The farnesoid X receptor (FXR) as a new target in non-alcoholic steatohepatitis

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Abstract

The farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily that is mainly expressed in liver, intestine, kidney and adipose tissue. On activation by bile acids, FXR regulates a wide variety of target genes that are critically involved in the control of bile acid, lipid and glucose homeostasis. Thus, FXR appears to be a promising target for the treatment of non-alcoholic steatohepatitis (NASH). Notably, FXR activation inhibits hepatic de novo lipogenesis, increases insulin sensitivity and protects hepatocytes against bile acid-induced cytotoxicity. More recent data also indicate a critical role of FXR in liver regeneration and hepatocarcinogenesis. For this reason, the development of FXR agonists and/or modulators (SBARMs) may prove to be clinically useful for treating NASH. While preclinical studies in rodents support this hypothesis, clinical studies are still warranted in humans.

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1. Introduction

The farnesoid X receptor (FXR, NR1H4) is an adopted member of the nuclear-receptor superfamily that is predominantly expressed in the liver, gut, kidneys and adrenals, with much lower levels in white adipose tissue. FXR is expressed from a single gene locus in humans (chromosome 12q23.1); (for reviews, see Cariou and Staels, and Lee et al. [1,2]). Two alternative promoters, in the presence of an internal cryptic splicing site, lead to the expression of four isoforms—FXRα1/FXRα2 and FXRα3/FXRα4—which are not equivalent in terms of gene transactivation [3]. FXR was originally named for its weak activation at supraphysiological concentrations by farnesol, an intermediary in the mevalonate biosynthetic pathway [4,5]. In 1999, three independent teams demonstrated
that bile acids (BAs) bind to and activate this nuclear receptor [6-8]. The hydrophobic BA chenodeoxycholic acid (CDCA) is the most effective activator of FXR, whereas hydrophilic ursodeoxycholic (UDCA) and muricholic acids are inactive. Several synthetic FXR ligands have been generated, especially the non-steroidal GW4064 compound, and have been extensively used both in vitro and in vivo in rodents [9]. Ligand-activated FXR binds to DNA elements called ‘FXR response elements’ (FXREs). It is worth noting that FXR can bind to and activate or repress a large variety of FXREs either as a classical FXR/RXR heterodimer or as a monomer [10]. Although BAs can also influence gene expression via FXR-independent pathways, it is now well established that FXR activation by BAs results in the regulation of several genes controlling BA metabolism, thereby acting as the ‘master intracellular BA sensor’. In this review, we specifically focus on the role of FXR in liver steatosis and fibrosis. The potential therapeutic value of pharmacological modulation of FXR in non-alcoholic fatty liver diseases (NAFLD) is also discussed.

2. FXR and bile acid metabolism

The main physiological role of FXR is to act as a BA sensor in enterohepatic tissues. FXR activation regulates the expression of various transport proteins and biosynthetic enzymes crucial to the physiological maintenance of BA and lipid homeostasis. BAs are actively secreted by the liver into bile and discharged into the intestinal lumen upon ingestion of a meal. BAs exhibit detergent-like properties that are crucial for their physiological functions in hepatic bile formation, and absorption of dietary lipids and fat-soluble vitamins from the small intestine. Efficient reabsorption of BAs in the terminal ileum results in the accumulation of a certain mass of BAs within the body, referred to as the ‘BA pool’, which cycles \( \approx 12 \) times between intestine and liver in the enterohepatic circulation. Only \( \approx 5\% \) of the pool escapes reabsorption per cycle and is lost via the large intestine in the feces [11]. This fecal loss of BAs, which is compensated for by de novo BA biosynthesis in the liver to maintain the pool size, represents a major route for cholesterol removal in humans. On the other hand, the physical characteristics of BAs, which allow them to form micelles, also impose a certain risk to cells that are exposed to high concentrations of these natural detergents. When present at high concentrations, BAs can become cytotoxic. In particular, hepatocytes and bile duct cells are at risk, for instance, in conditions of cholesterol, and protective mechanisms appear to become active when intracellular BA concentrations are elevated. BAs themselves are directly involved in the regulation of gene expression in liver and intestine via interaction with FXR, which provides a sensory function (Fig. 1).

