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The liver X receptor modulator 22(S)-hydroxycholesterol exerts cell-type specific effects on lipid and glucose metabolism

Nina Pettersen Hessvik[a], Siril Skaret Bakke[a,1], Robert Smith[a,1], Aina Westrheim Ravna[b], Ingebrigt Sylte[b], Arild Christian Rustan[a], G. Hege Thoresen[a], Eili Tranheim Kase[a,*]

[a] Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Oslo, Norway
[b] Medicinal Pharmacology and Toxicology, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway

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ABSTRACT

The aim of this study was to explore the effects of 22(S)-hydroxycholesterol (22(S)-HC) on lipid and glucose metabolism in human-derived cells from metabolic active tissues. Docking of T0901317 and 22(S)-HC showed that both substances fitted into the ligand binding domain of liver X receptors (LXR). Results show that while several lipogenic genes were induced by T0901317 in myotubes, HepG2 cells and SGBS cells, effect of 22(S)-HC varied more between cell types. In myotubes, most lipogenic genes were downregulated or unchanged by 22(S)-HC, whereas a more diverse pattern was found in HepG2 and SGBS cells. Treatment with 22(S)-HC induced sterol regulatory element binding transcription factor 1 in SGBS and HepG2 cells, but not in myotubes. Fatty acid synthase was downregulated by 22(S)-HC in myotubes, upregulated in SGBS and unchanged in HepG2 cells. *De novo* lipogenesis was increased by T0901317 in all cell models, whereas differently affected by 22(S)-HC depending on the cell type; decreased in myotubes and HepG2 cells, whereas increased in SGBS cells. Oxidation of linoleic acid was reduced by 22(S)-HC in all cell models while glucose uptake increased and tended to increase in myotubes and SGBS cells, respectively. Cholesterol efflux was unaffected by 22(S)-HC treatment. These results show that 22(S)-HC affects LXR-regulated processes differently in various cell types. Ability of 22(S)-HC to reduce lipogenesis and lipid accumulation in myotubes and hepatocytes indicate that 22(S)-HC might reduce lipid accumulation in non-adipose tissues, suggesting a potential role for 22(S)-HC or a similar LXR modulator in the treatment of type 2 diabetes.

1. Introduction

Liver X receptors (LXR) are important regulators of cholesterol, lipid and glucose metabolism. These receptors are members of the nuclear receptor superfamily and regulate gene expression through interaction with LXR-responsive elements [1]. The LXRβ isoform is ubiquitously expressed in adults [2], whereas the expression of LXRα is mainly restricted to tissues known to play an important role in lipid metabolism, such as liver, adipose tissue, macrophages, kidney, skeletal muscle and small intestine [1,3,4]. Agonists for LXRs include naturally occurring oxidized cholesterol derivatives (oxysterols) [5] and the synthetic compound T0901317 [6], whereas the synthetic 22(S)-hydroxycholesterol (22(S)-HC) has been shown to act as a LXR antagonist on certain genes [7,8]. LXR agonists have been proposed as therapeutic agents for metabolic and cardiovascular diseases due to their effects on cholesterol [6,9] and glucose metabolism [10]. However, LXR activation mediates some undesirable effects, such as increased lipogenesis, hypertriglyceridemia and hepatic steatosis [6,10].

Increasing obesity promotes insulin resistance, but the location of body fat seems to be even more important than generalized adiposity for the disease development [11]. Recently, intrahepatic triacylglycerol content was shown to be a better predictor than visceral adipose tissue for metabolic disorders [12]. Ectopic fat deposition might lead to impaired organ function, and intracellular lipid content in skeletal muscle and liver correlates with insulin resistance and type 2 diabetes [11]. Therefore, antagonizing LXRs' effects on ectopic lipid accumulation might be beneficial in the treatment of type 2 diabetes.

