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# Differential anti-atherosclerotic effects in the innominate artery and aortic sinus by the liver X receptor agonist T0901317

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**Abstract**

Activation of liver X receptors (LXRs) has been reported to reduce atherosclerosis in mouse models. However, this can be associated with enhanced liver de novo lipogenesis and elevation of plasma triglyceride-rich VLDL, which may limit its clinical use. In this study, we administered orally the LXR agonist T0901317 to male LDLR−/− mice fed a Western diet. This induced a persistent enhanced hypertriglyceridemia by largely increasing plasma triglyceride-rich VLDL. T0901317 treatment decreased atherosclerosis with a much more pronounced response and dose dependence in the innominate artery than in the aortic sinus. Lesions in the innominate artery were less complex containing mostly macrophage foam cells in T0901317-treated mice. However, in the aortic root, a significant reduction of atherosclerosis was seen only in the right coronary-related aortic sinus (RC) of T0901317-treated mice. Increasing the dose of T0901317 did not extend atheroprotection to the other sinuses of the aortic root. Lesions in the RC were less complex both in T0901317 and vehicle-treated mice with macrophage foam cells predominating. On the other hand, in T0901317-treated mice, the left coronary-related sinus (LC) lesions while not reduced in size, were more complex with a large fibrous cap and necrotic core, more collagen-positive areas, and variable macrophage foam cell content compared to vehicle-treated mice. These data suggest that activation of LXR by T0901317 had differential anti-atherosclerotic effects in two arterial regions in mice with hypertriglyceridemia.


*Keywords:* Hypertriglyceridemia; Atherosclerosis; LXRs

## 1. Introduction

LXRs, members of the nuclear receptor superfamily, are activated by oxysterols and regulate lipid and glucose metabolism and inflammation [1]. Activation of LXRs in macrophages promotes the efflux of free cholesterol (CH) and phospholipids from cells to nascent and mature HDL by upregulating the LXR target genes ABCA1, ABCG1 and apoE [2–4], which reduces lipid accumulation in macrophages and foam cell formation. LXR agonists also downregulate expression of inflammatory genes in macrophages [5], and have been shown to reduce atherosclerosis in mice [6–8]. However, activation of LXRs in liver also triggers the expression of sterol regulatory element binding protein 1c (SREBP-1c), a sterol-responsive transcription factor, which in turn induces some of the key enzymes involved in fatty acid synthesis, such as fatty acid synthase (FAS) and acetyl-coenzyme A carboxylase (ACC). Expression of these enzymes initiates de novo lipogenesis and triglyceride (TG) accumulation that eventually causes fatty liver and increases the blood level of large TG-rich lipoproteins [9,10].

Since elevation of plasma TG is an independent risk factor for coronary heart disease [11], the utility of LXR agonists in prevention of atherosclerosis has been questioned [10]. However, the effect of hypertriglyceridemia on the response of atherosclerotic lesions to LXR activation has not been studied in detail. Transient elevation of plasma TG by an

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LXR agonist was observed in low density lipoprotein receptor knockout (LDLR−/−) male mice fed an atherogenic diet containing sodium cholate. Cholate is a natural agonist of farnesoid X receptor (FXR) that can lower plasma TG by down-regulation of short heterodimer partner (SHP) and LXR-associated SREBP-1c expression [7,12]. Transient hypertriglyceridemia, induced by LXR activation, did not prevent a robust dose-dependent reduction of atherosclerosis in the aorta sinus [7]. In another study, a persistent elevation of plasma TG was induced by the same LXR agonist in the same mouse model fed a Western diet without cholate. In this case, regression of pre-existing atherosclerotic lesions in the aortic root was not observed [8]. The different outcomes of these two studies may be explained by two possibilities. LXR agonists may have less of an effect on pre-existing advanced plaques compared to early developing lesions. It is also possible that persistently elevated plasma TG induced by the activation of LXR in the liver may interfere selectively with LXR mediated effects in the aortic sinus.

