Liver X Receptor Agonists as Potential Therapeutic Agents for Dyslipidemia and Atherosclerosis

Erik G. Lund, John G. Menke, Carl P. Sparrow

Abstract—The recent identification of liver X receptors (LXR) as regulators of the cholesterol and phospholipid export pump ABCA1 has raised the possibility that LXR agonists could be developed as HDL-raising agents, possibly also acting on the artery wall to stimulate cholesterol efflux from lipid-laden macrophages. Presently several pharmaceutical companies are working to develop such compounds, which will require finding a path for separating these beneficial effects from the detrimental stimulation of triglyceride synthesis also inherent to LXR agonists. Other challenges to the drug development process include species differences, which makes prediction of in vivo effects of LXR agonists in humans difficult. This review summarizes the present state of knowledge on LXR as a drug target and discusses possible solutions for dissociating the favorable effects of LXR agonists from their unwanted effects. (Arterioscler Thromb Vasc Biol. 2003;23:●●●●●●●.)

Key Words: PLEASE ■ SUPPLY ■ KEY ■ WORDS ■

Atherosclerosis and its clinical sequelae, myocardial infarction and ischemic stroke, are a major cause of premature death and disability in the industrialized world. Multiple studies have shown that statins, drugs that inhibit the enzyme HMG-CoA reductase, improve clinical outcomes for patients with atherosclerosis. There are also reports of favorable clinical outcomes using fibrates, drugs that bind to and activate the nuclear receptor PPARα. Neither statins nor fibrates reduce the risk of a heart attack in dyslipidemic patients by as much as 50%, however, so there is clearly a need for additional drugs to combat atherosclerosis.

What biological effects might be desired in a new antiatherosclerosis drug? One possibility is HDL raising, which is supported by the strong epidemiological evidence linking low HDL levels to increased risk of disease. Although it is true that HDL raising can be achieved using fibrates or certain statins such as simvastatin, these effects are fairly modest (usually <20%). Niacin raises HDL more dramatically and has shown clinical benefits in placebo-controlled trials; however, adverse effects associated with this drug makes compliance poor. A second desired effect of a new drug would be to act on the cells of the artery wall to directly stimulate the exit of cholesterol from existing atherosclerotic lesions; available evidence suggests that lipid-rich lesions are more prone to rupture and thereby cause myocardial infarction. It may be possible to induce these two desirable effects, HDL raising and cholesterol efflux, through manipulation of a single drug target, the nuclear receptor liver X receptor (LXR).

Nuclear receptors are ligand-activated transcription factors; the first members of this superfamily to be described were the steroid hormone receptors. The binding of a small molecule ligand, such as estradiol for the estrogen receptor, to a nuclear receptor modulates transcription of target genes through the binding of the nuclear receptor/ligand complex to appropriate response elements in the proximal promoter of target genes. The nuclear receptors LXRα (NR1H3) and LXRβ (NR1H2) are important regulators of genes involved in lipid metabolism. Originally cloned as orphan nuclear receptors, it is now believed that both LXR isoforms are intracellular sensors of cholesterol excess. Both LXR subtypes are different from the classical steroid receptors in that they show a low level of constitutive activation in the absence of ligand.

LXR target genes include many of those known to be important in the reverse cholesterol transport pathway, from cellular cholesterol efflux through HDL metabolism to biliary cholesterol excretion. A partial list of LXR-induced genes, along with their presumed function, is given in Table 1. The apparent coordinate regulation of components of the reverse cholesterol transport pathway makes LXR an attractive target for novel pharmaceutical agents.

From a drug development point of view, perhaps the most interesting gene induced by LXR is ABCA1, which is a lipid pump that functions to remove excess cholesterol from cells. The critical role of ABCA1 in cellular cholesterol homeostasis and HDL metabolism is supported by the fact that genetic deficiency of ABCA1, which results in Tangier disease, leads to cholesterol accumulation in several tissues and vanishingly low HDL levels. The importance of ABCA1 in atherosclerosis was supported by the observation that patients heterozygous for ABCA1 deficiency have 3-fold increased risk for coronary artery disease. Therefore,
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logical ligands of LXR are cholesterol-derived oxysterols, 11 feasible.