Previously, we have shown that exposure to 22(S)-HC reduced synthesis of complex lipids, repressed certain genes involved in lipogenesis [7], and counteracted the T0901317-induced increase in lipid formation in human skeletal muscle cells [13]. 22(S)-HC reduced the activity of a reporter construct containing a LXR responsive element, supporting that 22(S)-HC

* Corresponding author at: Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, N-0316 Oslo, Norway. Tel.: +47 22856545; fax: +47 22854402.
E-mail address: e.t.kase@farmasi.uio.no (E.T. Kase).
[1] Both the authors contributed equally to this study.

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regulates lipogenesis through direct interaction with LXRs [7]. Furthermore, 22(S)-HC increased glucose uptake and oxidation [13]. Thus, 22(S)-HC might have a therapeutic potential in the treatment of type 2 diabetes. We therefore wanted to investigate the effects of 22(S)-HC in human cell models representing metabolic active tissues important for development of type 2 diabetes. Significant differences in the response to LXR activation have been observed between human and rat hepatocytes, questioning the relevance of rodent models [14]. Few studies on effects of LXRs have been performed in human cells, thus such investigations are highly needed. The aim of the present study was to explore gene regulatory effects of 22(S)-HC compared to T0901317 in human myotubes, SGBS and HepG2 cells, representing human skeletal muscle, white adipose tissue and liver, respectively. Furthermore, we aimed to elucidate whether treatment with 22(S)-HC affected lipogenesis, fatty acid oxidation, glucose uptake and cholesterol efflux in these cells.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM-Glutamax™, 5.5 and 25 mM glucose), DMEM without phenol red, foetal bovine serum, Ultroser G, penicillin–streptomycin–amphotericin B, and trypsin-EDTA were obtained from Gibco, Life Technologies (Paisley, UK). [1-^{14}C]acetic acid (54 mCi/mmol), [1-^{14}C]linoleic acid (56 mCi/mmol), [1-^{14}C]oleic acid (55 mCi/mmol), [1.2-^{3}H(N)]cholesterol (44.5 Ci/mmol) and 2-[^{3}H(G)]deoxy-D-glucose (6.0 Ci/mmol) were purchased from ARC (American Radiolabeled Chemicals, St. Louis, MO, USA). Insulin Actrapid was from Novo Nordisk (Bagsvaerd, Denmark). Linoleic acid, oleic acid, bovine serum albumin (BSA) (essentially fatty acid-free), extracellular matrix (ECM) gel, apoA1, DMEM/nutrient mix F-12 and 22(S)-hydroxycholesterol were purchased from Sigma–Aldrich (St. Louis, MO, USA). RNeasy Mini kit and RNase-free DNase were purchased from Qiagen Sciences (Oslo, Norway). Agilent Total RNA isolation kit was from Agilent Technologies (Santa Clara, CA, USA). The primers were purchased from Invitrogen (Paisley, Scotland, UK), while SYBR® Green and TaqMan® reverse-transcription reagents kit and TaqMan® Low Density Custom Arrays were from Applied Biosystems (Foster City, Canada). T0901317 was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Hydrophobic MultiScreen® HTS plates were from Millipore (Billerica, MA, USA). Corning® CellBIND® tissue culture plates were obtained from Corning Life-Sciences (Schiphol-Rijk, The Netherlands). OptiPhase Supermix and UniFilter®-96 GF/B were delivered by PerkinElmer (Shelton, CT, USA). Glass bottom plates were from MatTek (Ashland, MA, USA). The protein assay reagent was obtained from BioRad (Copenhagen, Denmark). MitoTracker®Red FM, Bodipy 493/503 (4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene) and Hoechst 33258 were obtained from Molecular Probes, Invitrogen (Carlsbad, CA, USA). All other chemicals used were standard commercial high purity quality.

2.2. Docking experiment

The Internal Coordinate Mechanics program [15] was used for docking of 22(S)-HC and T0901317 into the ligand binding domain of the human LXRα and human LXRβ. The X-ray crystal structures of the LXRα ligand binding domain (PDB entry: 1UHL) and the LXRβ ligand binding domain (PDB entry: 1PQ6) were used as targets for docking of 22(S)-HC. The agonist T0901317 has been crystallized in complexes with LXRα (PDB entry: 1UHL) and LXRβ (PDB entry: 1PQ9). T0901317 was also docked into the ligand binding domains of LXRα and LXRβ, and the docking modes were compared with the X-ray crystallographic complexes.

