A 15-ketosterol is a liver X receptor ligand that suppresses sterol-responsive element binding protein-2 activity^1

Robert J. Schmidt,* James V. Ficorilli,* Youyan Zhang,* Kelli S. Bramlett,* Thomas P. Beyer,* Kristen Borchert,* Michele S. Dowless,* Keith A. Houck,* Thomas P. Burris,* Patrick I. Eacho,* Guosheng Liang,* Li-wei Guo,† William K. Wilson,† Laura F. Michael,* and Guoqing Cao*,2

Lilly Research Laboratories,* Eli Lilly & Company, Indianapolis, IN 46285; and Department of Biochemistry and Cell Biology,† Rice University, Houston, TX 77005

Abstract Hypercholesterolemia is a major risk factor for coronary artery disease. Oxysterols are known to inhibit cholesterol biosynthesis and have been explored as potential antihypercholesterolemic agents. The ability of 3β-hydroxy-5α-cholest-8(14)-en-15-one (15-ketosterol) to lower non-HDL cholesterol has been demonstrated in rodent and primate models, but the mechanisms of action remain poorly understood. Here we show in a coactivator recruitment assay and cotransfection assays that the 15-ketosterol is a partial agonist for liver X receptor-α and -β (LXRs and LXRβ). The binding affinity for the LXRs was comparable to those of native oxysterols. In a macrophage cell line of human origin, the 15-ketosterol elevated ATP binding cassette transporter ABCA1 mRNA in a concentration-dependent fashion with a potency similar to those of other oxysterols. We further found that in human embryonic kidney HEK 293 cells, the 15-ketosterol suppressed sterol-responsive element binding protein processing activity and thus inhibited mRNA expression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, LDL receptor, and PCSK9. Our data thus provide a molecular basis for the hypocholesterolemic activity of the 15-ketosterol and further suggest its potential antiatherosclerotic benefit as an LXR agonist.—Schmidt, R. J., J. V. Ficorilli, Y. Zhang, K. S. Bramlett, T. P. Beyer, K. Borchert, M. S. Dowless, K. A. Houck, T. P. Burris, P. I. Eacho, G. Liang, L-w. Guo, W. K. Wilson, L. F. Michael, and G. Cao. A 15-ketosterol is a liver X receptor ligand that suppresses sterol-responsive element binding protein-2 activity. J. Lipid Res. 2006. 47: 1037–1044.

Supplementary key words hypercholesterolemia • statin • scintillation proximity assay

Hypercholesterolemia is a major risk factor for coronary artery disease. In recent years, success has been recorded with the development of the statin class of drugs, which inhibit 3-hydroxy-3-methyl glutaryl-CoA reductase (HMG-CoA reductase), resulting in inhibition of hepatic cholesterol biosynthesis, upregulation of LDL receptor (LDLR), and accordingly, the reduction of plasma LDL cholesterol (1). However, even with the widespread clinical utilization of statins, there is still an unmet medical need for agents that would further reduce hypercholesterolemia by complementary mechanisms. Certain oxysterols are known to be potent inhibitors of cholesterol biosynthesis (2, 3). The mechanism of this inhibition was not well understood until the recent discovery of a pair of transcription factors, sterol-responsive element binding proteins 1 and 2 (SREBP1 and -2), which primarily control cholesterol and fatty acid biosynthesis and LDL uptake (4). SREBPS are members of the basic helix-loop-helix leucine zipper family of transcription factors that are synthesized as inactive precursors. The inactive full-length protein, which is anchored to the endoplasmic reticulum by two transmem-
brane domains, contains an N-terminal transcription factor and a carboxyl-terminal domain. Under sterol-depleted conditions, the protein is escorted to the trans-Golgi complex and undergoes sequential proteolytic cleavages by two proteases, S1P and S2P. This process results in the release of the N-terminal transcription factor and upregulates LDLR expression (4). Oxysterols hinder the escort of SREBPs to the trans-Golgi apparatus, thus preventing the activation of cholesterol biosynthesis. Exactly how oxysterols exert their role in this process is not yet clear.

