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LXR-α antagonist *meso*-dihydroguaiaretic acid attenuates high-fat diet-induced nonalcoholic fatty liver

Woo-Cheol Sim [a], Sora Park [a], Kang-Yo Lee [a], Young-Tae Je [a], Hu-Quan Yin [a], You-Jin Choi [a], Sang Hyun Sung [a], So-Jung Park [b], Hyun-Ju Park [b], Kye Jung Shin [c], Byung-Hoon Lee [a,*]

[a] College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Gwanak-ro 1, Gwanak-gu, Seoul 151-742, Republic of Korea
[b] School of Pharmacy, Sungkyunkwan University, Seobu-ro 2066, Jangan-gu, Suwon 440-746, Republic of Korea
[c] College of Pharmacy, The Catholic University, Bucheon 420-743, Republic of Korea

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ABSTRACT

Collaborative regulation of liver X receptor (LXR) and sterol regulatory element binding protein (SREBP)-1 are main determinants in hepatic steatosis, as shown in both animal models and human patients. Recent studies indicate that selective intervention of overly functional LXRα in the liver shows promise in treatment of fatty liver disease. In the present study, we evaluated the effects of *meso*-dihydroguaiaretic acid (MDGA) on LXRα activation and its ability to attenuate fatty liver in mice. MDGA inhibited activation of the LXRα ligand-binding domain by competitively binding to the pocket for agonist T0901317 and decreased the luciferase activity in LXRE-*tk*-Luc-transfected cells. MDGA significantly attenuated hepatic neutral lipid accumulation in T0901317- and high fat diet (HFD)-induced fatty liver. The effect of MDGA was so potent that treatment with 1 mg/kg for 2 weeks completely reversed the lipid accumulation induced by HFD feeding. MDGA reduced the expression of LXRα co-activator protein RIP140 and LXRα target gene products associated with lipogenesis in HFD-fed mice. These results demonstrate that MDGA has the potential to attenuate nonalcoholic steatosis mediated by selective inhibition of LXRα in the liver in mice.

1. Introduction

The liver X receptors (LXR) are members of the nuclear receptor superfamily of transcription factors that regulate various gene transcriptions and biological events. LXRs, first isolated as sterol sensors [1], are now known to be involved in many physiological processes, including glucose and lipid homeostasis and immune and neurological function. Because of the various diseases with which LXRs are associated, these nuclear receptors are considered to be promising pharmacological targets [2]. LXRα agonists reduce peripheral cholesterol levels by activating reverse cholesterol transport and possess anti-inflammatory properties, which make them good candidates for anti-atherogenic drug development. One of the target genes of LXRα is sterol regulatory element binding protein 1 (SREBP-1), a transcription factor responsible for hepatic lipogenesis. Therefore, a number of LXR agonists have also been shown to induce hypertriglyceridemia and hepatic steatosis, which hinders the development of this class of compound for human use in atherosclerotic cardiovascular disease [3]. Because of the double-edged role of LXRα in the vascular and hepatic systems,

Abbreviations: ABCA1, ABCG1, ATP-binding cassette transporter A1 or G1; AMPK, AMP-activated protein kinase; ACC, acetyl-coenzyme A carboxylase; FAS, fatty acid synthase; HDL, high density lipoprotein; HFD, high fat diet; LBD, ligand binding domain; LXR, liver X receptor; MDGA, *meso*-dihydroguaiaretic acid; mTOR, mammalian target of rapamycin; PPAR, peroxisome proliferator activated receptor; PGC-1α, PPAR-γ coactivator-1 alpha; RIP140, receptor interacting protein 140; SCD-1, stearoyl-CoA desaturase-1; SREBP, sterol regulatory element binding protein; T0901317, N-(2,2,2-trifluoroethyl)-N-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]-benzenesulfonamide; TG, triglyceride; TR-FRET, time-resolved fluorescence resolution energy transfer.

* Corresponding author.
E-mail address: lee@snu.ac.kr (B.-H. Lee).

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only selective agonists or antagonists of this nuclear receptor have the potential for development as therapeutics. Recent studies have suggested that selective regulation of LXRα in target tissues may be applied to the treatment of many diseases, including metabolic syndromes [4,5].

Regulation of metabolic processes by nuclear receptors is determined by the recruitment of cofactors that are required to remodel the chromatin structure in the vicinity of the promoters and modulate the function of the basal transcription machinery [6]. Non-ligand-bound receptors interact with co-repressors to repress gene transcription. Upon ligand binding, receptors undergo a conformational change that results in dissociation of repressors and recruitment of co-activators. Peroxisome proliferator-activated receptor (PPAR)-γ co-activator (PGC)-1α, activating signal co-integrator 2 (ASC-2), steroid receptor co-activator 1 (SRC-1), or p300 interacts with LXRα to induce its transcriptional activity [7,8]. Receptor-interacting protein 140 (RIP140) acts as a co-repressor of LXRα, playing an important role in lipid accumulation in adipose tissue by suppressing gene expression associated with oxidative metabolism and mitochondrial biogenesis [9,10]. In the liver, on the other hand, RIP140 functions as a co-activator to stimulate the transcription of fatty acid synthase (FAS) [11].

