J Korean Soc Appl Biol Chem (2012) 55, 63−68
DOI 10.1007/s13765-012-0011-6

ORIGINAL ARTICLE

Inhibitory Effect of Galanolactone Isolated from *Zingiber officinale* Roscoe Extract on Adipogenesis in 3T3-L1 Cells

Eun-Kyung Ahn · Joa Sub Oh

Received: 7 October 2011 / Accepted: 16 November 2011 / Published Online: 29 February 2012

Abstract *Zingiber officinale* Roscoe commonly known as ginger, has been used in traditional medicine. Inhibtion effect of galanolactone isolated from *Z. officinale* Roscoe on adipogenesis in 3T3-L1 cells was evaluated. Effect of galanolactone on 3T3-L1 adipocyte differentiation was measured by Oil Red O staining, and cytotoxicity effect of galanolactone was analyzed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay. The expression of various genes involved in adipogenic action of galanolactone was determined by real-time PCR and Western blot. Peroxisome proliferator-activated receptor γ (PPARγ) luciferase transactivation assay was used to evaluate the PPARγ transcriptional activity of galanolactone in HEK 293T cells. Galanolactone inhibited lipid accumulation and expression of adipocyte fatty acid-binding protein (aP2) and resistin in a dose-dependent manner in 3T3-L1 cells. Treatment with 50 and 100 μM of galanolactone significantly decreased the troglitazone-induced PPARγ transcripitional activity in HEK 293T cells, and suppressed expressions of PPARγ and CCAAT-enhancer-binding protein α (C/EBPα) at mRNA and protein levels in 3T3-L1 cells. These findings suggest that galanolactone isolated from *Z. officinale* Roscoe exerts anti-obesity effect through downregulation of adipogenic transcription factors and adipogenic marker genes.

Keywords adipogenesis · adipogenic marker genes · galanolactone · peroxisome proliferator-activated receptor γ · 3T3-L1 cells · *Zingiber officinale*

E.-K. Ahn · J. S. Oh (✉)
Natural Products Research Institute, Gyeonggi Institute of Science & Technology Promotion; 864-1 Ieui-dong, Yeoungtong-gu, Suwon-si, Gyeonggi-do 443-766, Republic of Korea
E-mail : jsoh@dankook.ac.kr

J. S. Oh
College of Pharmacy, Dankook University, San #29, Anseo-dong, Dongnam-gu, Cheonan-si 330-714, Republic of Korea

Introduction

Zingiber officinale Roscoe, a well known herbal medicine, has been used in the treatment of a wide variety of ailments (Afzal et al., 2001; Chrubasik et al., 2005). In previous studies, *Z. officinale* has been shown to reduce plasma lipids in cholesterol-fed hyperlipidaemic rabbits and was found to inhibit low density lipoprotein (LDL) oxidation in atherosclerotic mice (Bhandari et al., 1998; Fuhrman et al., 2000). *Z. officinale* has been reported to have chemical constituents including gingerols, gingerones, and shogaols, and these chemical constituents have also shown anti-allergy and anti-inflammatory effects (Kikuzaki et al., 1992; Chen et al., 2009; Sang et al., 2009). Han et al. (2005) reported that *Z. officinale* extract shows anti-obesity effects in high-fat diet-induced obese mice. However, the possible anti-obesity effects of *Z. officinale* constituents remain undefined; thus, in the present study, the isolation of galanolactone from *Z. officinale* and the investigation of its potential anti-obesity effect in 3T3-L1 cells are reported.

Peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT-enhancer-binding protein α (C/EBPα) have been known to play a key role in the regulation of adipogenesis and modulation of fat cell function in adipose tissue. PPARγ is a highly fat-specific member of the PPAR subfamily of nuclear non-steroid hormone receptors (Tontonoz et al., 1994). C/EBPα, is a member of a large family of leucine zipper transcription factors, and is expressed during the differentiation of preadipocytes into adipocytes (Burn et al., 1996; Morrison and Farmer, 2000). PPARγ and C/EBPα induce adipocyte differentiation in a number of fibroblast cell lines and preadipocytes (Kubota et al., 1999). PPARγ and C/EBPα are expressed prior to the expression of most adipocyte genes and regulate expression of genes involved in creating and maintaining the adipocyte phenotypes, including adipocyte fatty acid-binding protein (aP2) and resistin (Lowell, 1999; Rosen et al., 2000; Jeon et al., 2004). Expression of antisense C/EBPα reduces the expression

of C/EBPα mRNA and protein, which effectively suppresses the expression of aP2 mRNA and protein, and blocks triglyceride accumulation (Mandrup and Lane, 1997).

