Intervention in hepatic lipid metabolism : implications for atherosclerosis progression and regression

Li, Z.

Chapter 6

LXR activation is essential to induce atherosclerotic plaque regression in C57BL/6 mice

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ABSTRACT

Background & Aims: LXR activation leads to a number of favorable changes in lipid metabolism. LXR agonists have been shown to attenuate the progression of atherosclerosis. It is thus interesting to examine whether rapidly improved plasma lipoprotein profiles combined with LXR activation can lead to atherosclerotic lesion regression.
Methods and Results: $LDLr^{-/-}$ and C57BL/6 mice were fed with atherogenic diet to induce atherosclerotic development, after which the diet was switched to chow diet with or without supplementation of LXR agonist T0901317. In $LDLr^{-/-}$ mice, LXR activation did not induce atherosclerotic plaque regression. In C57BL/6 mice, LXR agonist dramatically improved plasma lipoprotein profile, including large reductions in (V)LDL and brisk enhancements in HDL. T0901317 induced favourable hepatic gene expression profiles. T0901317 significantly increased hepatic expression of ABCA1, ABCG1, and SR-BI, suggesting enhanced reverse cholesterol transport. Subsequently, T0901317 supplemented in chow diet significantly reduced the pre-existing atherosclerotic plaque size (-43%).
Conclusions: The current study shows that 1) intact LDL receptor function is crucial to overcome LXR-induced hyperlipidemia; and 2) rapidly optimized plasma lipoprotein profiles combined with LXR agonist induced favorable gene expression profiles can induce regression of pre-existing atherosclerotic plaques.
Keywords: LXR, T0901317, lipoprotein, atherosclerosis, regression

INTRODUCTION

Hypercholesterolemia holds a critical role in the development of atherosclerosis[1]. Lowering of very-low-density lipoprotein- (VLDL-) and low-density lipoprotein- (LDL-) cholesterol levels leads to reduction in cardiovascular morbidity and mortality in individuals at risk for cardiovascular events[2,3]. High-density lipoprotein (HDL) is an anti-atherosclerotic lipoprotein based on its ability to mediate reverse cholesterol transport (RCT), which is a major protective system against atherosclerosis[4]. HDL can remove cholesterol from the periphery, allowing it to be cleared by the liver and then excreted into the bile[5]. Cholesterol efflux from macrophages to HDL is a crucial step in RCT[6]. Modulation of the major molecular mediators in RCT, such as ATP binding cassette transporter A1 (ABCA1), ATP binding cassette transporter G1 (ABCG1), and scavenger receptor class B1 (SR-B1) has consistent beneficial effects on RCT and atherosclerosis[7,8]. Agents that raise HDL and promote RCT, including statins, fibrates, nicotinic acids, and liver X receptor (LXR) agonists thus represent promising treatments for atherosclerotic disease[9].

LXRs play an important role in the maintenance of cellular and systemic cholesterol homeostasis[10]. Pharmacological activation of LXRs *in vivo* leads to a number of favorable changes in lipid metabolism[11,12]. Synthetic LXR agonists promote biliary sterol secretion, reduce cholesterol absorption, and increase fecal sterol excretion[13,14]. In addition, LXR activation significantly enhances the ABCA1- and ABCG1-mediated cholesterol efflux to nascent HDL particles and inhibits the atherosclerosis development in animal models[15,16].

While numerous studies have been dedicated to inhibit the progression of atherosclerosis, recent attention has been focused on reversing atherosclerosis, which is the regression of existing atherosclerotic plaque. The first evidence of dramatic atherosclerotic regression in mice was achieved by Trogan *et al* via robust surgical measures, such as aortic transplantation, to rapidly improve the plaque environment[17]. This study suggested that the essential prerequisite promoting regression of atherosclerosis lesion is the robust improvement in plasma lipoprotein profiles and plaque milieu, including large reductions in plasma atherogenic apoB-lipoproteins and brisk enhancements in efflux of cholesterol from plaques. Recently, the same group has demonstrated that LXR expression in the plaque is required for maximal effects on plaque macrophage egression during atherosclerosis regression in mice[18].

LXR activation has been shown to significantly up-regulate cholesterol efflux activity and inhibit development of atherosclerosis, providing direct evidence for an anti-atherogenic effect of LXR agonists[19,20]. However, in these studies, LXR agonists are only shown to attenuate the progression of atherosclerosis in mouse models, while their potential to abrogate pre-existing cardiovascular disease and to stabilize the established atherosclerotic lesions has not been widely addressed. It is thus interesting to examine whether rapidly improved plasma lipoprotein profiles combined with therapeutic LXR agonist-induced activation of RCT and cholesterol efflux could lead to atherosclerotic lesion regression. In the current study, we specifically evaluated the potential of LXR agonist T0901317 to regress diet-induced pre-existing atherosclerotic plaque in $LDLr^{-/-}$ and C57BL/6 mice.

