Liver X receptor in the cardiovascular system

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LXR Activation Attenuates Cardiac Remodeling

Liver X receptor activation attenuates cardiac remodeling after myocardial infarction

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ABSTRACT

Liver X receptors (LXRs) play a crucial role in lipid and cholesterol metabolism, but can also act as a modulator of the cardiovascular system. We hypothesized that LXR activation reduces adverse cardiac remodeling after a myocardial infarction. Male C57Bl/6j mice were subjected to myocardial infarction (MI) by permanent ligation of the left anterior descending coronary artery. Three experimental groups were studied; sham, MI, and MI treated with the synthetic LXR agonist T0901317 (T09). After 3 weeks, myocardial function was assessed and animals were sacrificed hereafter. Ejection fraction, fractional shortening and hypertrophic responses of the myocardium were significantly affected in MI mice compared to MI-sham mice. In MI-T09 mice, ejection fraction and fractional shortening were improved and hypertrophic responses were attenuated compared to MI mice. In conclusion, LXR activation improves cardiac function after MI, confirming a cardio-protective role for LXR.

INTRODUCTION

Cardiac (or ventricular) remodeling is the common term used for pathophysiological changes in size, shape, and function of the heart after cardiac injury.[1] Remodeling may occur after myocardial infarction, but also in response to chronic pressure overload (aortic stenosis, hypertension), volume overload (valvular regurgitation), inflammatory heart muscle disease (myocarditis), congenital heart disease (intracardiac shunting) or genetic cardiomyopathy. The initial remodeling response after cardiac injury is considered 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 adapative 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.

The nuclear hormone receptor LXR plays a central role in reverse cholesterol metabolism, which stimulates cholesterol efflux from tissues.[2] Endogenous ligands of LXRs are oxidized cholesterol particles (oxysterols); intermediate metabolites of cholesterol.[3] Activation of LXRs initiates the transcription of downstream genes, which stimulate the cholesterol efflux. Therefore LXR has been proposed as a potential target in the treatment of atherosclerosis. Recently, it has been shown that the liver X receptor (LXR)-α attenuates cardiac remodeling in models of pressure overload.[4,5]

Aside from their central role in cholesterol metabolism, LXRs modulate many other pathways of the cardiovascular system. It has been shown that LXR activation leads to decreased activity of the renin-angiotensin-aldosterone system (RAAS).[6,7] In addition, increased LXR activity leads to diminished inflammatory responses.[8] Macrophages are known to be critically involved in the inflammatory responses after MI.[9] Interestingly, macrophages, which express LXR abundantly, show altered inflammatory responses when treated with LXR agonists.[10] Myocardial infarction (MI) remains to be the main reason for left ventricular (LV) dysfunction in a clinical setting. This post-MI remodeling is highly influenced by inflammatory responses.[11]

In summary, LXRs have been shown to play a protective role in the cardiovascular system. In addition, they are capable of attenuating inflammatory responses. Therefore, we hypothesize that LXR activation would reduce adverse cardiac remodeling after MI.

METHODS

Animals and housing conditions

All experiments were approved by the local Committee on Animal Experimentation and were performed under international guidelines on animal experimentation. Male C57Bl/6j WT mice were obtained from Harlan (Netherlands) at the age of 9 weeks. During the entire experiment, animals were kept on a 12 hour light:12 hour dark cycle with ad libitum access to food and water.

