Liver X receptor agonist T0901317 reduces atherosclerotic lesions in \textit{apoE}^{-/-} mice by up-regulating \textit{NPC1} expression

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In this study, we studied the effect of liver X receptor (LXR) agonist T0901317 on Niemann-Pick C1 protein (NPC1) expression in \textit{apoE}^{-/-} mice. Male \textit{apoE}^{-/-} mice were randomized into 4 groups, baseline group ($n=10$), control group ($n=14$), treatment group ($n=14$) and prevention group ($n=14$). All of the mice were fed with a high-fat/high-cholesterol (HFHC) diet containing 15% fat and 0.25% cholesterol. The baseline group treated with vehicle was sacrificed after 8 weeks of the diet. The control group and the prevention group were treated with either vehicle or T0901317 daily by oral gavage for 14 weeks. The treatment group was treated with vehicle for 8 weeks, and then was treated with the agonist T0901317 for additional 6 weeks. Gene and protein expression was analyzed by real-time quantitative PCR, immunohistochemistry and Western blotting, respectively. Plasma lipid concentrations were measured by commercially enzymatic methods. We used RNA interference technology to silence \textit{NPC1} gene expression in THP-1 macrophage-derived foam cells and then detected the effect of LXR agonist T0901317 on cholesterol efflux. Plasma triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and apoA-I concentrations were markedly increased in T0901317-treated groups. T0901317 treatment reduced the aortic atherosclerotic lesion area by 64.2% in the prevention group and 58.3% in the treatment group. LXR agonist treatment increased \textit{NPC1} mRNA expression and protein levels in the small intestine, liver and aorta of \textit{apoE}^{-/-} mice. Compared with the normal cells, cholesterol efflux of siRNA THP-1 macrophage-derived foam cells was significantly decreased, whereas cholesterol efflux of LXR agonist T0901317-treated THP-1 macrophage-derived foam cells was significantly increased. Our results suggest that LXR agonist T0901317 inhibits atherosclerosis development in \textit{apoE}^{-/-} mice, which is related to up-regulating \textit{NPC1} expression.

Niemann-Pick C1 protein, liver X receptor agonist, atherosclerosis, plaque

Monocytes migrate into the blood vessel endothelium space and differentiate into macrophages. Macrophages turn into foam cells after acquiring a mass of lipoprotein-derived cholesterol\cite{1}. Cholesterol accumulation in macrophage-derived foam cells is one of the important events of atherogenesis\cite{2}. Mobilization to the plasma membrane and efflux to extracellular acceptors of intracellular cholesterol are important mechanisms of cells in regulating the cholesterol level. This is the first step of reverse cholesterol transport (RCT).

Niemann-Pick disease type C (NPC) is a fatal auto-

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somal recessive lipid storage disorder characterized by lysosomal accumulation of cholesterols and gangliosides\(^3\), caused by mutations in NPC1 or Niemann-Pick C2 protein (NPC2)\(^4,5\). Mutations in NPC1 account for 95% of all of the NPC cases, whereas mutations at NPC2 account for the remaining 5%\(^6\). NPC1 is expressed in almost every tissue\(^7\) and researched widely at the cellular and molecular level.

NPC1 gene of human and mouse has been identified by positional cloning methods\(^8\). NPC1 is expressed in gene spanning more than 47 kb and contains 25 exons\(^9\). NPC1 is a transmembrane glycoprotein localized in the late endosomal compartment with a sterol-sensing domain projected into the lumen\(^10\) and supervises the change of the cellular cholesterol level and regulates the intracellular lipid homeostasis by altering the manner of vesicle transport or participating in lipid transmembrane transportation directly. NPC2 is a soluble cholesterol-binding protein localized in the lysosome\(^11\). NPC1 and NPC2 control cholesterol trafficking to the plasma membrane\(^12\).

The livers X receptors (LXRs) are ligand-dependent transcription factors belonging to the nuclear hormone receptor superfamily, and they can regulate the expression of genes controlling lipid metabolism\(^13\). In small intestine, LXR can control sterol absorption by regulating expression of ATP-binding cassette transporter A1 (ABCA1), ABCG5 and ABCG8\(^14\). In macrophages, LXR ligands enhance apoA-I-mediated cholesterol efflux through up-regulating ABCA1 expression\(^15\). The natural ligands for LXR include oxidized derivatives of cholesterol, such as 22(R)-hydroxycholesterol and 27-hydroxycholesterol\(^16\). T0901317 is a synthetic LXR agonist broadly used for the research in LXR biology\(^17\). It is well known to similar efficacy to natural ligands, but it is significantly more potent and selectively bound to LXR\(^18\).

Recent research has shown that LXR agonists can enhance the mobilization of free cholesterol to the plasma membrane by inducing NPC1 and NPC2 gene expression in macrophages, resulting in enriched cholesterol content in the outer layer of the plasma membrane, thus becoming more available for efflux\(^12\). Because 95% of NPC cases are caused by NPC1 mutation, it is obvious that the role of NPC1 in intracellular lipid trafficking is much greater than that of NPC2. Furthermore, the effect of LXR agonists on NPC1 expression in vivo has not been clarified. In this study, we fed apoE\(^-/-\) mice with a high-fat/high-cholesterol (HFHC) diet with a synthetic nonoxysterol LXR agonist T0901317 and observed the influence of T0901317 on NPC1 expression and atherosclerotic lesions in the apoE\(^-/-\) mice. In addition, we explore the role of NPC1 in atherosclerosis initiation and development by investigating the influence of T0901317 on intracellular cholesterol efflux in NPC1 gene-silenced THP-1 macrophage-derived foam cells.

## 1 Materials and methods

### 1.1 Animals and diets

Male 8-week-old apoE\(^-/-\) mice and HFHC diet were purchased from Laboratory Animal Center of Peking University, China.

### 1.2 Materials

T0901317 was obtained in Cayman Chemical and dissolved in vehicle (PEG400: Tween 80, 4:1), stored at –20°C. Goat-anti-mouse NPC1 antibody and horseradish peroxidase (HRP)-conjugated rabbit-anti-goat antibody were purchased from Santa Cruz Biotechnology, Inc. Western Blotting Luminol Reagent (sc-2048) was obtained from Zhongshan Golden Bridge Company. The BCA protein assay kit and Blue Ranger pre-stained protein molecular mass marker mix were provided by Hyclone-Pierce. Ponceau S staining solution and Poly-L-Lysine Solution were from Sigma Company. Streptavidin-biotin-peroxidase (SABC-AP) kit was obtained from Boster Bioengineering Company. DyNAmoTM SYBR Green qPCR kit was bought from Finnish Finzymes Company. RevertAidTM First Strand cDNA Synthesis Kit (#k1622) was purchased from Fermentas. Pentobarbital sodium and neutral balsam were gained from Shanghai Chemistry Reagent Company in China National Group of Medicines. Pentobarbital sodium was dissolved in deionized water to turn into 1% solution, filtered through a filter membrane to get rid of bacteria and stored at room temperature. TRIzol reagent is the production of BBI Company, Canada. DEPC came from Ameresco Company, USA. PSilencer2.1-U6-Hygro was obtained from Ambion Company. LipofectamineTM 2000 was from Invitrogen Company. Other chemicals were commercially available.

### 1.3 Main apparatuses and equipments

Automatic biochemical analysys (CX717) was the production of Hitachi Company, Japan. Vacuum tissue