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 non-alcoholic steatohepatitis and finally cirrhosis and hepatocellular carcinoma. NAFLD pathogenesis is not completely understood but various risk factors such as 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.

Isosforms 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(+), for SHP (small heterodimer partner) it was FXRα1(-) > FXRα2(-) > FXRα1(+) = FXRα2(+). 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 Cyp8b1 and 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...
fetal cholesterol excretion by inhibiting intestinal cholesterol absorption [55,56]. Further, FXR activation decreases hepatic cholesterol uptake via increasing the expression of low density lipoprotein (LDL) receptor (LDLR), scavenger receptor class B type I and decreasing cluster differentiation protein 36 expression [54,57]. FXR activation also increases liver cholesterol excretion by increasing the expression of ATP-binding cassette G5/8 (ABCG5/G8), the cholesterol efflux transporter [58].

NAFLD patients exhibit high triglyceride levels due to the decreased FXR and increased SREBP–1c expression [29]. FXR activation significantly impacts lipid synthesis, mainly by decreasing the expression of the sterol regulatory element binding protein 1c (SREBP-1c) and its enzymes which are the main regulator in lipogenesis [59]. In addition, FXR activation increases the clearance of LDL, very low density lipoprotein (VLDL) and chylomicrons by activation of lipoprotein lipase [60], and increasing VLDL receptor expression [61]. Furthermore, FXR activation results in the induction of the peroxisome proliferator activated-α receptor which increases fatty acid oxidation [62]. Also it increases the secretion of FGF21 which decreases lipogenesis by inhibition of SREBP–1c [63,64].

Role of FXR in glucose homeostasis

FXR also plays a key role in a glucose homeostasis. FXR activation improves insulin sensitivity and decreases gluconeogenesis by suppression of phosphoenolpyruvate kinase and glucose-6-phosphatase which are the key enzymes required for gluconeogenesis [32, 65]. Further, by increasing FGF21 secretion, FXR induces the phosphorylation of glycogen synthase kinase which promotes glycogen synthesis and suppresses gluconeogenesis [66,67].

Anti-inflammatory and anti-fibrogenic properties

FXR is reported to exhibit anti-inflammatory and anti-fibrogenic properties. FXR activation decreases hepatic inflammation by suppressing the nuclear factor kappa B pathway [68]. Administration of the FXR agonist in a NAFLD animal model reduces various pro-inflammatory cytokines and growth factors [31]. FXR knockout mice have been shown to be more susceptible to lipopolysaccharide–induced liver injury, thus indicating that FXR has anti-inflammatory properties [68].

Anti-tumorigenic properties

FXR is a multi-functional receptor that also exhibits anti-tumorigenic properties. FXR knockout mice have been shown to develop liver tumours with ageing [69,70], and FXR expression has been found to be significantly decreased in many human tumour specimens [71–74]. In FXR knockout mice excessive BA accumulation has been considered to have cytotoxic effects, thus favouring tumorigenesis [69,70,75]. Also, sharply increased BA levels lead to activation of YAP protein and Hippo pathway which is a crucial promoter of hepatocarcinogenesis [76–78]. NASH, obesity and diabetes mellitus have been considered to 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–muc downstream regulated gene 2 (NDGR2– tumour suppressor gene). FXR knockout mice and human HCC patients have shown to have diminished levels of NDGR2 mRNA. FXR agonists or ectopic over-expression of FXR leads to the transcriptional induction of the NDGR2 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, CCL4, 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 more 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.
However, patient’s adherence to lifestyle 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-in-inflammatory and anti-fibrogenic properties. These actions of FXR make it a suitable therapeutic option for NAFLD 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, PX26606, has been shown to have anti-fibrotic and vasodilator properties and lowers portal hypertension [102]. A newly found non-bile stereoal dual ligand for FXR and GPBAR1 receptors, BAR502, reverses apoptosis in hepatic cell in culture and alleviates gut bacteria translocation [111]. Additional miR-21 ablation in the Zucker (fa/fa) rat, a NAFLD rat model, resulted in greater than CDCA [104, 105]. Preclinical studies 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 proatherogenic 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 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].

presented from various preclinical and clinical studies suggest that it can be a good therapeutic option in the prevention and treatment of NAFLD. However, several undesirable results such as a decrease in plasma HDL is concerning. Therefore, larger, long-term clinical trials are required to determine its efficacy and safety. Further, combination therapies with FXR agonist and agents that prevent atherosclerosis are warranted. Furthermore, we should continue to gain a better understanding of NAFLD pathogenesis such that additional molecular targets and cellular pathways could be identified for developing other novel therapeutic regimen(s) in the future.

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**References**