MATERIALS AND METHODS

Animals
For the study on LDLr$^{-/-}$ mice, male homozygous LDL receptor-deficient mice (LDLr$^{-/-}$; C57BL/6 background) of 12 weeks old were used. The animals were fed with semi-synthetic Western-type diet (WTD) containing 15% (w/w) fat and 0.25% (w/w) cholesterol (Diet W, Special Diet Services, Witham, UK) for 6 weeks to induce the development of atherosclerotic lesions. For the study on C57BL/6 mice, female C57BL/6 mice of 12 weeks old were used. The animals were fed with semi-synthetic cholate-containing cholesterol-enriched atherogenic diet containing 15% (w/w) cocoa butter, 1% (w/w) cholesterol, and 0.5% cholate (AB Diets, Woerden, The Netherlands) for 16 weeks to induce the development of atherosclerotic lesions. After the formation of initial atherosclerotic plaques in both studies, diet was switched to regular cholesterol-free chow diet containing 4.3% (w/w) fat (RM3, Special Diet Services, Witham, UK) for 3 weeks, with or without supplementation of LXR agonist T0901317 (10 mg/kg/day; MSD Oss, The Netherlands). After euthanization, mice were bled via orbital exsanguination, and perfused *in situ* through the left cardiac ventricle with ice-cold PBS (pH 7.4) for 20 minutes. Tissues were dissected and snap-frozen in liquid nitrogen. Heart and one lobe of the liver were dissected free of fat and stored in 3.7% neutral-buffered formalin (Formal-fixx, Shandon Scientific Ltd., UK) for histological analysis. Animal care and procedures were performed in accordance with the national guidelines for animal experimentation. All protocols were approved by the Ethics Committee for Animal Experiments of Leiden University.

Plasma lipid analysis
Plasma lipid analysis was performed at different time points throughout the experiments. At the endpoint, mice were not fasted prior to euthanization. Plasma concentrations of total cholesterol (TC) and triglycerides (TG) were measured using the enzymatic colorimetric assay (Roche Diagnostics, Mannheim, Germany). The distribution of cholesterol over different lipoproteins in plasma was determined by fast protein liquid chromatography (FPLC) through a Superose 6 column (3.2 x 30 mm; Smart-System, Pharmacia, Uppsala, Sweden). Cholesterol content of the lipoprotein fractions was determined as described above.

Hepatic lipid analysis
Lipids were extracted from the liver using the Folch method. Briefly, 100 mg of tissue was homogenized with chloroform/methanol (1:2). The homogenate was centrifuged to recover the upper phase, which was further washed with chloroform-0.9% NaCl (1:1, pH 1.0). After centrifugation, the lower chloroform phase containing lipids was evaporated and the retained lipids were resolubilized in 2% Triton X-100 by sonification. Protein contents were analyzed by BCA assay (Pierce Biotechnology, Thermo Fisher Scientific BV, IL, USA). Cholesterol content of lipid extracts was determined as described above. Data were expressed as milligrams of lipid per milligram of protein.

RNA isolation and gene expression analysis
Total RNA from the liver was isolated using acid guanidinium thiocyanate (GTC)-phenol-chloroform extraction. Briefly, 500 µL of GTC solution (4 M guanidine

isothiocyanate, 25 mM sodium citrate, 0.5% N-lauroylsarcosine) was added to each sample, followed by acid phenol:chloroform extraction. The RNA in aqueous phase was precipitated with isopropanol. The quantity and purity of the isolated RNA were examined using ND-1000 Spectrophotometer (Nanodrop, Wilmington, DE, USA). One microgram of the isolated RNA from each sample was converted into cDNA by reverse transcription with RevertAid™ M-MuLV Reverse Transcriptase (Promega, Madison, WI, USA). Negative controls without addition of reverse transcriptase were prepared for each sample. Quantitative real-time PCR was carried out using ABI Prism 7700 Sequence Detection system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. 36B4, Beta-actin, and GAPDH were used as internal housekeeping genes. The gene-specific primer sequences used are listed in Table 1. Amplification curves were analyzed using 7500 Fast System SDS software V1.4 (Applied Biosystems, Foster City, CA, USA). The relative expression of each gene was expressed as fold changes $2^{-(\Delta\Delta CT)}$ compared to baseline group. Standard error of the mean (SEM) and statistical significance were calculated using ΔΔCt formula.

Table 1. Murine primers for quantitative real-time PCR analysis

Gene	Forward primer	Reverse Primer
36B4	GGACCCGAGAAGACCTCCTT	GCACATCACTCAGAATTTCAATGG
Beta-actin	AACCGTGAAAAGATGACCCAGAT	CACAGCCTGGATGGCTACGTA
GAPDH	TCCATGACAACTTTGGCATTG	TCACGCCACAGCTTTCCA
ABCA1	GGAGTTCTTTGCCCTCCTGAG	AGTTTGCGAATTGCCCATTC
ABCG1	AGGTCTCAGCCTTCTAAAGTTCCTC	TCTCTCGAAGTGAATGAAATTTATCG
ABCG5	TGGCCCTGCTCAGCATCT	ATTTTTAAAGGAATGGGCATCTCTT
ABCG8	CCGTCGTCAGATTTCCAATGA	GGCTTCCGACCCATGAATG
SR-BI	GGCTGCTGTTTGCTGCG	GCTGCTTGATGAGGGAGGG
LPL	CCAGCAACATTATCCAGTGCTAG	CAGTTGATGAATCTGGCCACA
FAS	GGCATCATTGGGCACTCCTT	GCTGCAAGCACAGCCTCTCT
CYP7A1	CTGTCATACCACAAAGTCTTATGTCA	ATGCTTCTGTGTCCAAATGCC
SREBP-1c	GGAGCCATGGATTGCACATT	CCTGTCTCACCCCCAGCATA
CD68	CCTCCACCCTCGCCTAGTC	TTGGGTATAGGATTCGGATTTGA

Histological analysis

Accumulation of lipids in the liver and the atherosclerotic plaques at the aortic root in the heart was analyzed. The liver and heart was cut latitudinally and embedded in O.C.T™ Compound (Tissue-Tek, Sakura finetek, Tokyo, Japan), and subsequently sectioned using a Leica CM 3050S cryostat at 10 μm intervals. Cryostat sections were stained with Oil-red O (Sigma-Aldrich) to identify lipids, and counterstained with hematoxylin (Sigma-Aldrich) to assist in tissue visualization. Quantitative analysis of the atherosclerotic lesion area at the aortic root was performed in a blinded fashion. Mean lesion area (in μm^2 per aortic root per mouse) was calculated from ten Oil-red O stained cryostat sections, starting at the appearance of the tricuspid valves.

