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Pharmacological evidence for a role of liver X receptors in atheroprotection

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1. Liver X receptors (LXRs)

Nuclear receptors, such as LXR (liver X receptor), bind to DNA *cis* elements, known as hormone response elements, and activate transcription of their specific target genes [1]. LXR was originally termed orphan nuclear receptor because its natural ligand was unknown at the time that it was initially cloned. With the recent identification of physiological ligands that activate LXR, this orphan is now ‘adopted’ [2].

LXR was originally isolated from a human cDNA library [3], and later two isoforms were identified, LXR α and LXR β . Both isoforms bind RXR (9-*cis* retinoic acid receptor) to form a heterodimer, and the heterodimers bind to the hormone response element DR4. Oxidized forms of cholesterol (oxysterols), which are intermediary substrates in the rate-limiting steps of steroid hormone biosynthesis, of bile acid synthesis, and in the conversion of lanosterol to cholesterol, have been identified as the potential physiological ligands for LXRs [4]. These findings strongly suggest that LXRs play a role in the cholesterol homeostasis of the body. Indeed, several important genes encoding proteins involved in body cholesterol transport have been shown to be regulated by LXRs. In addition, LXRs have also been shown to affect major genes encoding proteins that control triglyceride metabolism. Thus, LXRs appear to provide peripheral tissues with fatty acids while bringing cholesterol back to the liver (Fig. 1).

2. LXRs in reverse cholesterol transport

LXRs appear to control all the major steps in a pathway called ‘reverse cholesterol transport’. In this process cholesterol is transported from extrahepatic tissues into the liver to be excreted as cholesterol or bile acids into the bile, and ultimately into the gut (for a recent review see [5]). LXRs induce expression of ABCA1 [6] and ABCG1 [7], which are involved in transport of cholesterol and phospholipids from cells to extracellular cholesterol acceptors, notably the lipid-poor apolipoproteins apoAI and apoE. Interestingly, LXRs also induce expression of apoE in macrophages and adipocytes [8], and so may enhance reverse cholesterol transport in a tissue-specific manner. The lipid transfer proteins regulated by LXRs include the phospholipid transfer protein (PLTP) [9] and cho-

lesterol ester transfer protein (CETP) [10]. PLTP is involved in the generation of efficient acceptors of cellular cholesterol (pre β -HDL (high density lipoprotein)). In this process, PLTP transfers excess lipoprotein surface phospholipids (surface remnants) to lipid-poor apolipoprotein A-I (apo A-I). The remnants are formed when lipoprotein lipase (LPL) hydrolyzes triglyceride-rich lipoproteins such as very low density lipoprotein (VLDL). In addition, PLTP generates pre β -HDL through remodeling of circulating HDL particles. CETP, in turn, transports cholesteryl esters from HDL particles to the apolipoprotein B-100-containing lipoprotein particles (VLDL, intermediate density lipoprotein or IDL, and low density lipoprotein or LDL). This leads to hepatic clearance of the cholesteryl esters when the IDL and LDL particles are taken up by the liver. Finally, in the mouse, but not in humans, LXRs also increase hepatic transcription of 7- α -hydroxylase [11], which is involved in bile acid synthesis and thus drives secretion of the cholesterol taken up by the liver into the bile.

3. LXRs in triglyceride metabolism

Recent studies have revealed that LXRs are involved in the regulation of triglyceride metabolism [12,13]. LXRs stimulate fatty acid synthesis in the liver, and the increased quantities of fatty acids in the liver cells then become available for the synthesis of triglycerides, which are subsequently secreted into the circulation as major components of the triglyceride-rich lipoproteins (VLDLs). The genes involved in fatty acid synthesis and activated by the LXRs include the gene encoding the sterol regulatory element-binding protein 1c (SREBP-1c) and the fatty acid synthase [14]. Moreover, LXRs also control the synthesis of lipoprotein lipase (LPL) [15], an enzyme located on the luminal surface of vascular endothelial cells. By hydrolyzing triglycerides in triglyceride-rich lipoproteins, LPL liberates fatty acids into adipose tissue for storage and into skeletal muscle for energy expenditure [16]. Thus, LXRs are involved in fatty acid metabolism by promoting both their hepatic synthesis and their peripheral uptake.

