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LXRs in the Cardiovascular System

Liver X receptors and the cardiovascular system – lipids and beyond

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Submitted

ABSTRACT

Liver X receptors (LXRs) are key players in cholesterol and lipid metabolism. In the last decade, several pleiotropic functions have been discovered, that suggested that LXRs may be potential targets in inflammatory response, hypertension, and cell proliferation. This review focuses on the role of LXRs in the cardiovascular system, discussing their effects on the development of atherosclerosis, inflammatory responses, cardiomyocyte hypertrophy, and cardiac remodeling.

INTRODUCTION

Although liver X receptors (LXRs) were originally identified as key players in cholesterol and lipid metabolism,¹ numerous pleiotropic functions of LXRs have been revealed since.² The importance of normal LXR functioning for maintaining homeostasis in many physiological processes is further strengthened by the observation that LXR functioning is associated with longevity.³ In this review we will discuss how LXR functioning, in both lipid metabolism and other physiological processes, affects the cardiovascular system.

Nuclear hormone receptors

LXRs are members of the nuclear hormone receptor family. Nuclear hormone receptors consist of a large group of nuclear receptors that exert a wide variety of actions.⁴ They directly bind to genomic DNA and act as transcription factors by binding onto specific elements in the DNA (hormone response elements; HREs), thereby regulating the transcription of genes downstream the HREs. Their natural ligands are small lipophilic molecules (e.g. hormones) or metabolic intermediates (sterols, prostaglandins). Several reviews have recently been published that cover contemporary knowledge on nuclear hormone receptors.^{5,6,7}

Nuclear hormone receptors are grouped into a large superfamily and are thought to be evolutionary derived from a common ancestor. Evolutionary analysis of the receptors has led to a subdivision in six different subfamilies, based on their similarities in areas of structural homology.⁴ One of these subfamilies is formed by a large class of ex-orphan receptors, including LXRs, thyroid receptors, vitamin D receptors (VDRs), peroxisome proliferator activated receptors (PPARs), and retinoic acid receptors (RARs). Although these receptors are mostly known for their roles in growth, differentiation, metabolism, reproduction, and morphogenesis of higher organisms and humans, recent studies have described several ancillary effects. For example, a large number of nuclear hormone receptors has been shown to influence the renin-angiotensin-aldosterone system (RAAS); the main regulator of blood pressure homeostasis.⁸

LXRs: discovery, ligands, and mechanisms of action

LXRs were first identified in 1994 and 1995 by independent groups.^{9,10,11} Because their biological ligands were initially unknown, they were originally classified as “orphan receptors”. In the following years it was discovered that endogenous oxysterols (oxidized cholesterol derivatives) serve as activators of LXRs - LXRs were thus “deorphanized”.¹²

LXRs form a heterodimer with the retinoid X receptor (RXR). This complex binds to specific parts of the genome called LXR response elements (LXREs). LXREs consist of direct repeats of the core sequence AGGTC A separated by 4 nucleotides, hence denoted DR4.¹¹ In the non-active state, the LXR/RXR complex is occupied by corepressor complexes. However upon ligand binding, the corepressor complexes are exchanged by coactivator complexes, which results in the transcription of specific target genes of LXR. The LXR/RXR complex is a “permissive heterodimer” that can be activated by ligands of either partner (as opposed to “nonpermissive heterodimers” that can also be formed with RXR, but are only activated by ligands specific for its binding partner). Ligand activation of LXRs can also result in

transcriptional regulation of genes that do not contain a LXRE. Through a process of trans-repression, LXR activation may inhibit the transcription of certain genes, e.g. proinflammatory cytokines.¹³ Finally, LXRs are capable of regulating gene transcription in a ligand-independent fashion.¹⁴ Activated by cAMP, LXRs have been shown to bind to a specific cAMP responsive element, called CNRE (cAMP negative responsive element). This responsive element, which is distinctly different from DR-4 elements, is found in the promoters of several cAMP responsive genes, such as c-myc, tyrosine aminotransferase, and renin. These modes of action all play a role in LXRs' impact on CV disease.

