1 β -Hydroxy-2-oxopomolic Acid Isolated from *Agrimonia pilosa* Extract Inhibits Adipogenesis in 3T3-L1 Cells

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In order to determine anti-adipogenic effect, this study investigated 1 β -hydroxy-2-oxopomolic acid (HOA) isolated from *Agrimonia pilosa* inhibits adipocyte differentiation and expression of adipogenic marker genes, such as peroxisome proliferator activated receptor γ (PPAR γ), CCAAT-enhancer-binding protein α (C/EBP α), glucose transporter 4 (GLUT4), adiponectin, adipocyte fatty acid-binding protein 2 (aP2), adipocyte determination and differentiation factor 1/sterol regulatory element binding protein 1c (ADD1/SREBP1c), resistin, and fatty acid synthase (Fas) in 3T3-L1 preadipocyte. We demonstrated that HOA induced a significant decrease in lipid accumulation and expression of adipogenic marker genes in a dose-dependent manner. In addition, HOA reduced the transcriptional activity of PPAR γ induced by troglitazone, a potent diabetes agent; it also suppressed expression of PPAR γ and C/EBP α protein levels. Our data suggest that HOA isolated from *Agrimonia pilosa* inhibits adipocyte differentiation through downregulation of various adipocytokines by blocking PPAR γ and C/EBP α expression.

Key words 1 β -hydroxy-2-oxopomolic acid; adipogenesis; adipogenic marker gene; 3T3-L1 cell line

Obesity is associated with other metabolic disorders, including type II diabetes, hypertension, and cardiovascular disease and has become a major problem worldwide. Adipose tissue has critical roles in energy homeostasis and lipid metabolism.^{1,2)} Adipocyte differentiation is known to lead to induction of adipogenesis. Peroxisome proliferator activated receptor γ (PPAR γ) and CCAAT-enhancer-binding protein α (C/EBP α) are important transcription factor in adipogenesis.³⁾ PPAR γ , one of the nuclear hormone receptors, has been defined as a component of a differentiation-dependent regulatory factor, adipocyte regulatory factor 6 (ARF6), fat cell specific enhancer of the adipocyte fatty acid-binding protein 2 gene (aP2).^{4,5)} It plays a major role in regulation of adipogenesis, as supported by the fact that it is expressed in adipocytes, where it stimulates lipoprotein lipase and promotes fatty acid uptake and storage in mature adipocytes. C/EBP α , one of a large family of leucine zipper transcription factors, plays an important role in induction of terminal adipocyte differentiation.^{6,7)} During adipocyte differentiation, PPAR γ and C/EBP α regulate expression of genes involved in making and maintaining adipocytes, including various adipogenic genes, such as aP2, adiponectin, adipocyte determination and differentiation factor 1/sterol regulatory element binding protein (ADD1/SREBP1c), fatty acid synthase (Fas), glucose transporter 4 (GLUT4), and resistin.^{8,9)} Adipose cells and tissues secrete a variety of adipokines and secreted cytokines may play a role in activation of various inflammatory responses that are considered to be important mediators of cardiovascular disease and Type 2 diabetes.^{3,10)}

Agrimonia pilosa LEDEB. (Rosaceae) is a perennial herbaceous flowering plant, which is distributed primarily in temperate regions of the Northern Hemisphere.¹¹⁾ The aerial parts of *Agrimonia pilosa* have been used as a traditional medicine for treatment of abdominal pain, sore throat, headaches, and heat stroke.¹²⁾ Several triterpenoids and phenolic constituents isolated from this plant have been found to have anticancer, antioxidant, anti-inflammatory, and hepatoprotec-

tive activities.^{13–15)} However, cellular molecular mechanisms of *Agrimonia pilosa* which might be responsible for differentiation and regulation of gene expression in adipocytes, have not been reported.

