Intestinal FXR: A New Therapeutic Target for Nonalcoholic Fatty Liver Disease

ABSTRACT 1

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Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance

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The systemic expression of the bile acid (BA) sensor farnesoid X receptor (FXR) has led to promising new therapies targeting cholesterol metabolism, triglyceride production, hepatic steatosis and biliary cholestasis. In contrast to systemic therapy, bile acid release during a meal selectively activates intestinal FXR. By mimicking this tissue-selective effect, the gut-restricted FXR agonist fexaramine (Fex) robustly induces enteric fibroblast growth factor 15 (FGF15), leading to alterations in BA composition, but does so without activating FXR target genes in the liver. However, unlike systemic agonism, we find that Fex reduces diet-induced weight gain, body-wide inflammation, and hepatic glucose production, while enhancing thermogenesis and browning of white adipose tissue (WAT). These pronounced metabolic improvements

suggest tissue-restricted FXR activation as a new approach in the treatment of obesity and metabolic syndrome.

ABSTRACT 2

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Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease

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Nonalcoholic fatty liver disease (NAFLD) is a major worldwide health problem. Recent studies suggest that the gut microbiota influences NAFLD pathogenesis. Here, a murine model of high-fat diet-induced (HFD-induced) NAFLD was used, and the effects of alterations in the gut microbiota on NAFLD were determined. Mice treated with antibiotics or tempol exhibited altered bile acid composition, with a notable increase in conjugated bile acid metabolites that inhibited intestinal farnesoid X receptor (FXR) signaling. Compared with control mice, animals with intestine-specific FXR disruption had reduced hepatic triglyceride accumulation in response to a HFD. The decrease in hepatic triglyceride accumulation was mainly due to fewer circulating ceramides, which was in part the result of a lower expression of ceramide synthesis genes. The reduction of ceramide levels in the ileum and serum in tempol- or antibiotic-treated mice fed a HFD resulted in downregulation of hepatic SREBP1C and decreased de novo lipogenesis. Administration of C16:0 ceramide to antibiotic-treated mice fed a HFD reversed hepatic steatosis. These studies demonstrate that inhibition of an intestinal FXR/ceramide axis mediates gut microbiota-associated NAFLD development, linking the microbiome, nuclear receptor signaling, and NAFLD. This work suggests that inhibition of intestinal FXR is a potential therapeutic target for NAFLD treatment.

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COMMENTS

With an epidemic of obesity and metabolic syndrome looming on the horizon, the burden of nonalcoholic fatty liver disease is increasing, and is expected to be the most common indication of Liver Transplantation in the USA.¹ NAFLD affects 20–30% of the adult population and up to 80% of obese people across the globe.² It is characterized by triglyceride accumulation in the liver. The clinical spectrum includes fatty liver, nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma.³ Insulin resistance, type 2 diabetes, coronary heart disease, and atherosclerosis are commonly associated with NAFLD.⁴ The current concept in pathogenesis is the ‘Two-hit’ or ‘Multiple-hit’ theory, accumulation of fat/insulin resistance being the first hit, and subsequent to this, cytokines, adipokines, endotoxins, endoplasmic reticulum stress represent the second hit.^{5,6} Till date, there is no effective drug therapy for NAFLD. A better understanding of the mechanism of pathogenesis is essential for developing future strategies in the management of NAFLD.

Gut microbiota have increasingly been implicated in the pathogenesis of NAFLD.⁷ Alteration in the gut microbiota may lead to changes in the host bile acid composition, which may in turn lead to metabolic dysfunction, including obesity and insulin resistance, through its action on the farnesoid X receptor (FXR).⁸ FXR is a bile acid receptor (BAR) belonging to the nuclear receptor superfamily. It is expressed in the liver, kidney, adrenal cortex, stomach, gall bladder, and intestine.⁹ When activated, FXR translocates to the cell nucleus, leading to reduced transcription of cholesterol 7 α -hydroxylase, which is the rate limiting step in the synthesis of bile acids (BAs), hence reducing synthesis of bile acids. FXR activation in the liver has been implicated in lipid, cholesterol, and glucose homeostasis.¹⁰ Recently FXR has been shown to be associated with dyslipidemia, obesity, and atherosclerosis.¹¹ The intestinal FXR, along with hepatic FXR possibly plays a role in BA synthesis and transport. The role of intestinal FXR has been explored as a potential target in the two studies published recently by Fang et al.¹² and Jiang et al.¹³

Fang et al.¹² in this mice experiment, used fexaramine (Fex) per orally to selectively activate intestinal FXR. In the Fex treated obese mice, the weight gain was less (as also the overall fat mass). In addition, inflammatory cytokines in the serum – including tumor necrosis factor- α , interleukin (IL)-1 α , IL-1 β , and IL-17 – were less. The benefit of Fex in preventing weight gain was explored by assessing the expression of genes involved in thermogenesis and fatty acid oxidation, and these were found to be overexpressed. To explore further how Fex contributes to the changes in energy expenditure, RNA-sequencing of intestinal tissues was done. Fgf15 was significantly elevated, which is known to activate thermogenesis in brown adipose tissue and also negatively regulate BA synthesis. As expected, the BA pool

was decreased. Further, there was a change in the composition of the BA pool – increase in lithocholic acid and decrease in taurocholic acid. Fex treatment also improved insulin sensitivity.

