Lesion Macrophages Are a Key Target for the Antiatherogenic Effects of LXR Agonists

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The Liver X Receptors (LXRα and LXRβ) are members of the nuclear hormone receptor superfamily. These sterol-responsive transcription factors regulate the expression of a number of genes involved in intestinal cholesterol absorption, conversion of cholesterol to bile acids, and reverse cholesterol transport. Activation of LXRs blocks cholesterol absorption and induces cholesterol efflux from lipid-loaded cells such as macrophages. Because increased cholesterol efflux is predicted to limit the transformation of macrophages into atherosclerotic foam cells, LXR activity is predicted to be antiatherogenic. This hypothesis is supported by several studies using mice genetically deficient in LXR expression. Previous work from Tangirala and colleagues established that selective elimination of macrophage LXRα and LXRβ expression in the bone marrow compartment results in accelerated disease in both the spontaneous (ApoE−/−) and Western-diet inducible (LDLR−/−) models of atherosclerosis. Furthermore, LXRαβ double knockout mice fed a normal chow diet exhibit increased cholesterol accumulation in the macrophages of their arterial walls. In addition to their effects on cholesterol metabolism, activation of the LXRs by synthetic agonists has an inhibitory effect on inflammatory gene expression in macrophages, pointing to a second potential antiatherogenic mechanism of these receptors. Finally, recent studies suggest that the ability of microbial infections to modify the development of atherosclerosis may be accomplished in part through interference with the LXR signaling pathway and macrophage cholesterol metabolism.

Collectively, these studies point to LXRs as potential therapeutic targets for the treatment or prevention of atherosclerosis. However, it should be noted that in addition to their beneficial effects on cholesterol metabolism, LXRs have been demonstrated to induce the expression of genes involved in hepatic lipogenesis, leading to elevated plasma triglyceride levels in agonist treated mice. This sobering observation called into question whether the net effect of chronic LXR activation would be beneficial or deleterious with respect to the development of atherosclerosis. Initial studies addressing this issue concluded that activation of LXRs by synthetic LXR agonists in fact protects against lesion development in mouse models. Joseph et al investigated the effect of the LXR agonist GW3965 in both ApoE−/− and LDLR−/− atherosclerotic mice. In each model, atherosclerotic lesion area was reduced by ~50% as determined by en face analysis. Furthermore, LXR agonist was shown to exert direct effects on vascular gene expression, increasing expression of the transporters ABCA1 and ABCG1 in the aortas of treated mice. In a subsequent study, Terasaka et al obtained comparable results in the LDLR−/− system using a different LXR agonist, T0901317.

These early studies provided clear evidence for an atheroprotective effect of LXRs in murine models. However, in order for LXR ligands to be useful as therapeutic agents, they would need to cause regression of established lesions in the artery wall and block de novo lesion development. In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Levin et al provide new data to address this issue. Specifically, they questioned whether the LXR agonist T0901317 had beneficial effects on preexisting lesions in male LDLR−/− animals. Hypercholesterolemia and atherosclerosis were induced in the mice by feeding them a Western diet for 8 weeks. At this time, mice were either harvested (baseline) or treated with vehicle or agonist along with the same diet for an additional 6 weeks. As expected, mice harvested at baseline demonstrated advanced lesions that evolved further in the vehicle group after an additional 6 weeks, as determined by en face analysis. Impressively, LXR ligand treatment led to a 70% and 63% reduction of lesion area in comparison to vehicle-treated controls and to mice harvested at baseline, respectively. These results provide a convincing demonstration that LXR agonist not only inhibits lesion development but also leads to regression of preestablished lesions. Consistent with previous work, the LXR agonist increased plasma triglyceride levels and reduced total cholesterol levels.

To characterize the effects of the LXR agonist on lesion composition in more detail, Levin et al conducted immunostaining and gene expression studies. They found a 48% reduction in macrophage-positive lesion area and a 45% reduction in the expression of the macrophage marker CD68 in the aortic lesions of mice treated with agonist. They also reported a 67% increase in ABCA1 mRNA expression. Additionally, Levin et al observed a 34% increase in collagen content in immunostained lesions. Earlier studies had established that LXR agonist decreased the expression of inflammatory mediators, including the matrix-degrading enzyme matrix metalloproteinase (MMP)-9 in atherosclerotic aortas.
Together with previous work, the data of Levin et al support the idea that LXR agonist treatment decreases the foam cell composition of the lesions, induces genes that promote cholesterol efflux, and may promote the stabilization of lesions that may otherwise be susceptible to rupture. It would be interesting to know whether agonist treatment under the conditions described by Levin et al also has a direct effect on the expression of inflammatory genes in the regressing aortic lesions.

A second unresolved issue addressed by Levin et al is the target cell type involved in the atheroprotective effects of LXR agonists. In theory, these effects could involve promotion of cholesterol excretion in the liver, inhibition of cholesterol absorption in the intestine, promotion of macrophage cholesterol efflux, or a combination of these mechanisms. In an elegant series of studies combining bone marrow transplantation experiments and synthetic ligand administration, Levin et al provide evidence that macrophage LXR activity is mandatory for inhibition of atherosclerosis progression by LXR agonist. As outlined above, previous bone marrow transplantation studies have reported that loss of LXR expression led to exacerbated atherosclerosis. In the present work the authors demonstrate that the ability of LXR agonist to inhibit atherosclerosis progression is lost in mice transplanted with bone marrow lacking macrophage LXR expression. Thus, although it remains possible that effects of LXR in other tissues are also relevant for atherogenesis, the macrophage appears to play an indispensable role.

In summary, Levin et al provide compelling evidence that the synthetic LXR ligand T0901317 exerts its antiatherogenic effects in large part through regulation of macrophage function (Figure). Ligand activation of LXRs in macrophages triggers multiple changes in gene expression, including induction of genes involved in cholesterol efflux and repression of genes involved in inflammation. Both of these actions are likely to contribute to the atheroprotective effects of LXR agonists in the artery wall. Although significant obstacles remain to be overcome, the present study provides further support for the potential utility of LXR modulators in the treatment of human cardiovascular disease. In particular, they suggest that LXR agonists that are active in macrophages but not in the liver may have optimal therapeutic profiles. Such agents would exert desirable effects on lesion macrophages without stimulating hepatic lipogenesis. The development of LXRβ-selective agonists represents one way of achieving this goal, because the LXRα isoform plays the dominant role in hepatic lipogenesis, whereas both LXRα and LXRβ are equally effective in promoting cholesterol efflux and inhibiting inflammation in macrophages.

LXR agonist treatment ameliorates atherosclerosis by modifying macrophage gene expression. Previous studies have demonstrated that LXR agonists inhibit atherosclerosis development and progression. New evidence presented by Levin et al suggests that the synthetic LXR ligand T0901317 can also induce regression and stabilization of preexisting lesions in the LDLR−/− mouse model. This is accomplished in part through the induction of ATP-binding cassette transporter (ABC) genes which promote cholesterol efflux from macrophages as well as through the reduction of the inflammatory composition of the lesions.

References
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