Statistical analysis

Statistical analyses were performed by the unpaired Student's t-test for independent samples (Instat GraphPad software, San Diego, USA). Statistical significance was defined as $p<0.05$. Data are expressed as means ± SEM.

RESULTS

LXR activation does not induce atherosclerotic plaque regression in LDLr$^{-/-}$ mice

LDLr$^{-/-}$ mice are an established mouse model to study atherogenesis[21,22]. To evaluate the potential of LXR agonists to regress pre-existing atherosclerotic lesions, we fed the LDLr$^{-/-}$ mice with high-fat high-cholesterol Western-type diet (WTD) for 6 weeks to develop atherosclerotic plaques. Subsequently, a group of mice was sacrificed to obtain baseline data, whilst the remainder of the mice was switched to a low-fat cholesterol-free chow diet without or with LXR agonist T0901317 supplementation for 3 weeks.

WTD markedly increased plasma total cholesterol from basal level 235 mg/dL to 1220 mg/dL. Subsequently, when the atherogenic WTD was switched back to cholesterol-free chow diet alone, plasma total cholesterol concentration decreased significantly within one week, and after 3 weeks dropped back to almost basal level (400 mg/dL; Figure 1A). In contrast, T0901317 treatment supplemented in chow diet further increased plasma total cholesterol level up to approximately 2000 mg/dL, which was 1.6-fold higher than baseline ($p<0.001$) and 5-fold higher than the group fed with chow diet alone ($p<0.001$) (Figure 1A). As determined by FPLC lipoprotein separation, the largely enhanced plasma total cholesterol concentration after T0901317 treatment was due to markedly elevated plasma VLDL-cholesterol (23-fold; $p=0.001$) and LDL-cholesterol levels (3-fold; $p=0.04$) (Figure 1B).

6 weeks of atherogenic WTD-feeding induced atherosclerotic lesion development to approximately $60x10^3$ μm^2 at aortic root as baseline, visualized by oil Red-O staining of cryosections (Figure 1C). After the diet was switched to regular chow diet alone, lesion size surprisingly progressed (3-fold; $p<0.001$) despite of the significantly normalized plasma lipoprotein profile in this group (Figure 1C). In the mice treated with T0901317, lesion size also increased 3-fold ($p<0.01$) compared to baseline. However, despite its 5-fold higher plasma total cholesterol level compared to mice fed with chow diet alone, the increase in the lesion size from T0901317-treated mice was not larger than the chow group (Figure 1C). It is interesting that the atherosclerotic lesion progression in LDLr$^{-/-}$ mice was not concomitant with the changes in plasma lipoprotein profiles. We thus measured the ratio between the increase in lesion size and the plasma total cholesterol concentration. T0901317 induced 7-fold less lesion progression per unit of plasma cholesterol concentration compared to mice fed with chow diet alone ($p<0.001$) (Figure 1D).

Taken together, switching atherogenic WTD to cholesterol-free chow diet rapidly normalized plasma lipoprotein profiles, but this measure alone was not enough to attenuate atherosclerotic lesion progression or induce lesion regression in LDLr$^{-/-}$ mice. LXR agonist treatment in LDLr$^{-/-}$ mice failed to normalize plasma lipoprotein profiles, and thus failed to induce regression of pre-existing aterhosclerotic lesion. This may be due to the absence of hepatic LDL receptors, thus LDLr$^{-/-}$ mice exhibit prolonged half lives of plasma VLDL and LDL[23]. Furthermore, the absence of LDL receptor-mediated uptake leads to increased (V)LDL synthesis, which is further enhanced by LXR activation[24,25]. However, T0901317 significantly attenuated the lesion progression per increase of plasma cholesterol concentration.

