Biochemical Pharmacology 98 (2015) 453–464

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Estrogen receptor mediates simvastatin-stimulated osteogenic effects in bone marrow mesenchymal stem cells

Shu-Chun Chuang[a], Chung-Hwan Chen[a,b,c,d], Yin-Chin Fu[a,b,c,e], I-Chun Tai[a,f,g], Ching-Ju Li[a], Li-Fu Chang[a], Mei-Ling Ho[a,f,g,h,*], Je-Ken Chang[a,b,c,d,**]

[a] Orthopedic Research Center, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[b] Department of Orthopedics, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[c] Department of Orthopedics, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[d] Department of Orthopedics, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[e] Department of Orthopedics, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[f] Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[g] Department of Physiology, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[h] Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung, Taiwan

ARTICLE INFO

Article history:
Received 29 June 2015
Accepted 22 September 2015
Available online 26 September 2015

Keywords:
Simvastatin
Osteogenesis
Mesenchymal stem cells
Estrogen receptor
Estrogen response element

ABSTRACT

Simvastatin, an HMG-CoA reductase inhibitor, is known to promote osteogenic differentiation. However, the mechanism underlying simvastatin-induced osteogenesis is not well understood. In this study, we hypothesize that the estrogen receptor (ER) mediates simvastatin-induced osteogenic differentiation. ER antagonists and siRNA were used to determine the involvement of the ER in simvastatin-induced osteogenesis in mouse bone marrow mesenchymal stem cells (D1 cells). Osteogenesis was evaluated by mRNA expression, protein level/activity of osteogenic markers, and mineralization. The estrogen response element (ERE) promoter activity and the ER–simvastatin binding affinity were examined. Our results showed that the simvastatin-induced osteogenic effects were decreased by treatment with ERα antagonists and ERα siRNA but not by an antagonist specific for the G protein-coupled estrogen receptor (GPER-1). The simvastatin-induced osteogenic effects were further increased by E2 treatment and were reversed by ERα antagonists or siRNA treatment. Luciferase reporter gene assays demonstrated that simvastatin increase ERα-dependent transcriptional activity that was suppressed by ERα antagonists. Furthermore, the ERα–simvastatin binding assay showed that IC_{50} value of simvastatin is 7.85 μM and that of E_2 is 32.8 nM, indicating that simvastatin is a weak ligand for ERα. These results suggest that simvastatin-stimulated osteogenesis is mediated by ERα but not GPER-1. Moreover, this is the first report to demonstrate that simvastatin acts as an ERα ligand and a co-activator to enhance ERα-dependent transcriptional activity and thus promotes osteogenesis. These results indicate that simvastatin-induced osteogenesis is mediated via an ERα-dependent pathway.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Simvastatin, a hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is known to inhibit cholesterol biosynthesis [1,2]. Recent studies have reported that simvastatin stimulates bone formation *in vitro* and *in vivo* [3–5]. Our previous study indicated that simvastatin promotes osteoblast proliferation by enhancing mitochondrial function [6]. Statins have been reported to enhance expression of bone morphogenic protein-2 (BMP-2), which is an important growth factor for osteogenic differentiation. Simvastatin also enhances BMP-2 expression *via* the Ras/Smad/Erk/BMP-2, Ras/PI3K/Akt/BMP-2, and MAPK/BMP-

Abbreviations: ALP, alkaline phosphatase; BMP-2, bone morphogenic protein-2; BMSC, bone marrow mesenchymal stem cell; C2C12 cells, mouse myoblast cells; DMEM, Dulbecco modified Eagle medium; D1 cells, mouse bone marrow mesenchymal stem cells; ECM, extra-cellular matrix; ER, estrogen receptor; ERE, estrogen response element; GPER-1, G protein-coupled estrogen receptor; MG63 cells, human osteoblastic cells; OC, osteocalci.