Recent extensive reviews have focused on the role of FXR in BA metabolism [10,12]. Briefly, FXR induces the small heterodimer partner (SHP) in liver that, in turn, down-regulates the expression of both Cyp7a1 and Cyp8b1 genes, encoding enzymes that synthesize BAs from cholesterol, and represses the Na+-taurocholate pump (NTCP) that transports BAs from serum to the liver. In addition, FXR also induces the expression of transporters, such as the bile salt export pump (BSEP), to transport BAs from the liver into the bile canaliculi. In the intestine, FXR represses the expression of the ileal apical sodium-dependent BA transporter (ABST; also called ‘intestinal BA transporter’ or IBAT), and induces both ileal bile-acid-binding protein (IBABP), and the organic solute and steroid transporters (OST) \( \alpha/\beta \) that serve to transport BAs from the gut to the circulation, where they are then transported back to the liver. In addition, in response to BA flux in the intestine, FXR activates fibroblast growth-factor 15/19 (FGF15/19) gene expression in the enterocyte. Once secreted, FGF15/19 is transported to the liver where, through the FGF receptor-4 (FGFR4) signal-transduction pathway, it
downregulates Cyp7a1 and Cyp8b1 expression [13]. In summary, FXR activation suppresses de novo BA synthesis, and accelerates hepatic biliary excretion and detoxification, while simultaneously reducing their importation from the portal vein in a tightly coordinated fashion [2,10]. Thus, FXR protects liver cells from the deleterious consequences of cellular BA overload.

3. FXR and liver steatosis

Besides its classical role in BA and lipid homeostasis, recent data have underlined an unexpected function of FXR in glucose metabolism (for review, see Cariou and Staels [1]). The first indication came from the observation that hepatic FXR expression is reduced in several rodent models of diabetes [14] and varies during nutritional changes: it is increased during fasting and decreased on refeeding [15,16]. Interestingly, FXR can impact several steps of the pathophysiological process of liver steatosis by modulating insulin sensitivity and the rate of de novo lipogenesis.

3.1. FXR and insulin sensitivity

Three independent teams simultaneously identified a role for FXR in regulating insulin sensitivity [17-19]. FXR deficiency leads to impaired glucose tolerance and insulin resistance. While hyperinsulinenic-euglycaemic clamp studies clearly concluded that FXR+/− mice display peripheral insulin resistance, reflected by reduced peripheral glucose disposal [17,18], there are discordant data concerning the level of hepatic insulin sensitivity in FXR−/− mice. While some studies found a reduced inhibition of hepatic glucose output during a low-dose insulin clamp [18], FXR deficiency was also shown to be associated with normal hepatic insulin sensitivity [15,17]. The reason for this discrepancy is unclear, but may be linked to differences in the genetic backgrounds of the mice and/or the insulin dose used during the clamp. If FXR acts as an insulin sensitizer, then FXR activation would be expected to promote insulin sensitivity. In support of this hypothesis, treatment with GW4064 improved insulin sensitivity in vivo in both db/db, KK-A(y) [19] and ob/ob [17] diabetic mice.

Nevertheless, the molecular mechanisms behind the insulin-sensitizing effects of FXR remain poorly defined. Insulin signalling was found to be impaired in peripheral insulin-sensitive tissues such as skeletal muscle and white adipose tissue, whereas liver data remain conflicting [17,18]. As FXR is not expressed in skeletal muscle, it is conceivable that FXR deficiency indirectly alters insulin signalling in this tissue. One hypothesis is that FXR deficiency promotes ectopic lipid deposition in insulin target tissues, a phenomenon usually referred to as ‘lipotoxicity’ [20]. Indeed, FXR+/− mice have elevated circulating FFA levels [17,18], and increased intramuscular triglyceride and FFA contents [18]. A similar mechanism could also operate in liver as hepatic triglyceride content is increased in FXR−/− mice [15,18].