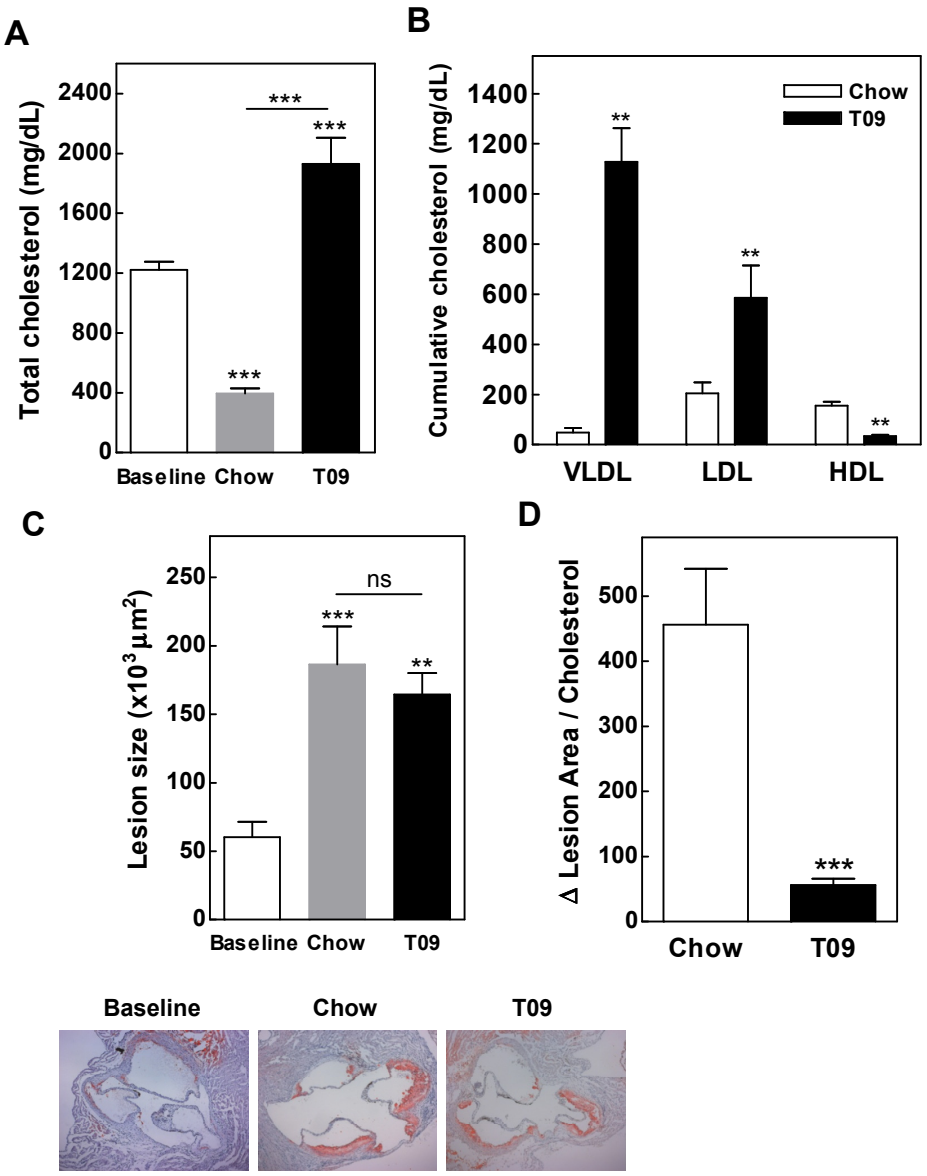

Figure 1. Effects of LXR agonist in LDLr$^{-/-}$ mice. LDLr$^{-/-}$ mice were fed WTD for 6 weeks, after which a group of mice was sacrificed (Baseline), whilst the remainder of the mice was switched to regular chow diet without (Chow) or with LXR agonist T0901317 supplementation (T09) for 3 weeks. Plasma total cholesterol concentration was measured (A). Plasma lipoproteins were separated by FPLC and cholesterol level was measured in each fraction. VLDL represents the sum of cholesterol concentrations from fraction 2 to 7 (VLDL fractions); LDL represents the sum of cholesterol concentrations from fraction 8 to 14 (LDL fractions); HDL represents the sum of cholesterol concentrations from fraction 15 to 22 (HDL fractions) (B). Cryostat sections of the aortic root in heart were stained with oil-red O to identify lipids, and the lesion size was quantified (C). The ratio between the change in lesion size and the plasma total cholesterol concentration was calculated (D). Values are means ± SEM (8 mice per group). **P<0.01; ***P<0.001.

LXR agonist dramatically improved plasma lipoprotein profile in C57BL/6 mice

Because the plasma basal level of (V)LDL-cholesterol in C57BL/6 mice is lower than in LDLr$^{-/-}$ mice, we set out to evaluate the effects of LXR agonist on plaque regression in C57BL/6 mice. The C57BL/6 mouse model has been used to study diet-induced atherosclerosis[26,27,28]. This murine model of atherogenesis represents an alternative to the use of genetically modified mice with impaired lipoprotein clearance, i.e. LDLr$^{-/-}$ mice, for the evaluation of anti-hyperlipidemic agents, including LXR agonists[29]. Here we fed C57BL/6 mice with cholate-containing cholesterol-enriched atherogenic diet for 16 weeks to induce atherosclerotic plaque development. Subsequently, a group of mice were sacrificed to obtain baseline data, whilst the remainder of the mice was switched to low-fat cholesterol-free chow diet without or with LXR agonist T0901317 supplementation for 3 weeks.

Atherogenic diet markedly increased plasma total cholesterol level from basal 30 mg/dL to approximately 300 mg/dL (10-fold; $p<0.001$). When diet was switched to chow diet alone, plasma total cholesterol level decreased significantly within one week, and after 3 weeks dropped back to 65 mg/dL (-80%, $p<0.001$), close to its basal level (Figure 2A). In this mouse model, the presence of T0901317 in chow diet did not elevate the cholesterol levels, and actually a similar 3-fold reduction in plasma total cholesterol concentration was noticed as compared to chow group ($p<0.001$) (Figure 2A). T0901317 did not significantly increase plasma triglycerides levels as compared to chow group (Figure 2B). As determined by FPLC lipoprotein separation, the largely reduced plasma total cholesterol concentration was majorly due to markedly decreased plasma VLDL- and LDL-cholesterol levels. Switching to regular chow diet alone significantly reduced plasma (V)LDL-cholesterol concentration (4-fold, $P < 0.001$). T0901317 treatment in C57BL/6 mice did not induce elevated (V)LDL-cholesterol levels, and actually reduced plasma (V)LDL-cholesterol concentration dramatically (28-fold compared to baseline, $P < 0.001$) (Figure 2C).

