Food and Chemical Toxicology 106 (2017) 17–24

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Regulatory effects of 4-methoxychalcone on adipocyte differentiation through PPARγ activation and reverse effect on TNF-α in 3T3-L1 cells

Younho Han, Sung Ho Lee, Ik-Soo Lee, Kwang Youl Lee*

College of Pharmacy & Research Institute of Drug Development, Chonnam National University, Gwangju, Republic of Korea

ARTICLE INFO

Article history:
Received 24 June 2016
Received in revised form
11 May 2017
Accepted 15 May 2017
Available online 18 May 2017

Keywords:
4-Methoxychalcone
Adipocyte differentiation
PPARγ
TNF-α

ABSTRACT

Chalcones, the biosynthetic precursors of flavonoids and isoflavonoids abundant in edible plants, possess a number of pharmacological properties, and there is growing evidence that chalcone derivatives inhibit TNF-α mediated insulin resistance. The aim of the present study was to define the effects of 4-methoxychalcone (4-MC) on adipocyte differentiation and to determine the underlying molecular mechanism. We investigated the effects of 4-MC on adipocyte differentiation and lipid accumulation, and expression of adipogenic genes in 3T3-L1 cells. Additionally, treatment with 4-MC significantly increased the PPARγ-induced transcriptional activity and 4-MC also enhanced the DNA binding affinity of PPARγ to the proliferator-activated receptor response elements (PPRE) at target promoters. Next, we tested the effect of 4-MC on the inhibition induced by TNF-α on adipocyte differentiation. Treatment with 4-MC enhanced the lipid accumulation and strongly up-regulated the expression of adipogenic markers, including PPARγ, aP2, FAS, and adiponectin during adipocyte differentiation. Finally, 4-MC attenuated the inhibitory effect of TNF-α on adipocyte differentiation and adiponectin expression and subsequently regulated the expression and secretion of various adipokines that are involved in insulin sensitivity. This study clearly demonstrates that 4-MC enhanced adipocyte differentiation, in part, by its potent effects on PPARγ activation and by its reverse effect on TNF-α.

1. Introduction

Chalcone, a constituent of natural compounds that are especially abundant in fruits, vegetables, and numerous plants and spices, is an open-chain flavonoid, bearing two aromatic rings, connected by three carbons (Ju et al., 1995; Orlikova et al., 2011). Chalcone derivatives have shown diverse pharmacological properties, including anti-tumor and anti-inflammatory activities. (Fogaca et al., 2017; Lin et al., 2002; Nowakowska, 2007). In spite of the high structural similarity between chalcone derivatives, the mechanisms of their cellular activities are considerably different (Yadav et al., 2011). Even during carcinogenesis, different chalcones regulate completely different steps, from the very early stages of tumor initiation, progression, angiogenesis, and invasion, to the very late stages that lead to metastasis (Kamei et al., 1997; Orlikova et al., 2011). Moreover, the molecular mechanisms and bioactive principles responsible for the adipogenic activities of chalcone derivatives remain to be elucidated.

Obesity is a common metabolic disorder and medical condition that is defined as excess fat mass in the body, to the extent that it negatively affects health and potentially accelerates to type 2 diabetes. One of the main causes of type 2 diabetes is impaired insulin action in adipose tissue, skeletal muscle, and the liver, coupled with insufficient secretion of insulin to overcome this resistance (Aberer et al., 2015; Kotronen et al., 2008; Wallia and Molitch, 2014). In the obese state, various adipokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), and monocyte chemotactic protein-1 (MCP-1), are highly expressed in adipocytes, leading to increased insulin resistance. Additionally, in the obese state, the expression of adiponectin, which is responsible for increasing insulin sensitivity, is reduced in adipocytes (Andrade-Oliveira et al., 2015). Peroxisome proliferator-activated receptor (PPAR) γ, as a master regulator of adipocyte differentiation, regulates adipogenic genes during differentiation

Abbreviations: 4-MC, 4-methoxychalcone; aP2, fatty-acid binding protein 2; FAS, fatty acid synthase; GluT1, glucose transporter type 1; GluT4, glucose transporter type 4; IL-6, interleukin-6; MCP-1, monocyte chemotactic protein-1; PAI-1, plasminogen activator inhibitor-1; PPARγ, peroxisome proliferator-activated receptor gamma; PPRE, proliferator-activated receptor response elements; TNF-α, tumor necrosis factor-alpha.

* Corresponding author.
E-mail address: kwanglee@chonnam.ac.kr (K.Y. Lee).

http://dx.doi.org/10.1016/j.fct.2017.05.032
0278-6915/

and controls lipid metabolism. PPARγ agonists, especially thiazolidinedione derivatives such as rosiglitazone, pioglitazone, and troglitazone, increase the number of adipocytes that produce adiponectin, while decreasing the number of adipocytes that express TNF-α, IL-6, and other adipokines that negatively regulate insulin sensitivity (Buras et al., 2005; Kim et al., 2006; Rasouli et al., 2006). Rosiglitazone was approved for the treatment of type 2 diabetes by the Food and Drug Administration (FDA) in 1999. However, because rosiglitazone caused or exacerbated congestive heart failure, and several other serious side effects, the FDA has put restrictions on its use (Roughead et al., 2015). Although pioglitazone is currently available in clinical use, safety concerns are still existing (Govindan and Evans, 2012). Thus, safe agents that target PPARγ activation are needed for the prevention and treatment of type 2 diabetes.