In recent years, certain oxysterols have been identified as liver X receptor (LXR) ligands (5, 6). LXRα and LXRβ were initially isolated as orphan nuclear receptors and are now recognized to play critical roles in multiple physiological processes, including cholesterol catabolism (7). LXRs regulate the expression of several ATP binding cassette (ABC) transporters (8–10), HDL modifying enzymes (11, 12), and apolipoproteins (13–15) that are involved in cholesterol homeostasis. Synthetic LXR agonists have been shown to elevate HDL cholesterol in rodent models and to exert antiatherogenic properties (12, 16–18). However, LXR agonists also activate SREBP1c expression, which controls the entire fatty acid biosynthetic pathway and promotes hypertriglyceridemia and hepatic steatosis (16). Thus, selective LXR modulators have potential pharmacological benefits. The mechanism of oxysterol regulation of cholesterol metabolism was not known in late 1970s, when Schroepfer and colleagues (19, 20) pioneered the effort to develop synthetic oxysterols as hypocholesterolemic agents. One promising candidate, 3β-hydroxy-5α-cholest-8(14)-en-15-one (15-ketosterol, referred to as compound 1 in the following text), was studied extensively and was demonstrated to have hypocholesterolemic efficacy in rodent and primate models (20–24). However, the mechanisms for the observed hypocholesterolemic effects were not clearly defined. In this report, we present evidence that compound 1 interferes with SREBP processing and is a partial agonist for LXRα and LXRβ. These effects may explain the ability of compound 1 to lower LDL and raise HDL cholesterol in animal models.

**METHODS**

Materials

15-Ketosterol was prepared as described previously (25) and showed >99% purity by 500 MHz NMR. The LXR radioligand binding assay was performed using scintillation proximity assay (SPA) technology as previously described (26). We utilized 800 ng of baculovirus-expressed, His-tagged human LXRα ligand binding domain (LBD) protein (aa 162-447) or 600 ng of LXRβLBD protein (aa 202-461), 25 nM [3H]25-hydroxycholesterol (Amerham; Piscataway, NJ), 0.05 mg polylysine-coated yttrium silicate SPA beads (Amersham), and varying concentrations of competitor per well of a 96-well OptiPlate (Packard; Meriden, CT). Protein, radioligand, and competitor were added to the plate. SPA beads were then added to the assay plate, followed by 10 min of gentle shaking at room temperature protected from light. The plates were incubated in the dark at room temperature for 2 h prior to reading in a TopCount plate reader (Packard).

Coactivator interaction assay

Interaction between the LXRα/LXRβ and the coactivator SRC-1 was assayed using AlphaScreen™ (amplified luminescent proximity homogenous assay) technology (PerkinElmer; Wellesley, MA). The assay was performed in white, low-volume, 384-well plates (Greiner Bio-One; Germany) utilizing a final volume of 10 μl containing final concentrations of 10 nM of affinity-purified, His-tagged, baculovirus-expressed human LXRαLBD or LXRβLBD

---

**Fig. 1.** Compound 1 is a liver X receptor (LXR) ligand as measured by a radiolabeled scintillation proximity assay (A) chemical structure of compound 1. (B) Radiolabeled 25-hydroxycholesterol was incubated with LXR ligand binding domain, and competition of compound 1. With radiolabel was assessed with the scintillation proximity assay as described in Methods.
protein, 5 nM of GST-SRC-1 protein that contained the entire nuclear receptor interacting domain of SRC-1 fused to GST, and 10 μg/ml of both Ni²⁺ chelate AlphaScreen donor beads and anti-GST AlphaScreen acceptor beads. The assay buffer contained 25 mM HEPES (pH 7.0), 100 mM NaCl, 0.1% BSA, and 2 mM DTT. All manipulations involving assay beads were done in ambient light. Assay plates were covered with a clear seal and incubated in the dark for 4 h, after which the plates were read for 1 s/well in a Packard AlphaQuest microplate analyzer using the manufacturer’s standard AlphaScreen detection protocol.