In contrast to a decreased plasma HDL-cholesterol level in C57BL/6 mice fed with chow diet alone, T0901317 significantly increased the plasma HDL-cholesterol concentration (+30%; $p<0.05$), which was 2-fold higher ($p<0.01$) than group with chow diet alone (Figure 2C). The ratio between HDL- and non-HDL-cholesterol concentration was dramatically increased by T0901317 treatment (13-fold; $p<0.001$) (Figure 2C).

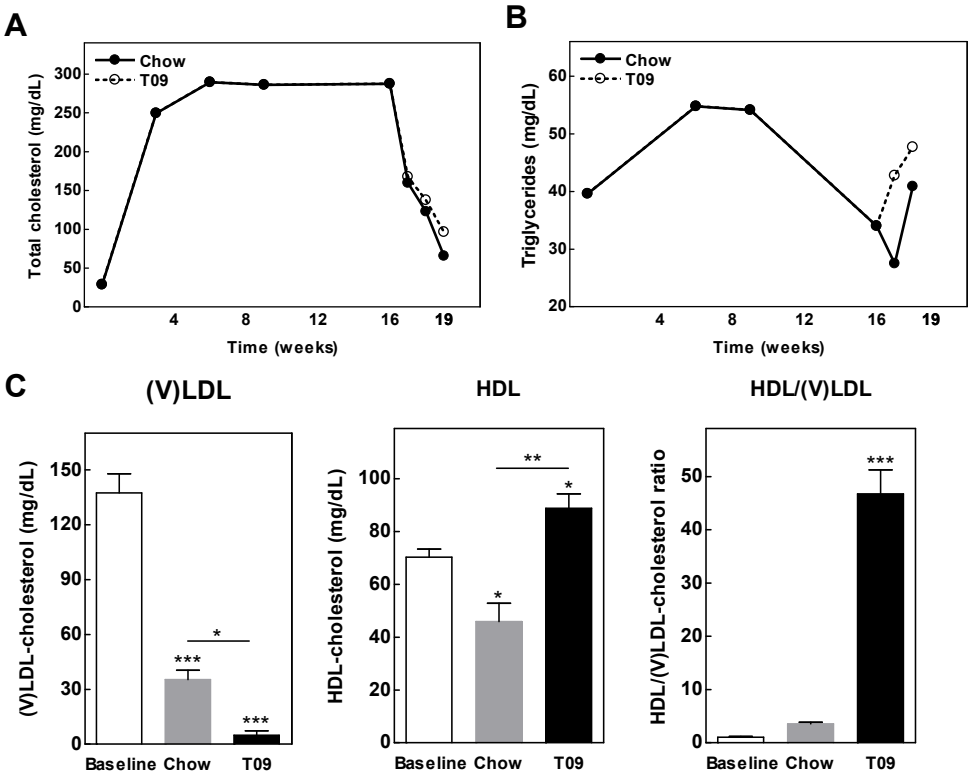

Figure 2. Effects of LXR agonist on plasma lipids in C57BL/6 mice. C57BL/6 mice were fed atherogenic diet for 16 weeks, after which a group of mice was sacrificed (Baseline), whilst the remainder of the mice was switched to regular chow diet without (Chow) or with LXR agonist T0901317 supplementation (T09) for 3 weeks. Plasma total cholesterol (A) and triglycerides (B) concentration were measured. Plasma lipoproteins were separated by FPLC and cholesterol level was measured in each fraction. VLDL represents the sum of cholesterol concentrations from fraction 2 to 7 (VLDL fractions); LDL represents the sum of cholesterol concentrations from fraction 8 to 14 (LDL fractions); HDL represents the sum of cholesterol concentrations from fraction 15 to 22 (HDL fractions) (B). The ratio between HDL- and non-HDL-cholesterol concentration was calculated (C). Values are means ± SEM (8 mice per group). *P<0.05; **P<0.01; ***P<0.001.

LXR agonist dramatically reduced hepatic cholesterol concentration in C57BL/6 mice

Atherogenic diet feeding induced severe liver steatosis in C57BL/6 mice, indicated by enlarged liver size (figure not shown) and intensive accumulation of lipids shown by oil-Red O staining (Figure 3B). However, switching to regular chow diet rapidly reduced liver steatosis. Chow diet alone reduced the hepatic cholesterol content significantly (-27%, P<0.05), while T0901317 supplemented in chow further reduced hepatic cholesterol content (-63%, P<0.001 compared to baseline; -50%, P<0.01 compared to chow group) (Figure 3A). The reduction of cholesterol content was visualized as attenuated neutral lipid staining in liver cryosections (Figure 3B). In addition to the reduced hepatic cholesterol content, hepatic gene expression of macrophage marker CD68 also decreased (-70%, P<0.001) compared to baseline (Figure 3C), indicating largely attenuated hepatic macrophage content and inflammation under chow diet conditions[30,31,32].

