target genes.8 FXR is highly expressed in liver and intestine8 and was cloned during a search for new Retinoid X receptor (RXR or NR2B1) heterodimers.9,10 Originally described as a farnesol-activated receptor interacting with RXR, and accordingly named, FXR was later identified as a
receptor that is activated by bile acids (Figure 1). Subsequently, triterpenoids like forskolin were shown to increase FXR activity in a cell-based assay. More recently, polyunsaturated fatty acids (PUFA) like arachidonic, linolenic, or docosahexaenoic acid, as well as intermediates of the bile acid synthetic pathways, were shown to be FXR ligands and modulators in vitro. Bile acid intermediates could be important FXR ligands during cholestasis or inborn metabolic disorders when these compounds can potentially be present in large amounts. In addition, several pharmacological FXR ligands have been generated.

After ligand binding, FXR binds to DNA elements called FXR response elements (FXREs). Interestingly, FXR can bind to and activate or repress through a large variety of FXREs, either as a monomer or as a FXR/RXR heterodimer (Figure 2). The recent identification of several FXR cofactors, together with the description of the ligand-binding domain structure, will help to elucidate how the FXR complex is stabilized and interacts with RNA polymerase II to modulate transcriptional activity.

FXR is expressed from a single gene locus in humans (chromosome 12q23.1). Two alternative promoters, with the presence of an internal cryptic splicing site, lead to the expression of 4 isoforms called FXRα1/FXRα2 and FXRα3/FXRα4 (referred to as FXRβ1/FXRβ2) that are not equivalent in term of gene transactivation. Interestingly, the organization of the FXR locus is conserved in rodents, but because it is a pseudo-gene in primates and because its expression pattern in rodents is confined to the reproductive tract, it is not discussed in this review.

**FXR Regulates Bile Acid Synthesis, Transport, and Detoxification**

Bile acids are synthesized from cholesterol exclusively by the liver. The biosynthetic steps that collectively accomplish the conversion of water-insoluble cholesterol molecules into more water-soluble compounds also confer detergent properties to the bile acids that are crucial for their physiological functions in bile formation and intestinal fat absorption. On conjugation with glycine or taurine, bile acids are actively secreted by the hepatocytes into the bile canaliculi that drain into intrahepatic bile ducts, stored in the gallbladder, and expelled into the intestinal lumen in response to a fatty meal. In the small intestine, bile acids act as detergents to emulsify and facilitate the absorption of dietary fats and lipid-soluble vitamins. Subsequently, they are reabsorbed from the terminal ileum by specific transporter proteins: 95% return to the liver to be secreted again into the bile, completing the so-called enterohepatic circulation, whereas 5% escape re-absorption and are lost via the feces. The fraction that is lost per cycle is compensated for by hepatic synthesis from cholesterol, which maintains the bile acid pool size constant. Although the fractional loss per cycle is relatively small, daily bile acid synthesis in adult humans amounts up to 500 mg, which accounts for 50% of total cholesterol turnover.

Two major pathways, generally referred to as the neutral and the acidic pathway, are involved in bile acid synthesis. CYP7A1 is the first and rate-controlling enzyme of the neutral pathway and partly controlled by a negative bile acid feedback loop, whereas CYP27A1, the main enzyme of the acidic pathway, is not regulated by bile acids (Figure 3). CYP7A1 was known for many years to be under a feedback control at the transcriptional level, but it appeared that FXR itself does not bind the putative bile acid response elements (BARE) in its promoter. Two groups independently demonstrated that FXR activation induces expression of the atypical nuclear receptor small heterodimer partner (SHP or NR0B2). SHP, in turn, interacts with 2 other nuclear receptors that transactivate CYP7A1 expression via the BARE region, ie, the hepatic nuclear factor 4 (HNF4 or NR2A1) and the liver receptor homolog-1 (LRH-1 or NR5A2) (Figure 4). SHP repression of CYP7A1 gene transcription occurs by promoting the dissociation of coactivators linked to HNF4 and LRH-1, as well as by histone deacylation of the promoter.
FXR also modulates CYP7A1 expression by induction of fibroblast growth factor-19 (FGF-19) expression. On its secretion, FGF-19 activates the hepatic FGF receptor-4, which, in turn, downregulates CYP7A1 through c-Jun N-terminal kinase activation (Figure 4). Several other FXR-independent mechanisms are involved in the regulation of CYP7A1 expression by bile acids, but they are beyond the scope of the present review. CYP8B1, the enzyme controlling 12α-hydroxylation and thereby the hydrophobicity of the bile acid pool, was also suggested to be under negative bile acid regulation, possibly via a SHP/HNF4α-independent mechanism (Figure 4).

