Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of fatty liver, characterized by the accumulation of fat in the hepatocytes in the absence of alcohol consumption. The spectrum of this disease ranges from steatosis to hepatitis and finally cirrhosis and hepatocellular carcinoma. NAFLD pathogenesis is not completely understood but various risk factors like obesity, insulin resistance, and metabolic syndromes have been identified. With the rapid increase in obesity and diabetes during the past decade, the incidence of NAFLD is on the rise and is predicted to become the most common indication for liver transplantation in the future.

Context of the study: The treatment option for NAFLD is limited and mainly focuses on risk factor modification like dietary changes and exercise. A major shortcoming of this approach is the lack of adherence and non-compliance over time. Other therapeutic options are available but are limited in number and have questionable efficacy and safety profiles. Thus, new target-oriented therapies are needed.

Results: One such option is using agonists of the farnesoid X receptor (FXR) which are nuclear receptors abundantly expressed in the liver and shown to play a key role in various metabolic pathways such as bile acid, cholesterol, lipid and glucose metabolism.

Main focus and conclusions: In this review, we mainly discuss the role of FXR in the pathophysiology of NAFLD and how it can be a useful treatment target for such patients.

Abbreviations

NAFLD: Non-Alcoholic Fatty Liver Disease; FXR: Farnesoid X Receptor; NASH: Non-Alcoholic Steatohepatitis; HCC: Hepatocellular Carcinoma; CYP7A1: Cholesterol-7α-hydroxylase; LDLR: LDL Receptor; SREBP-1c: Sterol Regulatory Element Binding Protein 1c; LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein; FGF: Fibroblast Growth Factor; HDL: High Density Lipoprotein; Apo A-1: Apolipoprotein A-1; NASH: Non-Alcoholic Steatohepatitis; CDCA: Chenodeoxycholic; OCA: Obeticholic Acid

Background

The incidence and prevalence of non-alcoholic fatty liver disease (NAFLD) is on the rise with each passing decade and at present 25-35% and 5-15% of the general population of Western and Asian countries, respectively, are affected by this disease [1-3]. The spectrum of NAFLD ranges from benign steatosis to non-alcoholic steatohepatitis (NASH) to cirrhosis and finally to hepatocellular carcinoma (HCC). The exact pathophysiology of this disease is not completely understood but various risk factors such as obesity, type 2 diabetes mellitus and metabolic syndrome have been identified. The prevalence of NAFLD is much higher in patients with obesity (75-92%) and diabetes (60-70%) compared to the general population [4-7]. Most of the NAFLD patients have benign steatosis and are asymptomatic. However, 15-40% of such patients may progress to NASH which can be life threatening [8]. 15% of NASH patients can progress to cirrhosis in 10-15 years [9] and cirrhosis increases the risk of HCC by 10% [10,11]. In addition, NAFLD increases the risk for various other cancers, particularly in the gastrointestinal tract (colon, oesophagus, stomach, and pancreas) and extra-intestinal sites (kidney, prostate, breast) [12]. With the increase in incidence of NAFLD, the incidence of liver transplantation in such patients is also increasing. NASH is currently the second leading reason for liver transplantation and it is predicted that it will be the leading cause in the future [13,14]. With the increasing incidence of NAFLD, it has also been reported that hospitalisation and mortality in these
patients is not mainly due to liver related causes but also
due to cardiovascular and renal causes [15-19]. Thus NAFLD
poses a serious health problem and up until now, no proper
pathophysiological targeting treatment has been found.
Treatment is mainly directed towards weight loss and risk
factor reduction. A weight loss of 3–5% by diet modification
and exercise has been shown to reduce steatosis while ≥5–7% drop
in weight has shown to resolve NASH. Greater reductions in
weight ≥10% may also improve hepatic fibrosis [20]. However,
the shortcoming of this approach is the lack of adherence and
non-compliance with time. [20-23]. Thus, an effective and
safe therapeutic regimen is critically needed.

Farnesoid X receptor (FXR) is a nuclear hormone receptor,
which is expressed in various organs and tissues, mainly in
the liver, intestine, kidney, and adrenal cortex [24,25]. It is
a ligand activated transcription factor, with bile acid being
the natural ligand to these receptors [26]. These receptors
are involved in regulating various metabolic pathways such
as bile acid, cholesterol, and lipid and glucose metabolism
[27,28]. The expression of FXR is reduced in the liver of NAFLD
patients [29], and various FXR knockout animal models exhibit
hepatic steatosis, bile acid accumulation, hyperlipidaemia,
hyperglycaemia and fibrosis [30-32]. Importantly, these
conditions are improved by increasing FXR expression [33,34],
indicating that the FXR agonist could be an effective therapeutic
option for NAFLD patients.