There are two known isoforms of LXR; LXR- α (NR1H3) and LXR- β (NR1H2).¹⁵ The two isoforms share a close homology and have approximately 77% identical amino acid sequences in both DNA- and ligand-binding domains. The LXR- α isoform is predominantly expressed in cells and tissues that play a role in cholesterol metabolism (such as the liver, intestine, and in macrophages) but also in considerable quantities in organs less importantly involved in cholesterol metabolism, such as the kidney and the heart. The LXR- β isoform is expressed in all tissues examined.¹⁶

CHOLESTEROL AND LIPID METABOLISM

Since lipid metabolism and lipid disorders are quintessential in CV disease, the role of LXRs in lipid metabolism is herein highlighted. When oxysterols were identified as potent LXR ligands,¹² their role in cholesterol metabolism soon became evident. The creation of mice with genetic disruption of LXR- α (LXR- $\alpha^{-/-}$ mice) showed that LXR- α is crucial in cholesterol clearance from tissues. When fed with a high cholesterol diet, LXR- $\alpha^{-/-}$ mice exhibited a decreased metabolism of cholesterol to bile acids and a dramatic accumulation of cholesterol esters in the liver.¹ Since these effects are far less pronounced in LXR- $\beta^{-/-}$ mice,¹⁷ it is assumed that LXR- α is the dominant isoform in so-called reverse cholesterol transport (RCT).

Target genes

One of the first genes identified as a LXR- α target gene is *Cyp7a1*, which encodes the rate-limiting enzyme in the synthesis of bile acid from cholesterol.¹⁸ The absence of hepatic *Cyp7a1* expression in LXR- $\alpha^{-/-}$ mice causes a near complete disability to metabolize cholesterol to bile acids, and this contributes to the accumulation of cholesterol esters in the liver. Subsequent studies identified LXR as a regulator of the expression of ATP-binding cassette (ABC) membrane transporters, such as ABCA1 and ABCG1. These transporters mediate the efflux of cholesterol from cells (in the form of high-density lipoproteins [HDL] back to the liver to be excreted in the bile, the process known as RCT. In addition to this, LXRs are also important regulators of hepatic lipogenesis. LXRs regulate the expression of sterol regulatory element binding protein (SREBP)-1c;¹⁹ a transcription factor that regulates the expression of all genes involved in hepatic fatty acid biosynthesis, e.g. acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase (SCD).

ATHEROSCLEROSIS

Development of atherosclerosis and role of lipids

Because of their eminent role in cholesterol metabolism, the potentially beneficial role of LXRs in the

treatment and development of atherosclerosis has been studied extensively. LXR activity is important during (almost) all stages of atheroma formation, and LXRs are therefore an attractive therapeutic target. Atherosclerosis is multifactorial, however, high plasma concentrations of cholesterol, particularly low-density lipoprotein (LDL), form a major risk factor.²⁰ Early lesions consist mainly of monocyte-derived macrophages, filled with cholesterol, that are formed after monocytes adhere to the damaged vessel wall.²¹ They further differentiate into macrophages that exhibit massive accumulation of cholesterol ('foam cells'). Progression of the lesion involves inflammatory processes: influx of T-cells, macrophages, and smooth muscle cells, production of cytokines. Death of the lipid-laden foam cells may lead to the formation of a necrotic core enclosed in a fibrous cap. The rupture of an advanced lesion can lead to thrombus formation that may occlude a vessel lumen, inducing ischemia in the heart, brain, or extremities, resulting in infarction.

