Atherosclerosis 222 (2012) 382–389

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Pharmacological LXR activation reduces presence of SR-B1 in liver membranes contributing to LXR-mediated induction of HDL-cholesterol☆

Aldo Grefhorst[a,1], Maaike H. Oosterveer[a], Gemma Brufau[a], Marije Boesjes[a], Folkert Kuipers[a,b], Albert K. Groen[a,b,*]

[a] Department of Pediatrics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[b] Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 23 December 2011
Received in revised form 2 February 2012
Accepted 10 February 2012
Available online 3 March 2012

Keywords:
Liver X receptor
LXR
T0901317
Scavenger receptor-1B
SR-B1
TICE
HDL

ABSTRACT

Objective: Pharmacological LXR activation has anti-atherosclerotic actions in animal models. Part of these beneficial effects may be explained by accelerated reverse cholesterol transport since both plasma high density lipoprotein (HDL) cholesterol and fecal neutral sterol secretion are higher upon LXR activation. Mechanisms underlying these LXR-mediated effects have not been fully elucidated.
Methods: We investigated the roles of the isoforms LXRα and LXRβ and the HDL cholesterol uptake receptor SR-B1 in modulation of cholesterol metabolism upon treatment of mice with the LXR ligand T0901317.
Results: HDL cholesterol was maximally 60% increased in a time-dependent fashion due to appearance of more and larger HDL particles. Fecal neutral sterol secretion was maximally induced after 1 week treatment. T0901317 treatment induced fecal neutral sterol secretion by ~300% in wild-type but not in *Lxrα* deficient mice. Surprisingly, LXR activation reduced SR-B1 protein amount in hepatic membranes, suggesting that this might contribute to elevated HDL cholesterol. However, T0901317 still elevated plasma HDL cholesterol in *Sr-b1* deficient mice, suggesting that SR-B1 is not the only step involved in LXR-mediated induction of plasma HDL cholesterol. In addition, SR-B1 is not essential for LXR-induced cholesterol removal from the body.
Conclusion: Induction of fecal neutral sterol secretion by T0901317 critically depends on LXRα but not on LXRβ. LXR activation reduces SR-B1 in hepatic membranes, probably partly contributing to elevated HDL cholesterol. SR-B1 is not required to enhance fecal neutral sterol secretion.

1. Introduction

Liver X receptors (LXR) are nuclear receptors that are activated by oxysterols, *i.e.*, oxygenated cholesterol metabolites [1]. Two LXR isoforms have been identified. LXRα (NR1H3) is mainly expressed in the liver and to a lesser extent in intestine and adipose tissue, while LXRβ (NR1H2) is ubiquitously expressed [2,3]. From *in vitro* and *in vivo* studies employing pharmacological LXR ligands like T0901317 [4] it is evident that LXR ligands are potentially promising for treatment of cardiovascular diseases. Pharmacological LXR activation reduces atherosclerotic plaque development in animal models of atherosclerosis (reviewed in [4]). Plasma high density lipoprotein (HDL) cholesterol concentrations were increased upon pharmacological LXR activation [4] and it has therefore been proposed that this treatment induces 'classical' reverse cholesterol transport, *i.e.*, the transport of cholesterol from peripheral tissues (*e.g.*, macrophages) *via* HDL to the liver. Recently, however, van der Veen et al. [5] showed that the increase in fecal neutral sterol secretion induced by LXR activation in mice was mainly due to stimulation of transintestinal cholesterol excretion (TICE), *i.e.*, the direct transfer of cholesterol from the plasma across the intestinal wall into the feces [6].

So far, the mechanism(s) underlying the LXR-mediated increase in plasma HDL cholesterol concentration and the associated changes in HDL composition have not been fully elucidated. We [7] and others [8,9] have shown that LXR activation by pharmacological

Abbreviations: ABC, ATP binding cassette; ApoAI, apolipoprotein AI; FPLC, fast protein liquid chromatography; HDL, high density lipoprotein; LXR, liver X receptor; SR-B1, scavenger receptor-1B; SREBP-1c, sterol-regulatory element-binding protein-1c; TICE, transintestinal cholesterol excretion.