In this study, we gained compound 1 isolated from *Agrimonia pilosa* and it identified as 1 β -hydroxy-2-oxopomolic acid (HOA). We investigated the effect of HOA isolated from *Agrimonia pilosa* on adipocyte differentiation in 3T3-L1 cells. Our results showed that HOA had potent anti-adipogenesis activity and that treatment with HOA resulted in decreased levels of PPAR γ , C/EBP α , GLUT4, adiponectin, aP2, ADD1/SREBP1c, resistin, and Fas in 3T3-L1 cells. These findings suggest that HOA can be developed as an effective anti-obesity agent by inhibition of adipocyte differentiation modulated through downregulated expression of adipogenic transcription factors and adipocyte-specific genes.

MATERIALS AND METHODS

Preparation of Natural Product Extracts The dry material of *Agrimonia pilosa* L. was purchased from the Kyungdong Oriental Herbal Market in Korea (August 2007) and identified by one of the authors (Prof. Joa Sub Oh). A voucher specimen (GPRC-196) of this plant has been deposited at the Gyeonggi Pharmaceutical Research Center, Suwon, Korea. The dry material of *Agrimonia pilosa* L. (2 kg) was pulverized and extracted with EtOH (3 $\times$ 18 L) at room temperature (24 h).

Extraction and Isolation of 1 β -Hydroxy-2-oxopomolic Acid The extract was filtered and concentrated, *in vacuo*, and suitably diluted with water, then successively partitioned with *n*-hexane (3 $\times$ 1 L) and CH₂Cl₂ (3 $\times$ 1 L). Column chromatography of the CH₂Cl₂-soluble layer (10 g) over silica gel using a CH₂Cl₂-MeOH mixture with increasing polarity yielded eight fractions (G21C-9-1–8). Fraction G21C-9-2 (1.8 g) was further applied to a silica gel column chromatography, eluting with CH₂Cl₂-acetone (1:0, 50:1, 20:1, 10:1, 5:1, acetone),

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Fig. 1. Effect of HOA on Lipid Accumulation in 3T3-L1 Cells

(A) Structure of 1 β -hydroxy-2-oxopomolic acid (HOA) isolated from *Agrimonia pilosa*. (B) 3T3-L1 cells were treated with HOA at various concentrations (6.25–100 μ M) for 24 h. Cell viability was determined by the MTT assay. Post-confluent 3T3-L1 cells were differentiated in the absence or in the presence of HOA for 8 d. (C) Triglyceride content was quantified by measuring absorbance. Data are expressed as means $\pm$ S.D. where $p < 0.05$ was considered statistically significant. (D) Lipid droplets were measured by Oil Red O staining.

affording six fractions (G21C-11-1–6). Among the above six fractions, 1 β -hydroxy-2-oxopomolic acid (46 mg) was isolated from fraction G21C-11-4 by flash column chromatography on RP-18 using MeOH–H₂O (30:70 to 80:20). ¹H- and ¹³C-NMR spectrum was recorded on a Varian 500 NMR spectrometer using pyridine-*d*₅ as a solvent. Mass spectrum was obtained on a Waters Q-time-of-flight (TOF) micro mass spectrometer.

1 β -Hydroxy-2-oxopomolic Acid White amorphous powder; ¹H-NMR (pyridine-*d*₅, 500 MHz) δ : 5.71 (1H, t-like, H-12), 4.38 (1H, s, H-1), 4.34 (1H, s, H-3), 3.17 (1H, m, H-16a), 3.09 (1H, s, H-18), 1.82 (3H, s, H-27), 1.46 (3H, s, H-29), 1.36 (3H, s, H-23), 1.15 (3H, s, H-26), 1.14 (3H, d, $J = 7.0$ Hz, H-30), 0.97 (3H, s, H-25), 0.91 (3H, s, H-24); ¹³C-NMR (pyridine-*d*₅, 125 MHz) δ : 212.3 (C-2), 180.8 (C-28), 139.0 (C-13), 129.1 (C-12), 85.4 (C-1), 82.0 (C-3), 72.8 (C-19), 54.6 (C-18), 52.0 (C-5), 49.6 (C-10), 48.7 (C-9), 48.4 (C-17), 45.9 (C-4), 42.3 (C-20), 42.3 (C-14), 41.3 (C-8), 38.6 (C-22), 33.5 (C-7), 29.5 (C-15), 29.4 (C-23), 27.4 (C-11), 27.2 (C-29), 27.1 (C-21), 26.5 (C-16), 24.8 (C-27), 18.8 (C-6), 17.2 (C-24, 26), 16.9 (C-30), 12.6 (C-25); electrospray ionization (ESI)-MS m/z 501 [M–H][–]. The structure of 1 β -hydroxy-2-oxopomolic acid is presented in Fig. 1.