Bile acids are conjugated to taurine or glycine, by sequential actions of the enzymes bile acid coenzyme A (CoA) synthetase (BACS) and the bile acid-CoA amino acid N-acetyltransferase (BAT), to increase their hydrophilicity in a process regulated by FXR. Conjugated bile acids require a transporter network to cycle between liver and intestine, which is to a certain extent also under FXR control. Bile acids are secreted by hepatocytes into the bile canaliculi by the bile salt export pump (BSEP or ABCB11) via an ATP-dependent process; BSEP mutations underlie progressive familial intrahepatic cholestasis type II (PFIC II), an inborn cholestatic liver disease. BSEP expression is induced by FXR at the transcriptional level. Because relatively hydrophobic bile acids are potentially toxic, protective mechanisms such as oxidation by CYP3A4, sulfation by dehydroepiandrosterone-sulfotransferase SULT2A1, or glucuronidation catalyzed by uridine glucuronosyltransferase 2B4 all have evolved (Figure 3). SULT2A1, UGT2B4, and CYP3A4 all are positively regulated by FXR by means of a noncanonical inverted repeat 0 (IR-0), a monomeric site, and 2 response elements (an ER-8 and another IR-1/DR-3 site), respectively. Interestingly, FXR also induces the expression of the multidrug resistance-associated protein 2 (MRP2 or ABCC2), a multispecific ABC transporter able to excrete sulfated and glucuronidated bile acids into the bile, via an everted repeat-8 (ER-8) site (Figure 3).

In the ileum, bile acids are efficiently taken up by enterocytes via the ileal apical sodium-dependent bile acid transporter (ASBT, also called intestinal bile acid transporter [IBAT] or SLC10A2). FXR indirectly influences the expression of ASBT, but directly induces the expression of the intestinal bile acid binding protein (IBAB-P or FABP6) (Figure 3). It is generally assumed that IBAB-P provides a shuttle allowing bile acids to traffic from the apical to the basolateral side of the enterocytes during their absorption. However, the fact that FXR-deficient mice were found to display enhanced intestinal bile acid absorption despite an extremely low IBAB-P expression demonstrates that the real physiological function of IBAB-P remains unresolved. At the basolateral side, bile acids are believed to be secreted into the portal vein either by a truncated form of ASBT (tASBT), or by the multidrug resistance-associated protein 3 (MRP3 or ABCC3) or by the newly described Oat/sβ transporters (Figure 3).

The uptake of bile salts that return to the liver after intestinal absorption is mainly mediated by the Na+-taurocholate cotransporting polypeptide (NTCP or SLC10A1). Bile acids downregulate NTCP expression via a FXR-dependent mechanism, but NTCP expression is not changed in FXR-deficient mice compared with wild-type controls. A potential mechanism involves SHP activation that inhibits RXR/RAR (retinoic acid receptor or NR1B1) transactivation of the promoter (Figure 3). Approximately 75% of uptake occurs by this Na+-dependent process; yet, another family of transporters is also involved, the organic anion transporter polypeptides (OATPs). OATP-C (or SLCO1B1) is the most ubiquitously expressed OATP transporter in human hepatocytes.
cytes, but it is currently unknown whether FXR is involved in its regulation (Figure 3). In mice, it has been reported that bile acids regulate OATP-1 (or Slco1a1) expression,71,73 but the basal expression of OATP-1 was not modified in FXR-deficient mice.65,71

Taken together, FXR activation in hepatocytes will suppress de novo bile acid synthesis, accelerate their biliary excretion and detoxification, and simultaneously limit their import. Therefore, FXR can be considered as a bile acid sensor that has evolved to maintain the functional unit of the enterohepatic circulation of bile acids and to protect liver (and perhaps also intestinal) cells from potential deleterious consequences of cellular bile acid overload.

