Review

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Future therapeutic perspectives in nonalcoholic fatty liver disease: a focus on nuclear receptors, a promising therapeutic target

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a major public health problem worldwide, with an increasing incidence, secondary to the increasing incidence of obesity and diabetes, from a very young age. It is associated with metabolic and cardiovascular disorders, as components of the metabolic syndrome (MS). NAFLD is the hepatic manifestation of MS. The pathogenesis of the disease is multifactorial and complex, involving genetic, metabolic, but also environmental factors. Currently, nuclear receptors (NRs) represent a promising therapeutic target in the treatment of non-alcoholic steatohepatitis (NASH). Of these, the most studied receptor was the liver X receptor (LXR), which would have great potential in the treatment of metabolic diseases, namely hypercholesterolemia, atherosclerosis, and NAFLD. However, the therapeutic use of NRs is restricted in medical practice for two reasons: limited knowledge of the structure of the receptor and its inability to modulate certain actions in the target organs and genes. One problem is the understanding of the function and structure of the N-terminal domain which has a major transcriptional activation function (AF1).

Keywords: non-alcoholic fatty liver disease, nuclear receptors, liver X receptor, treatment

Introduction

NAFLD is the main cause of chronic liver disease worldwide with a current incidence of 47 cases per 1,000 inhabitants, the incidence being much higher among men. Over time, the prevalence of the disease has increased, currently the global prevalence is 32%, higher among men (40%) compared to the prevalence in women (26%). In the future, it is estimated that the prevalence of NAFLD will increase significantly in certain regions until the year 2030, along with the increase in the prevalence of obesity and diabetes [1]. Currently, NASH is the main worldwide cause of hepatocellular carcinoma (HCC) [2,3] and in the United States of America it is the main indication for liver transplantation [4]. Primary steatosis and steatohepatitis, in the absence of other etiologies such as chronic alcohol consumption, hepatitis C virus infection, various drugs (glucocorticoids, amiodarone, methotrexate, etc.), endocrine disorders, are part of the NAFLD spectrum [5]. Simple or "mild" hepatic steatosis (HS) is considered a benign form of the disease due to extremely low progression to cirrhosis, while NASH is a severe form of the disease, characterized by simple steatosis, inflammatory infiltrate, hepatocellular lesions, and fibrosis, which may eventually progress to cirrhosis [6-8]. The term NASH was first described in 1980 to describe HS associated with inflammation in patients without a history

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of chronic alcohol consumption, with moderate obesity, and having histopathological characteristics similar to alcoholic steatohepatitis [9]. NAFLD associated with cardiovascular and metabolic disorders, such as high blood pressure, obesity, insulin resistance (IR), type 2 diabetes (T2DM), and dyslipidemia are components of MS. NAFLD is known as the hepatic manifestation of MS [10], being the most common liver disease worldwide [11-13].

The pathogenesis of the disease is multifactorial. Genetic factors, together with metabolic and environmental factors, stimulate the accumulation of lipids in the hepatocyte. The first theory proposed in the last decade of the twentieth century was the "two-hit" theory. The "first hit" of this theory is represented by the appearance of IR that favors the deposition of hepatic triglycerides, triggering the appearance of HS. The "second hit" is characterized by the appearance of oxidative stress, depletion of ATP reserves and endotoxin activity, which acts on a field already affected, making it more susceptible, ultimately causing inflammation and fibrosis that can develop up to the appearance of hepatocellular carcinoma. Nowadays, the two-hit theory has been replaced by the "multiple hit" theory. This theory suggests that pathogenic factors act in parallel or sequentially and somewhat synergistically in a subject with a genetic predisposition to NAFLD [14]. Some patients will develop steatosis and consequently NASH, while other subjects may develop inflammation and fibrosis in the first stage, most likely due to the influence of epigenetic and genetic factors [15]. One-third of patients diagnosed with NAFLD will develop inflammation and fibrosis as a result of NASH, increasing the risk of cancer [15-17]. The role of NR in the pathogenesis of NASH has been analyzed by numerous authors. Willett and Mellor together with collaborators [18,19], as well as Evans [20], studied the role of other organs in the pathogenesis of NASH, such as the gut-liver axis, which is involved in both the pathogenesis and progression of liver disease. Recently, Yang's study [21] suggests that the receptors through their multiple roles in the appearance of the disease have a particular impact on the treatment of fatty liver disease, both in preventing and treating the disease. However, the mechanisms underlying NRs signaling in the onset and development of the disease are not fully known, and further studies are needed to demonstrate the role of receptors in the development and progression of liver disease. The incidence of a continuous increase of NAFLD and the lack of adequate treatment determines further research in the field to obtain a treatment for NASH that stops the evolution of the disease.