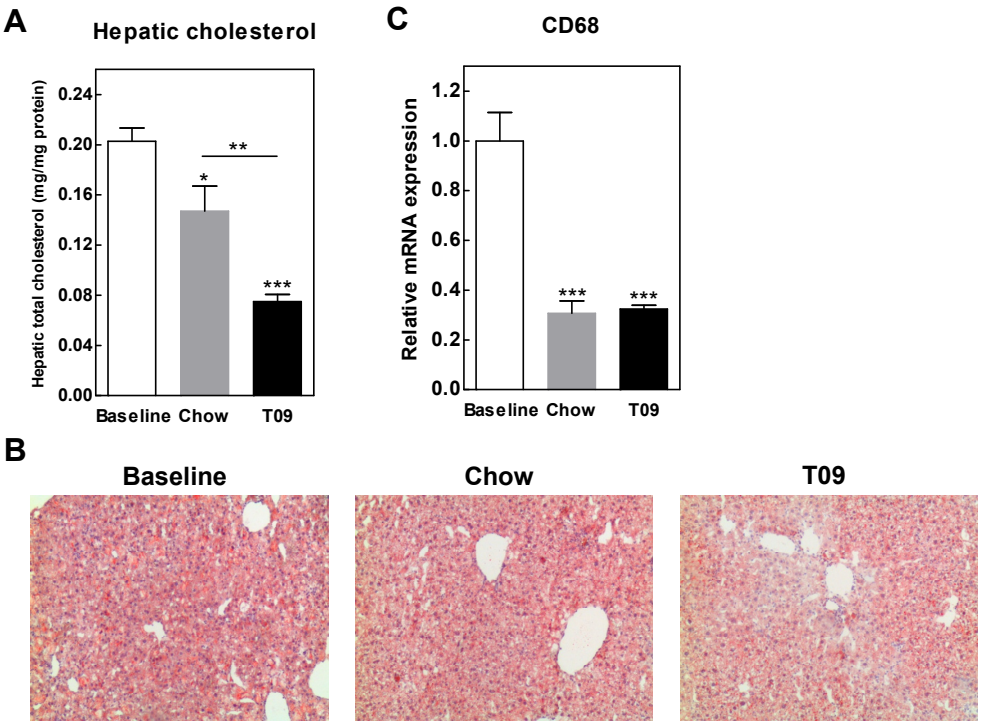

Figure 3. Effects of LXR agonist on hepatic lipids in C57BL/6 mice. Lipids were extracted from liver and hepatic cholesterol concentration was measured (A). Cryostat sections of the liver were stained with oil-red O to identify lipids, and counterstained with hematoxylin to assist in tissue visualization (original magnification: x4) (B). Total RNA was extracted from liver and relative mRNA expression of CD68 was assessed by quantitative PCR and presented as fold-change relative to baseline group (C). Values are means ± SEM (8 mice per group). *P<0.05; **P<0.01; ***P<0.001.

LXR agonist regulated hepatic gene expression in C57BL/6 mice

As expected, T0901317 treatment strongly up-regulated the hepatic expression of the LXR target genes FAS (p<0.001), SREBP-1c (p<0.001) (Figure 4A), and the hepatic triglyceride level (data now shown). This LXR agonist-induced hepatic lipogenesis has been well established as a positive control for LXR activation in mice[33,34].

T0901317 significantly up-regulated the hepatic expression of LDL-receptor compared to group fed chow diet alone (1.7-fold, *P*<0.05) (Figure 4B), suggesting enhanced LDL uptake by the liver in C57BL/6 mice. In addition, T0901317 significantly up-regulated the hepatic expression of ABCG5 (4-fold, *P*<0.05), ABCG8 (2.6-fold, *P*<0.01), and CYP7A1 (1.7-fold, *P*<0.01) compared to group with chow diet alone (Figure 4B), suggesting promoted biliary cholesterol secretion after LXR activation.