An Emerging Role for FXR in Control of Lipid Metabolism

Several older clinical studies already suggested a role of bile acids in the control of lipid metabolism (Table 1). It is well-established that bile acid sequestrants like cholestyramine and colestipol, as well as ileal resection decrease plasma total and low-density lipoprotein (LDL) cholesterol. Interestingly, patients on bile acid supplementation display low high-density lipoprotein (HDL) cholesterol.22 In these transgenic mice, feeding of the FXR agonist GW4064 and natural bile acids reduced apoA-I expression both in human primary and immortalized hepatocytes22 (Figure 4). The apoA-I promoter analysis demonstrated that FXR binds the so-called C footprint as a monomer.22 Recently, another article suggested that LRH-1 is able to upregulate human apoA-I gene expression by the C-site.85 Because SHP is able to interact with LRH-1 and to decrease its activity (Figure 4), it was suggested that FXR by induction of SHP could repress human apoA-I expression. Nevertheless, the fact that SHP-deficient mice display no lipid phenotype86,87 without any change in total cholesterol levels87 (which is mainly carried in HDL in the mouse), and the fact that apoA-I was not identified in the microarray study performed on livers from SHP-deficient mice86 show that the suggested mechanism, if it exists, is not important to maintain basal levels of apoA-I expression. Intriguingly, the LRH-1 binding site mapped was not identical to the FXR binding site. Because several species-differences were reported with respect to the role of LRH-1 in bile acid synthesis regulation,85,87–91 it is unfortunate that the authors used mouse LRH-1 to investigate

<table>
<thead>
<tr>
<th>Study (ref)</th>
<th>Total Cholesterol</th>
<th>LDL Cholesterol</th>
<th>HDL Cholesterol</th>
<th>Triglyceride</th>
<th>Comments and/or Results</th>
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<td>Shepherd76</td>
<td>–32%</td>
<td>–32%</td>
<td>HDL chol unchanged; HDL2/HDL3 apoA-I synthesis increased; apoA-II not changed</td>
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<td></td>
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<td>–17%</td>
<td>–26%</td>
<td>+8%</td>
<td>+28%</td>
<td>Moderate chol-lowering diet; –24% CAD; and –19% nonfatal CHD</td>
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<tr>
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<td>–20%</td>
<td>+2%</td>
<td>+10%</td>
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<tr>
<td>Betteridge82</td>
<td>–23%</td>
<td>–31%</td>
<td>No change with pravastatin or cholestyramine</td>
<td>+18%</td>
<td>Comparison pravastatin/cholestyramine</td>
</tr>
<tr>
<td>POSCH77</td>
<td>–23%</td>
<td>–37%</td>
<td>+4% apoA-I increased</td>
<td>+19% and VLDL chol+18 %</td>
<td>Intestinal resection decreased chol absorption and increased fecal sterol excretion, decreased BA reabsorption</td>
</tr>
</tbody>
</table>

BA indicates bile acid; CAD, coronary artery disease; CHD, coronary heart disease; Chol, cholesterol; VLDL, very-low-density lipoprotein.