An interesting alternative pathway to explain the insulin-sensitizing effects of FXR is its role in white adipose tissue. FXR expression increases progressively during adipocyte differentiation in vitro, both in 3T3-L1 cells and mouse embryonic fibroblast (MEFs) cells [17,21]. Using MEFs as a model system, it has been shown that FXR deficiency leads to impaired adipogenic processing with defective triglyceride accumulation [17]. Conversely, the synthetic FXR ligand 6α-ECDDA/INT-747 promotes adipocyte differentiation and lipid storage in 3T3-L1 adipocytes [21]. Consistent with these in vitro data, FXR−/− mice exhibit a moderate lipoatrophic phenotype that may contribute to their impaired insulin sensitivity. Moreover, GW4064 treatment improves insulin signalling and insulin-induced glucose uptake in 3T3-L1 differentiated adipocytes [17,21]. Recently, FXR has been shown to directly stimulate the expression of the insulin-responsive glucose transporter GLUT4 [22].

Although beyond the scope of this review, it should be noted that BAs can also modulate metabolic homeostasis in a FXR-independent manner. The addition of cholic acid (CA) to the diet increases energy expenditure in brown fat in mice, and prevents the development of high-fat diet-induced obesity and insulin resistance. This metabolic effect of BAs was found to be critically dependent on induction of type 2 iodothyronine deiodinase (DIO2), and was suggested to be mediated by cAMP production induced by BAs binding to the G-protein-coupled receptor TGR5 (or Gpbar1) [23]. In addition, a recent study indicates that taurine-conjugated UDCA (tUDCA) can act as a molecular chaperone, thereby protecting hepatocytes against endoplasmic reticulum (ER) stress. As a consequence, in vivo treatment with tUDCA protects mice against diet-induced obesity and insulin resistance as well as fatty liver disease [24]. The physiological relevance of these signalling pathways, however, remains to be established in humans.

3.2. FXR and lipogenesis

FXR is involved in the control of hepatic de novo lipogenesis, one source of the fatty acids used for the assembly of very low-density lipoproteins (VLDL) (Fig. 2). FXR activation by BAs or synthetic agonists represses the expression of the transcription factor SREBP-1c and its lipogenic target genes in mouse primary hepatocytes and in liver, at least in part, in an SHP-dependent manner [16,25]. In addition, FXR modulates the kinetics of the response to dietary carbohydrate intake, as the maximum induction of glyco-
cholesterolemia, feeding a high-fat diet. In addition to LDL receptor knockout mice, a mouse model of hypertriglyceridemia induces non-alcoholic steatohepatitis (NASH) in mouse fatty-acid oxidation [27,28].

α expression of PPARα and PDK-4, both of which are known to promote carbohydrate-rich diet [15]. Finally, FXR ligands induce the significantly increased upon refeeding FXR –/– mice with a carbohydrate-rich diet [15]. At the molecular level, FXR therefore leads to enhanced glycolytic flux, which provides substrates for lipogenesis [15]. At the molecular level, FXR activation by GW4064 attenuated glucose-induced mRNA expression as well as promoter activity of several glucose-regulated genes, such as L-pyruvate kinase and acetylCoA carboxylase-1, in rodent primary hepatocytes [15]. Triglycerides derived from de novo lipogenesis efficiently mobilize apolipoprotein B and induce VLDL assembly [26]. Consistent with this observation, hepatic VLDL production is significantly increased upon refeeding FXR –/– mice with a carbohydrate-rich diet [15]. Finally, FXR ligands induce the expression of PPARα and its target gene pyruvate dehydrogenase kinase-4 (PDH-4), both of which are known to promote fatty-acid oxidation [27,28].