Considering that currently no treatment for NAFLD is approved, this review aims to briefly present the role of NRs in NAFLD. Among the NRs, the most promising is LXR with great therapeutic potential in numerous metabolic diseases, including NASH. In carrying out the review, we used the most relevant scientific works published in databases such as PubMed, Scopus, and Wiley Online Library.

Nuclear receptors

NRs are ligand-activated transcription factors involved in both physiology and liver pathophysiology. In humans, the NR superfamily contains 48 members, of which at least a quarter participate in liver functions. [22]. Most NRs can be activated or deactivated by numerous molecules, quickly becoming important therapeutic targets in various pathologies. Understanding the mechanisms of receptors has been intensively studied since 1980 with the cloning of the first receptors. During the last 20 years, research has focused on understanding hormonal signaling, which has contributed to the inclusion of NRs in the standard of care of various endocrine pathologies, breast and prostate cancer [23].

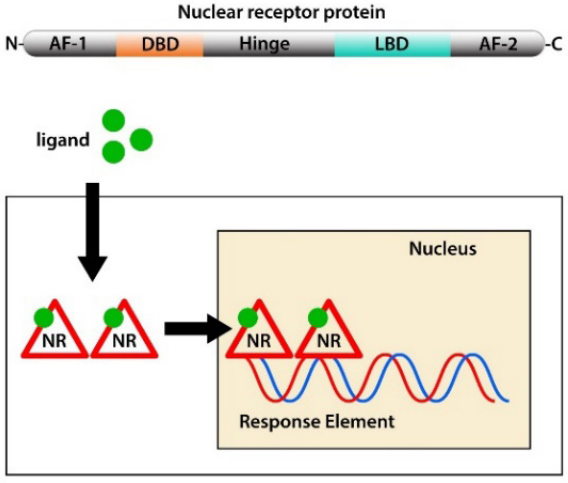

Figure 1. Transcriptional regulation and nuclear receptor protein structure.
AF-1: N-terminal activation function 1, DBD: DNA binding domain, LBD: ligand binding domain, AF-1: C-terminal activation function 2, NR: nuclear receptor.

Proteins in the structure of NRs bind to specific deoxyribonucleic acid (DNA) sequences and act as an on-off switch in the transcription process, being called ligand-activated proteins. These proteins control the process of development and differentiation of bones, skin, and nerve centers in the brain, and intervene in the process of continuous regulation of reproductive tissues. Nuclear receptor proteins consist of several domains with different roles [24]: a DNA binding domain (DBD), a variable N-terminal domain (NTD), a major region (hinge), a ligand binding domain (LBD), and a variable C-terminal domain (Figure 1). The most important domains in the

structure of receptors are DBD and LBD. In the DBD structure, there are two zinc bonds, which act as a hook that allows its binding to the chromatin in the nucleus [5]. The affinity and specificity of LBD differ depending on the ligands [25,26], because not all receptors are activated by ligands; orphan receptors are without ligands. The ligands of each class of receptors have similar structures, the activation taking place when the ligand binds to LBD [26,27].