FXR and HDL Metabolism

HDL carries cholesterol from the peripheral organs to the liver, where it can be excreted into the bile as either free cholesterol or after conversion into bile acids. FXR-deficient mice are hypercholesterolemic because of an increase in HDL cholesterol levels71 and show elevated plasma apoA-I concentrations. In human apoA-I transgenic mice, FXR activation decreased total cholesterol because of a reduction of HDL cholesterol.22 In these transgenic mice, feeding of the FXR agonist taurocholic acid reduced mouse as well as human apoA-I gene expression by the C-site.85 Because SHP is able to interact with LRH-1 and to decrease its activity (Figure 4), it was suggested that FXR by induction of SHP could repress human apoA-I expression. Nevertheless, the fact that SHP-deficient mice display no lipid phenotype86,87 without any change in total cholesterol levels87 (which is mainly carried in HDL in the mouse), and the fact that apoA-I was not identified in the microarray study performed on livers from SHP-deficient mice86 show that the suggested mechanism, if it exists, is not important to maintain basal levels of apoA-I expression. Intriguingly, the LRH-1 binding site mapped was not identical to the FXR binding site. Because several species-differences were reported with respect to the role of LRH-1 in bile acid synthesis regulation,85,87–91 it is unfortunate that the authors used mouse LRH-1 to investigate
FXR and Triglyceride Metabolism

Chow-fed FXR-deficient mice are clearly hypertriglyceridemic, suggesting, in line with the clinical studies mentioned, a role for FXR in the control of triglyceride metabolism.

Because serum triglyceride levels reflect the balance between production and clearance of triglyceride-rich lipoproteins such as very-low-density lipoprotein and chylomicrons, and because lipoprotein lipase (LPL) is a key enzyme involved in the lipolysis of these particles, several groups have explored the effects of FXR activation on LPL cofactors. 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. However, natural and synthetic FXR agonists were found to repress hepatic apoC-III expression in mice and in human primary hepatocytes. Interestingly, promoter analysis, together with chromatin immunoprecipitation assays, revealed that the FXR/RXR heterodimer represses apoC-III expression. Moreover, FXR induces the expression of the very-low-density lipoprotein receptor, a protein that plays a major role in the metabolism of postprandial lipoproteins by enhancing LPL-mediated triglyceride hydrolysis. In addition, expression of syndecan-1, a transmembrane protein that binds remnant particles before their transfer to receptors, was found to be FXR-sensitive. Thus, FXR controls a variety of genes crucially involved in triglyceride metabolism in the blood compartment.

Surprisingly, FXR also regulates the expression of peroxisome proliferator-activated receptor (PPAR)-α in humans. PPARα is a nuclear receptor that is activated by fatty acids and by fibrates, a class of synthetic hypolipidemic drugs. Because PPARα activation decreases plasma triglyceride levels, probably by enhancing fat oxidation, but also modulates bile acid composition and synthesis, this implies that FXR controls bile acid and triglyceride metabolism both directly and indirectly and that a coordinated regulation of fatty acid and bile acid metabolism occurs also in humans.

Several studies have shown expression of "the" FXR target gene CYP7A1 to be reduced in rats and piglets during fasting, ie, a situation in which PGC1α and FXR are activated in vivo. In addition, the synthetic FXR agonists were found to repress hepatic apoC-III expression in mice and in human primary hepatocytes. Intriguingly, whereas 2 groups demonstrated that PGC1α was recruited in a ligand-dependent manner and through the interaction between a charge clamp (helix 3 and 12) on FXR and the LXXLL motif of PGC1α, another group showed that FXR interacts with PGC1α in a ligand-independent manner via its DNA binding domain without requirement of the LXXLL domain of PGC1α. These latter investigators suggested a specific role for FXR in triglyceride metabolism during fasting, because triglyceride levels were increased in fasted FXR-deficient mice instead of being decreased as it is observed in fasted wild-type mice. This was mechanistically ascribed to inhibition of SREBP1c expression because of induction of SHP expression by FXR/RXR/PGC1α. The fact that, according to these authors, PGC1α and FXR interact in a ligand-independent manner implicates that bile acids are not directly involved in the control of these pathways. In contrast, other investigators demonstrated that SREBP1c expression is negatively regulated by bile acids via SHP induction. These discrepancies could be caused by the fact that some investigators studied human whereas others studied murine FXR in vitro systems. In addition, the synthetic agonist used in one of these studies is known not to regulate the same genes as natural bile acids do, probably because of the so-called specific bile acid receptor modulator (SBARM) effect that postulates that FXR adopts a different conformation on binding of chemically distinct ligands.
with respect to the FXR/PGC1α pathway, other groups studying the consequences of fasting in mice\textsuperscript{112,113} showed that \textit{Cyp7A1} expression is stimulated by PGC1α.\textsuperscript{112} This would suggest that induction of PGC1α during fasting is not linked to FXR signaling. The events that dictate the specificity of PGC1α interactions with FXR or other nuclear receptors in complex situations like fasting need to be addressed in more detail in the future. Taken together, FXR decreases triglyceride levels by: (1) increasing their clearance by modulating LPL activity; (2) inducing PPARα in humans; and probably (3) inhibiting SREBP1c in mice.