In a previous medicinal chemistry report, the structure-activity relationship data revealed that several chalcone derivatives exhibits the highest activity with glucose medium concentration compared to rosiglitazone and suggested the possibility to develop chalcone derivatives as an anti-diabetic agent (Hsieh et al., 2012). In the present study, we attempted to investigate the regulatory effects of small molecule compounds including three chalcone derivatives (*trans*-chalcone, 4-methoxychalcone, hesperidin-methyl chalcone, Fig. 1) and biologically active compounds (ginkgolide A, ginkgolide B, bilobalide, artemisinin) on adipogenic activity and metabolic syndrome, and to evaluate the relevant molecular mechanisms. To this end, we focused on the adipogenic activity of 4-MC on adipocyte differentiation and lipid accumulation and determined the underlying molecular mechanisms.

2. Materials and methods

2.1. Cell culture and differentiation conditions

Murine, preadipocyte 3T3-L1 cells were maintained in a 5% CO_2 incubator at 37 °C in Dulbecco's modified Eagle medium (DMEM, Life Technologies, Carlsbad, CA, USA) containing 10% bovine calf serum (BCS, Gibco Invitrogen, Carlsbad, CA, USA). For adipocyte differentiation, a mixture of 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, and 10 μg/mL insulin (MDI) was used. 3T3-L1 cells were grown to full confluence for 2 days in 24-well plates, and the medium was subsequently replaced with DMEM supplemented with 10% fetal bovine serum (FBS, Gibco Invitrogen) and MDI, with or without 4-methoxychalcone (day 0) for 2 days. Thereafter, the MDI was changed to adipogenic maintenance media consisting of DMEM, 10% FBS, and 10 μg/mL insulin, with or without 4-methoxychalcone (day 2) and the cells were fed DMEM containing 10% FBS, with or without 4-methoxychalcone every other day. The cells were allowed to accumulate lipid droplets until used for further experiments.

2.2. Antibodies and reagents

Antibodies against C/EBPβ (04-1153, Upstate Biotechnologies, Lake Placid, NY, USA), PPARγ (MAB3872, Chemicon International Inc., Temecula, CA, USA), HA (12CA5, Roche Applied Science, Basel, Switzerland), and α-tubulin (sc-53646, Santa Cruz Biotechnology, Dallas, TX, USA) were used. Insulin (I2643), dexamethasone (D4902), 3-isobutyl-1-methylxanthine (I5879), GW9662 (M6191), rosiglitazone (R2408), *trans*-chalcone (TC, 136123), 4-methoxychalcone (4-MC, S456748), hesperidin-methyl chalcone (HMC, H5006), ginkgolide A (51863), ginkgolide B (94970), bilobalide (79593), and artemisinin (69532) were purchased from Sigma-Aldrich (St. Louis, MO, USA). TNF-α (GF027, recombinant mouse protein) was purchased from Merck Millipore (Darmstadt, Germany). GW9662, rosiglitazone, *trans*-chalcone, 4-methoxychalcone, hesperidin-methyl chalcone, ginkgolide A, ginkgolide B, bilobalide, and artemisinin were dissolved in DMSO. TNF-α was reconstituted with sterile distilled water. Vehicle cultures received the DMSO and the final concentration of DMSO in all experiments was 0.1% in all samples.

2.3. Transient transfection and luciferase reporter assays

Transient transfection was performed using polyethyleneimine (PEI; Polysciences, Warrington, PA, USA) by adding 0.5 μg of total plasmid DNA per well. Total amounts of transfected plasmids in each group were equalized by adding empty vector. 3T3-L1 cells were transfected with a luciferase reporter plasmid that contained the promoter for the PPAR responsive element (PPRE) of aP2, pCMV-β-gal, or PPARγ expression plasmids. Twenty-four hours after transfection, the cells were treated with the indicated reagents for 12 h. Luciferase activities were measured using the Luciferase Reporter Assay Aystem (Promega, Madison, WI, USA) and luciferase activity was normalized to β-galactosidase activity to determine transfection efficiency.

2.4. Oil red O staining

At the end of differentiation (day 8), differentiated 3T3-L1 cells were washed twice with phosphate-buffered saline (PBS). Monolayer cells were fixed with 10% formalin for 30 min, and stained with oil red O (0.5% oil red O, 6:4 isopropanol:water) for 30 min at room temperature on a shaker. After washing the cells three times with PBS, the stained cells were allowed to air dry and then were visualized with a light microscope and photographed with a digital camera. The stained dye was dissolved in isopropanol, and the lipid accumulation was quantified by measuring the absorbance at 530 nm using a spectrophotometer.

2.5. mRNA expression analysis

RNA was extracted using RNAiso Plus (Total RNA extraction reagent, Takara Bio, Japan), and oligo (dT) primers and reverse transcriptase (Promega) were used to synthesize cDNA. The mRNA expression of several adipogenic markers and inflammatory adipokines, including adiponectin, aP2, FAS, GluT1, GluT4, PPARγ, IL-6, PAI-1, and MCP-1 was quantified by real-time PCR using SYBR Premix Ex Taq kit (TaKaRa Bio, Japan) and CFX96 real-time PCR

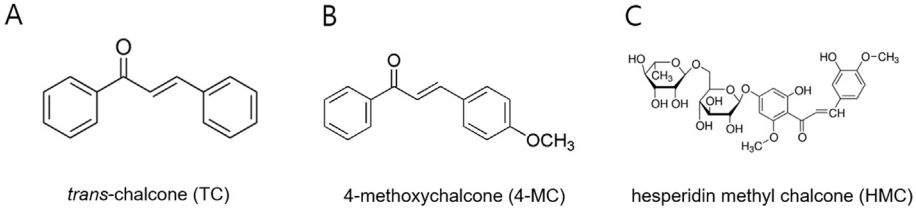

Fig. 1. Structure of chalcone derivatives examined in this study. (A) *trans*-chalcone, (B) 4-methoxychalcone, and (C) hesperidin-methyl chalcone.