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lation of ABCA1 mRNA and the stimulation of choles-
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phages.21 We confirmed previous observations22,23 that cho-

lesterol loading increased mRNA levels for ABCA1 (2-fold)

and ABCG1 (5-fold; Table 1). The synthetic LXR agonist

APD was more effective, increasing these mRNA levels by 4-

and 17-fold, respectively.21 The combination of cholesterol

loading plus APD produced changes similar to those seen

with APD alone. A similar superiority of APD was seen in

assays of cholesterol efflux.21 This work strongly implies that

synthetic LXR agonists are more effective than cholesterol

loading at inducing ABCA1 expression and cholesterol efflux

in human macrophages. Similar conclusions for mouse mac-

rophages were published by Joseph et al.24 Joseph et al also

showed that the synthetic LXR agonist GW3965 decreased

atherosclerosis in mice.24 This promising data must be inter-

preted cautiously given the significant species differences in

LXR-responsive genes between mice and humans (see dis-

cussion below). Nevertheless, taken together, these results

suggest that LXR agonists may be effective in the treatment

of human disease.

The observation that upregulation of ABCA1 expression

by synthetic LXR agonists is more effective than cholesterol

loading is consistent with our recent findings implicating

27-hydroxycholesterol as a major endogenous LXR ligand in

human monocyte-derived macrophages.25 We demonstrated

that cholesterol loading such cells leads to the production of

micromolar levels of 27-hydroxycholesterol and its metabo-

lites cholesterol acid (another LXR agonist26) with little or no

concomitant formation of other suggested natural LXR li-

gands.25 Furthermore, human fibroblasts deficient in CYP27,

the enzyme responsible for synthesis of 27-hydroxychole-

terol, did not upregulate LXR-regulated genes on cholesterol

loading.25 Although 27-hydroxycholesterol is a bona fide

LXR ligand,25,27,28 the maximal activation of LXR achieved

by this ligand is less than the maximal activation obtained by

synthetic ligands even at saturating conditions21,25,29 (and data

not shown), thus mirroring the effects elicited by cholesterol

loading.

induction of the ABCA1 gene by LXR agonists might lead to

a decreased cholesterol burden in the artery wall as well as

increased HDL levels. The exciting possibilities for LXR

agonists as new therapeutics, however, are counterbalanced

by one significant liability: the induction of genes of fatty

acid synthesis and the accumulation of triglyceride. This

liability is, at least in part, mediated by LXR-induced tran-

scription of SREBP-1c, a member of the sterol regulatory

element-binding protein family of transcription factors.16

SREBP-1c induces the transcription of many lipogenic genes,

including fatty acid synthase and acetyl-CoA carboxylase,

which are the 2 key enzymes of de novo fatty acid synthesis.17

Clearly the successful development of LXR agonists into

pharmaceuticals will require that a favorable balance be

found between the potentially beneficial effects of induction

of ABCA1 and the potentially detrimental effects of induc-

tion of SREBP-1c.

Can LXR Agonists Help When and Where

They Are Really Needed?