**Role of FXR in Glucose Homeostasis**

A first piece of evidence for a link between bile acid and glucose metabolism came from a short-term study in patients with noninsulin-dependent diabetes mellitus (NIDDM) or type II diabetes.\textsuperscript{114} Patients with high LDL cholesterol but normal triglyceride levels, using either glyburide or insulin to control glycemia, were treated with cholestyramine or placebo. Cholestyramine treatment lowered plasma glucose by 13% and decreased urinary glucose excretion, with a tendency toward lower glycosylated hemoglobin concentrations. At the same time, cholestyramine reduced total and LDL cholesterol and increased triglyceride levels. This study therefore identified bile acid sequestrants, which are not absorbed, as a potential option to treat type II diabetes. It will be of interest to determine whether the ASBT inhibitors, like S 8921115 or SC 435,\textsuperscript{116} that selectively interfere with bile acid reabsorption also regulate glycemia in diabetic patients. Nevertheless, because an increase of unbound bile acids entering the colon might have adverse consequences, it will be necessary to evaluate the intestinal effects of this treatment in detail before assessment of their potential impact in terms of metabolism.

More recently, studies demonstrated a link between triglyceride levels and gallbladder diseases.\textsuperscript{117,118} Because FXR controls triglyceride metabolism, because hypertriglyceridemia is associated with type 2 diabetes, and because bile composition is altered in diabetic patients,\textsuperscript{119,120} evaluation of a potential link between FXR and glucose metabolism was initiated. First, FXR was identified as a gene positively regulated by glucose.\textsuperscript{121} In cultured hepatocytes, glucose was shown to induce \textit{Fxr} gene expression, probably via metabolites of the pentose phosphate pathway, whereas insulin counter-regulated this effect. Moreover, \textit{apoC-III} gene expression was additively repressed by glucose and the synthetic FXR agonist GW4064 in cultured cells and \textit{Cyp7A1} gene expression was inversely correlated with \textit{Fxr} gene expression in livers of diabetic rats. Given the fact that hypertriglyceridemia is a common feature in diabetes, it is tempting to speculate that FXR dysregulation by glucose participates in the development of the diabetic phenotype or, conversely, that FXR modulation could reverse part of the lipid abnormalities associated with this condition. Interestingly, a patient with homozygous familial hypercholesterolemia (LDL receptor deficiency) was found to have increased synthesis rates of cholesterol and bile acids when fed a normal diet.\textsuperscript{122} On a high-glucose diet, this patient showed a decrease in total, HDL, and LDL cholesterol levels, whereas plasma triglyceride levels increased. At the same time, at least one large cutaneous xanthoma disappeared, demonstrating that the decline in plasma cholesterol was not caused by accelerated storage in peripheral organs. Fecal balance studies showed that the high cholesterol and bile acid synthesis rates in this patient decreased on ingestion of the high-glucose diet.\textsuperscript{122} Because FXR expression is induced by glucose,\textsuperscript{121} because FXR activation decreases HDL cholesterol,\textsuperscript{22} and because FXR suppresses bile acid synthesis,\textsuperscript{123} it is tempting to speculate that the high-glucose diet increased hepatic FXR expression, which, in turn, was followed by repression of \textit{apoA-I} and \textit{CYP7A1} expression. Finally, the fact that not only homozygous familial hypercholesterolemic patients\textsuperscript{123–125} but also normocholesterolemic men\textsuperscript{126,127} on total parenteral nutrition (a diet extremely rich in glucose) display a significant reduction in plasma cholesterol and a decreased excretion of bile acids suggest that glucose interference with FXR signaling is likely to constitute a novel mechanism involved in the control of plasma cholesterol levels.