Importance of reverse cholesterol transport (RCT)

The RCT is an endogenous system to clear cholesterol from tissues, and is thought to be essential to prevent atherosclerosis. Patients that lack the gene encoding ABCA1, who have a defective RCT, develop accelerated atherosclerosis.²² LXRs are key mediators of the RCT. LXRs (and other nuclear hormone receptors) do not only influence cholesterol metabolism systemically but also regulate lipid homeostasis and inflammatory responses in macrophages, endothelial cells, and smooth muscle cells within the artery wall. LXRs are expressed abundantly in macrophages and are involved in the scavenger function of macrophages. LXRs in macrophages are activated by oxysterols that are produced in proportion to cellular cholesterol content.²³ Furthermore, apoptotic cells in atherosclerotic plaques are internalized by macrophages, also initiating the RCT pathway, which protects the cell from lipid overload and its cytotoxic effects. When activated, LXRs stimulate expression of several genes involved in lipid metabolism and reverse cholesterol transport like ABCA1 and ABCG1.

On a functional level, these mechanisms were confirmed by murine studies that showed that activation of LXR with synthetic ligands inhibits the development of atherosclerosis in apoE^{-/-} and LDL-receptor^{-/-} mice.^{24,25} The significant induction of ABCA1 in atherosclerotic lesions of apoE^{-/-} mice suggested that a direct effect of the LXR agonist on macrophages in the arterial wall could have contributed to the anti-atherosclerotic effect of the LXR ligand. Subsequent studies using bone marrow transplantations confirm the key role of macrophages and LXR in this process: transplantation of apoE^{-/-} and LDL-receptor^{-/-} mice with bone marrow from LXR^{-/-} mice resulted in an *increase* of atherosclerosis.²⁶ Mechanistic studies showed that the anti-atherosclerotic effects of LXR activation in macrophages seem to be largely attributable to the anti-inflammatory effects of LXR activity²⁷ (and reviewed by²⁸).

LXR and the inflammatory response

Macrophages are key players in inflammatory processes, including in lipid metabolism and atherosclerosis.²⁸ Working as scavengers of pathogens and apoptotic cells, they coordinate inflammatory responses by the release of cytokines and chemokines. The expression of these inflammatory mediators is, like with LXRs, mediated by oxidized low-density lipoprotein (oxLDL) particles. Lipid disorders and other CV risk factors, like diabetes, lead to the accumulation of oxLDL particles in macrophages and increased susceptibility to atherosclerosis.²⁹ This leads to subsequent activation of LXRs, which

should be regarded as an endogenous response to neutralize the increased lipid and inflammatory stimuli. LXRs have been shown to inhibit expression of genes involved in the innate immune response in activated macrophages *in vitro*.²⁷ *In vivo*, activation of LXRs leads to diminished inflammatory gene expression also, whereas LXR^{-/-} mice exhibit enhanced reactions to inflammatory stimuli in a murine model of contact dermatitis.³⁰ Similarly, LXR^{-/-} mice are highly susceptible to infection when challenged with the gram-positive bacteria *Listeria monocytogenes*.³¹ LXR^{-/-} mice were unable to recover from an infection with *L. monocytogenes* and did not survive the challenge, whereas wildtype mice were able to effectively clear the same dose of *L. monocytogenes*.

The anti-inflammatory capacity of LXRs is an important feature for the scavenger function of macrophages. When apoptotic cells are internalized by macrophages, inflammatory responses are not appropriate. The cholesterol derivatives produced by these apoptotic cells activate LXRs, resulting in a repression of inflammatory gene expression. When pathogens are internalized however, an inflammatory response is warranted. Since most pathogens lack cholesterol derivatives (the natural ligands for LXRs), they are not likely to activate LXRs and thus repress immune responses.

It is believed that a major part of the anti-inflammatory effects of LXRs are mediated via nuclear factor-kappa (NF-κ)B.²⁷ LXR agonists repress the expression of multiple NF-κB target genes, although the upstream signaling pathway controlling NFκB activation is not altered by LXR agonists, suggesting that LXR agonists may exert their effects downstream of NFκB binding to DNA.³² In contrast to NF-κB, LXR activation leads to increased expression of tumor necrosis factor (TNF)-α. Transfection analysis and inhibitor studies demonstrated that the TNF-α promoter contains an LXRE, which makes it a direct target for transactivation by LXR/RXR heterodimers.³³ This seems in contrast with the anti-inflammatory capacities of LXR signaling. In this process however, the absence of the other primary cytokines normally associated with inflammation suggests that LXR activation does not elicit a generalized pro-inflammatory response. In addition to the regulation of the specific inflammatory markers NFκB and TNF-α, LXR activity protects macrophages from apoptotic signalling pathways.³⁴