In the present study, the effect of galanolactone isolated from *Z. officinale* Roscoe on adipocyte differentiation and expression of adipokine genes in 3T3-L1 cells were examined. Results showed that galanolactone has potent anti-obesity effects and treatment with galanolactone caused reduction in the expression of PPARγ, C/EBPα, aP2, and resistin in 3T3-L1 cells.

Materials and Methods

Preparation of natural product extracts. Dried rhizomes of *Z. officinale* were purchased from Kyungdong Oriental Herbal Market in Korea (August 2009) and identified by one of the authors (Prof. Joa Sub Oh). A voucher specimen (GPRC-198) of this plant has been deposited in the Gyeonggi Institute of Science & Technology Promotion Natural Products Research Institute, Suwon, Korea. The dried rhizomes (2 kg) were pulverized and extracted with EtOH (3×15 L) at room temperature (24 h).

Extraction and Identification of galanolactone. The extract was filtered and concentrated, *in vacuo*, suitably diluted with water, and partitioned with *n*-hexane (3×1.5 L) and CH_2Cl_2 (3×1.5 L). The *n*-hexane extract (24 g) was subjected to column chromatography on silica gel (70–230 mesh, 9×25 cm; Merck, Darmstadt, Germany) by eluting with $CHCl_3$/EtOAc (1:0) in increasing proportion of acetone, to yield ten fractions (ZR-101-1–10). ZR-101-3 (1.5 g) was subjected to vacuum liquid chromatography (VLC) on RP-18 (3×20 cm, 40–63 μm), eluting with MeCN/water (20, 40, 60, 70, 80, 90, and 100% MeCN) to afford nine fractions (ZR-104-1–9). Fraction ZR-104-2 (0.3 g) was subjected to flash column chromatography on RP-18 (2×30 cm, 40–63 μm; YMC, Allentown, PA), eluting with MeCN/water (20, 40, 50, 60, 70, 80, 90, and 100%), and affording six fractions (ZR-112-1–6). Fraction ZR-112-4 (300 mg) was further purified by means of semi-preparative HPLC (Shimadzu, Kyoto, Japan), eluting with MeCN/water (55:45) at 20 mL/min to yield galanolactone (20.3 mg). ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian 500 NMR spectrometer (Agilent, Palo Alto, CA) using $CDCl_3$ as a solvent. Mass spectrum (MS) was obtained on a Waters Q-TOF Micro mass spectrometer (Milford, MA).

Galanolactone. Colorless needles; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.64 (1H, m, H-12), 4.39 (2H, t, *J*=7.5 Hz, H-15), 2.83 (2H, m, H-14), 2.44 (1H, dd, *J*=3.5, 1.5 Hz, H-17), 2.31 (1H, dd, *J*=4.0, 1.5 Hz, H-17), 2.09 (1H, m, H-11), 0.93 (3H, s, H-20), 0.92 (3H, s, H-19), 0.88 (3H, s, H-18); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 171.3 (C-16), 142.9 (C-12), 124.7 (C-13), 65.4 (C-15), 57.5 (C-8), 54.9 (C-9), 52.3 (C-5), 49.0 (C-17), 41.8 (C-3), 39.6 (C-10), 39.3 (C-1), 35.7 (C-7), 33.5 (C-4), 33.48 (C-18), 25.3 (C-14), 22.7 (C-11), 21.7 (C-19), 20.0 (C-6), 18.6 (C-2), 14.5 (C-20); Electrospray ionization (ESI)MS *m/z* 317 $[M-H]^-$ (Molita and Itokawa, 1998). The structure of galanolactone is presented in Fig. 1A.

Cell culture and adipocyte differentiation assay. 3T3-L1 preadipocytes were purchased from the American Type Culture Collection (CL-173). Cells were maintained in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% Bovine Calf Serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin (Invitrogen, Carlsbad, CA) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. On day 0, the cells were induced with initiation medium [10 μg/mL insulin, 1 μM dexamethasone, and 0.5 mM 3-isobutyl-1-methyxanthine in 10% Fetal Bovin Serum (FBS)-DMEM]. On day 2, the initiation medium was replaced with progression medium (10 μg/mL insulin in 10% FBS-DMEM). On days 4 and 6, the progression medium was replaced with maintenance medium (10% FBS-DMEM). From days 0 to 6, the cells were treated with various concentrations of galanolactone.