Recently, in vitro and in vivo data showed that bile acids modulate gluconeogenesis by regulating the expression of the rate-controlling enzyme phosphoenolpyruvate carboxykinase (PEPCK), as well as of glucose-6-phosphatase (G6Pase) and fructose-1,6-biphosphatase (FBP1).\textsuperscript{113,128} Bile acid treatment in mice reduced gene expression of PEPCK.\textsuperscript{113} Subsequently, De Fabiani et al demonstrated that FXR was not required to downregulate PEPCK expression in vitro, because incubation of cells with bile acids reduced hepatic nuclear factor 4α (HNF4α) transactivation of the PEPCK promoter, whereas a synthetic FXR agonist did not. A similar interference of bile acids with HNF4α transactivation potential was already described to explain the negative regulation of \textit{CYP7A1} without SHP requirement.\textsuperscript{120} HNF4α is a major regulator of PEPCK expression and a master regulator of hepatic gene expression\textsuperscript{130} and is also involved in the maturity onset diabetes of the young type 1 (MODY1).\textsuperscript{131} More recently, Yamagata et al showed that PEPCK, FBP1, and G6Pase are negatively regulated by bile acid treatment in vivo, effects that they ascribed to induction of SHP, which subsequently interacts with HNF4α, to repress PEPCK and FBP1, or Foxo1 to repress G6Pase, respectively.\textsuperscript{128} Nevertheless, the mechanism is still hypothetical because these authors did not eliminate SHP from cells and did not use \textit{SHP}-deficient mice. Moreover, the physiological consequences of activation of these regulatory pathways under physiological (fasting/feeding transition) or pathophysiological (insulin resistance) conditions of altered glucose homeostasis have not yet been studied.\textsuperscript{132}

During the fasting period when gluconeogenesis is induced, \textit{Cyp7A1}, \textit{Pepck}, \textit{Ppara}, and, paradoxically, \textit{Fxr} gene expression are upregulated.\textsuperscript{113,133} Because \textit{Fxr}-repressed genes such as \textit{Cyp7A1} and \textit{Pepck} are upregulated, this implies that FXR control is weak in this condition, probably because bile acids are not circulating and are stored in the gallbladder. At the same time, it is important to note that \textit{SHP} gene expression is not changed during fasting.\textsuperscript{133} Therefore, not only is the induction of FXR is important but also is the
TABLE 2. FXR Modulators Approved or in Clinical Development

<table>
<thead>
<tr>
<th>Research Name</th>
<th>Status</th>
<th>Use</th>
<th>Comments and Side Effects</th>
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<tr>
<td>AGN-34</td>
<td>Phase 3 clinical trial</td>
<td>Hypercholesterolemia or colon cancer</td>
<td>Liver toxicity and metabolism unknown</td>
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<tr>
<td>Guggulsterone</td>
<td>Traditional herbal medicine</td>
<td>Hypercholesterolemia</td>
<td>Efficiency questioned; able to activate several signaling pathways, rash</td>
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<td>CDCA (eg, chenofalk)</td>
<td>Not approved in European Union, safety concern, extract from bovine bile</td>
<td>Originally gallstone dissolution, cerebrotendinous xanthomatosis</td>
<td>Liver toxicity, diarrhea, transient effects</td>
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<tr>
<td>6-Ethyl CDCA</td>
<td>(INT-647)</td>
<td>?</td>
<td>Liver fibrosis</td>
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<tr>
<td>CA (eg, cholifalk)</td>
<td>Not approved in European Union, safety concern, extract from bovine bile</td>
<td>C27 3beta-hydroxy steroid oxidoreductase deficiency or delta^3-3 oxo-reductase deficiency</td>
<td>Liver toxicity and pruritus</td>
</tr>
<tr>
<td>UDCA (eg, ursofalk, ursodiol)</td>
<td>approved</td>
<td>Gallstone dissolution, cholestatic liver diseases, and PBC</td>
<td>No side effects</td>
</tr>
</tbody>
</table>

