Liver X receptors (LXRα and LXRβ) are oxysterol-activated nuclear receptors. These sterol-responsive transcription factors regulate the expression of genes involved in intestinal cholesterol absorption and conversion of cholesterol to bile acids [1].

Since the macrophage plays an important role in host defense and immuno-inflammatory responses, particular interest has been paid to the role of LXRs in the control of macrophage gene expression and function. Altered macrophage functions contribute to the pathogenesis of many infectious, immunological and inflammatory disease processes, including atherosclerosis. Research reported over the last few years revealed important roles for LXRs in macrophage inflammation and cholesterol homeostasis with consequences in atherosclerosis development.

**LXRs and Macrophage Cholesterol Homeostasis**

Macrophage cholesterol homeostasis maintenance is the result of a balance between influx, endogenous synthesis, esterification/hydrolysis, and efflux of cholesterol.

In macrophages, LXR ligand activation enhances the expression of the ATP-binding cassette transporter ABCA1 and, consequently, apoAI-mediated cholesterol efflux [2,3], the first step of the reverse cholesterol transport from peripheral tissues to the liver. In addition they regulate the expression of the transporters ABCG1 and ABCG4 [4] which mediate cholesterol efflux to larger HDL particles [5]. Moreover, phospholipid transfer protein (PLTP), another modulator of HDL metabolism with a potential role in reverse cholesterol transport, is also a direct target gene for LXR in macrophages [6].

One important determinant governing cholesterol availability for efflux to extra-cellular acceptors is the mobilization of cholesterol from intracellular pools to the plasma membrane. Recently, it has been demonstrated that LXRs also control intracellular cholesterol trafficking in human macrophages [7]. LXR activation enhances the mobilization of free cholesterol to the plasma membrane resulting in a relative enrichment in cholesterol content of the outer layer, where it becomes readily available for efflux. Incubation of macrophages with synthetic LXR activators increases the amount of free cholesterol in the plasma membrane by inducing the gene expression of the Niemann-Pick C proteins NPC1 and NPC2, both involved in cholesterol trafficking from late endosome/lysosomes to the plasma membrane [7]. NPC1, a trans-membrane glycoprotein localized in the late endosomal compartment, contains a sterol-sensing domain projected into the lumen, whereas NPC2 is a soluble cholesterol-binding protein localized in the core of late endosomes [8].

LXR regulation of NPC1 and NPC2 occurs in a species-specific manner, since no induction is observed in murine bone marrow-derived macrophages upon stimulation [7]. These observations provide further evidence for the existence of species-differences in response to LXR agonists, which may result in distinct regulation of cholesterol homeostasis. Such species-differences have already been reported for cholesterol 7alpha-hydroxylase (Cyp7A1), which is only induced in rodents, but not in human liver [9].
The enrichment of cholesterol in the plasma membrane and induction of apoAI-specific cholesterol efflux by LXR activation is drastically reduced in the presence of progesterone [7], which blocks cholesterol mobilization from the late endosome/lysosome and mimics a phenotype comparable to the one observed in NPC-deficient cells [10,11]. Interestingly, simultaneous repression of NPC1 and NPC2 expression by a siRNA approach, leads to a drastic reduction of basal as well as LXR-induced cholesterol efflux, without affecting ABCA1 gene expression. However, LXR activation still results in an, albeit smaller, induction of cholesterol efflux suggesting the existence of other pathways involved in cholesterol movement regulated by LXR. It thus appears that stimulation of post-lysosomal cholesterol mobilization to the plasma membrane by LXR activation via NPC1 and NPC2 induction is an important step upstream of cholesterol efflux [7]. Recently, fibroblasts isolated from NPC patients were shown to display a reduced accessibility of plasma membrane cholesterol to cholesterol oxidase [12] associated with an impaired cholesterol efflux to apoAI [13]. In line, NPC1-deficient subjects have decreased plasma HDL cholesterol levels [13].

The induction of NPC1 and NPC2 gene expression after LXR activation and the subsequent induction of cholesterol efflux, could have physiological consequences in vivo in cells other than macrophages. Of particular interest, recent studies suggest a role of cholesterol metabolism in the development of Alzheimer’s disease [14]. In neuronal cells, which express ABCA1 [15], stimulation of cholesterol efflux decreased amyloid β-peptide secretion [14].