**LXR cotransfection assay**

For transient transfection of HEK 293 cells, 6 × 10³ cells were plated into 96-well dishes. Each well was transfected with 25 ng 5×UAS-luciferase reporter (pG5lac) and 25 ng of pM human LXRα (AA 162-447) or LXRβ (AA 153-461) LBD plasmid using Fugene 6 reagent (Roche; Indianapolis, IN). The chimeric protein was assessed for the ability to transactivate a Gal4-responsive luciferase reporter plasmid in a concentration-responsive manner to compound 1 (0.001–30 μM). Luciferase activity at each dose concentration was measured in triplicate using standard substrate reagents (BD Biosciences; San Diego, CA), and data are expressed as relative light units.

**ABCA1 branched-DNA assay**

The branched-DNA assay was performed according to the manufacturer’s protocol for the QuantiGene® High Volume Kit (Genospectra; Fremont, CA). After treatment of the cells with compounds, cells were lysed with QuantiGene® lysis buffer containing the ABCA1 mRNA oligonucleotides as described (27). After a 15 min incubation at 37°C, 100 μl of the lysis buffer from each well was transferred to the corresponding wells of the capture plate. The capture plate was incubated overnight at 53°C. The capture plate was then washed twice with QuantiGene® wash buffer, followed by the addition of 100 μl/well QuantiGene® amplifier working reagent. The plate was incubated for 60 min at 46°C, followed by two washes. The mRNA to be measured was then labeled by the addition of 100 μl QuantiGene® label probe working buffer, followed by a 60 min incubation at 46°C. The capture plate was then washed twice, followed by the addition of 100 μl/well QuantiGene® substrate plus QuantiGene® enhancer reagent. The plates were incubated at 37°C for up to 30 min and then read on a luminometer to detect the luminescent signal. The induction of ABCA1 mRNA expression was calculated as a ratio of compound-treated luminescent levels compared with untreated control levels.

**Western blot**

HEK 293 cells were seeded to 100 mm dishes and grown to confluency in 3:1 DMEM/F12 (Invitrogen) + 10% FBS supplemented with 20 mM HEPES, l-glutamine, and Penicillin/Streptomycin. The dishes were washed twice with 10 ml PBS-Mg²⁺/Ca²⁺, and the cells were then incubated with media under different culture conditions (see Figure legends). After 48 h, the cells were washed once with PBS and nuclear extracts were prepared for Western blot analysis (28). Equal concentrations of protein (40 μg/lane) were run on a 4–20% SDS-PAGE gel. Proteins were then transferred to Nitrocellulose membranes (0.45 μm; Novex), which were blocked for 90 min in Odyssey Blocking Buffer (Li-Cor Biosciences). Anti-SREBP1 antibody 2A4 (abcam AB3259) or SREBP2 antibody 1C6 (BD Pharmingen 557037) or the antibody raised against human LDLR (RDI-PRO61099, Research Diagnostics) was then added at a concentration of 1 μg/ml (SREBPs) or 10 μl/10 ml blotting solution (LDLR) and incubated overnight at 4°C in blocking buffer. The blots were quickly washed three
times in PBS + 0.1% Tween 20 (PBST) followed by three 5 min washes in PBST. Goat anti-mouse IgG was added to a concentration of 0.5 mg/ml in blocking buffer + Tween 20 (Molecular Probes Alexa Fluor 680 goat anti-mouse IgG, A-21057). After 1 h, the blot was quickly washed in PBST, followed by three 5 min washes in PBST. A final wash in PBS was performed, and the blots were visualized in the Li-Cor infrared imaging system.