To extend these studies, we fed LDLR−/− male mice a Western diet without cholate, a diet that has been found to induce persistent hypertriglyceridemia [13]. Corn oil was also not included in the diet studied here, since polyunsaturated fatty acids have several potential atheroprotective effects including an ability to inhibit the transcription of SREBP-1c by antagonizing ligand-dependent activation of LXR [14]. A potent LXR agonist T0901317, which induces liver lipogenesis, was administered at different doses leading to variations in plasma TG in LDLR−/− mice. Atherosclerotic lesions in two separate arterial regions as exemplified by the aorta sinus and innominate artery were analyzed in these mice.

## 2. Methods

### 2.1. Mice, diets and LXR agonist treatment

To examine the effects of LXR agonist on gene expression, 15-week-old male LDLR−/− mice in the C57BL/6 background obtained from Jackson Laboratory were fed a Western diet (Harlan TEKLAD, TD 88137) and treated by gavage with the LXR agonist T0901317 (Cayman Chemical Company) in a 20% microemulsion [15] at a dose of 0.5 mg/kg per day for 2 weeks. The mice in the control group (vehicle-treated) were treated with microemulsion without T0901317. Peritoneal macrophages flushed with ice-cold PBS from the peritoneal cavity were collected and pelleted by centrifugation. Cell pellets were resuspended in RNAlater (Ambion) and stored at −20 °C. Intestine samples were also stored in RNAlater at −70 °C.

To examine the effects of T0901317 on atherosclerosis, 8-week-old male LDLR−/− mice in a C57BL/6 background were fed a Western diet for 12 weeks. Mice also were given LXR agonist T0901317 by gavage at doses of 0.2, 0.5, 1 and 2 mg/kg daily in a 20% microemulsion. Mice from the control group were given microemulsion without T0901317. This treatment was initiated at the time of introduction of the Western diet.

### 2.2. Analysis of atherosclerotic lesions

After 12 weeks of LXR agonist treatment, mice were fasted for 4 h, anesthetized with ketamine and xylazine, and bled from the retro-orbital plexus. The mice were then perfusion fixed and the heart and aorta with its principal branches were isolated, embedded, and sectioned as described previously [16]. Lesions in the innominate artery were quantified from four digitally captured oil red O-stained 10-μm sections, each separated by 100 μm, and located between 150 and 450 μm distal to the branch point of the innominate artery from the aortic arch. Aortic sinus lesions were evaluated from three sections, each separated by 100 μm, beginning at the site of appearance of the coronary artery. Aortic roots were always oriented in the same way using a slice of liver as an orientation marker, so that individual sinuses could be separately evaluated. Hematoxylin and eosin (H&E), trichrome staining, sirius red staining, or immunostaining for macrophages using MoMa-2 was also performed on the sections. OpenLab software version 3.1.5 was used in the quantification.

### 2.3. RNA isolation and analysis of gene expression

Total RNA from mouse macrophages and intestine was isolated by using the RNeasy Mini System (QIAGEN). First strand cDNA was synthesized by utilizing the SuperScript III System (Invitrogen Life Technologies). Quantitative real time PCR was performed as described in the TaqMan Universal PCR Master Mix kit from Applied Biosystems. Specific mRNA levels were normalized to 36B4 mRNA levels and presented relative to the controls.

### 2.4. Analysis of lipids and lipoproteins

Plasma was prepared from blood and a 200 μl sample of plasma was fractionated on tandem Superose 6 FPLC columns [16]. CH and TG in the even-numbered fractions were measured by using commercial assay kits (Stanbio Laboratory). Liver tissue was homogenized and lipids extracted as described [17]. Extracted lipids were measured as described above.

### 2.5. Statistical analysis

Values are presented as means ± standard error of the mean (S.E.M.). Differences between means were analyzed for statistical significance by using one-way ANOVA and Fisher's PLSD post hoc test or unpaired Student's *t*-test. A statistically significant difference was set at $p < 0.05$.