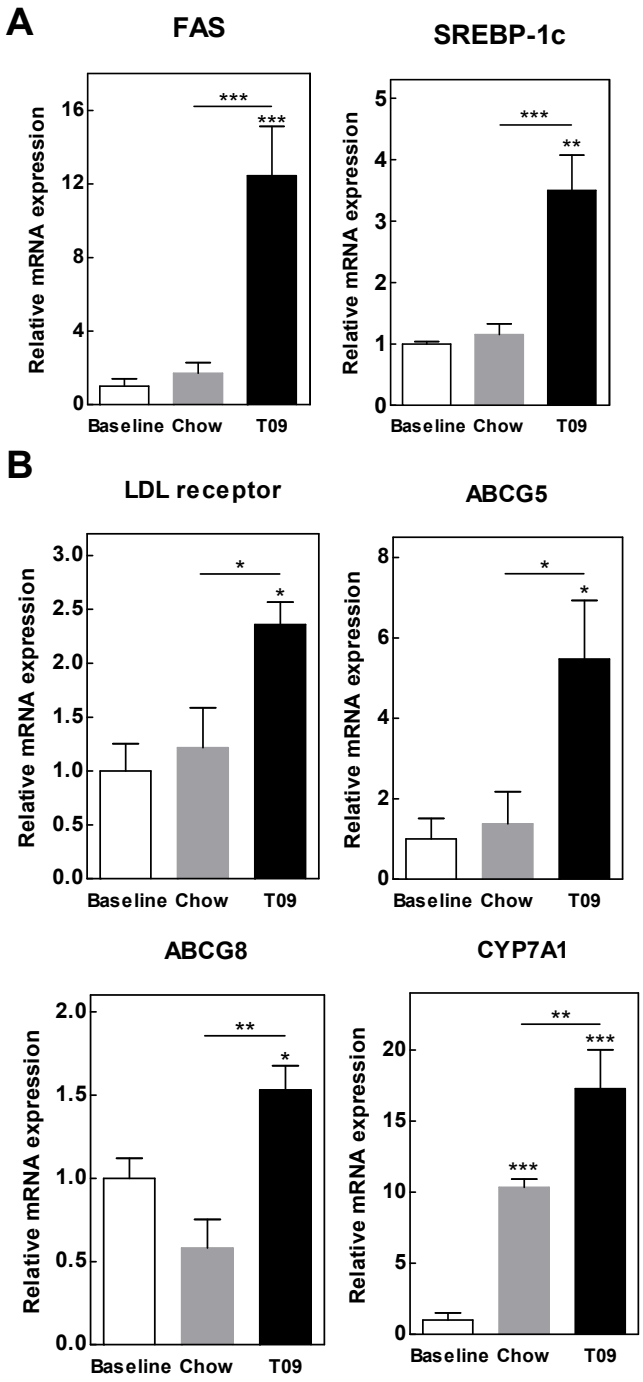

Figure 4. Effects of LXR agonist on hepatic gene expression profiles in C57BL/6 mice. Total RNA was extracted from liver, and relative mRNA expression of FAS, SREBP-1c (A), LDL receptor, ABCG5, ABCG8, and CYP7A1 (B) were determined by quantitative PCR and presented as fold-change relative to baseline group (C). Values are means ± SEM (8 mice per group). *P<0.05; **P<0.01; ***P<0.001.

In line with the elevated plasma HDL-cholesterol level, T0901317 significantly increased the hepatic expression of ABCA1 (+75%, *P*<0.05), ABCG1 (2.3-fold, *P*<0.01), and SR-BI (+70%, *P*<0.05) compared to chow group (Figure 5), suggesting that LXR activation promoted cholesterol efflux and reverse cholesterol transport. T0901317 also significantly increased the hepatic expression of lipoprotein lipase (LPL) (4.5-fold, *P*<0.001) (Figure 5), indicating an enhanced hydrolysis capacity of triacylglycerol component of circulating chylomicrons and VLDL.

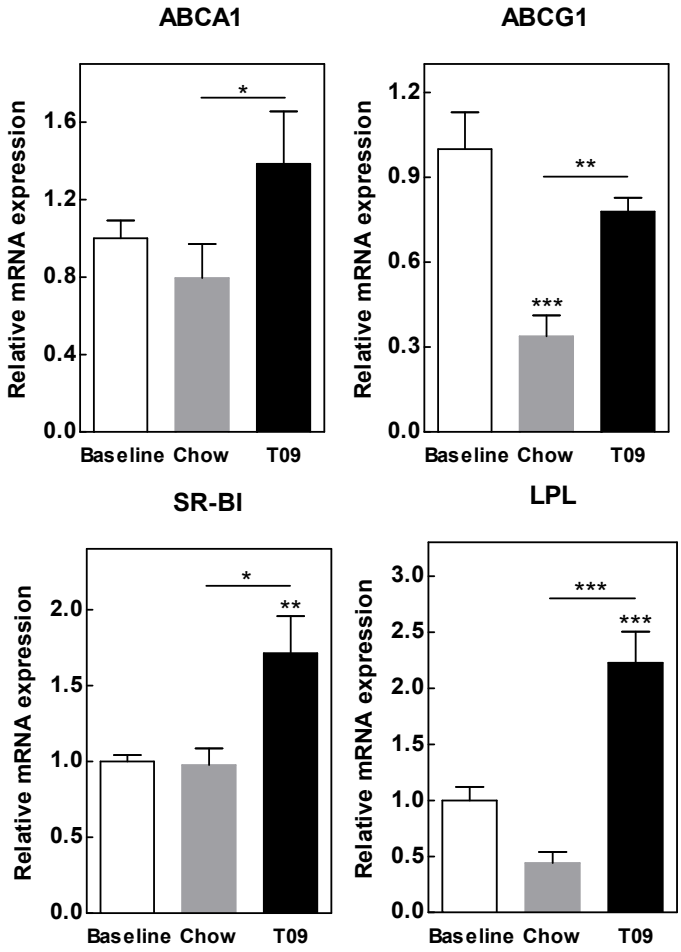

Figure 5. Effects of LXR agonist on hepatic expression of reverse cholesterol transport-related genes in C57BL/6 mice. Total RNA was extracted from liver, and relative mRNA expression of ABCA1, ABCG1, SR-BI, and LPL were determined by quantitative PCR and presented as fold-change relative to baseline group (C). Values are means ± SEM (8 mice per group). *P<0.05; **P<0.01; ***P<0.001.

LXR agonist induced atherosclerotic plaque regression in C57BL/6 mice

After atherogenic diet feeding, C57BL/6 mice developed initial atherosclerotic lesion of approximately $43x10^3$ μm^2 at aortic root as baseline (Figure 6). Three weeks after the diet was switched to chow diet alone, the lesion size stayed

unchanged at approximately $50x10^3$ μm^2, despite of a significantly normalized plasma lipoprotein profile. In contrast, T0901317 treatment supplemented in chow diet significantly reduced the pre-existing atherosclerotic plaque to approximately $25x10^3$ μm^2 (-43%, $P<0.05$), indicating that LXR agonist treatment successfully induced atherosclerotic lesion regression in C57BL/6 mice.