4. Effect of LXRs on murine atherogenesis

In atherosclerosis, lipoprotein-derived cholesterol accumulates in the arterial intima, especially in monocyte-derived macrophages, which become cholesterol-filled and then are called foam cells. To prevent such accumulation, cholesterol must be efficiently removed from the macrophages. LXR is

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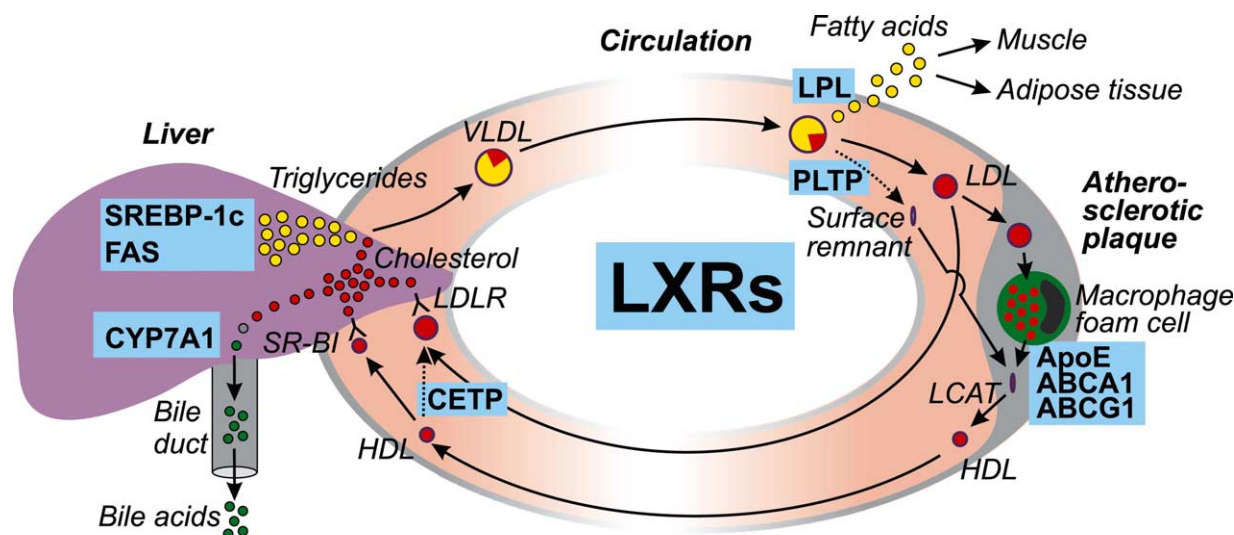


Fig. 1. LXRs in the regulation of lipid homeostasis of the body. The figure shows the various proteins whose regulation is under the control of LXRs (blue background). LXRs stimulate hepatic fatty acid synthesis by upregulating the SREBP-1c and the fatty acid synthase (FAS). The fatty acids formed are incorporated into VLDL particles in the form of triglycerides. The triglycerides of VLDL are hydrolyzed into free fatty acids by LPL in the capillary bed. The resulting LDL particles, the major cholesterol-containing lipoproteins in the circulation, accumulate in the arterial wall, notably in macrophages, causing the formation of foam cells. Efflux of unesterified cholesterol from the macrophages to the apolipoprotein (apo) AI- or E-containing small phospholipid disks (surface remnants) is facilitated by the ATP-binding cassette transporters A1 and G1 (ABCA1 and ABCG1). The surface remnants are derived from the surface material of the VLDL particles upon action of LPL and PLTP. Once enriched with unesterified cholesterol, the surface remnants mature into spherical HDL particles as the unesterified cholesterol is esterified by the enzyme lecithin cholesterol acyl transferase (LCAT). The cholesteryl esters of the HDL particles can be selectively taken up by the scavenger receptor BI (SR-BI) in the liver or, alternatively, be transported to larger apoB-100-containing lipoprotein particles (LDL) by CETP. The LDL particles are taken up by the hepatic LDL receptors (LDLR). Cholesterol in the liver can be used for synthesis of VLDL particles, or can be secreted into the bile, either as such or after being converted into bile acids. In this conversion the enzyme 7- α -hydroxylase (CYP7A1) is rate-limiting.

strongly expressed by arterial macrophages, and, as described above, plays a major role in controlling cellular cholesterol efflux, the initial step in reverse cholesterol transport. To explore the role of macrophage LXR in atherogenesis, bone marrow transplantation has been used in two murine models of atherosclerosis, the apoE^{-/-} and the LDLR^{-/-} mice. ApoE^{-/-} mice lack apolipoprotein E, and hence the receptor-mediated hepatic clearance of apoE-containing lipoproteins VLDL and IDL is retarded. Therefore, the apoE^{-/-} mice accumulate large quantities of these lipoproteins in plasma even on normal chow diet and develop atherosclerosis. Reconstitution of apoE^{-/-} mice with wild-type bone marrow leads apoE^{+/+} macrophages to populate arterial wall and other tissues. Such macrophages secrete apoE, which then binds to the apoE-deficient lipoproteins and promotes their clearance from the circulation, ultimately protecting these atherosclerosis-susceptible mice from atherogenesis. LDLR^{-/-} mice lack LDL receptors, and, due to absent receptor-mediated hepatic clearance of LDL particles, these animals accumulate apoB-containing lipoproteins (VLDL, IDL and LDL) in the plasma. However, these mice develop significant atherosclerosis only when fed a high-fat 'western' diet. Since in the LDLR^{-/-} mice, (i) an increase in plasma cholesterol is due to elevation of mainly LDL levels, (ii) the LDL receptor, in contrast to apoE, plays only a minor role in macrophage function, and (iii) both genetic and dietary factors are required to induce atherosclerosis, the LDLR^{-/-} mice are thought to reflect human cardiovascular disease better than the apoE^{-/-} mice. Tangirala and coworkers [17] transplanted wild-type or LXR $\alpha\beta$ ^{-/-} bone marrow into apoE^{-/-} mice and into LDLR^{-/-} mice. They found that transplantation of