CA indicates cholic acid; CDCA, chenodeoxycholic acid; PBC, primary biliary cirrhosis; UDCA, ursodeoxycholic acid.

bioavailability of its ligands crucial to obtain a physiological effect on gene expression.

Finally, it is remarkable that glucose is almost absent in human bile despite the fact that hepatocytes do secrete glucose into bile. Therefore, glucose is effectively re-absorbed from the biliary tract by an active mechanism in a so-called bilohepatic circulation. Because FXR deactivation controls glycemia and glucose controls FXR expression, it will be of interest to determine whether FXR is involved in control of this glucose bilohepatic cycle.

FXR: Therapeutic Implications

Dyslipidemia

Increasing HDL and lowering LDL cholesterol and triglyceride levels by dietary or pharmacological means remain the most important goals to reach in dyslipidemic patients. Currently, statins are the first choice drugs for the treatment of hypercholesterolemia. Given the fact that “resin effects” (cholestyramine, colestipol, colesvelam) may be partly caused by FXR deactivation, a mechanistic basis for the additive responses on statin and resin combination therapy could be solved by FXR modulation changes triglyceride and HDL cholesterol levels in the same direction (Figure 5), a simple FXR agonist or antagonist will have undesired side effects from a therapeutic point of view. The recent description of FXR partial agonists or BARMs that do not activate the entire spectrum of classical FXR targets suggests that such compounds, like AGN-34, can be generated (Table 2). Nevertheless, it is impossible that a single BARM will emerge to cover the wide range of therapeutic goals to treat hypertriglyceridemia, mixed dyslipidemia, or hyperlipidemia, diseases that all have causes that could be solved by FXR modulators.

Recently, much attention has been paid to natural BARM E and Z-guggulsterones, which are the active components of guggulipid, a tree resin extract used in traditional Indian medicine to treat obesity and lipid disorders. In vitro experiments showed that guggulsterone acts as a FXR antagonist and a pregnane X receptor (PXR) agonist. Wild-type mice fed high-cholesterol diet and treated with guggulsterone displayed a decreased hepatic cholesterol content, an effect that was not observed in FXR-deficient mice. Therefore, it was suggested that the hypolipemic properties of guggulsterone were caused by FXR antagonism. However, despite the fact that coactivator assays confirmed that guggulsterone can act as a FXR antagonist, in vivo and in vitro studies showed that guggulsterone is a partial FXR agonist, which induces Bsep and Shp expression but does not downregulate Cyp7a1 or Cyp8b1. Interestingly, guggulsterone raised HDL cholesterol and lowered triglyceride levels in rats, a highly desirable therapeutic goal in humans. Unfortunately, a recent study performed to address guggulsterone effects in humans showed that the compound did not decrease cholesterol levels in patients (Table 2). This discrepancy could be caused by species-specific differences in FXR biology.

Based on available data, however, it is plausible to assume that FXR modulators could provide a new class of drugs for the treatment of specific aspects of the metabolic syndrome and perhaps provide alternatives or complements to existing therapy in the future.