Jiang et al.¹³ explored the effect of changes in the gut microbiota on liver fat accumulation and inflammation with an interesting mice experiment. In their study, mice fed on a high fat diet were used as a model for NAFLD. Antibiotics like streptomycin, neomycin, and bacitracin were used to eliminate certain strains. In addition, Tempol – an antioxidant – was used to modulate the gut microbiota. The 16S rRNA sequencing showed that tempol treatment lead to decreased firmicutes and increased proteobacteria. Mice treated with a chow diet and a high fat diet were given antibiotics and tempol. Liver biopsy was done to assess the status of NAFLD. It was found that mice fed with a high fat diet had lipid accumulation in the liver. On giving them antibiotics and tempol, the gut microbiota was altered, which resulted in significant reduction in the fat content of the liver. The authors thus concluded that tempol and antibiotic treatment resulted in change in gut microbiota and a subsequent improvement in NAFLD.

How did the gut microbiota change cause a change in liver fat? To answer this question the authors looked at the bile acid milieu in the mice ileum using mass spectrometry. A quantification of the all bile acids was done. Levels of taurine conjugated β -muricholic acid (T- β -MCA) were high in mice treated with antibiotics and tempol. Antibiotics and tempol altered gut microbiota and reduced the bacterial enzyme bile salt hydrolase (BSH), which was directly quantified and found to be less. There was reduced deconjugation due to the reduction of BSH and this lead to an increase in T- β -MCA.

T- β -MCA is a natural antagonist of the FXR. The authors thus proposed that it is this increase in bile acid levels which antagonizes the FXR, and thus mediates the effects of gut microbiota change on the liver. To prove this, FXR target gene mRNAs small heterodimer partner (Shp) and fibroblast growth factor 15 (Fgf15) were measured. T- β -MCA treatment significantly blunted Shp and Fgf15 mRNA induction by taurocholic acid (TCA) in the ileum of antibiotic-treated mice on a high fat diet. TCA enhances FXR signaling which was blocked by T- β -MCA.

To prove that it is actually the antagonism of this receptor that leads to improvement of NAFLD in antibiotic or tempol treated mice, the authors used farnesoid X receptor knockout mice. When the receptor was knocked out, a high fat diet did not result in fat accumulation in the liver. But this did not occur due to reduction in absorption of free fatty acids, which were measured in FXR knockout mice. Thus, the authors concluded that FXR antagonism does modulate fat accumulation in the liver but this did not occur due to reduced absorption of fatty acids.

All the lipids in the mice ileum were measured and it was found the ceramide levels were significantly reduced in

mice treated with antibiotics/tempol and in FXR knockout mice. To investigate whether the decrease in ceramide is a major contributing factor in improving HFD-induced NAFLD development in antibiotic-treated mice, daily intraperitoneal injections of purified C16:0 ceramide were administered to HFD-fed and antibiotic-treated mice. This reversed the decrease in hepatic lipid droplets in antibiotic-treated mice fed a high fat diet for 7 weeks. The liver weights, liver/body mass ratios, and hepatic triglyceride content were also significantly increased. This was accompanied by an up regulation of SREBP1C (sterol response element-binding protein 1c)/CIDEA (DNA fragmentation factor α -like effector A) signaling and gene expression in the livers of antibiotic-treated mice. SREBP1 and CIDEA are genes which code for enzymes of hepatic lipogenesis. The authors postulated that ceramide increases hepatic lipogenesis and thus results in NAFLD. In mice treated with antibiotics, or FXR knockout mice, ceramide levels decreased and therefore decreased hepatic lipogenesis.

Both these studies in mice models elucidate the various mechanisms which can be targeted for future therapeutics, but it needs to be seen whether these stand true in humans also. T- β -MCA has not been shown in humans as yet. One study showing FXR agonism and another showing antagonism effective in NAFLD raises issues regarding which one is effective. It remains to be seen which mechanism and targets stand the test of time.

In summary, these two studies provide more knowledge regarding pathogenesis and new concepts regarding therapeutic strategies, which may be of benefit in patients with NAFLD.

CONFLICTS OF INTEREST

The authors have none to declare.

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