LXR $\alpha\beta$ ^{-/-} bone marrow cells (macrophages) into atherosclerosis-susceptible mice of either type led to increased atherosclerosis as compared with transplantation with wild-type bone marrow cells [17]. These experiments nicely demonstrated macrophage LXRs as inhibitors of atherosclerosis.

The physiological relevance of LXR in lipid homeostasis and atherosclerosis has also been tested in chow-fed LXR α ^{-/-}, LXR β ^{-/-}, and LXR $\alpha\beta$ ^{-/-} mice without other genetic defects influencing lipoprotein metabolism [18]. Interestingly, it was found that, in 18-month-old LXR $\alpha\beta$ ^{-/-} animals, cholesterol accumulated in the macrophages of the spleen, lung, and arterial wall. The levels of all the major plasma lipoproteins in the double-mutant mice were also affected. Thus, in addition to the expected strong reduction in the levels of serum triglycerides (VLDL), a subtle decrease in HDL cholesterol and a significant increase in LDL cholesterol were observed in the LXR $\alpha\beta$ ^{-/-} animals. Since foam cells were formed in spite of the low triglyceride levels, this finding would suggest that the LXR target genes encoding the proteins involved in cholesterol metabolism are the critical components in the observed atheroprotection of the LXRs. The data also reveal the potential of the full complement of LXRs in the mammalian body, i.e. when expressed in the macrophages and the liver, they may act in concert to protect the animals from atherosclerosis.

In the current issue of *FEBS Letters*, Terasaka et al. [19] provide pharmacological evidence of a role for LXR in atheroprotection. They found that administration of T-0901317, a synthetic LXR ligand, to LDLR^{-/-} mice on an atherogenic diet did not affect the total cholesterol levels, induced a transient increase in the triglyceride levels, and to some extent

corrected the decrease in the HDL levels induced by the atherogenic diet. Most importantly, T-0901317 induced macrophage ABCA1 expression in the aortic root and dose-dependently inhibited formation of atherosclerotic lesions (up to 71%). These results are consistent with those of Joseph et al. [20], who studied the effect of another synthetic LXR ligand, GW395, on atherosclerosis in both apoE^{-/-} and LDLR^{-/-} mice. These workers found that, in the LDLR^{-/-} mice on a high-fat diet, GW395 decreased the total cholesterol levels without affecting the triglycerides, whereas in apoE^{-/-} mice on a chow diet, GW395 did not affect the cholesterol levels but significantly increased the triglycerides. Despite the differential responses in the plasma lipid levels in the two murine models of atherosclerosis, formation of atherosclerotic lesions was decreased in all the mice; by ~50% in both the apoE^{-/-} and the LDLR^{-/-} male mice and by 35% in the female LDLR^{-/-} mice.

5. LXRs – good targets for the treatment of atherosclerosis?

Although the effects of LXR agonists on triglyceride metabolism have raised concerns over the use of these agonists in the treatment of atherosclerosis, the recent long-term experiments by Joseph et al. [20] and Terasaka et al. [19] with two different synthetic LXR ligands appear promising, at least in mice. These experimental studies suggest that the potentially harmful LXR-dependent changes in triglyceride metabolism (elevation of plasma triglyceride levels) are effectively counteracted by the potentially beneficial LXR-dependent variations in cholesterol metabolism (a decrease in the plasma LDL cholesterol level and an increase in the plasma HDL level). Although human studies with LXR agonists are not available, it is reasonable to assume that the basic physiological principles of LXR action also apply to the human liver, and that the opposing effects of LXR agonists on lipid metabolism also apply to humans. Thus, it is a challenge for the pharmaceutical industry to develop LXR agonists that specifically activate macrophage LXRs without influencing the activity of the LXRs in the liver. Perhaps the identification of peroxisome proliferator-activated receptors as LXR activators [21] will generate novel pharmacological approaches to stimulate ABCA1-dependent cholesterol efflux from macrophages without increasing the levels of plasma triglyceride-rich lipoproteins, or, ideally, lowering their levels.

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