Quantitative real-time PCR

Dishes treated similarly to those described for Western blot analysis were washed two times in PBS and lysed in Trizol (Invitrogen; 10 ml Trizol/100 mm dish), and RNA was isolated according to the manufacturer’s specifications. Molecular biology-grade water (0.5 ml) was used to resuspend the isolated RNA, which was then subjected to an additional purification using a Qiagen RNAeasy kit. Five micrograms of total RNA was converted to cDNA using the ABI High Capacity cDNA kit (ABI# 4322171). This cDNA was then used for Taqman RT-PCR. ABI Assays on Demand supplied the human LDLR and HMG-CoA reductase primer pairs (LDLR-Hs00181192, HMG-CoA Red-Hs0168352). Taqman analysis was performed in an ABI Prism 7100HT system.

RESULTS

As a first step in determining whether compound 1 (Fig. 1A) is an LXR ligand, we performed a radioligand binding assay for both LXRα and LXRβ in the format of a scintillation proximity assay. As shown in Fig. 1B, compound 1 effectively competed with radiolabeled 25-hydroxycholesterol, which is a natural ligand of LXRα. The binding affinity for compound 1 binding to LXRα [inhibition constant (Ki) 0.60 μM] or LXRβ (Ki 0.76 μM) is slightly less potent than that of another natural oxysterol ligand, 22-(R)-hydroxycholesterol (Ki 0.25 μM for LXRα and 0.49 μM for LXRβ) (26). To discriminate between agonist and antagonist activity, we first used a biochemical coactivator recruitment assay. The purified ligand binding domain of either LXRα or LXRβ was used to examine the interaction of the protein with the coactivator SRC-1.

Fig. 3. Agonist activity in a cotransfection assay. Gal-LXR fusion proteins were used in assessing compound 1 (A) or T0901317 (B) agonist activity. HEK 293 cells were cotransfected with DNA constructs encoding Gal-LXR fusion proteins and a reporter construct. Luciferase reporter activity was measured by standard methods as described.

Fig. 4. ABCA1 mRNA regulation by compound 1 in THP-1 cells. Monocytic THP-1 cells were induced to differentiate into macrophage cells, and the activity of compound 1 in regulating ABCA1 was assessed via the branched-DNA method as described.
upon the addition of the compound. Compound 1 demonstrated concentration-dependent agonist activity (Fig. 2A) in the assay for both LXRα and LXRβ. The EC50 concentrations for activating LXRα or LXRβ in this assay were 0.62 μM and 0.40 μM, respectively, with a maximum activation of 5.2-fold (LXRα) and 2.3-fold (LXRβ) over the baseline. We considered compound 1 to be a weak agonist, compared with the prototypic agonist T0901317, which demonstrated approximately 8.8-fold activation over the baseline for both LXRα and LXRβ (Fig. 2B). To further confirm its agonist activity, we then performed a cotransfection assay using Gal-human LXR fusion proteins and a 5×UAS-luciferase reporter. As expected, in both LXRα and LXRβ cotransfection assays, compound 1 displayed concentration-dependent agonist activity, with EC50 concentrations at 15.4 μM and 0.74 μM, respectively, with approximately 2-fold activation over the baseline (Fig. 3A). In the same assay, the prototypic LXR agonist T0901317 demonstrated potent agonist activity (Fig. 3B). We also examined the possibility of antagonist activity of compound 1 in both the coactivator recruitment assay and cotransfection assays and found no antagonist activity (data not shown). To explore whether compound 1 could regulate endogenous gene expression by modulating LXR, we examined ABCA1 mRNA expression in human macrophage cell line THP-1 cells. Compound 1 elevated ABCA1 mRNA expression in a concentration-dependent manner more than 3-fold (Fig. 4). This finding was consistent with the results obtained in both coactivator recruitment assay and cotransfection assays.

