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Editorial

Sulfated oxysterols as candidates for the treatment of nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) has emerged as a significant medical and socioeconomic burden. Its prevalence has been reported to be up to 45% of the general population in the USA [1] and 6%–35% in the rest of the world [2], reaching a prevalence of 60%–95% and 28%–55% in obese and diabetic patients, respectively [3]. The prevalence of NAFLD continues to increase in parallel with the growing epidemic of obesity and type 2 diabetes mellitus, and thus it is currently evolving as the most common liver disease worldwide [4]. However, no treatment has yet been established for NAFLD [5].

Although the pathogenesis of NAFLD is not fully understood, we do realize that it represents a complex process. The development of NAFLD is regarded as the consequence of "multiple-hits" leading initially to hepatic steatosis or simple nonalcoholic fatty liver (NAFL) and, subsequently, to nonalcoholic steatohepatitis (NASH) and NASH-related cirrhosis with complications [6]. NAFL is a prerequisite for the development of NASH. The homeostasis of hepatic lipid is determined by the dynamic balance of multiple pathways leading to lipid entry into or removal from hepatocytes. These include fatty acid uptake, de novo lipogenesis, lipid oxidation and lipoprotein formation and secretion [7]. The liver is the major site for de novo lipogenesis, which usually occurs when glucose supply is high. This is considered as a key-process in the pathogenesis of NAFLD by promoting hepatic triglyceride build-up; defects in lipid utilization via mitochondrial oxidation and lipid export also contribute to hepatic lipid accumulation. Lipogenesis is traditionally considered to be under the control of specific transcription factors, including sterol regulatory element binding protein (SREBP)-1c, activated by insulin, and carbohydrate response element binding protein (ChREBP), activated by glucose [8]. SREBP-1c and ChREBP upregulate lipogenic enzymes, such as acetyl-coenzyme A carboxylase, fatty acid synthase and stearoyl-CoA desaturase-1 [6]. The expression of both SREBP-1c and ChREBP is further upregulated by other transcription factors, such as the liver X receptors (LXRs).

Two isoforms of LXRs have been currently identified: LXRα, which is highly expressed in the liver, small intestine, kidney, macrophages and adipose tissue, and LXRβ, which is ubiquitously expressed [9]. LXRs form heterodimer with retinoid X receptors (RXRs). Only the heterodimer LXR/RXR binds to the DNA on LXR responsive element [10]. In the absence of the ligand, LXR/RXR binds to the DNA in the promoter of target genes and interacts with co-repressors [11]. Upon ligand binding, co-repressors are released and co-activators are recruited [10]. LXRs lie at a more central position than SREBP and seem to mediate the action of diverse factors in de novo lipogenesis, including insulin, glucose and oxysterols [10]. LXRs can upregulate lipogenic enzymes directly [10] or indirectly, through the upregulation of SREBP-1c [12] and ChREBP [13]. Therefore the LXR/SREBP-1c and LXR/ChREBP signaling pathways appear to play a crucial role in NAFLD pathogenesis [14,15].

The natural ligands for LXRs have been shown to be oxysterols, molecules largely derived from enzymatic or non-enzymatic oxidation of cholesterol by a variety of biochemical routes [16]; oxysterols are structurally similar to cholesterol, but with one or more additional oxygen containing functional groups, such as hydroxyl, carbonyl or epoxide group [10,17]. Oxysterols greatly vary according to their structure and their putative effects and possess many potent and diverse biological activities. Certain oxysterols classically serve as sensors and regulators of cholesterol excess. They may limit cholesterol synthesis through degradation of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, the rate-limiting enzyme in cholesterol synthesis [18], may inhibit intestinal cholesterol absorption, increase cholesterol efflux from cells, and may promote storage of cholesterol as esters [17,19]. Other oxysterols, by acting as ligands to LXRs, upregulate hepatic de novo lipogenesis [19,20].

In this issue, Bai et al. [21] demonstrate that 5-cholesten-3β-25-diol-3-sulfate (25HC3S), which is synthesized by the

Abbreviations: 25HC, 25-hydroxycholesterol; 25HC3S, 5-cholesten-3β-25-diol-3-sulfate; HMG, 3-hydroxy-3-methylglutaryl; ChREBP, carbohydrate response element binding protein; LXR, liver X receptor; NAFL, simple nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; RXR, retinoid X receptor; SREBP, sterol regulatory element binding protein.

