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1α-Hydroxy derivatives of 7-dehydrocholesterol are selective liver X receptor modulators

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ABSTRACT

The nuclear receptors liver X receptor (LXR) α and LXRβ are involved in the regulation of lipid metabolism, inflammation, immunity, cellular proliferation, and apoptosis. Oxysterols are endogenous LXR ligands, and also interact with other nuclear and membrane receptors. We previously reported that a phytosterol derivative with a 1α-hydroxy group acts as a potent LXR agonist with intestine-selective action and that 25-hydroxy and 26/27-hydroxy metabolites of 7-dehydrocholesterol (7-DHC) exhibit partial LXR agonism. In this study, we report that 1α-hydroxy derivatives of 7-DHC, 1α-OH-7-DHC and 1,25-$(OH)_2$-7-DHC, act as LXR modulators. Luciferase reporter gene assays showed that 1α-OH-7-DHC activates LXRα and LXRβ and that 1,25-$(OH)_2$-7-DHC activates both LXRs and vitamin D receptor. Examination of cofactor peptide association showed that the 1α-hydroxy derivatives, specifically 1,25-$(OH)_2$-7-DHC, induce association of coactivator/corepressor peptide in a different manner from the agonist T0901317. Docking modeling and alanine mutational analysis of LXRα demonstrated that 1,25-$(OH)_2$-7-DHC interacts with LXRα residues in a manner distinct from potent agonists, such as T0901317 and 24(*S*),25-epoxycholesterol. 1α-OH-7-DHC and 1,25-$(OH)_2$-7-DHC induced expression of LXR target genes in a cell type- and gene-selective manner. 1,25-$(OH)_2$-7-DHC effectively suppressed lipopolysaccharide-stimulated proinflammatory gene expression in an LXR-dependent manner. Therefore, 1α-hydroxy derivatives, such as 1,25-$(OH)_2$-7-DHC, are unique LXR modulators with selective agonistic activity and potent transrepression function. These oxysterols have potential as LXR-targeted therapeutics for inflammatory disease.

1. Introduction

The nuclear receptors liver X receptor (LXR) α and LXRβ are ligand-dependent transcription factors of the nuclear receptor superfamily that regulate several physiological processes, including lipid metabolism and immunity [1,2]. Oxysterols, such as 24(*S*),25-epoxycholesterol (24,25-EC) and 22(*R*)-hydroxycholesterol (22R-HC), activate LXRα and LXRβ [3,4], while 22(*S*)-hydroxycholesterol and fatty acids, such as arachidonic acid and linolenic acid, are LXRα/β antagonists [5,6]. LXRα is expressed in the liver, adipose tissue, small intestine and macrophages, while LXRβ is ubiquitous [1,2]. Liganded LXR forms a heterodimer with retinoid X receptor (RXR) and binds to an LXR-responsive element that consists of a two-hexanucleotide motif (AGGTCA or a related sequence) on specific target genes, such as genes involved in cholesterol and fatty acid metabolism. LXRα stimulates conversion of cholesterol to bile acids in the rodent liver by inducing cholesterol 7α-hydroxylase. LXRα and LXRβ are involved in intestinal and biliary excretion of cholesterol by increasing expression of the ATP-binding cassette (ABC) transporters ABCG5 and ABCG8, and also in reverse cholesterol transport by inducing ABCA1 and ABCG1. The regulatory effects on cholesterol metabolism lead to anti-atherosclerotic actions of synthetic LXR ligands in mouse models. Hepatic LXRs, specifically LXRα, stimulate lipogenesis by inducing lipogenic genes, such as sterol regulatory element-binding protein 1c (SREBP-1c) and fatty

Abbreviations: LXR, liver X receptor; 24,25-EC, 24(*S*),25-epoxycholesterol; 22R-HC, 22(*R*)-hydroxycholesterol; RXR, retinoid X receptor; ABC, ATP-binding cassette; SREBP-1c, sterol regulatory element-binding protein 1c; 7-DHC, 7-dehydrocholesterol; 1,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; VDR, vitamin D receptor; LCA, lithocholic acid; CDCA, chenodeoxycholic acid; FBS, fetal bovine serum; GST, glutathione *S*-transferase; TR-FRET, time-resolved fluorescence resonance energy transfer; GAPDH, glyceraldehyde dehydrogenase; IL, interleukin; Nos2, nitric oxide synthase 2; siRNA, small interfering RNA; LXR-KO, LXR-knockout; WT, wild-type; ELISA, enzyme-linked immunosorbent assay; FXR, farnesoid X receptor; GPBAR1, G protein-coupled bile acid receptor 1; DRIP205, vitamin D-interacting protein 205; SRC-1, steroid receptor coactivator 1; SMRT, silencing mediator for retinoic acid and thyroid hormone receptor; N-CoR, nuclear receptor corepressor