We also reasoned that compound 1 may exert its hypocholesterolemic activity by inhibition of SREBP processing, and thus we explored its activity in SREBP regulation. Using the HEK 293 model, cells grown under suppressed conditions (with 25-hydroxycholesterol, lane 1, Fig. 5) had significantly diminished mature SREBP2 levels as mea-

![Image](image-url)

Fig. 5. Inhibition of sterol-responsive element binding protein 2 (SREBP2) processing activity in cell culture models. A and B: HEK 293 cells were cultured under either induced conditions (lane 2, lipoprotein-deficient serum with 50 μM compactin and 50 μM mevalonate) or suppressed conditions (lane 1, induced conditions with 1 μg/ml 25-hydroxycholesterol and 10 μg/ml cholesterol) and treated with various concentrations of compound 1 (lanes 3–5). Twenty-four hours after the treatment, cells were collected and extracts were subjected to Western blot analysis to assess the extent of SREBP processing using 2A4 antibody for SREBP1 and 1C6 antibody for SREBP2. Lane 1, suppressed conditions. Lane 2, induced conditions. Lanes 3–5, induced conditions with increasing concentrations of compound 1 at 0.3, 1, and 10 μM. C: HepG2 cells with transfected SRE-luciferase reporter were treated with various concentrations of compound 1, and the luciferase activity was measured as described in Methods. D: HEK 293 cells were cultured and treated similarly to those described in A, and RT-PCR quantitation was carried out as described in Methods. Similar results were obtained in two other experiments.
sured by Western blot analysis using a monoclonal antibody against the C terminus of SREBP2 in comparison to cells grown under induced conditions (lipoprotein-deficient serum plus compactin, an inhibitor of HMG-CoA reductase) (Fig. 5A, lane 1 vs. lane 2). Addition of compound 1 under induced conditions (lanes 3–5) led to a significant reduction of mature SREBP2 levels (Fig. 5A, lanes 2–5), whereas nuclear SREBP1 levels (detected by an antibody against the SREBP1 N-terminal) did not display any observable change (Fig. 5B), suggesting that com-

Fig. 5. Continued.

Fig. 6. Compound 1 regulates PCSK9 mRNA and LDL receptor (LDLR) protein in HEK 293 cells. HEK 293 cells were cultured and treated similarly to those described in A, and PCSK9 mRNA was quantified through RT-PCR. The LDLR protein was examined using Western blot analysis (B).
compound 1 specifically repressed SREBP2 processing in HEK 293 cells. To further confirm the repression of SREBP activities by compound 1, HepG2 cells with a 3×SRE-luciferase reporter were treated with compound 1 and the luciferase activity was measured. As shown in Fig. 5C, compound 1 significantly decreased SRE reporter activity in a concentration-dependent manner, with an IC50 of 1 μM. Consistent with these observations, quantitative real-time PCR analysis of the genes regulated by SREBP2, including HMG-CoA reductase and LDLR, demonstrated expression changes that paralleled SREBP2 proteolytic processing (Fig. 5D). Compound 1 appeared to slightly repress SREBP2 mRNA expression and trend toward elevating SREBP1 mRNA. These data demonstrate that compound 1 effectively suppresses SREBP2 activity and its downstream target genes in cell culture models.

Recently, PCSK9 (proprotein convertase subtilisin/kexin type 9) has been reported as an SREBP2 target gene that intimately regulates LDLR levels through protease degradation of LDLR (29–31). Therefore, we further monitored PCSK9 mRNA regulation by 15-ketosterol. As anticipated, compound 1 dramatically reduced PCSK9 mRNA expression (Fig. 6A). Western blot analysis showed that compound 1 effectively suppressed LDLR protein levels (Fig. 6B), even with the dramatically reduced expression of PCSK9, thus suggesting a predominant regulation of LDLR at the transcription level by compound 1.

DISCUSSION

In previous studies, synthetic oxysterols, notably compound 1, showed potent hypocholesterolemic effects in animal models. To date, the mechanisms for the observed effects have remained unclear. Here we report that compound 1 is an LXR agonist and also effectively suppresses SREBP2 proteolytic processing and activity in cell culture models.

The sterol nature of compound 1, along with its similarity in binding affinity to natural oxysterols, suggests that compound 1 binds to both LXRα and LXRβ in a fashion similar to the binding affinity of native oxysterols. The lack of any preferential binding to either isoform is also characteristic of natural sterol ligands (6). The agonist activity identified in both the coactivator recruitment assay and the cotransfection assay is modest compared with the prototypic potent synthetic LXR agonist T0901317 (Figs. 2, 3) (26). However, compound 1 effectively regulates ABCA1 mRNA in macrophage cells (Fig. 4), suggesting additional potential athero-protective effects.