Treating Cerebrotendinous Xanthomatosis and Inborn Errors in Primary Bile Acid Synthesis

Originally, Chenodeoxycholic acid (CDCA), the most potent natural agonist of human FXR (Figure 1), was evaluated to treat either gallstone disease or hypertriglyceridemia (Table 2). However, side effects like liver toxicity and diarrhea led to the withdrawal of the compound from clinical application.
Moreover, with respect to the hypertriglyceridemia-lowering effects, CDCA was shown to be only transiently effective, i.e., hypertriglyceridemia was back to initial levels between 6 months and 1 year after the initiation of the treatment.144,145 A potential application for CDCA in humans might be in the normalization of lipid levels in cerebrotendinous xanthomatosis (Cyp27A1-deficiency) patients in conjunction with statin treatment.75 Nevertheless, because FXR induces CYP3A4 expression57 and because most of the statins are metabolized by CYP3A4, safety issues should be carefully monitored for this combination. It must also be noted that reported improvements of the lipid profile were not translated into gain in brain functions146,147 and that the levels of various lipid parameters (cholesterol, lathosterol) remained supraphysiological during treatment.75 Therefore, it could be of interest to study the influence of more potent FXR agonists than CDCA, like 6-ECDC (Table 2). Finally, another natural bile acid, cholic acid, was recently tried as an orphan drug (EU/3/02/127) to treat inborn errors in primary bile acid synthesis (Table 2).148 The first results are promising but will need to be confirmed.

Treating and Preventing Gallstones

Ursodeoxycholic acid is a weak FXR agonist (Figure 1), and so far the only bile acid with a clear therapeutic application. Ursodeoxycholic acid is used to treat gallstone disease and cholestatic liver diseases (Table 2). Because it was estimated that up to 800,000 patients in the US had gallstones diagnosed during the year 2000 only,149 the potential market for a better drug to treat this disorder is enormous.

Very recently, Moschetta et al150 proposed that nonbile acid FXR agonists might be used to prevent or treat patients at risk for cholesterol gallstone disease and acute microlithiasis pancreatitis. This recommendation was based on the presence of supersaturated gallbladder bile in FXR-null mice fed a lithogenic diet and on reduction of biliary cholesterol saturation index in gallstone-prone C57L mice fed the synthetic FXR agonist GW4064. The effects were explained by FXR-mediated induction of BSEP (ABCB11)50,51 and MDR3 (ABCB4),151 allowing for increased bile acid and phospholipid secretion into bile in the absence of changes in the expression of ABCG5/ABCG8 that control biliary cholesterol secretion. Apart from the fact that the presence of bile supersaturated with cholesterol is a prerequisite for gallstone formation but not by definition leads to gallstone formation, there are a number of issues that require critical evaluation. First, it should be noted that biliary bile acid secretion rates/concentrations are not controlled by BSEP, but rather by the magnitude of the bile acid pool and its cycling frequency. This is illustrated by the facts that bile acid secretion is not impaired in heterozygous BSEP-deficient mice152 or in heterozygous human PFIC2 patients who carry a mutation in ABCB11.153 Furthermore, bile acid secretion is actually increased by 15% in FXR-deficient mice in which BSEP expression is reduced by 40%.65 The latter mice, when fed standard chow diet, were shown to have a 2-fold increase in bile acid pool size and actually a reduced cholesterol/bile acid ratio in comparison to wild-type controls.65 In addition, and most importantly, it is anticipated that treatment of patients with an effective synthetic FXR agonist will reduce the size of the circulating bile acid pool and, thereby, adversely affect bile composition, i.e., lead to a relative increase in biliary cholesterol content. A reduced bile acid pool size has frequently been reported in human gallstone patients154–157 and might contribute to gallstone development.157 In view of these issues, together with the reported HDL-lowering effects of FXR agonists, it seems reasonable to state that application of FXR agonists for treatment of gallstone disease is by no means self-evident and will at least require development of highly selective BARMs.

In conclusion, given the various roles exerted by FXR in energy and bile acid metabolism, FXR will be an attractive target to design new drugs to treat dyslipidemia and liver disorders using highly specific modulators.

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