Induction of MI

Mice (n=6-8) were anesthetized with isoflurane, and intubated using a 20-gauge intravenous catheter with a blunt end. Mice were artificially ventilated at a rate of 150 strokes/min, using a rodent ventilator (Harvard Apparatus Rodent Ventilator Model 845) with isoflurane (2% in O2). The thorax was opened in the third intercostal space and the left anterior descending (LAD) coronary artery in mice was permanently ligated with a 6-0 non-absorbable prolene suture above the branching of the LAD. Sham-operated controls underwent the same procedure, except for induction of MI. Immediately after the surgical procedures, mice were fed either standard laboratory chow or chow supplemented with the synthetic LXR agonist T09 (50 mg/kg/day) throughout the entire experiment. This dose was previously shown to cause a strong, sustained activation of LXRs *in vivo*.[12]

Echocardiographic measurements

Three weeks after MI or sham operation cardiac dimensions were measured using transthoracic echocardiography with a 14 MHz transducer (Vivid 7, GE Healthcare, Diegem, Belgium). Mice were anesthetized as described above and body temperature was maintained by placing the mouse on a heating pad. Short-axis view and M-mode tracings were used to determine end-diastolic LV internal diameter (LVIDd), posterior wall thickness (LVPWd), and interventricular septal thickness (IVSd). From M-mode tracings fractional shortening and ejection fraction (Teichholz's formula) were calculated. Mitral valve Doppler signals were used to establish heart rate (HR) and left ventricular cardiac output (LVCO), from which the stroke volume was calculated.

Statistical analysis

Measured values are presented as means ± standard error of the mean (SEM), unless stated otherwise. Statistical analysis was performed using an analysis of variance (ANOVA) with post hoc comparisons (Tukey's test). A p-value of <0.05 was considered statistically significant.

RESULTS

LXR activation attenuates MI-induced cardiac hypertrophy

Three weeks after MI, mice were sacrificed and the LV and liver were weighed. The T09 treatment caused a significant increase in liver weights in the MI+T09 group (table 1). This is caused by accumulation of triglycerides in the liver; a known side effect of LXR agonism.[13] These data confirm that the T09 diet stimulated LXR activation.

LV weights were increased after MI compared to the LV weights of sham-operated mice (table 1).

Table 1

animal characteristics 3 weeks after MI or sham operation

	sham	MI	MI + T09
body weight (g)	30.0±0.8	28.5±0.5	26.9±1.5
LV weight (mg)	142±7	179±5 *	155±13
Liver weight (mg)	1310±55	1227±163	2077±124 *†

* Indicates statistically significant difference compared to corresponding sham-operated mice (P<0.05)
† Indicates statistically significant difference compared to corresponding MI-operated mice (P<0.05)

However, LXR activation by T09 treatment attenuated the increase of LV weight after MI. The mean LV weight of the MI+T09 mice was still higher than that of sham-operated mice, but the difference did not reach statistical significance (p=0.53).

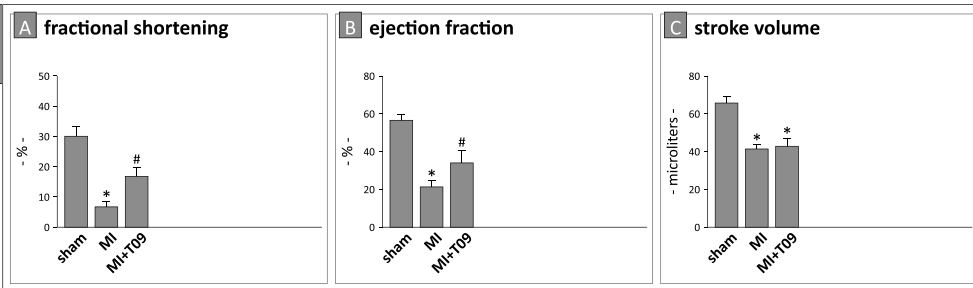
LV dimensions, assessed by echocardiography were increased after MI due to cardiac dilation (table 2): diastolic and especially systolic LV internal diameters were dramatically increased. Treatment with T09 attenuated the increase of the LV diameters. Wall thickness of the anteroseptal wall (IVS) was substantially thinner in MI mice, due to scarring. Posterior wall was not hypertrophied, possibly because of the increased wall stress due to the LV dilatation. Posterior wall thickness was actually significantly thinner in MI mice, whereas treatment with T09 resulted in a posterior wall thickness equal to sham operated mice.