Two SREBP isoforms, SREBP2 and SREBP1c, are present primarily in vivo (4). Their distinct roles in regulating lipid metabolism have been defined in recent years. SREBP1c has been identified as the master transcription factor regulating the entire fatty acid biosynthetic program, whereas SREBP2 is primarily responsible for mediating cholesterol homeostasis (4). Although fatty acids regulate SREBP1c by negative feedback, certain oxysterols specifically suppress SREBP2 processing activity (32). Our data indicate that compound 1, like natural oxysterols, also specifically represses SREBP2 proteolytic processing and thus its transcriptional activity.

Plasma LDL cholesterol is balanced by hepatic VLDL secretion and LDL cholesterol uptake through receptor-mediated endocytosis (33). Previously, it was not known whether inhibition of both cholesterol biosynthesis (and thus, accordingly, VLDL secretion) and LDLR expression by sterols would result in overall LDL cholesterol lowering. Recent genetic data have demonstrated that in S1P- or SREBP cleavage-activating protein-deficient mice, SREBP processing was severely impaired and both plasma triglyceride and cholesterol levels were reduced (34, 35). Thus, although the expression of the proteins of both cholesterol biosynthesis and LDL uptake is inhibited, the overall effect is a reduction in plasma lipid, owing primarily to a reduction in lipid synthesis. These results suggest that pharmacological inhibition of the SREBP pathway may represent an alternative path for further lipid lowering. Thus, if the hypocholesterolemic effect of compound 1 is a result of reduced SREBP processing, our data could be viewed as pharmacological validation for this approach.

In summary, our cell culture results point to regulatory mechanisms that may explain the beneficial effects of compound 1 on lipoprotein cholesterol in intact animals. Consistent with its reported lowering of LDL + VLDL cholesterol (20), compound 1 effectively suppressed SREBP2 cleavage and transcription of its target genes (encoding HMG-CoA reductase, PCSK9), with little effect on SREBP1. Compound 1 is a weak partial agonist for LXRα and LXRβ and increases transcription for ABCA1 that may contribute to the observed HDL cholesterol elevation in primates (20).

REFERENCES

1. Brown, M. S., and J. L. Goldstein. 1996. Heart attacks: gone with the century? Science. 272: 629.
2. Kandutsch, A. A., and H. W. Chen. 1973. Inhibition of sterol synthesis in cultured mouse cells by 7alpha-hydroxycholesterol, 7beta-hydroxycholesterol, and 7-ketocholesterol. J. Biol. Chem. 248: 8408–8417.
3. Brown, M. S., and J. L. Goldstein. 1974. Suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and inhibition of growth of human fibroblasts by 7-ketocholesterol. J. Biol. Chem. 249: 7306–7314.
4. Brown, M. S., and J. L. Goldstein. 1999. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proc. Natl. Acad. Sci. USA. 96: 11041–11048.
5. Janowski, B. A., P. J. Willy, T. R. Devi, J. R. Falck, and D. J. Mangelsdorf. 1996. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. Nature. 383: 728–731.
6. Janowski, B. A., M. J. Grogan, S. A. Jones, G. B. Wisely, S. A. Kliewer, E. J. Corey, and D. J. Mangelsdorf. 1999. Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. Proc. Natl. Acad. Sci. USA. 96: 266–271.
7. Tontonoz, P., and D. J. Mangelsdorf. 2003. Liver X receptor signaling pathways in cardiovascular disease. Mol. Endocrinol. 17: 985–993.
8. Costet, P., Y. Luo, N. Wang, and A. R. Tall. 2000. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. J. Biol. Chem. 275: 28240–28245.
9. Repa, J. J., K. E. Berge, C. Pomajzl, J. A. Richardson, H. H. Hobbs, and D. J. Mangelsdorf. 2002. Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors α and β. J. Biol. Chem. 277: 18793–18800.
10. Kennedy, M. A. A. Venkateswaran, P. T. Tarr, I. Xenarios, J. Kudoh, N. Shimizu, and P. A. Edwards. 2001. Characterization of the human ABCG1 gene: liver X receptor activates an internal promoter that produces a novel transcript encoding an alternative form of the protein. J. Biol. Chem. 276: 39438–39447.