MTT viability assay. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was used for determination of cell proliferation in 3T3-L1 cells *in vitro*. Cells were plated at 5×10^3 per well in 100 μL culture medium. One day after plating, a time zero control plate was made. Compounds were applied directly, and the cells were incubated for additional 24 h in a humidified 5% CO_2 atmosphere at 37°C; cell proliferation was then determined. MTT (5 mg/mL in PBS) was added to each well, followed by incubation for 3 h. The medium was removed from the wells by aspiration. Subsequently, 0.1 mL of buffered DMSO was added to each well, and the plate was shaken. Absorbance was measured on a microtiter plate reader at 540 nm.

Oil Red O staining. Eight days after induction of differentiation, the cells were stained with Oil Red O (Sigma, St. Louis, MO). Cells were washed twice with phosphate-buffered saline (PBS), fixed with 3.7% formaldehyde in phosphate-buffered saline for 30 min, and distilled washed twice with water. Cells were stained with 0.35% Oil Red O dye in isopropyl alcohol for 30 min. Excess stain was removed by washing with 70% ethanol and distilled water. Stained lipid droplets were dissolved in isopropyl alcohol containing 4% Nonidet P-40 and quantified by ELISA at 510 nm.

Luciferase reporter assay. HEK 293T cells (ATCC, CRL-11268) were seeded into 24-well plates and cultured for 24 h prior to transfection. Cells were transfected with 200 ng PPRE-luciferase reporter plasmid, 50 ng pcDNA3- hPPARγ, and 20 ng pRL-SV40 each using lipofectamine LTX reagent (Invitrogen, Carlsbad, CA). Twenty-four hours after transfection, the cells were treated with troglitazone, in the presence or absence of materials for 24 h. The Dual-Luciferase Reporter Assay System (Promega, Madison, WI), with a luminometer, was used for determination of luciferase activity. Relative luciferase activity was normalized to Renilla luciferase activity.

Quantitative real-time PCR. Total RNA was extracted using a total RNA extraction kit (Quiagen, Hilden, Germany). One microgram of RNA was used as a template for each reverse transcription polymerase chain reaction (RT-PCR) using SuperScript™ III One-Step RT-PCR System (Invitrogen, Carlsbad, CA). Newly synthesized cDNA from 3T3-L1 control