Genetics

In patients, there is a correlation between the severity of coronary atherosclerosis and the blood cellular LXR-α genomic profile.³⁵ Human blood mononuclear cells show a gradual increase in LXR-α gene transcription with respect to severity of coronary occlusion in patients suffering from coronary heart disease. This seems in contrast with the observed protective role of LXR-α in the development of atherosclerosis. However, functional genomics of the LXR-α mRNA revealed three critical mutations in the ligand binding domain of the blood cellular LXR-α gene in these patients.³⁶ *In vitro* studies show that these mutations render the LXR-α protein unable to bind to its natural ligands. These studies suggest that humans having a mutated LXR-α gene may develop a predisposition towards coronary heart disease. A defective LXR-α gene can affect cardiac function through several mechanisms.

EFFECTS OF LXRS BEYOND LIPIDS: CARDIAC REMODELING

Cardiac (or ventricular) remodeling is the common term used for pathophysiological changes in size, shape, and function of the heart after cardiac injury.³⁷ Remodeling may occur after myocardial in-

fraction, in response to chronic pressure overload (aortic stenosis, hypertension), volume overload (valvular regurgitation), inflammatory heart muscle disease (myocarditis), congenital heart disease or genetic cardiomyopathy. The initial remodeling response after cardiac injury is considered to be beneficial and is aimed to cope with the increased loading and work load of the heart. The cellular re-arrangement of the ventricular wall and activated neurohormonal systems results with maintained (or improved) cardiac output. However, the very adaptive mechanisms that are initially beneficial become adverse on the long term and progressive remodeling occurs, which ultimately may lead to heart failure. The time course and the extent of remodeling are influenced by many factors, such as the severity of the insult, secondary events (recurrent ischemia or infarction), neurohormonal activation, hemodynamic load, and genetic factors.

Recently, we and others showed that activation of LXRs in a model of pressure overload leads to attenuated cardiac hypertrophy.^{38,39} The exact mechanism underlying this effect is still unclear, but it has been shown that LXRs are involved in several factors that influence the development of cardiac remodeling, such as inflammatory responses and the RAAS.

Inflammatory responses

Injury to the heart triggers a reparative response that activates the innate immune system, resulting in an inflammatory reaction. Central player in both hypertrophy and inflammatory responses resulting from cardiac injury is NFκB.⁴⁰ Although the exact role of NFκB in cardiac inflammatory responses is still under investigation, it has been shown that blocking NFκB activity in a rat model of myocarditis prevents the progression of the cardiac inflammation.⁴¹ Also in patients, increased activation of NFκB is related to inflammatory processes in myocarditis.⁴² In addition to this, NFκB has proven to be a crucial player in the development of cardiac remodeling.⁴³

So far, little is known about the effects of LXR activation on cardiac inflammatory responses. However, LXR has been identified as a negative regulator of NFκB.²⁷ This might explain why activation of LXR results in attenuated cardiac remodeling in a model of cardiac pressure overload.³⁹ Indeed, Wu *et al.* showed that in cultured cardiomyocytes, activation of LXRs by a synthetic agonist suppressed both angiotensin II and lipopolysaccharide induced upregulation of NFκB activity, resulting in decreased expression levels of TNF-α, interleukin-6, and monocyte chemoattractant protein-1.³⁸ After the challenge with angiotensin II or lipopolysaccharide, the hypertrophic response of these cultured cardiomyocytes was attenuated when the cells were treated with the LXR agonist. We confirmed these data in neonatal rat derived ventricular cardiomyocytes and in the mouse atrial cell line HL-1 challenged with endothelin-1 or angiotensin II.³⁹ In both cell types, treatment with a LXR agonist attenuated the endothelin-1 or angiotensin II induced hypertrophic response. Continuous research will have to determine the exact role of LXR induced blunting of NFκB signaling and how this affects both inflammatory and hypertrophic responses in cardiomyocytes.