☆ Grants: This research was performed within the framework of project T2-110 of the Dutch Top Institute Pharma.

* Corresponding author at: Department of Pediatrics, Center for Liver Digestive and Metabolic Diseases, Room Y2145, University Medical Center Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands. Tel.: +31 50 363 2669; fax: +31 50 3611746.
E-mail address: a.k.groen@med.umcg.nl (A.K. Groen).
[1] Current address: Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands.

0021-9150/
doi:10.1016/j.atherosclerosis.2012.02.014

means results in the appearance of large HDL particles in mice. In the present study, we therefore have evaluated the effects of LXR activation for specific periods of time on plasma HDL-cholesterol metabolism and fecal neutral sterol output in relation to the presence and activity of scavenger receptor B1 (SR-B1) since the plasma HDL cholesterol profiles of LXR agonist-treated wild-type mice closely resemble those observed in non-treated *Sr-b1* deficient mice [10]. In addition, we clarified the respective roles of the two LXR isoforms LXRα and LXRβ in induction of fecal neutral sterol secretion and TICE by employing *Lxrα* deficient mice.

2. Materials and methods

2.1. Animals and in vivo experimental procedures

All mice were housed in a light- and temperature-controlled facility (lights on 6:30 AM–6:30 PM, 21 °C), were fed a standard laboratory chow diet (RMH-B, Abdiets, Woerden, The Netherlands) and had free access to drinking water. Experimental procedures were approved by the Ethics Committees for Animal Experiments of the University of Groningen.

To study the time-dependent effects of LXR activation, male C57Bl/6J mice (Charles River, L'Arbresle Cedex, France) received standard chow containing T0901317 (0.015%, w/w) for 1 day, 1 week or 2 weeks. Untreated controls received non-supplemented laboratory chow. On the last treatment day, 4-h fasted (8–12 AM) animals were sacrificed by cardiac puncture under isoflurane anesthesia. Livers were quickly removed, freeze-clamped and stored at −80 °C. Blood was centrifuged (4000 × *g* for 10 min at 4 °C) and plasma was stored at −20 °C. Feces were collected for 24 h prior to termination.

Cholesterol fluxes were measured in male *Lxrα*$^{-/-}$ mice and their wild-type littermates on a Sv129/OlaHsd C57Bl/6J mixed background [11] as described previously [5,12]. In short, at start of the 2-week T0901317 treatment period, mice received an intravenous dose of 0.3 mg (0.73 μmol) cholesterol-D7 dissolved in Intralipid (20%, Fresenius Kabi, Den Bosch, The Netherlands) and an oral dose of 0.6 mg (1.535 μmol) cholesterol-D5 dissolved in medium-chain triglyceride oil. Blood spots were collected from the tail on filter paper (Schleicher & Schuell No2992, 's Hertogenbosch, The Netherlands) daily for 14 days. At the end of the 2 weeks treatment, mice were anesthesized by intraperitoneal injection with Hypnorm (fentanyl/fluanisone, 1 ml/kg) and Diazepam (10 mg/kg). The gallbladder was cannulated and bile was collected for 30 min. Subsequently, mice were sacrificed by cardiac puncture and the liver and the small intestine were excised. Feces were collected for 48 h prior to termination. Cholesterol was extracted from the blood spots for gas chromatography/mass spectrometry (GC/MS) analysis as described previously [12]. Biliary bile acids were determined by an enzymatic fluorimetric assay [13]. Total cholesterol concentrations in plasma and bile were determined by gas chromatography, as described below.