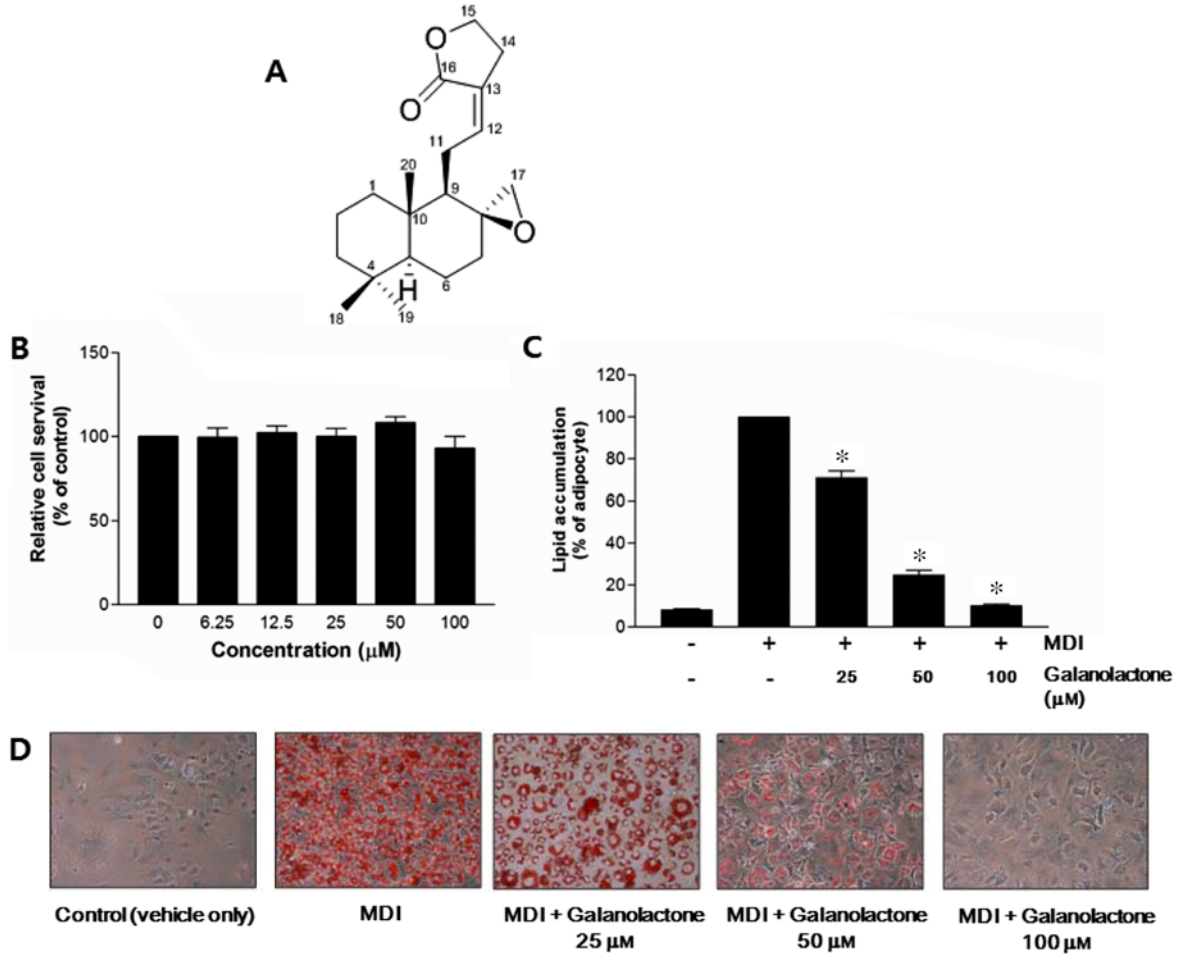

Fig. 1 Effect of galanolactone on lipid accumulation in 3T3-L1 cells. (A) Structure of galanolactone isolated from *Z. officinale* Roscoe. (B) 3T3-L1 cells were treated with galanolactone at various concentrations (6.25–100 μM) for 24 h. Cytotoxicity of galanolactone was determined by MTT assay. Post-confluent 3T3-L1 cells were differentiated in the absence or presence of galanolactone for 8 days. (C) Triglyceride content was quantified by measuring absorbance. Data are expressed as means ± SD, where $p < 0.05$ was considered statistically significant. (D) Lipid droplets were measured by Oil Red O staining.

cells and galanolactone-treated cells was amplified using specific primers and the SYBR Premix Ex Taq system (TaKaRa, Shiga, Japan). The primers used in the experiments are shown in Table 1. Glyceraldehyde 3- phosphate dehydrogenase (GAPDH) was used as the RNA loading control.

Western blot analysis. The cells were harvested and washed with PBS and collected by centrifugation at 13000 rpm for 1 min at 4°C. To obtain the cellular lysate, the cells were lysed on ice for 30 min in RIPA buffer [50 mM Tris–HCl, pH 7.5, 0.15 M NaCl, 1% NP-40, 0.1% SDS, 1 mM DTT, and 1 mM phenylmethylsulfonyl

Table 1 Primer sequences used for real-time PCR

Target	Primer sequences	Accession No.
GAPDH	5'-GAGTCAACGGATTTGGTCGT-3' (sense) 5'-GACAAGCTTCCCGTTCTCAG-3' (antisense)	BC083080
PPARγ	5'-CGCTGATGCACTGCCTATGA-3' (sense) 5'-AGAGGTCCACAGAGCTGATTCC-3' (antisense)	NM_011146
C/EBPα	5'-AGGTGCTGGAGTTGACCAGT-3' (sense) 5'-CAGCCTAGAGATCCAGCGAC-3' (antisense)	BC058161
aP2	5'-CATGGCCAAGCCCAACAT-3' (sense) 5'-CGCCCAGTTTGAAGGAAATC-3' (antisense)	NM_024406
Resistin	5'-TCAACTCCCTGTTTCCAAATGC-3' (sense) 5'-TCTTCACGAATGTCCCACGA-3' (antisense)	NM_022984

fluoride (PMSF)], which contained a mixture of protease inhibitors (Roche, Mannheim, Germany). Insoluble materials were removed by centrifugation at 13000 rpm for 10 min at 4°C. Fifty micrograms of the supernatants were separated using a 10% polyacrylamide gel containing 10% sodium dodecyl sulfate (SDS), 1.5 M Tris–HCl, 0.035% *N,N,N,N'*-tetra-methylenediamine, and 7 mg ammonium persulfate. The separated proteins were transferred to a nitrocellulose membrane (Whatman, Dassel, Germany) at 36 mA in a transfer buffer containing 39 mM glycine, 48 mM Tris base, 0.037% SDS, and 20% methanol. The membranes were sequentially incubated with anti-PPARγ, anti-CCAAT-enhancer-binding protein α (CEBPα), and rabbit polyclonal immunoglobulin antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:1000 dilutions and with anti-actin, mouse monoclonal immunoglobulin antibodies (Sigma, St. Louis, MO) at 1:2000 dilutions. Horseradish peroxidase-conjugated anti-rabbit and anti-mouse immunoglobulin antibodies (Santa Cruz Biotechnology) were used as secondary antibodies at a dilution of 1:1500. All detections were performed using an electro-chemiluminescence detection reagent (Amersham Biosciences, Uppsala, Sweden).