11. Luo, Y., and A. R. Tall. 2000. Sterol upregulation of human CETP expression in vitro and in transgenic mice by an LXR element. J. Clin. Invest. 105: 513–520.

12. Cao, G., T. P. Beyer, X. P. Yang, R. J. Schmidt, Y. Zhang, W. R. Bensch, R. F. Kaufman, H. Gao, T. P. Ryan, Y. Liang, et al. 2002. Phospholipid transfer protein is regulated by liver X receptors in vivo. J. Biol. Chem. 277: 39561–39565.

13. Laffitte, B. A., J. J. Repa, S. B. Joseph, D. C. Wilpitz, H. R. Kast, D. J. Mangelsdorf, and P. Tontonoz. 2001. LXRα controls lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. Proc. Natl. Acad. Sci. U.S.A. 98: 507–512.

14. Mak, P. A., B. A. Laffitte, C. Desrumaux, S. B. Joseph, L. K. Curtiss, D. J. Mangelsdorf, P. Tontonoz, and P. A. Edwards. 2002. Regulated expression of the apolipoprotein E/C1/C4/C7/C8/C9 gene cluster in murine and human macrophages. A critical role for nuclear liver X receptors α and β. J. Biol. Chem. 277: 31900–31908.

15. Liang, Y., X. C. Jiang, R. Liu, G. Liang, T. P. Beyer, H. Gao, T. P. Ryan, S. Dan Li, P. I. Eacho, and G. Cao. 2004. Liver X receptors (LXRs) regulate apolipoprotein AIV-implications of the antiatherosclerotic effect of LXR agonists. Mol. Endocrinol. 18: 2000–2010.

16. Schultz, J. R., H. Tu, A. Luk, J. J. Repa, J. C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D. J. Mangelsdorf, et al. 2000. Role of LXRs in control of lipogenesis. Genes Dev. 14: 2853–2838.

17. Joseph, S. B., E. McKillip, L. Fei, M. A. Watson, A. R. Collins, B. A. Laffitte, M. Chen, G. Noh, J. Goodman, G. N. Hagger, et al. 2002. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. Proc. Natl. Acad. Sci. U.S.A. 99: 7604–7609.

18. Terasaka, N., A. Hiroshima, T. Koieyama, N. Ubukata, Y. Morikawa, D. Nakai, and T. Inaba. 2003. T-0901317, a synthetic liver X receptor ligand, inhibits development of atherosclerosis in LDL-receptor-deficient mice. FEBS Lett. 536: 6–11.

19. Gibbons, G. F. 2002. From gallstones to genes: two hundred years of sterol research. A tribute to George J. Schroepfer Jr. Lipids. 37: 1153–1162.

20. Schroepfer, G. J., Jr. 1996. Design of new oxysterols for regulation of cholesterol metabolism. Curr. Pharm. Des. 2: 103–129.

21. Schroepfer, G. J., Jr., D. Monger, A. S. Taylor, J. S. Chamberlain, E. J. Parish, A. Kisic, and A. A. Kandutsch. 1977. Inhibitors of sterol synthesis. Hypocholesterolemic action of dietary 5α-cholest-8(14)-en-3β-ol-15-one. Chem. Phys. Lipids. 12: 227–1227.

22. Brabson, J. S., and G. J. Schroepfer, Jr. 1988. Inhibitors of sterol synthesis. The effects of dietary 5α-cholest-8(14)-en-3β-ol-15-one on the fate of [4,14C]-cholesterol and [2,4,3H]5α-cholest-8(14)-en-3β-ol-15-one after intragastric administration to rats. Chem. Phys. Lipids. 47: 1–20.