**Isoforms of FXR**

Until now, four FXR isoforms have been identified in
humans. These four isoforms are derived from a single gene
(NR1H4) in humans because of differential promoter usage and
splicing at exon 5. These isoforms are classified as FXRα1 (+),
FXRα1(-), FXRα2(+) and FXRα2(-). FXRα1 and FXRα2 differ
in amino acid sequence at their amino terminus and both FXRα1(+) and
FXRα2(+) contain a four amino acid (MYTG) insertion in the
hinge region immediately adjacent to the DNA binding
domain. This affects their ability to bind to FXR response
elements (FXRE), thus making them less transcriptionally
active [35,36]. All four isoforms occur in many tissues but
FXRα1 is predominantly expressed in the liver and adrenals,
whereas FXRα2 is mainly found in the intestine and kidney.
In most cell types the strongest response was found to be that of
FXRα1 (-). When the response of all four isoforms were studied,
it was found that in liver cells, FXR induced BSEP (bile salt
export pump) stimulating response was FXRα1(-) > FXRα2(-)
> FXRα1(+) > FXRα2(+) [38]; for SHP (small heterodimer partner) it
was FXRα2(-) > FXRα2(+) > FXRα1(+) = FXRα1(-). However, all
of the isoforms showed the same efficiency for OST β (organic
solute transporter β) expression. Also, the differential response
for all the isoforms in intestinal cells for FGF19 (fibroblast
growth factor 19) and IBABP (intestinal bile acid binding
protein) expression was found to be somewhat similar to BSEP,
with FXRα1 (+) and FXRα2(+) displaying same potency i.e.,
the order of magnitude for up regulation was FXRα1(-) > FXRα2(-)
> FXRα1(+) = FXRα2(+) [37]. In a mouse model study addressing
the role of FXRα1 (-) and FXRα2(-) on bile and lipid metabolism
showed that these most active isoforms differentially regulate
Cyp8btand SHP expression. Both isoforms have been shown to
reduce the elevated total plasma cholesterol levels, with FXRα1
(−) being more effective than FXRα2(−), but neither completely
normalized cholesterol levels to those seen in wild type mice
[38-40]. FXRα2(−) was shown to differ from FXRα1(−) in their
N-terminal parts with a 37 amino acid extension which must
have contributed to conformational changes in the FXR protein
and its transcriptional activity. Despite the identification
of the four FXR isoforms, their detailed physiological roles,
coregulator recruitment and DNA-binding in different tissues
are still not clearly understood. Thus, for the purpose of this
review, FXR will refer to all four isoforms.

**Effects of FXR on multiple metabolic pathways**

In addition to regulating various metabolic pathways
as indicated above [27, 28], FXR also affects inflammation,
fibrosis, liver regeneration and atherosclerosis [41,42].

**Role of FXR in bile acid metabolism**

The main role of FXR is to protect the hepatocytes by
preventing accumulation of bile acid by inhibiting bile acid
synthesis, reabsorption, and accelerating its excretion mainly
at the hepatocytes and enterocytes level. Bile acid is a natural
ligand for FXR and upon binding causes FXR activation
which, in turn, leads to the suppression of cholesterol-7α-
hydroxylase (CYP7A1), a key enzyme in bile acid synthesis.
CYP7A1 is not directly suppressed by FXR, rather FXR increases
the expression of the small heterodimer partner (SHP), which
in turn inhibits the CYP7A1 gene [43,44]. FXR in enterocytes,
upon activation by bile acid, induces fibroblast growth factor
19 (FGF 19) which upon binding to FGF4 receptors, causes
inhibition of CYP7A1 via the JNK pathway [45-47]. FXR also
regulates the enterohepatic circulation of bile acid. It does so
by inhibiting the Na+-dependent taurocholate transporter
which is responsible for bile acid transport, thus reducing
uptake by the hepatocytes as well as up regulates the bile salt
export pump, thus increasing bile acid export. FXR activation
in enterocytes reduces the expression of apical sodium-
dependent bile salt transporter which is mainly responsible
for bile acid absorption at the terminal ileum, thus inhibiting
its reabsorption. Moreover, the activation of FXR increases
the expression of the cytosolic intestinal bile acid–binding protein
(1-BABP), an important transport protein in the intestine
which transports the BAs across the enterocytes and portal
circulation to the liver [48,49]. Also it increases the expression
of the organic solute transporter αβ (OST αβ), thus secreting
bile acid into systemic circulation to be excreted via the kidney
[50]. Thus, FXR activation in hepatocytes and enterocytes
protect the hepatocytes from toxic accumulation of bile acids.