Statistical analysis. Data are expressed as the means ± SD. Experimental results were analyzed for statistical significance (Student's *t*-test and one way ANOVA). $p < 0.05$ considered statistically significant.

Results and Discussion

Effects of galanolactone on cytotoxicity and adipocyte differentiation in 3T3-L1 preadipocytes. The chemical structure of galanolactone is shown in Fig. 1A. To detect the cytotoxicity of galanolactone, 3T3-L1 cells were treated with various concentrations (6.25–100 μM) of galanolactone, and cell viability was measured by the MTT assay. Treatment with 6.25–100 μM galanolactone did not cause any significant cytotoxic effects in 3T3-L1 cells (Fig 1B). In our preliminary study, treatment with 6.25–500 μg/mL of ethanolic extract of *Z. officinale* was shown not to induce significant cytotoxic effects in 3T3-L1 cells (data not shown). Subsequently, the adipogenic effect of galanolactone on adipocyte differentiation was examined using a differentiation mixture (insulin, dexamethasone, 3-isobutyl-1-methyxanthine) to induce differentiation of 3T3-L1 cells. 3T3-L1 cells were treated with 25, 50, and 100 μM of galanolactone during differentiation. After 8 days, cells were stained with Oil Red O. Treatment of 3T3-L1 cells with galanolactone suppressed adipocyte differentiation in a