The family of NRs consists of over 500 members, which in turn are subclassified into four classes according to key characteristics, such as the dimerization process, the specificity of the DNA binding domain, and the ligand binding. The four classes of receptors are steroid receptors (class I), retinoid X receptor heterodimers (class II), orphan homodimeric receptors (class III), and orphan monomeric receptors (class IV) [26].

Liver X receptor

LXR has been described as an "orphan" member of the nuclear receptor transcription factor family because its ligands were unknown [28]. It was first described in 1990 [29] and has been extensively studied to date, with great potential in the treatment of metabolic diseases such as atherosclerosis, hypercholesterolemia, and NAFLD. Numerous clinical trials are also underway that include LXR in the treatment of other diseases, such as diarrhea, cancer, and atopic dermatitis [30].

LXR structure

Two subunits of the LXR receptor are known, namely LXR-α and LXR-β. The gene encoding the LXR-α subunit is located on chromosome 11p11.2, and the gene encoding LXR-β on chromosome 19q13.3 [31,32]. LXR-α (NR1H3, also known as RLD-1) was first described in 1995 by Willy as being well expressed in the liver, intestine, adipose tissue, and kidney [29] and LXR-β (NR1H2, also known as NER1) being well expressed in all tissues was described in 1995 by Thebes [33]. LXR is a ligand-activated receptor that binds both endogenous and exogenous ligands [34]. Major endogenous receptor ligands are cholesterol derivatives, such as oxysterols [35], biosynthetic cholesterol intermediates called desmosterols [34,36], and polyunsaturated fatty acids [37]. The LXR consists of 4 functional domains: the N-terminal AF-1 activation domain, the DNA binding domain, a ligand-bearing domain, but also an AF-2 C-terminal domain that interacts with the coactivator acting as regulation of transcriptional activity [38].

The main role of these receptors is the transcriptional one, which regulates the activity of enzymes but also of other proteins involved in maintaining the homeostasis of energy metabolism [39].

LXR in NAFLD

The progression of liver disease towards NASH, fibrosis and later cirrhosis is a consequence of the metabolic changes that occur concomitantly at the level of liver cells and the various tissues involved. A growing body of evidence claims that LXR is directly involved in disease progression as a result of its significant roles in lipid metabolism and inflammatory signaling [40]. LXR is directly involved in cholesterol metabolism, especially LXR-α which is well expressed in the liver [41,42]. Once activated, LXR induces the expression of a group of genes involved in lipid metabolism, with a role in the absorption, transport, efflux, and excretion of cholesterol [43-45]. In addition to this metabolic role, LXR also modulates anti-inflammatory and immune responses in macrophages [46].

Also, the receptor is involved in both lipid and bile acid metabolism [41,47]. In lipid metabolism, LXR acts by regulating a group of genes that participate in the transport of excess cholesterol in the form of high-density lipoprotein (HDL) from peripheral tissues in the liver, a process called reverse cholesterol transport. In „vivo", activation of the receptor by a high-affinity synthetic ligand increases both HDL levels and cholesterol secretion [48].

LXR is also involved in regulating the activity of several enzymes of lipoprotein metabolism, such as lipoprotein lipase (LPL), cholesterol ester transport protein, and phospholipid transfer protein [49]. Also, in lipid metabolism, LXR regulates the activity of the enzyme involved in the synthesis of bile acid, CYP7A1. In animals, this enzyme increases LXR levels in response to excess cholesterol in the diet. Thus, enzymatic activation as well as the conversion of cholesterol into bile acids is a key mechanism in treating excess dietary cholesterol [50-52]. Besides its ability to modulate lipid metabolism and bile acid metabolism, LXR is also a key regulator of hepatic lipogenesis. Its lipogenic activity is secondary to the regulation of the main element of hepatic lipogenesis, sterol regulatory element binding protein-1c (SREBP-1c), which increases the level of intrahepatic lipids, being an etiological agent involved in the pathogenesis of NAFLD [53,54].