**Role of FXR in cholesterol and lipid metabolism**

Previous research has shown that bile can modulate
cholesterol and lipid metabolism [51, 52]. The expression
of FXR is reduced in the liver of NAFLD patients [29]. The
relevance of FXR in modulating cholesterol homeostasis is
evident from FXR knockout mice that exhibit increased hepatic
and serum cholesterol levels [53,54]. FXR activation increases

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folic acid can increase the risk of HCC; thus, by maintaining the homeostasis of glucose, lipid and by antagonizing the hepatic inflammation and fibrosis, FXR is believed to impede the progression of NASH to cirrhosis to HCC [60]. FXR also promotes liver regeneration by activating FoxM1b transcription factor [79]. FXR deficient mice display defective repair ability and delayed liver regeneration in an already damaged liver [79,80]. Moreover, it causes the inhibition of inflammatory signalling pathways like NFκB and STAT3 which play a key role in hepatic damage, fibrosis and act as a promoter of liver carcinogenesis [81–83]. Another FXR targeted gene is N–myc downstream regulated gene 2 (NDRG2– tumour suppressor gene). FXR knockout mice and human HCC patients have shown to have diminished levels of NDRG2 mRNA. FXR agonists or ectopic over-expression of FXR leads to the transcriptional induction of the NDRG2 gene [84]. Also, FXR has been shown to have a chemoprotective response on liver cells by changing the expression of several genes like ABCB4, TCEA2, CCL14, CCL15 and KRT13 which may be involved in drug efflux, DNA repair, and cell survival. This characteristic is shared by both healthy and tumour cells, thus playing an important role in the chemoprotection of healthy hepatocytes against genotoxic compounds and at same time reducing the response of liver tumor cells to certain pharmacological treatments [85].

Due to the FXR deficiency, hepatocytes are exposed to an environment which favours malignant transformation. Therefore, changing the FXR silencing or activation of remnant FXR may be potential strategies for liver cancer patients.

**Pro-atherosclerotic properties**

However, FXR activation has some concerning side effects. It increases the susceptibility to atherosclerosis by inhibiting the removal of cholesterol from peripheral cells via suppressing the expression of apolipoprotein A–1 (Apo A–1), a main constituent of high density lipoprotein (HDL) [86,87]. FXR activation also suppresses the paraoxonase 1 enzyme which plays a key role in inactivation of pro–atherogenic lipids [88,89]. Finally, FXR suppresses the action of proprotein convertase subtilisin/kexin 9 that promotes degradation of LDL [90,91]. Two phase I studies conducted in healthy individuals looking at the effects of FXR activation by OCA reported a decrease in HDL and increase in LDL cholesterol, regardless of the dose of OCA (5, 10 or 25 mg daily) after 14–20 days of treatment [92]. Similarly, treatment of NAFLD patients with OCA caused a 10% increase in total cholesterol, a 20% increase in LDL cholesterol and a 5% decrease in HDL cholesterol. Comparable reduction in HDL cholesterol was also reported in PBC patients treated with OCA. These effects are reversible after drug discontinuation [93–95]. These adverse side effects of FXR activation raise concern for its utility in treating NAFLD patients. The significance of these changes on cardiovascular outcomes needs to be explored in any OCA based treatment strategy.

**Role of FXR agonist in NAFLD treatment**

At present there is no effective therapy for NAFLD and the treatment options are mainly directed towards lifestyle modification in the form of diet modification, weight loss and exercise as these factors improve obesity and insulin sensitivity.

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However, patient’s adherence to life style modification and compliance falls with time [96–98]. Liver transplantation is the only option left for NASH patients with cirrhosis. However, even after transplantation there is risk of recurrence of disease and cardiovascular complications [99].

As discussed, FXR play a key role in bile acid, cholesterol, lipid and glucose homeostasis; and also it is shown to have anti-inflammatory and anti-fibrogenic properties. These actions of FXR make it a suitable therapeutic option for NASH patients.

FXR agonist (GW4064) treatment in a preclinical study conducted in a genetically obese mouse with insulin resistance improved insulin sensitivity and glucose clearance when compared to controls [100]. Further, treatment of FXR+/+ and FXR−/− mice with GW4064 showed a significant decrease of plasma glucose and fatty acids in FXR+/+ mice [67]. Similar efficacy of the FXR agonist was observed in a diabetic mouse model [67]. GW4064 increases the expression of p62/SQSTM1 and nuclear factor erythroid 2–related factor 2 (Nrf2) resulting in the induction of various antioxidant and anti-apoptotic molecules [101]. Furthermore, administration of an FXR agonist (WAY 362450) to a methionine and choline deficient, diet-induced animal model of NASH, exhibited a significant reduction in liver transaminases enzymes. Also, a significant decrease in hepatic fibrosis and inflammatory cell infiltration and cytokines were observed [34]. Recently, a novel, non-steroidal FXR agonist, PX20606, has been shown to have anti-fibrotic and vasodilator properties and lowers portal hypertension [102]. A newly found non-bile steroidal dual ligand for FXR and GPBAR1 receptors, BAR502, reverses fibrosis and in an animal model with thioacetamide-induced cirrhosis, OCA reduced hepatic transaminases, enzymes. Also, the FXR agonist could be an effective treatment option for NAFLD patients.

