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Liver X receptor in the cardiovascular system

Kuipers, Irma

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Document Version

Publisher's PDF, also known as Version of record

Publication date:
2010

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Kuipers, I. (2010). *Liver X receptor in the cardiovascular system*. s.n.

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Summary and Future Perspectives

SUMMARY

Liver X receptors (LXRs) are nuclear hormone receptors that act as ligand-activated transcription factors.¹ They are critically involved in cholesterol metabolism² and their natural ligands are oxysterols; oxidized cholesterol derivatives.³ There are two known isoforms of LXRs; LXR- α (mainly expressed in liver, spleen, intestine, macrophages, heart and kidney) and LXR- β (expressed ubiquitously).⁴ Over the last decade, research on LXRs was mainly focused on their role in cholesterol metabolism. It was discovered that LXRs play a crucial role in the process of 'reverse cholesterol metabolism'; the efflux of cholesterol from peripheral tissues towards the liver for excretion.² More recent studies show that LXRs also exert pleiotropic properties. They have been shown to attenuate proliferative pathways,⁵ blunt inflammatory responses,⁶ and influence the renin-angiotensin-aldosterone system (RAAS).⁷

Metabolic and inflammatory pathways highly influence the onset and development of cardiovascular disease. Both pathways are controlled by complex mechanisms, in which LXRs play a critical role.⁸ However, knowledge of the direct effects of LXRs on the cardiovascular system remains scarce. In this thesis, several models of cardiac remodeling and cardiac failure have been used to investigate the role of LXRs in the pathophysiology of these conditions. **Chapter 1** provides an extensive overview of previous studies on LXR functioning in the cardiovascular system, extended with our own findings described in detail in the following chapters.

The RAAS

The RAAS has been identified as the main system involved in blood pressure regulation, renal hemodynamics, and sodium volume homeostasis. Furthermore, the RAAS directly affects vascular and cardiac remodeling through proliferative and inflammatory signaling. Pharmacological targeting of the RAAS is a consolidated and evidence-based approach in the treatment of various aspects of cardiovascular disease. An exploding number of recent studies have provided novel insights into nuclear receptor biology in relation to cardiovascular (patho)physiology. **Chapter 6** reviews the potential effects of nuclear hormone receptors (NHRs) on the RAAS, summarizing the extensive body of evidence from experimental, animal, and clinical studies, suggesting that NHRs and the RAAS are closely intertwined.

A specific interaction of LXRs and the RAAS is described in **chapter 2**. Here we report that activation of LXR with the specific agonist T09 leads to a decrease in renal and cardiac RAAS activation due to isoproterenol (ISO) infusion. The LXR agonist T09 blunts ISO induced increases in renin, angiotensin converting enzyme (ACE) and angiotensin type 1 receptor (AT₁R) expression *in vitro* and *in vivo*, and competes with the binding of LXR- α to the CNRE in the renin promoter. The observed effects are completely absent in LXR- α ^{-/-} mice, suggesting LXR- α dependency. On the basis of these data, we postulate that LXR agonists may serve as inhibitors of the RAAS.

Chapter 2 describes the first long-term *in vivo* study focussing on LXR signalling in the RAAS. Previously, LXR- α was denoted as a cAMP-dependent regulator of renin *in vitro*,⁹ and Morello *et al.*⁷ showed that immediately after administration of a synthetic agonist, LXR- α regulates renin transcription *in vivo*. The transcriptional regulation of ACE is largely unknown, whereas regulation of AT₁R is tight, complex and well described.¹⁰ Our findings of decreased renal AT₁R mRNA expression are in line with

those of Imayama *et al.*,¹¹ who demonstrated LXR to be a negative regulator of AT₁R in cultured vascular smooth muscle cells. In line with this, Leik *et al.* showed that LXR activation by GW3965 leads to reduced angiotensin II-mediated pressor responses in rats.

After identifying LXRs as negative regulators of the RAAS, we hypothesized that LXR activation protects the heart from disadvantageous effects of hypertension.

Cardiac remodeling

Cardiac hypertrophy

The hypothesis that LXR activation protects the heart from disadvantageous effects of hypertension was tested in **chapter 3**. This study demonstrates that the activation of LXRs attenuates cardiac hypertrophy. Using an *in vitro* model of cultured cardiomyocytes, we show that LXR activation attenuates cardiomyocyte hypertrophy. In addition, we show in an *in vivo* model of cardiac hypertrophy that LXR activation decreases cardiac remodeling along with hypertension and improves diastolic parameters. These effects were substantially blunted in LXR- $\alpha^{-/-}$ mice, indicating that these are LXR- α -dependent effects.

Recently, Wu *et al.*¹² reported that LXR activation in cultured rat cardiomyocytes reduces hypertrophy in response to angiotensin II treatment. Here we confirm and extend these results; we prove that this is indeed a LXR-dependent effect. Employing siRNA interference, which causes knock-down of LXR expression, we showed that the attenuating effect of T09 is absent in this condition.