Furthermore, LXR can activate the protein that binds the carbohydrate response element (ChREBP) [55]. At the same time, ChREBP may be a target of LXR and a glucose-sensitive transcription factor involved in the hepatic conversion of carbohydrates to lipids. The LXR-mediated hypertriglyceridemic effect involves several proteins, the most specific being angiopoietin-specific protein 3 (Angplt3) [56], a protein secreted by the liver that causes increased plasma triglyceride levels secondary to inhibition of LPL activity in various tissues and secondary free fatty acids activation of lipolysis in adipocytes or apolipoprotein AV (ApoAV). Secondary LXR activation increases Angplt3 expression while ApoAV expression decreases [57].

The second important stage in NAFLD is the

appearance of proinflammatory molecules, whose expression is accelerated by LXR. These include cyclooxygenase 2, interleukin-6 (Il-6), interleukin-1b (IL-1b), chemokine monocyte-3 chemotactic protein, and monocyte-1 chemokine chemoattractant protein [58]. Activation of these pathways by LXR plays a central role in lipid metabolism and the whole body, so further investigation into synthetic LXR antagonists and/or specific agonists may represent therapeutic options for patients with NAFLD [59].

The role of LXR in NASH

NRs possesses numerous functions in terms of metabolism and the inflammatory process; in recent years, scientific efforts have focused on the role of NRs in the progression of steatosis to NASH [60]. The receptors are expressed in immune cells such as macrophages, which allows them to intervene in the inflammatory process. Taking into account this function of NRs, their pharmacological targeting could positively influence the evolution of NASH by modulating one or more pathways of disease progression, especially by reducing the metabolic stress at the level of hepatocytes [61]. In NAFLD, LXR signaling influences energy storage by activating the synthesis of fatty acids and triglycerides. Also, LXR stimulates cholesterol efflux, reducing its synthesis and absorption. Thus, LXR seems to have two opposite roles: LXR expression increases with the severity of steatohepatitis, but LXR is able to suppress inflammation and improve hypercholesterolemia. Therefore, the role of LXR in steatohepatitis remains ambiguous [61,62].

LXR-α activation has an important role in hepatic lipogenesis and the development of NAFLD, its role in the occurrence of HS being well known. However, the role of LXR-α is not very clear in the occurrence of inflammation and intrahepatic fibrosis. LXR-α stimulates intrahepatic lipid accumulation but also has anti-inflammatory properties in various tissues and cells. Wouters et al. [63] found that despite the occurrence of steatosis, pharmacological activation of this receptor decreases hepatic inflammation in parallel with intrahepatic cholesterol levels. Moreover, the study by Liu and colleagues [64] demonstrated that LXR-α activation can decrease the inflammatory lesions caused by lipopolysaccharides in NAFLD, in addition to the inhibition of pro-inflammatory macrophages. The role of LXR in endotoxemia was demonstrated by Wang [65] who states that receptor activation protects against liver damage as a result of secondary inhibition of Kupffer cell activity. All these studies from the specialized literature support the important role of LXR in the development and progression of the disease as well as the need to introduce therapy based on LXR that can influence the evolution of the disease.

Deposition of intrahepatic lipids is the first stage involved in inflammation and the process of fibrosis, however, intrahepatic triglycerides, which are the main component of liver lipids, do not influence inflammation and intrahepatic fibrosis [66]. The accumulation of intrahepatic triglycerides itself is a relatively benign and reversible condition, the consequences being secondary to the inflammation process. This is why clinicians are more interested in treating NASH than treating simple steatosis.