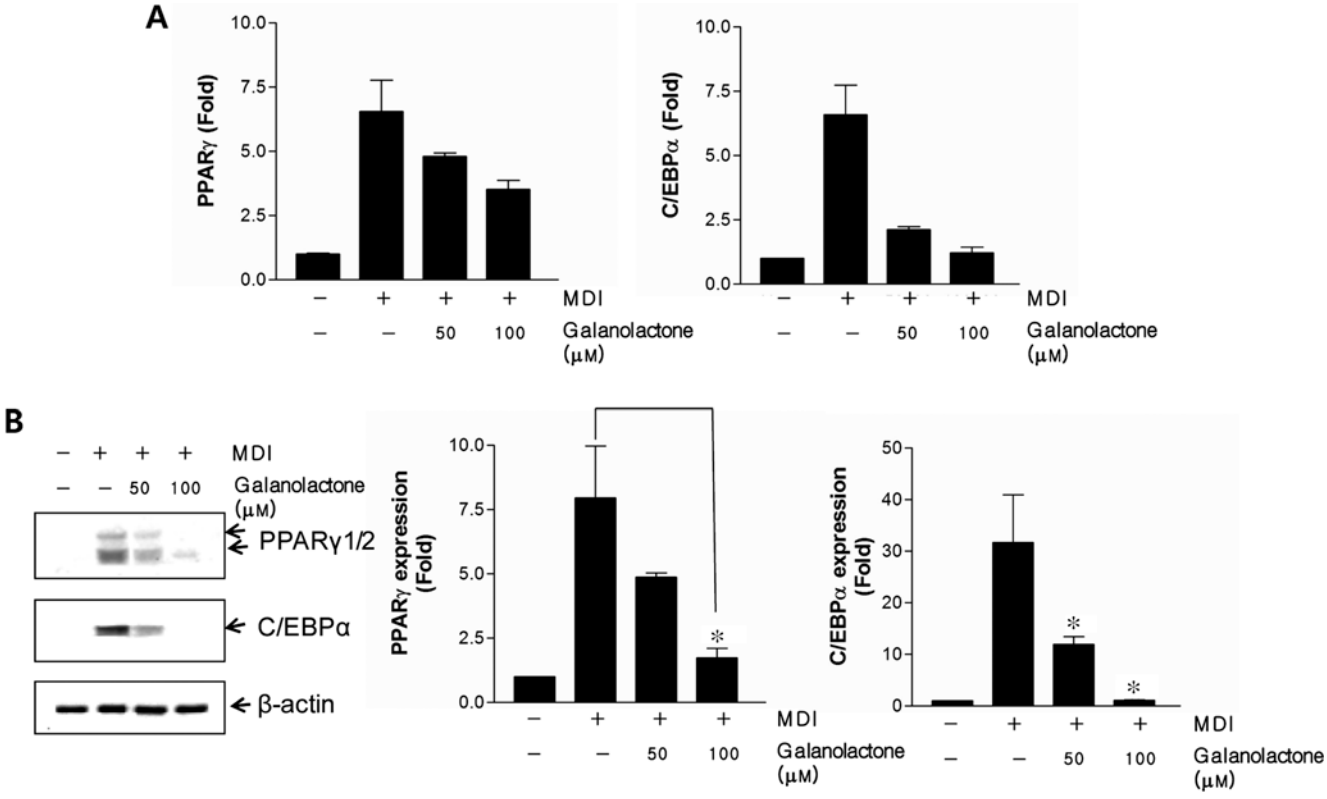

Fig. 2 Effects of galanolactone on expression of PPARγ and CEBPα in 3T3-L1 cells. Post-confluent 3T3-L1 cells were differentiated in the absence or presence of galanolactone for 8 days. (A) PPARγ and CEBPα mRNA expression were evaluated by quantitative real-time PCR. (B) PPARγ and CEBPα protein expressions were analyzed by Western blotting. Data are expressed as the means ± SD, where $p < 0.05$ was considered statistically significant.

dose-dependent manner (Fig. 1C). Lipid droplet accumulation of 3T3-L1 adipocytes treated with 25, 50, and 100 μM of galanolactone were 76, 25, and 9%, respectively. Hwang et al. (2009) reported that Ginsenoside Rg3, rich in red ginseng, inhibits adipocyte differentiation and lipid accumulation of 3T3-L1 adipocytes treated with 80 μM Ginsenoside Rg3 was approximately 60%. The present study demonstrates for the first time that galanolactone can inhibit fat accumulation of adipocytes. In addition, cells treated with 50 and 100 μM galanolactone showed a significant reduction of lipid accumulation through inhibited differentiation of 3T3-L1 preadipocytes (Fig. 1D). These results directly indicate that galanolactone isolated from *Z. officinale* inhibits lipid deposition in a dose-dependent manner without causing cytotoxic effects.

Effect of galanolactone on the expression of PPARγ and CEBPα in 3T3-L1 adipocytes. To clarify whether galanolactone inhibits adipogenesis through the PPARγ and CEBPα pathways, 3T3-L1 adipocytes were evaluated by quantitative real-time PCR and Western blot analysis after treatment of fully differentiated cells (day 8) with 50 and 100 μM galanolactone. Adipocytes develop from preadipocytes by a specific signaling pathway, which leads to an up-regulation and activation of PPARγ (Hu et al., 1996). PPARγ induces the expression of CEBPα and also increases its own expression. Similarly, CEBPα induces PPARγ expression as well as its own expression. These cooperative functions help in maintaining high levels of PPARγ and CEBPα, and then PPARγ stimulates adipocyte differentiation (Tontonoz et al., 1995). In the present study, expression of PPARγ and CEBPα was suppressed by galanolactone at the mRNA level (Fig. 2A), and galanolactone treatment resulted in dose-dependent suppression of PPARγ and CEBPα at the protein levels. Protein levels of PPARγ and CEBPα were reduced by up to 73 and 97% respectively, by treatment with 100 μM of galanolactone (Fig. 2B).

Effect of galanolactone on PPARγ transcription activity in HEK 293T cells. Inhibitory effect of galanolactone on PPARγ transcriptional activity was evaluated. PPARγ is one of the most important transcription factors regulating fat cell differentiation (Gregoire et al., 1998). Using a reporter assay system, PPARγ transcriptional activity was evaluated after galanolactone treatment. HEK 293T cells were transfected with the pGL3-PPRE-Luc and PPARγ plasmids after treatment with galanolactone or troglitazone, an anti-diabetic agent as a PPARγ ligand (Lehmann et al., 1995). Like most members of the nuclear receptor family, PPARγ activity is regulated by ligands. Pharmaceutical ligands include thialzolidinediones (TZDs) such as troglitazone, BRL 49653 (rosiglitazone), and pioglitazone, and these compounds are very effective at promoting adipogenesis (Rosen et al., 2000). Furthermore, galanolactone treatment dose-dependently inhibited not only the PPARγ transcriptional activity but also the troglitazone-induced PPARγ transcriptional activity (Fig. 3). In particular, treatment with 50 and 100 μM of galanolactone significantly decreased the troglitazone-induced activation of PPARγ-mediated transactivation. These results suggest that galanolactone effectively inhibits mRNA expression by blocking

