Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Basic Science

Cascade regulation of PPARγ 2 and C/EBPα signaling pathways by celastrol impairs adipocyte differentiation and stimulates lipolysis in 3T3-L1 adipocytes

Seung Kug Choi[a,b], Sunmi Park[a,b], Subin Jang[a,b], Hun Hee Cho[a,b], Siwoo Lee[c], Seungkwon You[b,d], Sang-Hyuk Kim[c,*], Hyun-Seuk Moon[a,b,e,**]

[a] Laboratory of Metabolic Engineering, Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, South Korea
[b] Institute of Animal Molecular Biotechnology, Korea University, Seoul 02841, South Korea
[c] Mibyeong Research Center, Korea Institute of Oriental Medicine, Daejeon 34054, South Korea
[d] Cell Function Regulation Laboratory, Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, South Korea
[e] Biomedical Research Center of Guro Hospital, Research Driven Hospital, Korea University, Seoul 08308, South Korea

ARTICLE INFO

Article history:
Received 3 December 2015
Accepted 20 January 2016

Keywords:
Celastrol
Adipocytes differentiation
Lipolysis
PPARγ2
C/EBPα

ABSTRACT

Objective. Celastrol, a triterpene from the root bark of the Chinese medicinal plant *Tripterygium wilfordii*, has been shown to exhibit anti-oxidant, anti-inflammatory, anti-cancer and insecticidal activities. Also, it has been demonstrated that celastrol has obesity-controlling effects in diet-induced obesity mice. However, direct evidence that celastrol contributes to the development of adipocyte differentiation and lipolysis has not been fully elucidated. Moreover, no previous studies have evaluated whether celastrol may regulate adipogenic transcriptional markers in adipocytes.

Materials/Methods. In order to address the questions above, we extended previous observations and investigated *in vitro* celastrol signaling study whether celastrol may regulate differentiation, lipolysis and key adipogenic transcriptional pathways in 3T3-L1 adipocytes.

Results. Treatment of celastrol not only inhibited adipocyte differentiation (lipid accumulation, glyceraldehyde-3-phosphate dehydrogenase activity and triglyceride content) but also increased lipolysis (glycerol release and free fatty acid release) in 3T3-L1 adipocytes. In addition, all celastrol-regulated functional activities were controlled by PPARγ2 and C/EBPα signaling pathways in duration of celastrol's treatment in 3T3-L1 adipocytes.

Conclusion. Our initial data from *in vitro* celastrol signaling studies suggest novel insights into the role of PPARγ2 and C/EBPα as probable mediators of the action of celastrol in regulating adipocyte differentiation and lipolysis in 3T3-L1 adipocytes.

* Correspondence to: S.-H. Kim, Mibyeong Research Center, Korea Institute of Oriental Medicine, 1672 Yuseong-Daro, Yuseong-gu, Daejeon 34054, South Korea. Tel.: +82 42 868 9238; fax: +82 42 868 9480.
** Correspondence to: H.-S. Moon, Laboratory of Metabolic Engineering, Department of Biotechnology, College of Life Sciences & Biotechnology, Korea University, Seoul 02841, South Korea. Tel.: +82 2 3290 3004; fax: +82 2 3290 3040.
E-mail addresses: kataz@kiom.re.kr (S.-H. Kim), mhs72@korea.ac.kr (H.-S. Moon).

http://dx.doi.org/10.1016/j.metabol.2016.01.009
0026-0495/

1. Introduction

Obesity is relevant to a number of pathological disorders, such as type 2 diabetes, hypertension, hyperlipidemia, cardiovascular disease, respiratory complications, osteoarthritis, and cancer [1]. Given the global epidemic and its association with many life-threatening chronic diseases, obesity induces a main health problem [2–3]. Adipose tissue mass is determined by processes regulating adipocyte number and size [4–5]. The number of adipocytes increases because of increased adipogenesis, *i.e.*, proliferation and differentiation of pre-adipocytes, whereas adipocyte hypertrophy is determined by unbalanced lipogenesis and lipolysis [4].

Celastrol, a quinine methide triterpene extracted from the perennial vine *Tripterygium wilfordii* (Celastraceae), has been used in traditional Chinese medical science as a natural remedy for inflammation and a diversity of autoimmune diseases for hundreds of years [6–7]. Recently, it has been shown that the celastrol suppresses food intake, body weight in hyperleptinemic diet induced obese mice by increasing leptin sensitivity [8], suggesting that celastrol is a leptin sensitizer and a promising agent for the pharmacological treatment of obesity.