Apart from its role in lipid metabolism, LXR inhibits numerous pro-inflammatory signaling pathways both in various bacterial infections and during exposure to numerous cytokines in macrophages. Both LXR isoforms have pro-inflammatory effects, effects supported by numerous studies that show the inhibition of a subset of genes with an inflammatory role such as inducible nitric oxide synthesis, metalloproteinase-9 (MMP-9) and cyclooxygenase-2 (COX-2) by LXR ligands in derived macrophages from wild mice. Also, there is reciprocal onregulation between microbial ligands, LXR-dependent cholesterol metabolism and Toll-like receptor 3/4 (TLR3/4) signaling. All these evidences underline the regulatory functions of LXR between metabolism, inflammation and immunity [40]. Currently, the role of LXR-α in the development of intrahepatic inflammation and fibrosis is not clearly understood, as there are insufficient studies on the effects of LXR-α activation in humans. In this regard, Ahn et al. [67] evaluated the clinical and paraclinical characteristics of patients with NAFLD compared to control patients and demonstrated that LXR-α expression was correlated both with intrahepatic lipid deposition and the degree of liver inflammation and fibrosis in these patients as well. The authors also suggested that LXR is a potential therapeutic target for the treatment and reduction of liver inflammation and fibrosis.

Recently, Li's study [68] based on the update of the information known so far, concluded that some NR agonists had insufficient effects due to either low potency or reduced specificity at the target organ level. Further studies are needed to demonstrate the benefits of activation NR in the treatment of NAFLD.

Modulators of LXR receptors

Liver X receptors (LXRs) serve as critical regulators of lipid and cholesterol metabolism, providing potent anti-inflammatory properties [69]. In pathology, NRs serve as a vital link between metabolism, inflammation and regeneration in the liver. NAFLD/NASH is characterized by important problems such as obesity, abnormal hepatic lipid metabolism, increased inflammation and IR [61]. These NRs are essential in understanding liver diseases such as NAFLD, which is associated with altered function of NRs and disturbances along the gut-liver axis [70].

Numerous studies have highlighted the critical roles of NRs such as PPAR (α, β/δ, γ), FXR and LXR in maintaining nutritional and energy balance by influencing the gut-liver-adipose axis [71]. Modulation of NRs functions has been shown to be effective in reducing

NAFLD-related problems, including HS, inflammation, IR, fibrosis, and obesity. Understanding the significance of NRs in liver diseases is essential for the development of effective therapeutic approaches [72]. Modulation of these receptors presents an attractive strategy for addressing the multifaceted challenges presented by NAFLD and related conditions. In essence, NRs offer a promising avenue of intervention, potentially offering amelioration of a range of metabolic disorders related to disruption of nutrient and energy homeostasis [73].

To date, three classes of LXR receptor modulators have been developed. The primary categories of LXR modulators include agonists and antagonists. Agonists promote LXR activation, which results in the recruitment of coactivator proteins and increased expression of downstream target genes. Initially, selective LXR-β agonists were developed to avoid unwanted effects on hepatic lipogenesis, but the challenge lies in selectively modulating LXR-β activity over LXR-α due to their high sequence homology. Efforts have been made to develop tissue-selective LXR agonists to enhance their efficacy and safety. For example, gut-selective LXR agonists such as GW6340 have shown promise by increasing cholesterol efflux without affecting hepatic lipogenesis [34]. However, some pharmaceutical interventions have faced challenges, including unexpected adverse effects, leading to discontinuation of clinical trials [74-79].

Starting from the observations that LXR activation, on the one hand, can suppress inflammation and improve atherosclerosis, and on the other hand can promote the development of obesity and HS, the idea appeared that LXR antagonism leads to the attenuation of steatosis and liver fibrosis. So, specific LXR antagonists might be effective for NAFLD. LXR activation tends to reduce inflammation, because activated LXR can inhibit nuclear factor-κB (NF-κB) activity [80]. Cholesterol levels in the plasma membrane affect the function of Toll-like receptors (TLRs) and higher ABCA1 expression following LXR activation, which causes a reduction in membrane cholesterol content and a reduction in the sensitivity of TLRs. It has been observed that an important anti-inflammatory mechanism can occur following the expression of the ABCA1 gene and cholesterol depletion. Thus, an animal study showed a considerable reduction of steatosis, inflammation and hepatic collagen disposition following the use of an LXR antagonist, SR9238, in mice with NAFLD induced by a high-fat diet [81]. In contrast, LXR antagonists block agonist binding but have yet to demonstrate therapeutic efficacy [34].