* Corresponding author at: Orthopedic Research Center and Department of Physiology, College of Medicine, Kaohsiung Medical University, No. 100, Shih-Chuan 1st Road, Kaohsiung 807, Taiwan. Tel.: +886 7 3121101x2553; fax: +886 7 3219452.
** Corresponding author at: Orthopedic Research Center and Department of Orthopedics, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University, No. 100, Shih-Chuan 1st Road, Kaohsiung 807, Taiwan. Tel.: +886 7 3121101x2553; fax: +886 7 3219452.
E-mail addresses: homelin@kmu.edu.tw (M.-L. Ho), jkchang@kmu.edu.tw (J.-K. Chang).

http://dx.doi.org/10.1016/j.bcp.2015.09.018
0006-2952/© 2015 Elsevier Inc. All rights reserved.

2 signaling pathways to promote osteogenic differentiation [7,8]. Recent studies have shown that pathways that do not involve BMP-2 also contribute to statins' effects on osteogenesis [9,10].

Estrogen plays an important role in regulating bone remodeling by suppressing the activity of osteoclasts involved in bone resorption [11,12] and by enhancing osteoblast activities involved in bone formation [13–15]. Estrogen promotes osteogenic differentiation by promoting BMP-2 transcription, *RUNX2* gene expression, and Alkaline phosphatase (ALP) activity in osteoblasts and bone marrow mesenchymal stem cells (BMSCs) [14]. Furthermore, recent reports indicate that estrogen receptor mediation is required for osteogenesis in osteogenic lineage cells [14,16].

Two classes of estrogen receptor exist: (1) traditional estrogen receptors (ER), including ERα and ERβ, that belong to the nuclear steroid hormone receptor superfamily; and (2) G protein-coupled estrogen receptor-1 (GPER-1), a Gs-coupled receptor that promotes estrogen-dependent activation via extranuclear signaling pathways [17,18]. ERα has been reported to induce estrogen-dependent gene expression that promotes osteogenesis [16,19]. Although GPER-1 deficiency has been reported to cause increased bone mass, mineralization, and growth plate proliferative activity in male mice [20,21], another report showed that GPER-1 deficiency decreases bone mass and cortical bone thickness [21].

Our previous study showed that simvastatin increases bone formation in rats with estrogen-deficiency-induced osteopenia [22], indicating that simvastatin's effect on bone formation may be estrogen related. Several reports indicate that ER-mediated osteogenic differentiation does not require estrogen as a ligand [16,23,24]. A recent study showed that simvastatin stimulates osteogenic differentiation by increasing ER expression. However, the molecular mechanisms underlying the effect of simvastatin and the involvement of the estrogen receptor are unknown [25]. In this study, we hypothesize that ERs may be involved in simvastatin-stimulated osteogenic differentiation in osteogenic-linage cells. Here we use antagonists and siRNA transfection to examine whether ERs mediate simvastatin-stimulated osteogenic differentiation by ER blockade. Furthermore, we examine whether simvastatin acts as a ligand for ERα using an ERα competitor assay kit. We also evaluate estrogen response element (ERE) promoter activity using a luciferase gene reporter assay to clarify whether simvastatin affects binding between ER and ERE.

2. Materials and methods

2.1. Cell culture

Murine bone marrow mesenchymal stem cells (D1 cells, CRL-12424™, ATCC, Manassas, VA, USA), human osteoblastic cells (MG63 cells, CRL-1427™, ATCC, Manassas, VA, USA), and mouse myoblasts (C2C12 cells, CRL-1772™, ATCC, Manassas, VA, USA) were used in this study. D1 cells were cultured in bone medium (low-glucose Dulbecco modified Eagle medium [DMEM], Gibco BRL, Thermo Fisher Scientific Waltham, MA, USA) containing 10% fetal bovine serum, 100 U/mL penicillin (Gibco BRL, Thermo Fisher Scientific Waltham, MA, USA), 50 μg/mL ascorbic acid (Gibco BRL, Thermo Fisher Scientific Waltham, MA, USA), and 100 mg/mL nonessential amino acids solution (Gibco BRL, Thermo Fisher Scientific Waltham, MA, USA). C2C12 and MG63 cells were cultured in bone medium supplemented with 200 μM L-glutamine (Gibco BRL, Thermo Fisher Scientific Waltham, MA, USA). Cultures were incubated in a humidified atmosphere of 5% CO_2 at 37 °C [26–28]. During the drug treatment experiments, the DMEM in the bone medium was replaced with phenol-red-free DMEM to avoid the false estrogen effect [29].