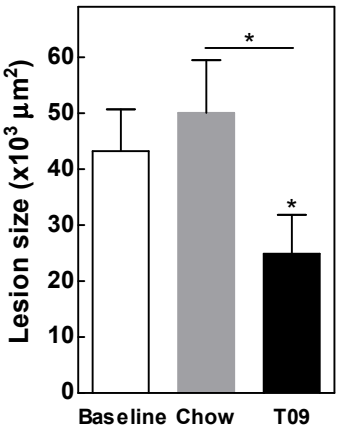

Figure 6. Effects of LXR agonist on atherosclerotic lesion development in C57BL/6 mice. Cryostat sections of the aortic root in heart were stained with oil-red O to identify lipids, and the lesion size was quantified. Values are means ± SEM (8 mice per group). *P<0.05.

DISCUSSION

In the current study, we evaluated the potential of LXR agonist T0901317 to regress diet-induced pre-existing atherosclerotic plaque in $LDLr^{-/-}$ and C57BL/6 mouse models. Our results show that LXR activation is crucial for atherosclerotic lesion regression. In C57BL/6 mouse with diet-induced lesion, LXR agonist supplemented in chow diet rapidly optimized plasma lipoprotein profiles and promoted reverse cholesterol transport, and subsequently induced regression of pre-existing atherosclerotic plaques.

The LXR agonist exhibited opposite effects on plasma lipoprotein and atherosclerotic lesion development in $LDLr^{-/-}$ and C57BL/6 mice. The unfavorable lipoprotein profile induced by LXR agonist in $LDLr^{-/-}$ mice prevented its beneficial effects on atherosclerotic lesion formation. The absence of the LDL receptor and thus impaired LDL clearance and increased VLDL secretion in $LDLr^{-/-}$ mice hampered the LXR agonist-induced lowering of plasma (V)LDL-cholesterol. The LDL receptor-mediated cholesterol uptake and feedback regulation are crucial when evaluating LDL-lowering effects of compounds[35]. Recent studies have shown that when LDL receptor gene was transferred back into hypercholesterolemic $LDLr^{-/-}$ mice to restore sustained expression of LDL-receptor protein in the liver, there was a drastic reduction in plasma cholesterol and non-HDL cholesterol levels and a pronounced regression of advanced atherosclerotic lesions[36,37]. Thus, it is proposed that intact LDL receptor is crucial for creating a lipoprotein profile which permits the regression of atherosclerotic lesions.

Cholesterol-enriched diets are often used to induce or accelerate atherosclerotic lesion progression in murine models. It appears that the induction of persistent hypercholesterolemia to levels higher than approximately 300 mg/dL is required for the development of experimental atherosclerosis in mouse[38]. In the current study, atherogenic diet feeding induced persistent hypercholesterolemia and initial atherosclerotic plaque development. After switching atherogenic diet to regular chow diet, there was a rapid and dramatic reduction in plasma total cholesterol level in both LDLr$^{-/-}$ and C57BL/6 mice, indicating that, with dietary cholesterol being the major proatherogenic component, low-fat cholesterol-free chow diet can largely improve plasma lipoprotein profile to achieve a regressive environment. However, despite the improved plasma lipoprotein profile, chow diet alone did not induce lesion regression in C57BL/6 mice, while in LDLr$^{-/-}$ mice the lesion size even increased. Similar observations were reported in literature where switching high-fat atherogenic diet to a standard chow diet led to markedly reduced plasma (V)LDL-cholesterol without significant reduction in lesion size[39], or with even significantly increased lesion size[40]. Taken together, our data further confirmed that improved plasma lipoprotein profile induced by chow diet alone is not enough to trigger atherosclerotic plaque regression.

The LXR agonist induced plaque regression is due to multiple metabolic effects. The LXR agonist T0901317 not only reduced plasma (V)LDL-cholesterol levels, but also significantly increased HDL-cholesterol level, leading to a 10-fold increased ratio between anti-atherogenic HDL and pro-atherogenic (V)LDL-cholesterol. This indicates that LXR agonist, supplemented in chow diet, rapidly optimizes the plasma lipoprotein profile and achieves a regressive plasma environment in C57BL/6 mice. In addition, the LXR agonist increased the hepatic expression of ABCG5, ABCG8, and CYP7A1, indicating down-regulated cholesterol absorption and up-regulated biliary sterol secretion[41]. Furthermore, the LXR agonist increased the hepatic expression of ABCA1, SRBI, and ABCG1, suggesting increased HDL synthesis, promoted reverse cholesterol transport, and diminished macrophage foam cell formation[42,43].

In conclusion, the current study shows that 1) intact LDL receptor function is crucial to overcome LXR-induced hyperlipidemia; and 2) rapidly optimized plasma lipoprotein profiles combined with LXR agonist induced favorable gene expression profiles can induce regression of pre-existing atherosclerotic plaques in C57BL/6 mice. In addition, our study shows that C57BL/6 mice with diet-induced atherosclerotic lesions may provide an alternative model to investigate plaque regression without robust surgical measures. Further investigations in more humanized animal models may similarly show the potential beneficial effects of LXR activation in the reversal of pre-existing atherosclerotic lesions.

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