A third class of modulators, called LXR inverse agonists, has been developed with the aim of favoring the recruitment of corepressor proteins by LXR, consequently suppressing the expression of LXR target genes, especially those related to de novo lipogenesis. A notable example is the development of two LXR inverse agonists, SR 9238 and SR 9243, which have shown potent activity for both LXR-α and LXR-β and efficiently recruit corepressor proteins. SR9238 demonstrated the ability to reduce the basal transcriptional activity of LXR-α and LXR-β and to reduce the expression of genes involved in de novo lipogenesis. Moreover, it has liver-specific effects due to its rapid metabolism [82]. In animal models, SR9238 showed impressive results. Administered to diet-induced obese (DIO) mice, it reduced HS, inflammation and hepatocellular injury while decreasing plasma LDL-cholesterol levels [81]. These benefits extended to models of NASH, where SR9238 decreased liver fibrosis. Furthermore, in the context of chronic ethanol-induced liver disease, SR9238 attenuated fat accumulation, inflammation, and fibrosis [83]. Furthermore, SR9238 revealed an interesting reduction in LDL-C levels by suppressing the expression of sterol O-acyltransferase 2 (Soat2), suggesting its potential for treating hypercholesterolemia [84,85].

The development of LXR inverse agonists sparked interest, and concurrently additional compounds with similar pharmacological profiles emerged, including cholestenoic acid analogs, fluorinated oxysterol agonists, and nonsteroidal LXR inverse agonists [86-89]. Oxysterols, derivatives of oxidized cholesterol, which can bind to LXRs are involved in various metabolic processes by binding to NRs, including LXRs [90-93]. Emerging evidence suggests that liver and serum levels of certain oxysterols are increased in NAFLD patients, but their precise roles in NAFLD pathogenesis remain unclear [94,95]. Fluorinated oxysterol agonists, developed as LXR inverse agonists, have a relatively low potency on LXR activation. Chen et al. [86] identified several fluorinated oxysterol agonists, known as nonsteroidal LXR inverse agonists, that exhibited a significant degree of LXR-β selectivity, approximately 3.5 times less potent than SR9238. Starting from this component, Phenex Pharmaceuticals developed additional LXR inverse agonists, known as TLC-2716, which is currently in phase I clinical trials for treatment of severe dyslipidemia [96]. Some oxysterols have conflicting effects on lipid accumulation, indicating the need for further investigation [97-99].

Recent studies have explored the potential benefits of combining LXR with other agents such as glucocorticoids [100]. Optimizing the development of tissue-selective LXR agonists holds promise for achieving potent, safe, and specific therapeutic efficacy without unintended side effects [40].

Although LXRs have historically been targeted for agonist development to promote reverse cholesterol transport (RCT), recent focus has shifted to LXR inverse agonists due to their ability to suppress LXR target genes involved in lipogenesis de novo. These inverse agonists have shown promising results in animal models, offering

potential treatments for NASH, hypercholesterolemia, and cancer. Several LXR inverse agonist chemicals have been identified, with some progressing to clinical trials, expanding the therapeutic landscape for metabolic disorders [82]. NRs are involved in both the prevention and treatment of NAFLD. However, the underlying mechanisms of NR signaling at onset as well as during disease progression remain unclear.

Conclusions

NAFLD is a major public health problem worldwide, for which there is currently no approved treatment. Currently, NR are one of the potential therapeutic targets for the treatment of steatohepatitis. LXR is the most studied receptor, due to its metabolic role and its ability to modulate anti-inflammatory and immune responses.

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