2.2. Drug treatment

Simvastatin (Merck Sharp & Dohme Corp., Rahway, NJ, USA), ER α and β antagonist ICI (No. 1047, Tocris Bioscience, Bio-Techne, Minneapolis, MN, USA), ERα-specific antagonist MPP (No. 1991, Tocris Bioscience, Bio-Techne, Minneapolis, MN, USA), and GPER-1 specific antagonist G15 (No. 3678, Tocris Bioscience, Bio-Techne, Minneapolis, MN, USA) were dissolved in DMSO as stock solutions. D1 cells were pretreated with the antagonists for 24 h and then were co-treated with simvastatin and the antagonists for 5 days. The simvastatin concentrations used in these experiments ranged from 0.1 to 1 μM. The concentrations of ICI, MPP, and G15 were 5 μM, 5 μM, and 10 μM, respectively. All reagents were diluted with phenol-red-free bone medium immediately before the treatments began. To reduce its influence on the cells, the final concentration of DMSO in each treatment was $<$0.1%. The cells were harvested to determine osteogenic gene expression on days 1, 3, and 5. After 5 days of drug treatment, the medium was changed to an osteo-induction medium (10 mM β-glycerophosphate disodium salt hydrate (Sigma–Aldrich, St. Louis, MO, USA) and 100 nM dexamethasone in bone medium, (Sigma–Aldrich, St. Louis, MO, USA) to induce osteogenic differentiation. Mineralization (calcium deposition) was evaluated using Alizarin red S staining.

2.3. siRNA transfection

D1 cells were incubated in antibiotic-free culture medium for 24 h before siRNA transfection. Lipofectamine RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA) was used to transfect ERα siRNA (Santa Cruz Biotechnology, Dallas, TX. USA) in D1 cells. The RNAi-negative universal control was used (Invitrogen, Carlsbad, CA, USA). Cells were cultured in nonserum Opti-MEM medium (Life Technologies, Eugene, OR, USA) during siRNA transfection. After transfection, the culture medium was changed to bone medium for 24 h, followed by treatment with simvastatin for 5 days in phenol-red-free bone medium to measure the mRNA expression of osteogenic genes and osteogenic differentiation.

2.4. RNA purification and real-time PCR

Total RNA from D1 cells was extracted using TRIzol Reagent (Cat. 15596026, Invitrogen Carlsbad, CA, USA). First-strand cDNA was synthesized from 1 μg of total RNA using a cDNA synthesis kit (Applied Biosystems, Foster City, CA, USA). Quantitative real-time PCR was performed in a Bio-Rad iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories Inc., Hercules, CA, USA) using iQ SYBR Green Supermix (Bio-Rad Laboratories Inc. Hercules, CA, USA). Reactions were performed in a 25-μL mixture containing cDNA, primers specific for each gene, and the iQSYBR Green Supermix. The mRNA level of BMP-2, ALP, osteocalcin (OC), and ERα were quantified using the following PCR primer pairs: BMP-2 (forward: AGC TGC AAG AGA CAC CCTTTG; reverse: CAA GTT GGC TGC TGC AGG CTT T); ALP (forward: AAC CCA GAC ACA AGC ATT CC; reverse: GTC AGT CAG GTT GTT CCG ATT CAA); OC (forward: CTT GGT GCA CAC CTA GCA GA; reverse: CTC CCT CAT CGT GTT GTC CCT); ERα (forward: AGG AGA CTC GCT ACT GTG C, reverse: ATC ATG CCC ACT TCG TAA CAC). The following cycling conditions were used: incubation at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 30 s, and annealing and extension at 59 °C for 30 s. The specific PCR products were detected by measuring the fluorescence of the SYBR Green, a double-stranded DNA-binding dye. The relative mRNA expression level was normalized to that of β-actin. The mean of the gene expression in the day 1 control group was assigned a value of 1, and the gene expression level of each experimental group was calculated relative to this control.

Download English Version:

https://daneshyari.com/en/article/2511868

Download Persian Version:

https://daneshyari.com/article/2511868

Daneshyari.com