The great hope for LXR agonists as novel therapeutic agents

is that such molecules will be able to increase expression of

ABCA1 in the artery wall, leading to enhanced cholesterol

efflux and resolution of the lipid overload in atherosclerotic

lesions. For this hope to be realized, synthetic LXR agonists

must stimulate ABCA1 expression and cholesterol efflux in

artery wall cells that are already cholesterol-loaded. On first

principles, however, it is not clear that it would be feasible to

additionally activate LXR in foam cells. Although not univer-

sally accepted,18 it is generally believed that the physiolo-

References are omitted for brevity.

roligands of LXR are cholesterol-derived oxysterols,11

although the relative importance of different oxysterols in this

role is still a matter of debate. Oxysterols are present in

atherosclerotic plaques in such large amounts that even if

only 10% are present in an unesterified form,19 27-

hydroxycholesterol in particular seems to be present at

saturating concentrations with respect to LXR activation.20 It

is therefore conceivable that the LXR’s may already be fully

activated in cholesterol-loaded cells such as those found in

atherosclerotic lesions in the artery wall and that no addi-

tional activation with an exogenous LXR agonist would be

feasible.

The authors’ laboratories have confirmed all of the effects listed in the table.

TABLE 1. Genes Regulated by LXR in Mice and Humans

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function of Gene Product</th>
<th>Humans Response to LXR Activation</th>
<th>Mice Response to LXR Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>Cellular cholesterol efflux and HDL formation</td>
<td>Induced</td>
<td>Induced</td>
</tr>
<tr>
<td>SREBP-1c</td>
<td>Master regulator of fatty acid synthesis</td>
<td>Induced</td>
<td>Induced</td>
</tr>
<tr>
<td>CYP7A1</td>
<td>Rate determining enzyme of bile acid synthesis</td>
<td>No effect</td>
<td>Induced</td>
</tr>
<tr>
<td>CETP</td>
<td>Transfer of neutral lipids between lipoprotein particles</td>
<td>Induced</td>
<td>Gene not present</td>
</tr>
<tr>
<td>PLTP</td>
<td>Phospholipid transfer; lipoprotein assembly</td>
<td>Weakly induced</td>
<td>Induced</td>
</tr>
<tr>
<td>LXRα</td>
<td>Sensor of cholesterol excess</td>
<td>Induced</td>
<td>No effect</td>
</tr>
<tr>
<td>ABCG1</td>
<td>Unknown</td>
<td>Strongly induced</td>
<td>Induced</td>
</tr>
<tr>
<td>ABCG5/ABCG8</td>
<td>Efflux of plant sterols and cholesterol into bile and intestinal lumen</td>
<td>Not known</td>
<td>Induced</td>
</tr>
</tbody>
</table>

We addressed this issue experimentally by studying the regulation of ABCA1 mRNA and the stimulation of cholesterol efflux in primary human monocyte-derived macrophages.21 We confirmed previous observations22,23 that cholesterol loading increased mRNA levels for ABCA1 (2-fold) and ABCG1 (5-fold; Table 1). The synthetic LXR agonist APD was more effective, increasing these mRNA levels by 4- and 17-fold, respectively.21 The combination of cholesterol loading plus APD produced changes similar to those seen with APD alone. A similar superiority of APD was seen in assays of cholesterol efflux.21 This work strongly implies that synthetic LXR agonists are more effective than cholesterol loading at inducing ABCA1 expression and cholesterol efflux in human macrophages. Similar conclusions for mouse macrophages were published by Joseph et al.24 Joseph et al also showed that the synthetic LXR agonist GW3965 decreased atherosclerosis in mice.24 This promising data must be interpreted cautiously given the significant species differences in LXR-responsive genes between mice and humans (see discussion below). Nevertheless, taken together, these results suggest that LXR agonists may be effective in the treatment of human disease.

The observation that upregulation of ABCA1 expression by synthetic LXR agonists is more effective than cholesterol loading is consistent with our recent findings implicating 27-hydroxycholesterol as a major endogenous LXR ligand in human monocyte-derived macrophages.25 We demonstrated that cholesterol loading such cells leads to the production of micromolar levels of 27-hydroxycholesterol and its metabolite cholesterol acid (another LXR agonist26) with little or no concomitant formation of other suggested natural LXR ligands.25 Furthermore, human fibroblasts deficient in CYP27, the enzyme responsible for synthesis of 27-hydroxycholesterol, did not upregulate LXR-regulated genes on cholesterol loading.25 Although 27-hydroxycholesterol is a bona fide LXR ligand,25,27,28 the maximal activation of LXR achieved by this ligand is less than the maximal activation obtained by synthetic ligands even at saturating conditions21,25,29 (and data not shown), thus mirroring the effects elicited by cholesterol loading.
Effects of LXR Agonists on Lipid Metabolism in Small Animals