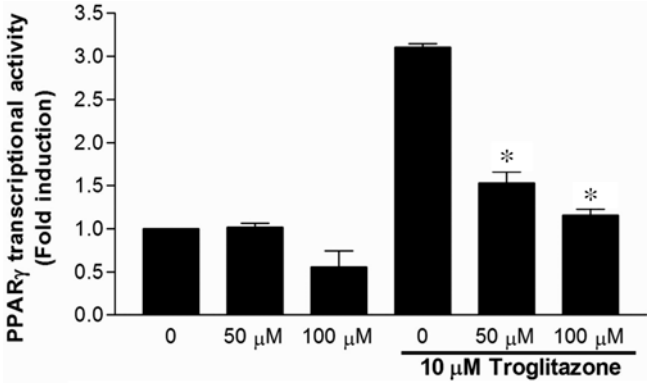

Fig. 3 Effects of galanolactone on PPARγ transcriptional activity in HEK 293T cells. After transfection, HEK 293T cells were treated with 50 and 100 μM of galanolactone in the absence or presence of troglitazone for 24 h. Luciferase assay was performed using Dual-Luciferase Reporter Assay System kit. Data are expressed as means ± SD, where $p < 0.05$ was considered statistically significant.

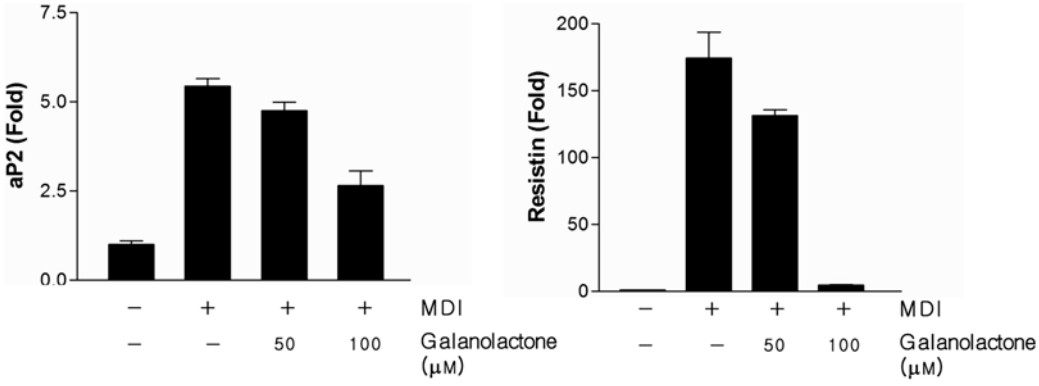

Fig. 4 Effects of galanolactone on expression of aP2 and resistin in 3T3-L1 cells. Post-confluent 3T3-L1 cells were differentiated in the absence or presence of galanolactone for 8 days. aP2 and resistin mRNA expression were evaluated by the quantitative real-time PCR.

the PPARγ transcriptional activity.

Effects of galanolactone on expression of aP2 and resistin in 3T3-L1 cells. Effects of galanolactone on the expression of adipogenesis relative genes, such as aP2 and resistin, in 3T3-L1 cells were examined. aP2, one of the adipocyte-specific genes, has binding sites for each of the two major adipogenic transcription factors, PPARγ and CEBPα. PPARγ plays a critical role in the adipocytes differentiation process and in the maintenance of the fully differentiated adipocyte by enhancing the expression of mature adipocyte marker genes such as aP2 (Gregoire et al., 1998; Lowell, 1999). Resistin, an adipocyte-secreted molecule, is known to be a critical link between obesity and insulin resistance, and plays a role in the regulation of glucose homeostasis and hepatic glucose production (Steppan et al., 2001; Rajala et al., 2003). Fully differentiated cells (day 8) were treated with 50 and 100 μM of galanolactone followed by extraction of total RNA for analysis using quantitative real-time PCR. Treatment with galanolactone decreased the expression of aP2 and resistin in a dose-dependent manner. In particular, the resistin mRNA level was reduced up to 98% by treatment with 100 μM of galanolactone (Fig. 4).

In conclusion, results of the present study support the findings that galanolactone inhibited adipocyte differentiation through down-regulation of adipogenic transcription factors, including peroxisome PPARγ and C/EBPα and reduced the expression of aP2 and resistin.

Acknowledgment This work was supported by a grant from the Next-Generation BioGreen 21 Program (No. PJ0081552011), Rural Development Administration, Republic of Korea

References

Afzal M, Al HD, Menon M, Pesek M, and Dharmi M (2001) Ginger an ethnomedical, chemical and pharmacological review. *Drug Metab Drug Interact* **18**, 159–190.