The targets were converted to Internal Coordinate Mechanics objects, hydrogen atoms were added, and Grid maps were calculated based on ligand positions in the X-crystal structure complexes. A model of 22(S)-HC was generated using the Internal Coordinate Mechanics molecule editor and interactive flexible ligand docking was performed into the ligand binding domain of LXRα and LXRβ. The docking poses were grouped based on similarities in binding mode, and interaction energies were calculated using the calcBindingEnergy macro of Internal Coordinate Mechanics.

2.3. Cell culturing

2.3.1. Culturing of human myotubes

Satellite cells were isolated as previously described [16] from the *Musculus obliquus internus* abdominis of 6 healthy donors, age 39.9 (±2.9) years, body mass index 23.5 (±1.4) kg/m^2, fasting glucose 5.3 (±0.2) mM, insulin, plasma lipids and blood pressure within normal range and no family history of diabetes. The muscle biopsies were obtained with informed consent and approval by the National Committee for Research Ethics, Oslo, Norway. The cells were cultured in DMEM-Glutamax™ (5.5 mM glucose), 2% foetal bovine serum, 2% Ultroser G, penicillin (100 units/ml) and streptomycin (100 μg/ml), and amphotericin B (1.25 μg/ml) for proliferation. At 70–80% confluence the growth medium was replaced by DMEM-Glutamax™ (5.5 mM glucose) supplemented with 2% foetal bovine serum, penicillin (100 units/ml) and streptomycin (100 μg/ml), amphotericin B (1.25 μg/ml), and insulin (25 pM) to induce differentiation. The cells were cultured in humidified 5% CO_2 atmosphere at 37 °C, and the medium was changed every 2–3 days. Experiments were performed after 7 days of differentiation.

2.3.2. Culturing of SGBS cells

Human SGBS cells were cultured and differentiated into adipocytes as previously described [17]. Briefly, cells were seeded at low passage 6–10, cultured in basal medium (DMEM/nutrient mix F-12 supplemented with biotin (33 mM), D-pantothenate (17 mM), L-glutamine (2 mM), penicillin (100 units/ml) and streptomycin (100 μg/ml)) supplemented with 10% non-inactivated foetal bovine serum to confluence. For adipocyte differentiation, cells were exposed to adipogenic medium (basal medium supplemented with human transferrin (10 μg/ml), insulin (20 nM), cortisol (100 nM) and T3 (0.2 nM)) supplemented with dexamethasone (25 nM), isobutylmethylxanthine (0.5 mM) and rosiglitazone (2 μM) to increase differentiation rate for 4 days, followed by continuous culturing in adipogenic medium. Cortisol and T3 were removed from the medium during incubation with LXR ligands. The cells were cultured in humidified 5% CO_2 atmosphere at 37 °C, and medium was changed twice weekly until experiments were started at days 15–17.

2.3.3. Culturing of HepG2 cells

The human hepatoblastoma cell line HepG2 (HB-8065, ATCC, Manassas, VA, USA) was cultured in DMEM-Glutamax™ (5.5 mM glucose) supplemented with 10% foetal bovine serum, streptomycin (100 μg/ml) and penicillin (100 units/ml) at 37 °C in 5% CO_2.

2.3.4. Culturing of CaCo-2 cells

CaCo-2 cells obtained from American Type Culture Collection (Rockville, MD, USA) were grown in DMEM-Glutamax™ (25 mM glucose) supplemented with 20% foetal bovine serum, insulin (10 μg/ml), L-glutamine (2 mM), penicillin (50 units/ml), streptomycin (50 μg/ml) and 1% non-essential amino acids (BioWhittaker) and maintained as previously described [18]. For experiments, cells were plated at a density of 2×10^5 cells/cm^2 on 24 mm diameter collagen-treated cell culture filter inserts with