The data described above demonstrate that LXR action induces ABCA1 expression and increases cholesterol efflux in cultured cells. Several studies also showed that synthetic LXR agonists can raise plasma HDL in experimental animals. Schultz et al.30 found a dose-dependent increase of HDL cholesterol in C57Bl/6 mice using the LXRα/β dual agonist T0901317, a result later confirmed by Grefhorst et al.31 and Cao et al.32 We have also observed HDL elevation using structurally unrelated LXR agonists (data not shown). Interestingly, the increase in HDL cholesterol chiefly occurred in a population of large HDL particles of low abundance in untreated animals.31,32 In contrast to the effects in C57Bl/6 mice, in mice deficient for either the LDL receptor or apolipoprotein E, prolonged treatment with the synthetic LXR agonist GW39655 did not raise HDL levels.24 The difference could possibly be explained by the disturbances of lipid metabolism induced by the gene deletions.

Encouragingly, recent studies have implicated LXR not only in HDL formation but also directly in the protection of the artery wall against atherosclerosis. Bone marrow transplantation from LXR double-knockout mice into mice deficient in either the LDL receptor or apolipoprotein E (apoE) led to a significant increase in atherosclerosis without significant effects on plasma lipids.33 Similarly, deficiency of LXRα, LXRβ, or both led to increased aortic lipid deposition in 18-month-old chow-fed mice, despite only minor changes in plasma lipids.34 These results imply that the absence of LXR signaling exacerbates atherosclerosis. The obverse of this, that increased LXR signaling caused by administration of an LXR agonist should decrease atherosclerosis, has recently been demonstrated by Joseph et al.24 who showed that the synthetic LXR agonist GW3965 decreases the formation of atherosclerotic plaques in mice deficient for apoE or the LDL receptor.24 In these studies, HDL cholesterol levels were not altered. We have confirmed the antatherosclerotic activity of LXR agonists in apoE-knockout mice using structurally unrelated compounds. An interesting difference in our study was that serum triglyceride levels were dramatically increased (unpublished data).

Enthusiasm over the favorable effects of LXR agonists on HDL metabolism and atherosclerosis may be tempered by the triglyceride-raising effects of the same compounds. Schultz et al.30 found a 2- to 3-fold increase in plasma triglycerides in C57Bl/6J mice treated with 5 to 50 mg/kg per day T0901317, coupled with an up to 5- to 6-fold elevation of hepatic triglyceride levels. These elevations were not seen in LXRα/β double knockout mice. The elevation of TG is at least in part attributable to increased biosynthesis, because Grefhorst et al.31 found a 2.5-fold increase in hepatic VLDL-TG secretion on treatment of C57Bl/6J mice with 10 mg/kg per day T0901317, an effect fully accounted for by the formation of larger as opposed to more VLDL particles. However, in that study, no elevation of plasma triglycerides was found, indicating a compensatory increase in particle clearance, a conclusion supported by the finding of massive hypertriglyceridemia in mouse models of defective lipoprotein clearance on treatment with T0901317. We have also measured varying degrees of hypertriglyceridemia and hepatic steatosis with different LXR agonists (not shown).