meso-Dihydroguaiaretic acid (MDGA), a dibenzylbutane lignan isolated from *Machilus thunbergii*, possesses anti-oxidative, anti-inflammatory and anti-neurotoxic properties [12–14]. Because it exhibits diverse pharmacological effects with no marked toxicity, *M. thunbergii* has been used in traditional medicine for the treatment of various disorders including leg edema, abdominal distension and pain. In vitro studies have suggested that MDGA inhibits the activation of hepatic stellate cells by downregulating transforming growth factor-β1 (TGF-β1) expression [15]. Recently, Lee et al. reported that MDGA inhibits the gene expression associated with fatty acid metabolism by activating AMP-activated protein kinase in HepG2 cells [16]. However, the effects of MDGA in vivo and the exact mechanisms by which MDGA inhibits lipid accumulation have not been described. In the present study, we evaluated the effects of MDGA on T0901317- and high fat diet (HFD)-induced fatty liver and elucidated its possible mechanisms of action in terms of inhibition of LXRα activation. Our findings demonstrate that MDGA acts as an antagonist, inhibiting ligand-activated LXRα co-activation and transcriptional expression of its downstream target genes involved in fatty acid synthesis, thereby reducing lipid accumulation in mice administered T0901317 or fed HFD.

2. Materials and methods

2.1. Materials

MDGA was synthesized and spectroscopically verified according to the published method (Fig. 1) [17,18]. The purity of MDGA used in this study was 99.6% as determined by high performance liquid chromatography using Agilent 1260 series (Agilent, Santa Clara, CA). Nile Red, Oil Red O, T0901317 (Fig. 1), 22S-hydroxycholesterol, rifampicin, GW4064, geranylgeranylpyrophosphate and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals used were of the highest pure grade available.

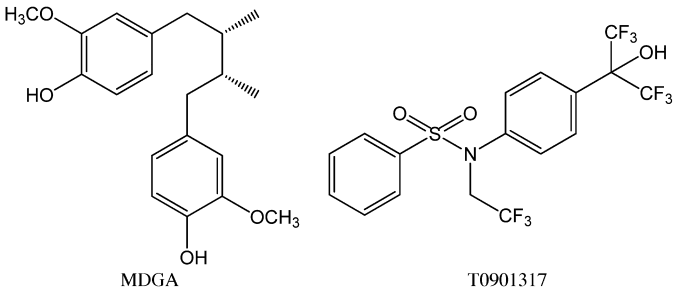

Fig. 1. Chemical structures of MDGA and T0901317.

2.2. Cell culture

HepG2 human hepatoma, THP-1 human monocytic leukemia, and RAW264.7 murine macrophage cell lines were obtained from American Type Culture Collection (Rockville, MD). Hepatoma cells were cultured in DMEM (Gibco-BRL, Rockville, MD) and the others in RPMI1640 (Gibco BRL) supplemented with 10% fetal bovine serum and 50 units/mL penicillin and streptomycin. Cells were incubated in a 37 °C incubator in an atmosphere of 5% in air. Cell viability was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay.

2.3. Time-resolved fluorescence resolution energy transfer (TR-FRET) LXRα co-activator recruitment assay

LanthaScreen™ TR-FRET LXRα co-activator assay kit (Invitrogen, Carlsbad, CA) was used according to the manufacturer's instructions. In this cell-free assay, ligands are identified by their ability to bind the recombinant ligand binding domain (LBD) of LXRα and induce a conformational change that results in recruitment of a fluorescein-labeled coactivator peptide. To test whether a test compound binds LXRα as an agonist, the compound diluted in DMSO was added to LXRα-LBD and coactivator peptide solutions. To test the ability of a compound to function as an antagonist, assays were performed in the same way except that each reaction contained 400 nM T0901317 (EC_{50} of the agonist as measured in this assay). The plates were incubated for 2 h at room temperature and read with a Molecular Device Spectrometer M5, with an excitation wavelength of 340 nm, emission wavelengths of 520 and 490 nm, a delay time of 100 μs, and an integration time of 200 μs. Emission ratios were calculated as the quotients of the emission values at 520 nm divided by the emission values at 490 nm.

2.4. Molecular docking

The 3D structures of MDGA and riccardin F were prepared using sketcher modules of Syblyx1.3 software package (Tripos Inc., St. Louis, MO) and saved in MOL2 format, and that of T0901317 was extracted from crystal structure of LXRα (PDB id: 1UHL) [19]. Tripos force field (distance-dependent dielectric) was used for energy minimization of the ligands, and Gasteiger–Hückel method was used for the calculation of atom charges of the ligands. The energy minimization was continued to reach the final convergence value of 0.001 kcal/(Å mol). For molecular docking performance, crystal structure of the LXRα (PDB id: 1UHL) was retrieved from PDB website. Hydrogen atoms and the Kollman-All atomic charges were added into the receptor used by biopolymer module [20]. Molecular docking was performed using Surflex-Dock program of Sybylx1.3. Surflex-Dock [21,22] is a fast, automatic flexible docking program which uses idealized active site named protomol for docking experiments. The bound ligand in the cocrystal structure of LXRα was used to generate protomol, leaving the threshold and blot parameters at their values of 0.5 and 0 Å respectively. All docking and scoring were performed with default parameters of Surflex-Dock program. As the result of Surflex-dock running, ranked scores of docked conformers were afforded to evaluate the dissociation constant (K_d), expressed in $-\log K_d$ unit. The top-scored conformer was selected as the docking study. In addition to ranked scores obtained from Hammerhead scoring function, also consensus score function (CScore) was considered.