Very recently, it has been demonstrated that FXR-deficiency induces non-alcoholic steatohepatitis (NASH) in LDL receptor knockout mice, a mouse model of hypercholesterolemia, feeding a high-fat diet. In addition to liver macrosteatosis, FXR-deficiency was specifically associated with inflammatory infiltrates [29,30]. Based on these results, it would be expected that FXR activation by its ligands would reduce hepatic steatosis. Data from rodent models appear to confirm this suggestion. CA lowers hepatic triglyceride accumulation, VLDL secretion and elevated serum triglycerides in KK-A(y) mice, a mouse model of hypertriglyceridemia [25]. Furthermore, GW4064 treatment reduces neutral lipid accumulation in the liver of db/db mice [19].

Altogether, these results suggest that FXR activation may have a beneficial role in NAFLD by decreasing hepatic de novo lipogenesis that constitutes the ‘first hit’ of the disease.

4. FXR and liver fibrosis

Inflammatory processes are the ‘second hit’ in the course of NAFLD, and lead to the development of hepatitis and subsequent liver fibrosis [29]. Hepatic FXR appears to be downregulated during the acute-phase response in rodents in a similar manner as seen in other nuclear receptors such as PPARα and LXR [31,32]. This indirectly suggests that FXR may also modulate the expression of genes participating in the inflammatory response. Treatment of mice with CA induces the expression of ICAM-1, VCAM-1, serum amyloid A2 and TNF-α. Moreover, in vitro experiments in human hepatocytes demonstrate that FXR increases the transcriptional activity of the human ICAM-1 promoter [33]. Based on these results, FXR activation in liver could be associated with a deleterious proinflammatory profile.

However, a recent study indicates that FXR activation inhibits the expression of inflammatory mediators in response to NF-κB activation in vitro in hepatoma cell line and in primary hepatocytes. Interestingly, FXR –/– mice are more prone to develop necrosis and severe inflammation after treatment with lipopolysaccharide (LPS) than wild-type mice [35,36]. In addition, FXR has been shown to be expressed in both rat and human stellate cells (HSCs) [34,35]. Activated HSCs are responsible for the deposition and accumulation of extracellular matrix in fibrotic liver. In chronic liver disease, HSCs undergo a progressive process of transdifferentiation from a resting, fat-storing phenotype, toward a myofi broblast-like phenotype characterized by increased expression of fibroblast cell markers such as α-smooth muscle actin [37]. Activation of FXR with its synthetic agonist 6E-CDCA reduces HSC transdifferentiation in vitro, thereby protecting the liver against fibrosis in rodent models of liver injury [34,38]. This effect is thought to be mediated by the induction of hepatic PPARγ [39]—and potentially PXR [40]—expression following FXR activation.
5. FXR and other liver diseases

5.1. Cholestasis

Due to its hepatoprotective action, FXR has been proposed as an attractive target for treatment of cholestatic liver diseases. FXR–/– mice were found to be less sensitive to bile duct ligated (BDL)-induced liver damage, a model for obstructive extrahepatic cholestasis [41, 42]. This is due, at least partly, to the fact that these mice, in contrast to wild-type mice, do not maintain expression of the transporter BSEP. In rat models of chemically induced intrahepatic cholestasis, activation of FXR with GW4064 resulted in significant reductions in serum alanine and aspartate aminotransferases as well as other markers of liver damage [43]. GW4064 also decreased the incidence and extent of necrosis, decreased inflammatory cell infiltration and bile duct proliferation. Based on analyses of gene expression profiles, the beneficial effects of FXR activation have been ascribed to the reduction of BA synthesis genes such as Cyp7a1, and the induction of genes involved in biliary transport such as the phospholipid transporter Mdr2/Abcc4 [43]. FXR also induces UGT2B4 expression and activity in human hepatocytes, indicating a feed-forward reduction of BA toxicity in humans by glucuronidation [44]. In addition, upregulation of Mrp2/Abcc2, Mrp4/Abcc4 and Ostα/Ostβ on the basolateral surface of renal tubular cells in the kidney will increase the overall elimination capacity for such hydrophilic BA metabolites from the body [45].