Speculation on Possible Effects of LXR Agonists in Humans

Potential effects of LXR agonists in humans are suggested by extrapolating from existing published evidence on the effects of LXR antagonists in humans. It has recently been demonstrated that n-3 fatty acids, the major constituents of fish oil, are LXR antagonists.25,26 Figure 1. Dietary n-3 fatty acids have long been known to decrease serum triglyceride in humans,37 an effect consistent with the triglyceride-raising effects of synthetic LXR agonists described above. In general, changes in VLDL triglyceride and HDL cholesterol are inversely correlated38,39 and, accordingly, most triglyceride-lowering regimens (exercise, weight loss, niacin, and fibrates) raise HDL. Interestingly, dietary n-3 fatty acids do not change HDL significantly in humans; this failure of dietary n-3 fatty acids to increase HDL may be attributable to their antagonism of LXR, which could lead to decreased ABCA1 expression. This model is consistent with our unpublished data showing that feeding fish oil to mice decreases both serum triglyceride and HDL.

Another clue to the possible effects of LXR agonists in humans comes from the study of patients taking certain CYP3A4-inducing anticonvulsants, including phenobarbital, carbamazepine, and phenytoin. It was recently shown that patients taking these drugs have highly elevated levels of plasma 4β-hydroxycholesterol. These authors propose that this is a result of 4β-hydroxylase activity of CYP3A4.40 In contrast, valproic acid, another anticonvulsant, does not induce CYP3A4 and did not raise 4β-hydroxycholesterol.40 Interestingly, 4β-hydroxycholesterol is a relatively potent LXR agonist in a cell-based transactivation assay.27 Numerous studies have been published describing effects on plasma lipids by anticonvulsants, and although some of these studies show an increase in HDL or triglyceride by the CYP3A4-inducing drugs but not valproic acid,41 others do not.42 Nevertheless, it was recently speculated that the effects of anticonvulsants on plasma lipids were attributable to LXR activation.43 Although caution must be exercised in interpreting these studies, taken together with the evidence on n-3 fatty acids, it is reasonable to expect that LXR agonists will increase both HDL and triglyceride in humans. Dissociating this expected dual effect is the great challenge for the development of LXR agonists as new therapeutics (see discussion below).

Other possible effects of LXR agonists should also be considered. Both isoforms of LXR are expressed in the brain, and disruption of the LXR genes44 as well as treatment with LXR agonist45 alters brain lipid homeostasis. In addition, a recent study demonstrated increased secretion of Aβ from neuronal cells treated with LXR agonists.46 In diabetic rodents, the LXR agonist T0901317 was recently shown to suppress genes involved in glucoseogenesis.47 Also recently, LXRβ was implicated in regulation of angiogenesis.48 It is at present not clear what the physiological importance of the above findings might be; however, it could be reasonably expected that a brain-penetrant LXR agonist would have
effects on cholesterol homeostasis in that organ. An extended list of genes regulated in liver and adipose tissue of mice fed an LXR agonist was recently published.49

Understanding the Multiple Effects of LXR Agonists

If we accept that LXR can detect cholesterol overload, it is possible to rationalize why LXR would increase the expression of the cholesterol pump ABCA1. More difficult to understand is why LXR activation should increase fatty acid synthesis through induction of SREBP-1c. Whereas requirement of fatty acyl-CoAs for the synthesis of cholesterol esters may provide a partial explanation,16,30 another part of the answer may reside in the proposed mechanism of action of ABCA1. LXR also stimulates the transcription of SREBP-1c precursor, but this effect can be expected to be balanced by suppressed SREBP-1c activation, and, therefore, the resulting increase in fatty acid synthesis is modest. Synthetic LXR agonists induce SREBP-1c transcription but do not suppress activation, leading to an overshoot in fatty acid synthesis. The activation of LXR can be antagonized by polyunsaturated fatty acids, which also suppress SREBP-1c processing.35,36,85,86 SREBP indicates sterol regulatory element binding protein; HMGCR, HMG-CoA reductase; LDLR, low-density lipoprotein receptor; FAS, fatty acid synthase; LCA, long chain fatty acyl-CoA elongase; TG, triglycerides; PL, phospholipids; and PUFA, polyunsaturated fatty acids.