Bhandari U, Sharma JN, and Zafar R (1998) The protective action of ethanolic ginger extract in cholesterol-fed rabbits. *J Ethnopharmacol* **61**, 167–171.

Burn RP, Kim JB, Hu E, Altiok S, and Spiegelman BM (1996) Adipocyte differentiation a transcriptional regulatory cascade. *Curr Opin Cell Biol* **8**, 826–832.

Chen BH, Wu PY, Chen KM, Fu TF, Wang HM, and Chen CY (2009) Antiallergic Potential on RBL-2H3 Cells of Some Phenolic Constituents of Zingiber officinale. *J Nat Prod* **72**, 950–953.

Chrubasik S, Pittler MH, and Roufogalis BD (2005) Zingiberis rhizoma a comprehensive review on the ginger effect and efficacy profiles. *Phytomedicine* **12**, 684–701.

Fuhrman B, Rosenblat M, Hayek T, Coleman R, and Aviram M (2000) Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation and attenuates development of atherosclerosis in atherosclerotic, apolipoprotein E-deficient mice. *J Nutr* **130**, 1124–1131.

Gregoire FM, Smas CM, and Sul HS (1998) Understanding adipocyte differentiation. *Physiol Rev* **78**, 789–809.

Han L, Gong X, Kawano S, Saito M, Kimura Y, and Okuda H (2005) Antiobesity actions of Zingiber officinale Roscoe. *Yakugaku Zasshi* **125**, 213–217.

Hu E, Kim J, Sarraf P, and Spiegelman B (1996) Inhibition of adipogenesis through MAP Kinase-mediated phosphorylation of PPAR gamma. *Science* **274**, 2100–2103.

Hwang JT, Lee MS, Kim HJ, Sung MJ, Kim HY, Kim MS, and Kwon DY (2009) Antiobesity effect of ginsenoside Rg3 involves the AMPK and PPAR-gamma signal pathways. *Phytother Res* **23**, 262–266.

Jeon T, Hwang SG, Hirai S, Matsui T, Yano H, Kawada T, Lim BU, and Ki D (2004) Red yeast rice extracts suppress adipogenesis by down-regulating adipogenic transcription factors and gene expression in 3T3-L1 cells. *Life Sci* **75**, 3195–3203.

Kikuzaki H, Tsai SM, and Nakatani N (1992) Gingerdiol related compounds from the rhizomes of Zingiber officinale. *Phytochemistry* **31**, 1783–1786.

Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K, Satoh S, Nakano R, Ishii C, Sugiyama T, Eto K, Tsubamoto Y, Okuno A, Murakami K, Sekihara H, Hasegawa G, Naito M, Toyoshima Y, Tanaka S, Shiota K, Kitamura T, Fujita T, Ezaki O, Aizawa S, Nagai R, Tobe K, Kimura S, and Kadowaki T (1999) PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol cell* **4**, 597–609.

Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, and Kliewer SA (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). *J Biol Chem* **270**, 12953–12956.

Lowell BB (1999) PPARγ: An essential regulator of adipogenesis and modulator of fat cell function. *Cell* **99**, 239–242.

Mandrup S and Lane MD (1997) Regulating Adipogenesis. *J Biol Chem* **272**, 5367–5370.

Morrison RF and Farmer SR (2000) Hormonal Signaling and Transcriptional Control of Adipocyte Differentiation. *J Nutr* **130**, 3116–3121.

Rajala MW, Obici S, Scherer PE, and Rossetti L (2003) Adipose-derived resistin and gut-derived resistin-like molecule-beta selectively impair insulin action on glucose production. *J Clin Invest* **111**, 225–230.

Rosen ED, Walkey CJ, Puigserver P, and Spiegelman BM (2000) Transcriptional regulation of adipogenesis. *Genes & Dev* **14**, 1293–1307.

Sang S, Hong J, Wu H, Liu J, Yang CS, Pan MH, Badmaev V, and Ho CT (2009) Increased growth inhibitory effects on human cancer cells and anti-inflammatory potency of shogaols from Zingiber officinale relative to gingerols. *J Agric Food Chem* **22**, 10645–10650.

Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, and Lazar MA (2001) The hormone resistin links obesity to diabetes. *Nature* **409**, 307–312.

Tontonoz P, Hu E, Devine J, Beale EG, and Spiegelman BM (1995) PPARγ2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. *Mol Cell Biol* **15**, 351–357.

Tontonoz P, Hu E, Graves RA, Budavari AI, and Spiegelman BM (1994) mPPAR72 tissue-specific regulator of an adipocyte enhancer. *Genes & Dev* **8**, 1224–1234.