5.2. Liver regeneration and hepatocarcinogenesis

During the past few years, accumulating data have indicated that FXR is involved in carcinogenesis (for review see Wang et al. [46]). The incidence of hepatocellular carcinoma (HCC) has doubled over the last two decades in the United States often as a complication of NAFLD. Partial hepatectomy experiments in mouse models have revealed that FXR is crucial for the control of liver regeneration [47], a critical process for restoring liver mass following liver injury. However, uncontrolled regeneration of hepatocytes, which occurs after repeated cycles of necrosis and regeneration in chronic hepatitis, appears to be an important factor in hepatocarcinogenesis. While elevated BAs stimulate liver growth after partial hepatectomy, BA sequestrants strongly decrease the rate of liver regeneration. As these effects are lost in FXR–/– mice, FXR appears to be the molecular link of the effect of BAs on liver regeneration [47]. FXR activation contributes to cell cycle entry of hepatocytes by inducing the expression of transcription factors that regulate cell cycling such as FoxM1b [48, 49]. Recently, it was also demonstrated that FXR protects liver cells from apoptosis induced by serum deprivation in vitro and starvation in vivo [50]. The protective role of FXR is strongly underlined by the increased prevalence of liver tumors in old (12-15 months) male and female FXR–/– mice, a tumorigenic response characterized by general liver injury, irregular regeneration and severe inflammation [48, 49]. These tumors include hepatocellular adenoma, carcinoma and hepatocellulargliocellular carcinoma. As FXR–/– mice display elevated BA pool size, the tumorigenic process is probably related to the cytotoxic effects of BAs. In accordance with this hypothesis, a CA-enriched diet favors chemically induced liver tumor progression. In contrast, BA sequestrants decrease tumors in treated mice [48, 49].

In summary, FXR exerts its hepatoprotective effect by tightly controlling the BA level in liver and promoting liver repair though controlled regeneration (Fig. 3). This dual effect of FXR helps the liver to protect against hepatocarcinogenesis.

Endogenous BA ligands (CDCA)
Synthetic agonists (GW4064, INT-747)
Activation of FXR

BA sensor
↓BA uptake & synthesis
↑BA excretion
↑BA detoxification
Hepatoprotection against BA-induced carcinogenicity

Induction of FoxM1b & other genes
Cell cycle progression
Liver regeneration

Maintenance of normal Liver homeostasis

Fig. 3. A model of the dual action of FXR in the control of hepatic carcinogenesis (adapted from Wang et al. [44]).

6. Human studies

To date, there is no identified mutation in the FXR gene related to human diseases. However, based on quantitative trait locus analyses, it has been proposed that polymorphisms of FXR are likely to be primary genetic determinants of cholesterol gallstone susceptibility [51]. Interestingly, we found recently that plasma BA concentrations are negatively correlated with insulin sensitivity—but not glycemic status—in a wide variety of subjects, including healthy volunteers, abnormally obese and type 2 diabetes patients [52]. While reinforcing the hypothesis of a link between BA metabolism and...
insulin sensitivity, these data remain correlative. Therefore, whether or not elevated circulating BAs are agents or markers of insulin resistance is still an unresolved issue. More direct evidence of a metabolic role of BAs is found in the results of human studies employing BA sequestrants, which disrupt the enterohepatic circulation of BA [53]. Indeed, in lipid-lowering trials, BA sequestrants have been shown to lower plasma glucose and HbA1c [54,55]. Future interventional clinical studies with BAs, especially CDCA, or FXR agonists [56] are clearly needed to ascertain their functional relevance in the treatment of obesity, type 2 diabetes and NAFLD.

7. Conclusion

FXR is a multipurpose nuclear receptor that interferes with BA and metabolic homeostasis. As a BA sensor, it exerts a liver-protective role, and is an interesting target in both liver inflammation and carcinogenesis. Accumulating data, at least in rodents, suggest that FXR acts as an insulin-sensitizer. Given this dual effect, it is tempting to speculate that FXR activation by natural or synthetic agonists, or FXR modulation with selective bile-acid receptor modulators (SBARMs), may have a beneficial action in the pathogenesis of NAFLD. The findings of ongoing studies in humans will help to definitively resolve this issue.

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