Of all the synthetically derived FXR agonists, the most clinically advanced is INT–747/Obezicholic acid (OCA) which is a semi–synthetic derivative of a natural bile acid analogue, chenodeoxycholic acid (CDCA), with an affinity 100 times greater than CDCA [104, 105]. Preclinical studies of OCA in the Zucker (fa/fa) rat, a NAFLD rat model, resulted in reduction of gluconeogenesis, lipogenesis and improvement of insulin resistance and hepatic steatosis [33]. In a rat model of thioacetamide–induced cirrhosis, OCA reduced hepatic inflammation and fibrosis and also decreased intrahepatic vascular resistance and improved portal hypertension [106]. In a rabbit model of high fat diet induced NAFLD, administration of OCA resulted in an improvement in visceral fat and plasma glucose levels [107]. In addition, OCA administration reduces liver transaminases, IFN-γ and TNF-α in an autoimmune hepatitis mouse model [108]. FXR activation has been shown to promote hepatic amino acid catabolism and ammonium clearance via ureagenesis and glutamine synthesis [109]. OCA also decreases intestinal inflammation in various colitis animal models [110]. In an animal model with advanced cirrhosis, treatment with OCA significantly reduced gut bacterial translocation [111]. Additional miR–21 ablation with FXR activation by OCA ameliorated NASH suggesting that a multi–receptor targeting therapy could be the most effective treatment strategy [112].

OCA is the only FXR agonist which has been examined in clinical trials on NAFLD patients. Its role has been investigated in two large randomized controlled trials (NCT00501592 and NCT01265498). The first trial was conducted on NAFLD and type 2 diabetes mellitus patients (NCT00501592), in which patients were randomly distributed in any of the three groups receiving placebo or 25 mg or 50 mg OCA for a period of 6 weeks. It was noticed that patients receiving 25 mg and 50 mg of OCA showed improvement in insulin sensitivity by 28% and 21%, respectively, while it worsened in the placebo arm by 5%. Weight loss was noticed in both the OCA groups but hepatic fibrosis improved only in patients on the 25 mg OCA regimen. An increase in alkaline phosphatase, with a decrease in alanine transaminase and γ-glutamyltransferase levels was noticed in both OCA–treated groups. While aspartate transaminase levels remained stable in all, a decrease in HDL and an increase in LDL were noticed in patients treated with 50 mg OCA [113].

Recently, OCA treatment was used in another large trial, the FLINT trial (NCT01265498), which included NASH patients with or without cirrhosis. In this multicentre trial, 283 patients were randomly distributed in either placebo or 25 mg OCA arm for 72 weeks. Here 45% of the patients in the OCA arm and 21% of the patients in the placebo arm met the primary outcome of the study which was determined to be a drop of 2 points in the NAFLD activity score. In addition to this, 35% of the patients in the OCA arm and 19% in the placebo arm showed a reduction in hepatic fibrosis. OCA group patients showed a reduction in body weight, liver transaminases and systolic blood pressure but an increase in plasma glucose levels and insulin resistance. Pruritus was noticed as the main side effect in the patients in the OCA group [114]. A Phase 3, Double-blind RCT Multicenter Study is ongoing to evaluate the safety and efficacy of OCA in NASH patients (ClinicalTrials.gov Identifier: NCT02548351). This trial evaluates the effect of OCA compared to placebo on liver histology in non-cirrhotic NASH patients with stage 2 or 3 fibrosis. 2065 patients are randomized in 1:1:1 to placebo, 10 mg or 25 mg OCA. An interim analysis is to be done at 18 months and the study is expected to end in 6 years (https://clinicaltrials.gov/ct2/show/NCT02548351).

All of the preclinical animal/human and clinical human studies suggest that FXR agonist/OCA can be a potential therapeutic option in NAFLD patients. However, OCA produces pro-atherogenic effects that can be a concern for NAFLD patients with a high risk for cardiovascular adverse events. Therefore, long term larger clinical trials are required to determine its efficacy and safety. Further, combination therapies with FXR agonist and agents that prevent atherosclerosis are warranted.

Conclusions

The FXR agonist appears to be an attractive drug due to its pleiotropic actions of regulating various metabolic pathways. They play a critical role in bile acid, lipid, cholesterol, and glucose homeostasis. In addition, they also have anti-inflammatory and anti-fibrogenic properties. The data
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