Figure 1. Regulatory pathways controlling intracellular cholesterol homeostasis. As a response to low intracellular cholesterol levels, SREBP-1c and SREBP-2 are activated by proteolytic processing.17 The mature form of SREBP-2 activates transcription of the LDL receptor and biosynthetic genes, including HMG-CoA reductase.17 Also the transcription of SREBP-2 is increased in a feedforward loop. The net result is an increase in cholesterol synthesis and import. Under conditions of cholesterol sufficiency or excess, SREBP-2 processing and cholesterol synthesis are inhibited.17 Instead, cholesterol loading leads to generation of oxysterols,25,84 activation of LXR, and increased transcription of ABCA1,22 which catalyzes efflux of excess cholesterol. LXR also stimulates the transcription of SREBP-1c precursor, but this effect can be expected to be balanced by suppressed SREBP-1c activation, and, therefore, the resulting increase in fatty acid synthesis is modest. Synthetic LXR agonists induce SREBP-1c transcription but do not suppress activation, leading to an overshoot in fatty acid synthesis. The activation of LXR can be antagonized by polyunsaturated fatty acids, which also suppress SREBP-1c processing.35,36,85,86 SREBP indicates sterol regulatory element binding protein; HMGCR, HMG-CoA reductase; LDLR, low-density lipoprotein receptor; FAS, fatty acid synthase; LCA, long chain fatty acyl-CoA elongase; TG, triglycerides; PL, phospholipids; and PUFA, polyunsaturated fatty acids.
Potential Strategies for Dissociating Antiatherosclerotic Effects From Triglyceride Raising

Based on the evidence described above, it is reasonable to believe that activation of LXR would decrease atherosclerosis in humans. The challenge to drug discovery, however, is to identify an LXR agonist that will not cause unacceptable triglyceride accumulation. Below we shall discuss 3 potential strategies for dissociating the HDL- versus triglyceride-raising properties of LXR agonists.

1. LXRβ-Selective Agonists

Evidence from gene expression studies in LXR knockout mice suggests that it may be possible to dissociate the HDL-raising effects from the triglyceride-raising effects of LXR agonists by identifying LXRβ-selective agonists, i.e., compounds that selectively bind and activate LXRβ but not LXRα. The 2 LXR subtypes, LXRα and LXRβ, have very similar sequences but probably do not play identical roles in vivo, as judged by their pattern of expression and the phenotypes of the knockout mice. LXRα is strongly expressed in a small number of tissues (liver, small intestine, adipose, and macrophages), whereas LXRβ is ubiquitously expressed. LXRα knockout mice, but not LXRβ knockout mice, massively accumulate hepatic cholesterol on cholesterol feeding. No observable phenotype has yet been ascribed to LXRβ knockout mice.

LXRα knockout mice show reduced plasma triglyceride levels as well as reduced hepatic mRNA levels for multiple enzymes of fatty acid synthesis, including fatty acid synthase. LXRβ knockout mice do not show these effects, implying that LXRα is the subtype most responsible for controlling SREBP-1c transcription in liver. Conversely, peritoneal macrophages from LXRβ knockout mice, but not LXRα knockout mice, show altered basal expression of ABCA1 mRNA, suggesting that LXRβ is the subtype most responsible for controlling ABCA1 transcription in macrophages. The possibility that the LXR subtypes control different genes can be rationalized by their tissue distribution, combined with the idea that both LXR subtypes are activated by oxysterols that signal cholesterol excess or high rates of cholesterol synthesis. LXRα is expressed in tissues that synthesize triglyceride. In these tissues, such as liver, a slight excess of cholesterol could activate LXRα, leading to enhanced triglyceride synthesis, which would drive lipoprotein assembly and secretion of the excess cholesterol. Conversely, in typical extrahepatic tissues, the appropriate response to excess cholesterol would be activation of LXRβ leading to increased expression of ABCA1, allowing export of the excess cholesterol. It is possible that these apparent gene selectivities of LXRα and LXRβ can be explained by different ratios of LXRα versus LXRβ expression in different tissues. At present, the only bona fide gene selectivity of the LXR subtypes that has been described is that the LXR response element controlling the lipoprotein lipase gene responds only to LXRα and not to LXRβ.