23. Schroepfer, G. J., Jr., E. J. Parish, A. Kisic, E. M. Jackson, C. M. Farley, and E. G. Mott. 1982. 5α-cholest-8(14)-en-3β-ol-15-one, a potent inhibitor of sterol biosynthesis, lowers serum cholesterol and alters distributions of cholesterol in lipoproteins in baboons. Proc. Natl. Acad. Sci. U.S.A. 79: 3042–3046.

24. Schroepfer, G. J., Jr., B. C. Sherrill, K. S. Wang, W. K. Wilson, A. Kisic, and T. B. Clarkson. 1984. 5α-cholest-8(14)-en-3β-ol-15-one lowers serum cholesterol and induces profound changes in the levels of lipoprotein cholesterol and apoproteins in monkeys fed a diet of moderate cholesterol content. Proc. Natl. Acad. Sci. U.S.A. 81: 6861–6865.

25. Wilson, D. K., W. K. Wilson, F. A. Quijano, and G. J. Schroepfer, Jr. 1988. Concerning the structure of 3β-benzoyloxy-5β-cholesta-8,14-diene, a major byproduct in the chemical synthesis of 5α-cholest-8(14)-en-3β-ol-15-one. Chem. Phys. Lipids. 47: 275–282.

26. Bramlett, K. S., K. A. Houck, K. M. Borchert, M. S. Dowless, P. Kulanthaivel, Y. Zhang, T. P. Beyer, R. Schmidt, J. S. Thomas, L. F. Michael, et al. 2003. A natural product ligand of the oxysterol receptor, liver X receptor. J. Pharmacol. Exp. Ther. 307: 291–296.

27. Zhang, Y., T. Beyer, K. Bramlett, S. Yao, T. Burreis, R. Schmidt, P. Eacho, and G. Cao. 2002. Liver X receptor and retinoic X receptor mediated ABCA1 regulation and cholesterol efflux in macrophage cells-messenger RNA measured by branched DNA technology. Mol. Genet. Metab. 77: 150–158.

28. Cao, G., J. L. Goldstein, and M. S. Brown. 1996. Complementation of mutation in acyl-CoA:cholesterol acyltransferase (ACAT) fails to restore sterol regulation in ACAT-defective sterol-resistant hamster cells. J. Biol. Chem. 271: 14642–14648.

29. Horton, J. D., N. A. Shah, J. A. Warrington, N. N. Anderson, S. W. Park, M. S. Brown, and J. L. Goldstein. 2003. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. Proc. Natl. Acad. Sci. U.S.A. 100: 12927–12932.

30. Rashid, S., D. E. Curtis, R. Garuti, N. N. Anderson, Y. Bashmakov, Y. K. Ho, R. E. Hammer, Y. A. Moon, and J. D. Horton. 2005. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9. Proc. Natl. Acad. Sci. U.S.A. 102: 5574–5579.

31. Dubuc, G., A. Chamberland, H. Wassef, J. Davignon, N. G. Seidah, B. A. Bensch, R. F. Kauffman, H. Gao, T. P. Ryan, Y. Liang, et al. 2002. Liver X receptors control lipid-inducible expression of the apolipoprotein E/C1/C4/C7/C8/C9 gene cluster in murine and human macrophages. A critical role for nuclear liver X receptors α and β. J. Biol. Chem. 277: 6–11.

32. Jahoor, F., A. S. Taylor, J. S. Chamberlain, E. J. Parish, A. Kisic, E. M. Jackson, C. M. Farley, and E. G. Mott. 1982. 5α-cholest-8(14)-en-3β-ol-15-one, a potent inhibitor of sterol biosynthesis, lowers serum cholesterol and induces profound changes in the levels of lipoprotein cholesterol and apoproteins in monkeys fed a diet of moderate cholesterol content. Proc. Natl. Acad. Sci. U.S.A. 79: 3042–3046.