The 3T3-L1 adipocytes, which were isolated from the 3T3 cell line of Swiss 3T3 mouse embryos, are the most generally characterized and most very often used preadipocyte cell lines [9–11]. In these lines, committed preadipocytes undergo cell proliferation, growth arrest and afterwards, terminal differentiation into mature adipocytes upon treatment with induction medium including insulin, dexamethasone (DEX), and isobutylmethylxanthine (IBMX). It has been well documented that several transcription factors [12], containing peroxisome proliferator-activated receptors (PPARs) and CCAAT/enhancer binding proteins (C/EBPs), are also critical for adipocyte differentiation. In addition, C/EBP alpha, beta, delta, and gamma have been shown to be correlated with adipogenesis [13]. Specifically, C/EBP beta and delta are induced by DEX and IBMX during adipocyte differentiation, which could induce PPARγ^2 and C/EBPα to activate the diverse adipocyte markers that are required for the adipocyte phenotype [14–16]. In fact, PPARγ^2 is mainly present in adipose tissue, regulating lipid metabolism [17–18]. Moreover, C/EBPα promotes adipogenesis during terminal stages of adipocyte differentiation, and adipocyte fatty acid binding protein 2 (aP2) plays the role of fatty acid transporter that is primarily expressed in adipocytes.

Although celastrol is one of the candidates for increasing leptin sensitivity and reversing obesity, it has to carry out experiments to identify celastrol's mechanism of action in adipocytes. Specifically, it has to be elucidated whether celastrol may regulate adipocyte differentiation and lipolysis through key transcriptional activity in adipocytes. In order to address these questions, we performed *in vitro* signaling studies to clarify the role of celastrol in activating metabolically important signaling pathways as well as their potential interaction with differentiation and lipolysis in 3T3-L1 adipocytes.

2. Materials and Methods

2.1. Materials

Celastrol is the red amorphous powder with purity of more than 98%, whose molecular formula is $C_{29}H_{38}O_4$ with a molecular weight of 450.61 Da, which was purchased from Sigma-Aldrich Korea (Seoul, South Korea). Celastrol was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich Korea, Seoul, South Korea) and DMSO-treated cells were used as a vehicle control. Oil red O (ORO), isopropyl alcohol, insulin (INS), DEX, IBMX, penicillin, streptomycin and Dulbecco's Modified Eagle's Medium (DMEM) were obtained from Sigma-Aldrich Korea (Seoul, South Korea). Fetal bovine serum (FBS) was provided by Gibco Life Technologies (NY). Glucose, aprotinin, formalin, phenylmethylsulfonyl fluoride, and Tween 20 were purchased from Samchun Chemical (Gwangyang, South Korea).

2.2. Cell Culture

The 3T3-L1 adipocytes were obtained from the American Type Culture Collection (VA) and were cultured as previously described [19] with minor modifications. Briefly, the cells were grown in DMEM with high glucose containing 10% (v/v) FBS, 100 U/ml penicillin and 100 μg/ml streptomycin in plates (10^5 cells/ml) at 37 °C in a humidified atmosphere of 10% CO_2.

2.3. Cell Viability Assay

The cell viability assay was performed using the MTT proliferation kit (Sigma-Aldrich Korea, Seoul, South Korea) as described by the supplier. Briefly, the cells were seeded in 96-well plates at a density of 5×10^3 cells/well. Cells were left to adhere overnight and then treated with celastrol for 24 h and 48 h, respectively. Cells were washed with PBS and incubated with 100 μl serum-free medium and 10 μl Vybrant MTT solution for 2 h. Formazan crystals were dissolved overnight at 37 °C with the addition of 100 μl of 10% SDS in 0.01 N HCl per well and absorbance was measured at an optical density of 570 nm in an EMax® Plus (Molecular device, Seoul, South Korea).

2.4. Real-Time (RT) PCR Analysis

RNA of cultured cells was extracted using EasyPure RNA kit (Transgen, ER101-01), and the cDNAs were synthesized using PrimeScript 1st Strand cDNA Synthesis kit (TAKARA, 6110A). RT PCR was performed with Platinum SYBR green qPCR SuperMix-UDG (Life Technologies, C11733-038) on Stratagene Mx3005P qPCR system. For RT-PCR detection of each gene, a pair of oligonucleotide primers was designed in the corresponding cDNA sequences as follows: PPAR gamma2 (sense: CATCCAAGACAACCTGCTGC, anti-sense: TGTGACGATCTGCCTGAGGT), C/EBP alpha (sense: ACTCCTCCTTTTCCTACCG, anti-sense: AGGAAGCAGGAATCCT CC), aP2 (sense: AGCATCATAACCCTAGATGG, anti-sense: GAAGT CACGCCTTTCATAAC), and perilipin (sense: ACGAGGCTGAGAC TGAGGTG, anti-sense: GAGCACGTTCTCCTGCTCA). Beta actin (sense: CTAAGGCCAACCGTGAAAAG, anti-sense: ACCAGAGGCA TACAGGGACA) was measured as internal controls.

2.5. Glycerol-3-Phosphate Dehydrogenase (GPDH) Activity

The GPDH assay was performed by a spectrophotometric method for determination of the disappearance of NADH during GPDH-catalyzed reduction of DHAP under zero-order condition [20]. Protein content was determined using the BCA protein assay.