cytosolic sulfotransferase SULT2B1b through sulfation of 25-hydroxycholesterol (25HC), substantially decreases serum and hepatic lipid levels in a mouse model, possibly by inhibiting the LXRα/SREBP-1c signaling pathway; 25HC3S action is counteracting the action of the precursor 25HC, which acts as LXRα activator. More specifically, hepatic SULT2B1b overexpression significantly increases sulfated oxysterols, especially 25HC3S, and decreases non-sulfated oxysterols, including 25HC; this is observed in the presence of 25HC, but not in the absence of 25HC. Consistently, SULT2B1b overexpression significantly decreases nuclear protein and mRNA levels of LXRα and SREBP-1 in mice fed with high cholesterol or high fat diet, only in the presence of 25HC. As a result, SULT2B1b overexpression has a favourable effect on lipid profile, characterized by a decrease in serum and liver triglyceride, a decrease in serum very low- and low-density lipoprotein cholesterol, an increase in serum high-density lipoprotein cholesterol, and a decrease in hepatic cholesterol. Remarkably, liver toxicity is not observed [21]. Importantly, SULT2B1b gene expression following adenovirus infection demonstrates tissue specificity, which is more prominent in the liver (increase by 20-fold), only modestly in the lung (2 fold) and aorta (1.5 fold), and negligible in the heart and kidney. Apparently, this is the first report regarding the effect of 25HC3S on serum and hepatic lipid levels in vivo. The same group had previously described a similar mechanism in hepatocytes [22,23], macrophages [24] and aortic endothelial cells [25] in vitro. According to their findings, the authors proposed that 25HC3S may represent a novel target for the treatment of dyslipidemia, atherosclerosis and NAFLD.

Other authors have also proposed oxysterols, but not 25HC3S, as pharmacological targets for dyslipidemia [19,26]. Their concept was based on the property of oxysterols to act as "cholesterol sensors", thus protecting the cell from cholesterol overload, as described above. However, this hypothesis has as a negative consequence the increase in de novo lipogenesis, because some oxysterols may also act as LXR ligands [19]. The work of Bai et al. [21] has modified the concept of therapeutic potential of oxysterols, because 25HC sulfation to 25HC3S has a dual consequence: the decrease in 25HC, which acts as an activator, and the increase in 25HC3S, which acts as an inhibitor of LXRα/SREBP signaling pathway. This is pathophysiologically translated as improvement rather than deterioration of hepatic steatosis. 25HC3S seems to be beneficial in early stages of NAFLD, thereby preventing hepatic steatosis, which is a prerequisite for NASH. In our opinion, this is significant and of apparent clinical importance, given that most agents have to-date targeted advanced NASH, and only limited targeted NAFL. Furthermore, other factors, including endocrine disruptors [27] (i.e. bisphenol A [28]), may affect NAFLD through LXRs. Therefore, LXR inhibition could become of potentially great clinical importance by diminishing the effect of these factors on LXRs and hepatic lipogenesis.

Other factors targeting the LXRα/SREBP-1c signaling pathway have also shown beneficial effects on hepatic steatosis in murine models, including Jiangzhi Granule [29] and rhein [30], compounds of traditional Chinese medicine, *Agaricus bisporus*, a white button mushroom [31], and isoflavones, including genistein [32] and daidzein [33]. These interventional studies provide further support to the potential role of LXRα/SREBP-1c signaling in NAFLD pathogenesis.

Nevertheless, there are several issues that should be clarified, before 25HC3S-based clinical trials could commence, including but not limited to the following:

First, one needs to consider the effect of 25HC decrease in hepatic and serum cholesterol levels; given that it could act as a cholesterol sensor, its decrease may consequently increase cholesterol synthesis through withdrawal of the inhibition on HMG-CoA reductase.

Second, one needs to also consider the effect of 25HC3S in other tissues; it has been shown that the absence of LXRs suppresses de novo lipogenesis in the liver, but stimulates de novo lipogenesis in adipose tissue, thus demonstrating opposing roles in these two tissues and a tissue-specific activity, which is crucial for drug development [34].

Third, one needs to consider the effect of 25HC3S on LXRβ; LXRα and LXRβ have overlapping, but also discrete functions, with LXRβ being a major regulator of glucose homeostasis, energy utilization and fat storage in muscle and white adipose tissue [35]. If 25HC3S was, similarly to LXRα, an LXRβ inhibitor, it would have therapeutic potential for glucose intolerance and diabetes, which usually co-exist with dyslipidemia and NAFLD. On the other hand, if 25HC3S acted as LXRβ agonist, it would have therapeutic implications for glucose metabolism, and probably for insulin resistance and NAFLD. More importantly, LXRβ inhibition has been associated with gallbladder carcinogenesis in a mouse model [36]. Oxysterols other than 25HC3S have also shown such opposing effect; for example 5α,6α-epoxycholesterol exerts both agonist and antagonist activities depending on the specific LXR target genes [37].

Fourth, despite the probable beneficial effect of 25HC3S on hepatic steatosis, its effect on hepatic inflammation and fibrosis, if any, remains to be elucidated.

Finally, LXRs have been implicated in diverse morbidity, including immunity, inflammation, thrombosis, reproduction, neurodegenerative diseases, like Alzheimer's disease or amyotrophic lateral sclerosis–Parkinson's dementia, and cancer [10]. Although SULT2B1b showed liver specificity in the Bai et al study [21], the overall safety of the potential 25HC3S administration or upregulation remains to be fully elucidated by future studies.

In conclusion, the LXRα/SREBP-1c signaling pathway is emerging as a chief regulator of de novo lipogenesis and NAFLD pathogenesis. Its selective hepatic inhibition could have beneficial effect on both dyslipidemia and early stage NAFLD. However, further experimental studies are required before 25HC3S-based clinical trials are justified.

Conflict of interest

The authors declare no conflict of interest relevant to this article.

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