Regardless of the true mechanistic origins of the apparent gene selectivities, the observations and rationales described above suggest the tantalizing possibility that agonists selective for LXRβ, with a low expression in liver, may offer a solution to the triglyceride-raising problems of the LXRα/β dual agonists that have been disclosed to date. Final proof that LXRβ-selective agonists will be superior must await the identification and description of such molecules. The search for LXRβ-selective agonists may be an arduous one, however, because of the high degree of sequence identity between the ligand-binding domains of LXRα and LXRβ. As shown in Table 2, the ligand-binding domain of these two receptors are more similar to each other than most other pairs of related nuclear receptors.

2. Sterol-Mimicking LXR Agonists

Another approach for dissociating the triglyceride-raising from HDL-raising effects of LXR agonists is to emulate cholesterol loading by developing compounds, perhaps steroid derivatives, that not only activate LXR but also suppress SREBP processing. As discussed above, this suppression of SREBP activation would be expected to counteract the upregulation of SREBP-1c synthesis by LXR and result in an attenuated triglyceride response compared with other LXR agonists. However, this is a problematic approach, taking into the account the mechanism of action of statins. This class of drugs acts by inhibiting HMG-CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis. When the hepatocyte experiences lower cholesterol levels because of the inhibition of biosynthesis, it responds by an increase in SREBP processing and, as a result thereof, LDL receptor expression. This increase in LDL receptors leads to the
desired result—an efficient removal of LDL particles from the circulation. Thus, an LXR ligand that also inhibits SREBP processing would also be expected to counteract statin action. This is clearly undesirable, especially because the intended patient population to a large extent overlaps with the patients who benefit from the statin class of drugs.

3. Tissue- or Gene-Selective Agonists

At least two more avenues toward suitable compounds can be envisioned. First, LXR agonists with suitable pharmacokinetic properties, allowing a substantial distribution in the extracellular tissues, may be identified. Such a compound may be able to exert favorable effects on the artery wall at doses sufficiently small to allow any increase in fatty acid synthesis to be manageable. A second possibility is that selective LXR modulators, analogous to the selective estrogen receptor modulators, can be found. The latter compounds are characterized by mixed agonist profile; for example, the estrogen receptor ligand tamoxifen functions as an estrogen agonist in bone, but it is an antagonist in breast tissue. At this point, however, it is unknown whether a similar behavior can be obtained with LXR agonists. Presently, LXRβ-selective agonists seem to offer the most promising path toward LXR agonists having the desired properties with respect to gene and tissue specificity.

Species Differences in the Genes Controlled by LXR: A Challenge to Drug Discovery

The development of new drugs requires the use of appropriate animal models. A suitable animal model should mimic the biochemistry and physiology of humans. One common obstacle to using small animals to develop novel therapeutics is that drugs that interact with signaling receptors often have dramatically different binding affinities for receptors from different species; well-studied examples include β-adrenergic receptors and PPARα. Significant species differences for LXR ligands have not been reported to date; however, it has become clear that there exist important species differences with respect to which genes are controlled by LXR. Some of these differences are summarized in Table 1, which compares the effects of LXR activation on gene expression in mice and humans. These two species were chosen because humans are of greatest interest for drug discovery, whereas mice have been studied most extensively with respect to LXR function. The two most dramatic differences between the species are CETP and CYP7A1 (see Table 1). Luo and Tall59 have shown that the human CETP gene contains a functional LXR response element; we have extended these results by demonstrating that synthetic LXR agonists increase CETP expression in primary human hepatocytes (unpublished data). The finding that CETP is an LXR-responsive gene thus explains the original observation in rabbits that CETP expression is increased by cholesterol feeding.60 This potentially important effect of LXR agonists in humans is not recapitulated in mice because the mouse genome does not contain CETP.