To corroborate these *in vitro* findings *in vivo*, we employed a mouse model to test the effects of LXR activation on myocardial hypertrophy. Here we show that also in this model, T09 exerts anti-hypertrophic effects. In addition to a decrease in cardiac remodeling after AC surgery, we observed a decrease in blood pressure following treatment of wildtype mice with T09, which is in line with our findings described in chapter 2.

The mechanism through which LXRs exert their anti-hypertrophic effect is still under investigation. It has been observed in clinical studies that changes in left ventricular hypertrophy (LVH) may not be exclusively explained by the blood pressure lowering effect of a drug.¹³ In addition to the blood pressure lowering effect, direct LXR activation in the myocardium may also explain the observed changes in LVH. Several *in vitro* studies have described anti-proliferative effects of LXR activation in various cell types, including VSMCs,⁵ pancreatic islet β cells,¹⁴ keratinocytes,¹⁵ and breast cancer cells.¹⁶ Our study demonstrates that LXR activation also inhibits cardiomyocyte hypertrophy. Wu *et al.*¹² recently suggested that nuclear factor (NF)- κ B may confer the antihypertrophic effects of LXR- α in cardiomyocytes. However, other cardiac transcription factors such as Sp1 or other kinases such as mitogen-activated protein kinase (MAPK) may be controlled by LXRs as well and thus be involved in remodeling pathways of the hypertrophic heart. We show here that like in other tissues or cell types,¹⁷ the transcription factor sterol regulatory element binding protein (SREBP)-1c is increased in the hearts of mice treated with T09. SREBP-1c has been shown to be involved in parasympathetic responses in the murine heart,¹⁸ however its role in the development of cardiac remodeling remains to be elucidated. In addition, we also show an increase in mRNA expression of other specific target genes of LXR, e.g. ATP-binding cassette transporter (ABC)A1 and ABCG1, combined with an increase in protein levels of

ABCG1. These changes in specific LXR target genes show increased activity in the myocardium of LXR as a transcription factor. Together with the activation of systemic LXR targets (as shown by serum cholesterol increases), these data strongly support the notion that LXR specific pathways are activated by T09 treatment, both systemically and locally (in the myocardium).

Myocardial infarction

In **chapter 5** we show that activation of LXR results in attenuated cardiac hypertrophy after a myocardial infarction (MI). This was demonstrated by the attenuation of LVH and LV dilatation observed in T09-treated mice compared to the MI mice without T09 treatment. Also, the decrease in wall thickening after MI was attenuated in MI+T09 mice compared to MI mice. In addition, fractional shortening and ejection fraction were higher in MI+T09 mice compared to MI mice. These results provide further proof for a potential role for LXR activating drugs with the aim to prevent cardiac remodeling.

In this study, we did not perform additional tissue analyses that would provide mechanistic clues as to how LXR exerts its cardio-protective effects. It seems plausible that LXR attenuates the complicated process of post-MI cardiac remodeling via various pathways, as LXR influences so many (pleiotropic) processes. Direct actions on cardiomyocytes,¹⁹ macrophages,⁶ and the RAAS⁷ enable LXRs to influence cardiac remodeling, inflammatory responses and blood pressure homeostasis. Because all these processes are critically involved in the process of cardiac remodeling post-MI, further research is warranted to establish through which mechanism(s) LXR influences this process.

Statins

In **chapter 4** we studied the interplay of statins with LXRs. Here we describe that in a model of pressure overload, treatment with pravastatin attenuates LVH in wildtype mice but not in LXR- $\alpha^{-/-}$ mice. Since we have previously showed that activation of LXRs by a synthetic agonist attenuates the development of LVH,¹⁹ these data strongly suggest an important role for LXRs in the pathway through which statins are able to attenuate the development of LVH.

That statins are able to attenuate LVH has been known for some time (reviewed in²⁰), but it remains unclear through which mechanism this effect is exerted. The data described in chapter 4 (as well as earlier publications²¹) show that in wildtype mice, statin treatment results in lowered MAP. This suggests that the attenuation of LVH in these mice might be exerted by the blood pressure lowering effects of statins. In LXR- $\alpha^{-/-}$ mice statins did not affect the MAP, showing a crucial role for LXRs in the blood pressure lowering effects of statins. Indeed, earlier studies show that activation of LXRs results in blunted activation of the RAAS and decreased blood pressure.^{22,23}

However, as mentioned above, clinical studies show that changes in LVH may not be solely explained by the blood pressure lowering effect of a drug.¹³ Previously we showed that *in vitro* (so without any systemic haemodynamic effects), LXR activation results in attenuation of cardiomyocyte hypertrophy.¹⁹ In addition we show in chapter 4 that statin treatment directly affects cardiac mRNA expression of several target genes of LXR. So on top of its blood pressure lowering effects, statins also directly affect LXR target gene transcription in cardiomyocytes. These findings confirm interplay of LXRs and statins and suggest that the protective role of statins in the development of LVH is mediated by LXR- α .

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 form adequate remedies in the treatment of several cardiovascular diseases. The main challenge remains to exploit these beneficial properties of LXRs while retaining side effects of LXR activation, such as hypertriglyceridemia and steatosis hepatic.²⁴ So far, the development of isoform, tissue, or gene specific LXR agonists has been proposed as potential solutions for these unwanted side effects.²⁵

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