LXRs also modulate the intracellular distribution of cholesterol. In fact, LXR activation decreases cholesterol esterification rates and reduces cholesteryl ester levels in human macrophage foam cells [7]. These actions of LXR are not due to a decreased gene expression of ACAT1, the enzyme responsible for cholesterol esterification in macrophages. ACAT1 enzyme activity and cholesterol esterification rate are also controlled by fatty acid (FA) availability which could depend partially on their catabolism via mitochondrial FA oxidation. Carnitine palmitoyltransferase 1 (CPT-1) is an enzyme, which is located in the outer mitochondrial membrane and which controls the entry of long chain FAs into the mitochondria where they are degraded by β-oxidation [16]. However, in contrast to PPARα, LXR activation did not affect CPT-1 mRNA levels in macrophages, thus rendering the possibility that reduced cholesterol esterification is due to lowered FA substrate availability unlikely. Moreover, LXR activators did not affect neutral cholesteryl ester hydrolase (NCEH) activity in macrophages, thus indicating that the effects of LXR occur rather via inhibition of cholesterol esterification than by stimulation of cholesteryl ester hydrolysis. Thus, it is likely that the stimulation of cholesterol mobilization to the plasma membrane by LXR activators, results in a reduced availability of cholesterol as substrate for ACAT1.

These data demonstrate a novel role for LXR in the control of cholesterol mobilization and distribution, an effect which, associated with the induction of ABCA1 and ABCG1/ABCG4, may contribute to enhanced liberation and efflux of free cholesterol and stimulation of the initial step of the reverse cholesterol transport pathway [17].

**LXRs and Atherosclerosis**

These studies point to a role of LXRs as potential therapeutic targets for the treatment or prevention of atherosclerosis. However, it should be mentioned that in addition to their beneficial effect on cholesterol metabolism, LXRs induce the expression of genes involved in hepatic lipogenesis causing elevation of plasma triglycerides in agonist-treated mice [18]. Moreover,
LXR agonists increase expression of CETP, which may result in a transfer of cholesterol esters from HDL to LDL [19]. Thus these observations raise the question whether the net effect of LXR activation would be beneficial or deleterious with respect to atherosclerosis development. Initial studies addressing this issue demonstrated that activation of LXRz by synthetic ligands protects against atherosclerosis development in apoE-/- or LDLR-/- mouse models of atherosclerosis [20,21]. However, in order to use LXR ligands as therapeutic agents, it would be desirable if such compounds would provoke regression of established atherosclerotic lesions. Recently, it has been reported that the LXR agonist T0901317 increases ATP-binding cassette transporter ABCA1 expression within pre-existing atherosclerotic lesions in hypercholesterolemic and atherosclerotic LDLR-/- mice, resulting in the regression of the lesions [22]. In addition, LXR agonist treatment may result in a remodeling of vulnerable to stable lesions with an increase of collagen and a reduction in macrophage content. A second unresolved issue is the target cell and tissue involved in the atheroprotective effects of LXR agonists. In theory, these effects could be either due to the promotion of cholesterol excretion in the liver, the inhibition of cholesterol absorption in the intestine, the induction of cholesterol efflux from macrophages or a combination of these mechanisms. Using macrophage-selective LXR-deficient mice created by bone marrow transplantation, it has been demonstrated that macrophage LXR expression is necessary for the atheroprotective actions of an LXR agonist [22]. These data are in line with previous bone marrow transplant studies reporting that loss of LXR expression leads to exacerbated atherosclerosis [23]. Thus, although it is possible that the effects of LXRz in other tissues are relevant for atherogenesis, the macrophage appears to play a necessary role.

In summary, LXR activation in macrophages triggers changes in gene expression, including induction of genes involved in cholesterol trafficking and efflux and repression of genes of the inflammatory response, thus contributing to the atheroprotective effects of its ligands.

These observations provide evidence for the utility of LXR modulators in the treatment of cardiovascular diseases. Selective LXR agonists, which act on macrophages but do not stimulate hepatic lipogenesis, may display the most optimal therapeutic properties. The development of LXRβ-selective agonists represents one possible way to achieve this objective, since the LXRα isoform is thought to play the dominant role in hepatic lipogenesis, whereas both LXRα and LXRβ are equally effective in promoting cholesterol efflux in macrophages [24].

References


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