Another important difference in LXR-mediated gene regulation between humans and mice concerns CYP7A1. The mouse CYP7A1 gene figure prominently in the early seminal work on LXR. Lehmann et al61 showed that the mouse CYP7A1 gene figured prominently in the early seminal work on LXR. Lehmann et al61 showed that the mouse CYP7A1 gene. This potentially important effect of LXR agonists in humans is not recapitulated in mice because the mouse genome does not contain CETP.

### Table 2. Sequence Similarities for Various Pairs of Nuclear Receptors

<table>
<thead>
<tr>
<th>Nuclear Receptor A (Ligand Selective for A→B)</th>
<th>Nuclear Receptor B (Ligand Selective for B→A)</th>
<th>Ligand That Binds Both A and B</th>
<th>% Amino Acid Identity Between A and B in DNA-Binding Domain, %</th>
<th>Ligand-Binding Domain, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>LXRα (hypocholamide)</td>
<td>LXRβ (?)</td>
<td>T09013170, APD21</td>
<td>75</td>
<td>77</td>
</tr>
<tr>
<td>PPARα (fenofibrate)</td>
<td>PPARα (GW501516)</td>
<td>F3meetylAA22</td>
<td>86</td>
<td>74</td>
</tr>
<tr>
<td>PPARγ (fenofibrate)</td>
<td>PPARγ (rosiglitazone)</td>
<td>LY-46560875</td>
<td>83</td>
<td>69</td>
</tr>
<tr>
<td>Estrogen receptor-α (PPT76)</td>
<td>Estrogen receptor-β (DPN73)</td>
<td>17β-Estradiol</td>
<td>96</td>
<td>60</td>
</tr>
<tr>
<td>Glucocorticoid receptor (prednisone)</td>
<td>Mineralocorticoid receptor (aldosterone)</td>
<td>Cortisol13</td>
<td>93</td>
<td>56</td>
</tr>
<tr>
<td>Glucocorticoid receptor (prednisone)</td>
<td>Progesterone receptor (progesterone)</td>
<td>Mifepristone (RU-486)79</td>
<td>90</td>
<td>53</td>
</tr>
<tr>
<td>Retinoic acid receptor-α</td>
<td>Retinoi-X-receptor (RXR)-α (Targrett43)</td>
<td>9-cis-retinoic acid46</td>
<td>60</td>
<td>31</td>
</tr>
</tbody>
</table>

knockout background did not increase expression of the transgene in response to cholesterol feeding.

Mice and humans show differences in LXR-mediated gene regulation other than CETP and CYP7A1 (see Table 1). For example, ABCG1 is very strongly induced in human cells by LXR agonists.64 In our experience, ABCG1 is often induced >20-fold by LXR agonists in human cells (data not shown). In mouse cells, however, and in mouse tissues in vivo, ABCGI is usually only induced 2- to 3-fold by LXR agonists21,24,65 (also data not shown). The differences between mice and humans may complicate predictions of outcomes in humans based on the effects of LXR agonists in mice.

Summary
The last few years have seen an explosion of new information on the orphan nuclear receptors, including LXR, and in our understanding of the critical roles played by LXR-responsive genes such as ABCA1 and SREBP-1c. It has very recently become clear that LXR activation and ABCA1 expression can mitigate atherosclerosis in animal models. Taken together, the evidence makes a compelling case for attempting to develop LXR agonists as novel therapeutics for the treatment of atherosclerosis and its clinical consequences. The great challenges ahead include identifying means to circumvent the LXR-mediated overproduction of triglyceride and identifying appropriate animal models for the testing of novel LXR agonists.96–98

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References


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