To study the role of SR-B1 in LXR-mediated effects on cholesterol metabolism, untreated male *Sr-b1*$^{-/-}$ mice on a C57BL/6J background [14] were fasted for 4 h and blood was collected by retro-orbital bleeding. Feces were collected for 24 h prior to retro-orbital bleeding. Next, these mice were fed the diet with 0.015% (w/w) T0901317 for 2 weeks. On the last treatment day, 4-h fasted (8–12 AM) animals were sacrificed by cardiac puncture under isoflurane anesthesia. Livers were quickly removed, freeze-clamped and stored at −80 °C. Blood was centrifuged (4000 × *g* for 10 min at 4 °C) and plasma was stored at −20 °C. Feces were collected for 24 h prior to termination.

2.2. Plasma lipoprotein analysis

Plasma lipoproteins were separated by fast protein liquid chromatography (FPLC) and the total cholesterol content of the collected fractions was determined using a commercially available kit (Roche Diagnostics, Mannheim, Germany).

The FPLC fractions were diluted 1:1 with 2× SDS loading buffer (100 mM Tris–chloride, pH 6.8; 4% SDS; 0.2% bromophenol blue; 20% glycerol; 200 mM dithiothreitol). To determine the apoA-I content, equal volumes of FPLC fractions in SDS loading buffer were subjected to SDS-PAGE on a 26-wells 12.5% gel (Criterion, Bio-Rad). ApoA-I was determined using an antibody against human apoA-I raised in rabbit (Calbiochem, San Diego, CA). Finally, horseradish peroxidase-conjugated anti-rabbit from donkey (Amersham Pharmacia Bioscience, GE Healthcare) and SuperSignal West Pico Chemiluminescent Substrate System (Pierce, Rockford, IL) were used.

2.3. Hepatic gene expression analysis

Total liver RNA was isolated using the TRI Reagent method (Sigma) according to manufacture's protocol. Integrity and concentration of RNA were determined with the Nanodrop spectrophotometer (NanoDrop™ 1000 Spectrophotometer, Thermo Scientific, Waltham, MA). cDNA was obtained using the reverse transcription procedure with Moloney Murine Leukemia Virus-RT (Sigma, St. Louis, MO) with random primers according to the protocol of the manufacturer. cDNA levels were measured in real-time quantitative PCR amplification using an ABI PRISM 7700 sequence detector (Applied Biosystems, Foster City, CA) against a calibration curve of pooled cDNA solutions. Expression levels were normalized for β-actin levels. The sequences of the primers and probes can be found on www.labpediatricsrug.nl and are deposited at RTPrimerDB (www.rtprimerdb.org).

2.4. Fecal neutral sterol and bile acid contents

Collected feces were dried and homogenized. Fifty micrograms of dried feces were boiled in 1 mL alkaline methanol (1 M NaOH/methanol, 1:3 vol/vol) at 80 °C for 2 h. After cooling down, the sample was extracted three times with 3 ml petroleum-ether to collect the neutral sterols. The remaining sample was diluted 1:9 with distilled water and this was used for bile acid isolation by reversed phase (C18) solid phase extraction. The neutral sterols and the bile acids were derivatized to the methyl ester-trimethylsilyl derivatives for gas chromatographic analysis [15]. The prepared fecal neutral sterol and fecal bile acid samples were determined by capillary gas chromatography using an Agilent gas chromatograph (HP 6890) equipped with a 25 m × 0.32 mm CP-Sil-19 fused silica column (Varian B.V., Middelburg, The Netherlands) and a Flame Ionization Detector.

2.5. Immunoblot analysis

Hepatic membrane fractions were prepared as described before [16] and the protein concentrations were determined using the BCA Protein Assay kit (Pierce). Individual samples were mixed with loading buffer, heated for 5 min at 96 °C and subjected to SDS-PAGE. SR-B1 was determined using an antibody against mouse SR-B1 raised in goat (Novus Biologicals, Littleton, CO), PDZK1 was determined using an antibody against mouse PDZK1 raised in rabbit (Abcam, Cambridge, UK). As loading controls, the concentration of the β-subunit of the Na/K-ATPase was determined using an antibody against Na/K-ATPase raised in goat [17]. Finally, horseradish peroxidase-conjugated anti-rabbit from donkey (Amersham Pharmacia Bioscience) or horseradish peroxidase-conjugated anti-goat