The RAAS

The RAAS plays a central role in the development of cardiac remodeling. Therefore, treatment strategies targeting the RAAS, including angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), and aldosterone receptor antagonists, have been very beneficial in the preven-

tion of progressive remodeling and heart failure.⁴⁴ Numerous nuclear hormone receptors are able to influence RAAS activation, among which LXR⁴⁵ and PPAR- γ .⁴⁶ The important role of LXR in the RAAS has been substantiated by several *in vitro* and *in vivo* observations. First, it was shown by Tamura *et al.* that LXR- α functions as a cAMP-responsive regulator of renin in As4.1 cells (cells resembling juxtaglomerular [JG] cells, which produce renin).¹⁴ LXR- α interacts with a specific DNA element in the renin promoter, a CNRE. These findings were confirmed in an *in vivo* study, showing that also in mice LXR- α (and LXR- β) binds to the CNRE, and mice deficient for LXRs lose their capacity to up-regulate renin under β -adrenergic stress.⁴⁷ LXR- α and renin show strict co-localization in the JG cells. Single administration of a LXR agonist caused increased renin transcription within a few hours after administration. However, this was transient and returned to normal within hours. Recently, a role for LXR- α was reported in the differentiation of mesenchymal cells into JG cells.⁴⁸ Still, it remained unclear whether LXR- α is a negative or positive regulator renin *in vivo*.

Using a long-term murine model of RAAS activation (isoproterenol infusion or 7 days), we have shown that concomitant activation of LXR leads to diminished mRNA expression of several RAAS components, including renin, but also ACE and angiotensin type I receptor (AT₁R) mRNA expression.⁴⁵ Using LXR- α ^{-/-} mice, we confirmed in our models that the isoproterenol-induced RAAS activation is LXR- α dependent. These findings were in line with an *in vitro* study of Imayama *et al.*, who demonstrated LXR to be a negative regulator of AT₁R expression in cultured vascular smooth muscle cells.⁴⁹ On the other hand, Leik *et al.* showed that LXR activation by GW3965 leads to a transient increase in vascular gene expression of the angiotensin type I receptor, which decreased over time.⁵⁰ These findings in fact resemble the reported short-term increase of renin transcription after a single dose of T09 (reported by Morello *et al.*)⁴⁷ and the decrease of renin gene expression we found after a long-term period of LXR activation.⁴⁵ For both AT₁R and renin transcription, it seems that LXR activation results in a transient increase, followed by gradual and sustained decrease over time at a transcriptional and translational level. This hypothesis is supported by the observation by Leik *et al.* and our group; that long term treatment with a LXR agonist results in significant reductions of blood pressure, although this has not unequivocally been linked to the level of RAAS activation. All together there are convincing data to show that LXRs regulate renin transcription and that, in long term *in vivo* experiments, LXR activation is associated with inhibition of the RAAS.

SUMMARY AND FUTURE PERSPECTIVES

Over the last decade, the knowledge on LXR functioning in (patho)physiological conditions has increased intensively. As inhibitors of inflammatory responses, hypertension, cardiomyocyte hypertrophy, etc., LXR agonists may present attractive remedies in the treatment of several cardiovascular diseases. The stimulatory effect of LXR activation on SREBP-1c forms a major challenge in the development of LXR-based therapeutics; increased SREBP-1c activity leads to hypertriglyceridemia and fatty liver (steatosis hepatis). The successful development of LXR-based therapeutics requires strategies to exploit the beneficial aspects of LXR activation while avoiding these unwanted side effects. So far, attempts to limit increases of hepatic and plasma triglycerides in response to LXR-agonist treatment remain unsuccessful.⁵¹

The main challenge remains to exploit these beneficial properties of LXRs while retaining side effects of LXR activation, such as hypertriglyceridemia and steatosis hepatic. So far, the development of isoform, tissue, or gene specific LXR agonists has been proposed as potential solutions for these unwanted side effects.⁵²

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