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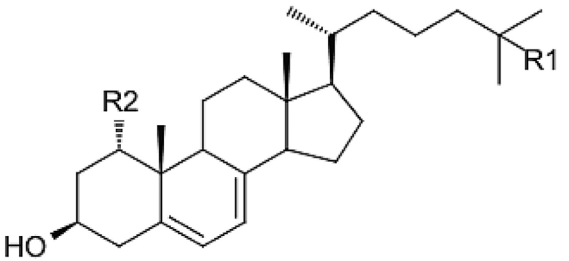
acid synthase. In addition, LXR activation modulates immune and inflammatory responses via transrepression of genes, such as proinflammatory genes in macrophages [1,7]. Furthermore, LXR ligand exhibits anti-cancer effects, which have been characterized in colon cancer, melanoma and glioblastoma [8–11]. Thus, LXR is a possible drug target in the treatment of cardiovascular disease, inflammatory and autoimmune disease, cancer, and neurodegenerative disease [2,12].

Recently, we demonstrated that sterol 27-hydroxylase-mediated metabolites of 7-dehydrocholesterol (7-DHC), 25-hydroxy-7-DHC (25-OH-7-DHC) and 26/27-OH-7-DHC, modulate LXR activity [13]. 7-DHC is generated from acetyl CoA by several enzymatic reactions, and is an intermediate precursor in both the vitamin D_3 and cholesterol biosynthetic pathways [14]. In vitamin D synthesis, 7-DHC is converted to vitamin D_3 (cholecalciferol) by an ultraviolet-induced photo-cleavage reaction in the skin [15]. Vitamin D_3 is metabolized to 25-hydroxyvitamin D_3 in the liver and further to 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] mainly in the kidney. 1,25$(OH)_2D_3$ is the active form of vitamin D and acts as a potent ligand for vitamin D receptor (VDR). In cholesterol synthesis, 7-DHC is converted to cholesterol by 3β-hydroxysterol 7-reductase (7-DHC reductase; DHCR7) [14]. Mutations in the *DHCR7* gene cause Smith-Lemli-Optiz syndrome, and patients with this disease accumulate 7-DHC, which is converted to 25-OH-7-DHC and 26/27-OH-7-DHC [13]. These 7-DHC metabolites suppress *SREBP-1c* expression in hepatic HepG2 cells and HaCaT keratinocytes but weakly increase *ABCA1* expression in HaCaT cells, indicating that these compounds are selective LXR modulators. We also reported that synthetic derivatives of natural steroids, such as the 1α-hydroxy-sterol (22E)-ergost-22-en-1α,3β-diol (YT-32), act as potent LXR agonists [16]. The 1α-hydroxy group of YT-32 is necessary for activation of LXRα and LXRβ, because its derivative lacking a 1α-hydroxy group (YT-33) loses LXR agonistic activity. In this study, we examined the effect of synthetic 7-DHC derivatives with the 1α-hydroxy group (Fig. 1) on LXR activity and found that 1α-hydroxy derivatives of 7-DHC are unique LXR ligands that bind to LXR in a manner distinct from natural ligands.