Together, these data suggest that activation of LXRs results in attenuated cardiac hypertrophy after a MI.

LXR activation improves cardiac function after a MI

From the cardiac dimensions assessed by echocardiography, the fractional shortening (FS) was calculated. MI caused a dramatic decrease in FS, but this was improved when animals were treated with T09 (figure A). This is reflected in the calculated LV ejection fraction; MI caused a decrease in LV ejection fraction compared to sham-operated mice, but treatment with T09 attenuated this decrease significantly (figure B). Stroke volume, calculated from the HR and LVCO, was not altered by LXR activation; MI caused a significant decrease in stroke volume, in both MI and MI+T09 mice (figure C).

Figure 1



Cardiac function as assessed by echocardiography 3 weeks after MI or sham operation. Fractional shortening (A) was calculated from cardiac dimensions, as was ejection fraction (B). Stroke volume (C) was calculated from HR and LVCO (assessed by mitral valve Doppler signals). (*) Indicates statistically significant difference compared to corresponding sham-operated mice (P<0.05) and (#) indicates statistically significant difference compared to corresponding MI-operated mice (P<0.05).

Table 2

left ventricular dimensions 3 weeks after MI or sham operation

	Diastole			Systole		
	sham	MI	MI + T09	sham	MI	MI + T09
LVID (mm)	3.88±0.12	5.84±0.27 *	5.17±0.26 *	2.73±0.19	5.61±0.35 *	4.49±0.31 *†
IVS (mm)	0.94±0.07	0.64±0.10	0.76±0.14	1.45±0.10	0.58±0.12 *	0.82±0.18 *
LVPW (mm)	0.82±0.06	0.77±0.10	0.83±0.08	1.31±0.08	0.89±0.14 *	1.39±0.03 †

* Indicates statistically significant difference compared to corresponding sham-operated mice (P<0.05)
† Indicates statistically significant difference compared to corresponding MI-operated mice (P<0.05)

DISCUSSION

In this pilot study we show that activation of LXR results in attenuated cardiac hypertrophy after a MI. This was demonstrated by the attenuation of LV hypertrophy 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, it is suggested that the attenuated cardiac hypertrophy and remodeling by LXR activation results in preserved cardiac function. Fractional shortening and ejection fraction were higher in MI+T09 mice compared to MI mice. These data suggest a cardio-protective role for LXR.

This is the first study focused on the role of LXR in cardiac remodeling post-MI. A few other nuclear hormone receptors have been shown to also exert a protective role in the heart post-MI. Activation of PPAR-α and PPAR-γ reduced MI size and improved contractile dysfunction caused by ischemia/reperfusion injury in rat models of cardiac failure[14,15] and blocking the mineralocorticoid receptor has been shown to improve LV function in experimental studies[16] and to reduce morbidity and mortality in patients with MI.[17] In addition to these studies, we now propose LXR as a new target in the treatment of cardiac remodeling after MI.

In this pilot study, we did not perform additional tissue analyses that would provide mechanistic clues 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. Recent studies form our group and others have reported the cardio-protective role of LXR in the process of cardiac remodeling in pressure overload models. This was associated with decreases in blood pressure, cardiomyocyte hypertrophy[2] and inhibition of the NK-kB pathway.[3] Because LXR plays a crucial role in cholesterol and lipid metabolism, most studies have focused on its ability to prevent atherosclerosis.[18] LXRs are however also able to influence the cardiovascular system via other means than by cholesterol and lipid metabolism. Direct actions on cardiomyocytes[2], macrophages[9], and the RAAS[5,6] 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.

In conclusion, this pilot study showed that activation of LXR attenuates adverse cardiac remodeling post-MI and improves cardiac function post-MI. The pathways through which LXRs exert these effects are yet to be elucidated. These results provide further proof for a potential role for LXR activating drugs with the aim to prevent cardiac remodeling.

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