2. Materials and methods

2.1. Compounds

T0901317 [N-(2,2,2-trifluoro-ethyl)-N-[4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-benzenesulfonamide]] was purchased from Cayman Chemical Company (Ann Arbor, MI), 24,25-EC was from Enzo Life Science (Farmingdale, NY), 22R-HC was from Steraloids (Newport, RI), 1,25$(OH)_2D_3$ was from Wako (Osaka, Japan),



7-DHC:	R1 = H,	R2 = H
25-OH-7-DHC:	R1 = OH,	R2 = H
1α-OH-7-DHC:	R1 = H,	R2 = OH
1,25-$(OH)_2$-7-DHC:	R1 =OH,	R2 = OH

Fig. 1. Chemical structures of 7-DHC derivatives.

lithocholic acid (LCA) and chenodeoxycholic acid (CDCA) were from Nacalai Tesque (Kyoto, Japan), and 7-DHC was from Sigma-Aldrich (St. Louis, MO). 25-OH-7-DHC (cholesta-5,7-diene-3β,25-diol), 1α-OH-7-DHC (cholesta-5,7-diene-1α,3β-diol), 1,25-$(OH)_2$-7-DHC (cholesta-5,7-diene-1α,3β,25-triol) (Fig. 1) and YT-32 were kindly provided from Dr. Yoji Tachibana (Nisshin Flour Milling Inc., Tokyo, Japan). Lipopolysaccharide (LPS) of *Escherichia coli* was from Sigma-Aldrich.

2.2. Plasmids

Expression plasmids, pCMX-LXRα, pCMX-LXRβ, pCMX-VDR, pCMX-FXR, pCMX-RXRα, pCMX-GPBAR1, pCMX-GAL4-LXRα, pCMX-GAL4-LXRβ, pCMX-GAL4-RXRα, pCMX-GAL4-SRC-1, pCMX-GAL4-DRIP205, pCMX-GAL4-SMRT, pCMX-GAL4-NCoR, pCMX-VP16-LXRα, pCMX-β-galactosidase, and pFLAG-CMV2-LXRα, were reported previously [13,16–18]. Expression plasmids of human LXRα mutants (E267A, M298A, E301A, H421A, and W443A) were generated by site-directed mutagenesis on pFLAG-CMV2-LXRα. Luciferase reporters, (rCyp7a1-DR4) × 3-tk-LUC, (hCYP3A4-ER6) × 3-tk-LUC, IR1 × 3-tk-LUC, Som-LUC, and MH100(UAS) × 4-tk-LUC, were used as reported previously [13,17,18].

2.3. Cell culture

Human kidney HEK293 cells (RIKEN Cell Bank, Tsukuba, Japan) were cultured in Dulbecco's modified Eagle medium containing 5% fetal bovine serum (FBS), 100 unit/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2 [18]. Human hepatic cancer-derived HepG2 (RIKEN Cell Bank), colon carcinoma HCT116, SW480, mouse macrophage RAW264.7 (American Type Culture Collection, Rockville, MD), and human immortalized keratinocyte HaCaT cells (kindly provided by Dr. Tadashi Terui, Department of Dermatology, Nihon University School of Medicine) were cultured in Dulbecco's modified Eagle medium containing 10% FBS. Human colon carcinoma Caco-2 cells (RIKEN Cell Bank) were maintained in minimum essential medium containing 10% FBS, and myeloid leukemia THP-1 (RIKEN Cell Bank) and breast carcinoma MCF-7 cells (American Type Culture Collection) were in RPMI 1640 medium containing 10% FBS.

2.4. Transfection and luciferase assay

Transfections in HEK293 cells were performed by the calcium phosphate coprecipitation method [18]. Briefly, 8 h after transfections, test compounds were added. Cells were harvested after an additional 20 h-culture, and were assayed for luciferase and β-galactosidase activity using a luminometer and a microplate reader (Molecular Devices, Sunnyvale, CA), respectively. Transfections used 50 ng of reporter plasmid, 10 ng of pCMX-β-galactosidase and 15 ng of each expression plasmid in a 96-well plate. Luciferase data were normalized to the internal β-galactosidase control.

2.5. Fluorescence polarization assay

Glutathione *S*-transferase (GST) fusion proteins of full-length LXRα (GST-LXRα) were prepared as described previously [13,19]. GST-LXRα proteins (2 μM) were incubated with test compounds for 15 min on ice and then with a fluorescence ligand, 2-(4-(4-methoxyphenethyl) phenyl)-5-(dimethylamino)isoindoline-1,3-dione [20], for 30 min on ice. The fluorescence polarization of the fluorescent ligand was measured with a fluorescence spectrometer (JASCO Corporation, Tokyo, Japan) [13,19]. Control GST proteins were also incubated with the fluorescent LXR ligand.