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's medium (DMEM) with 10% Bovine Calf Serum and 100 U/mL penicillin and 0.1 mg/mL streptomycin (Invitrogen, Carlsbad, CA, U.S.A.) in a humidified atmosphere of 95% air with 5% CO₂ at 37°C. One day 0,

the cells were induced with initiation media (10 μ g/mL insulin, 1 μ M dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine in 10% fetal bovine serum (FBS)-DMEM). On day 2, the initiation media was replaced with progression media (10 μ g/mL insulin in 10% FBS-DMEM). On day 4 and 6, the progression media was replaced with maintenance media (10% FBS-DMEM). From days 0 to 6, the cells were treated with various concentrations of HOA.

Viability Assay The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was used for *in vitro* determination of cell proliferation in 3T3-L1 cells. Cells were plated at 5×10^3 per well in 100 μ L culture medium. One day after plating, a time zero control plate was made. Compounds were applied directly, and the cells were incubated for a further 24 h in a humidified 5% CO₂ atmosphere at 37°C; cell proliferation was then determined. MTT (5 mg/mL in phosphate buffered saline (PBS)) was added to each well, followed by incubation for 3 h. The media was removed from the wells by aspiration; 0.1 mL of buffered dimethyl sulfoxide (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 At days 8 after induction of differentiation, the cells were stained with Oil Red O (Sigma, St. Louis, MO, U.S.A.). Cells were washed twice with PBS and fixed with 3.7% formaldehyde in phosphate-buffered saline for 30 min, and then washed twice with water. Cells were stained with 0.35% Oil Red O dye in isopropyl alcohol for 30 min. Excess of stain was removed by washing with 70% ethanol and water. Stained lipid droplets were dissolved in isopropyl

Table 1. The Primer Sequence Used for Real-Time PCR

Target	Primer sequences	Accession No.
GAPDH	5'-GAGTCAACGGATTGGTTCGT-3' (sense) 5'-GACAAGCTTCCCGTTCTCAG-3' (antisense)	BC083080
PPAR γ	5'-CGCTGATGCACTGCCTATGA-3' (sense) 5'-AGAGGTCCACAGAGCTGATTCC-3' (antisense)	NM_011146
C/EBP α	5'-AGGTGCTGGAGTTGACCAGT-3' (sense) 5'-CAGCCTAGAGATCCAGCGAC-3' (antisense)	BC058161
GLUT4	5'-CTTCTTTGAGATTGGCCCTGG-3' (sense) 5'-AGGTGAAGATGAAGAAGCCAAGC-3' (antisense)	NM_009204
Adiponectin	5'-AGCCTGGAGAAGCCGCTTAT-3' (sense) 5'-TTGCAGTAG A ACTTGCCAGTGC-3' (antisense)	NM_009605
aP2	5'-CATGGCCAAGCCCAACAT-3' (sense) 5'-CGCCAGTTTGAAGGAAATC-3' (antisense)	NM_024406
ADD1/SREBP1c	5'-CAAACCTGCCCATCCACCGAC-3' (sense) 5'-TGCTCTCTCCACTGCCACAA-3' (antisense)	NM_011480
Resistin	5'-TCAACTCCCTGTTTCCAAATGC-3' (sense) 5'-TCTTCACGAATGTCCACGA-3' (antisense)	NM_022984
Fas	5'-CTGAGATCCCAGCACTTCTTGA-3' (sense) 5'-GCCTCCGAAGCCAAATGAG-3' (antisense)	NM_007988

alcohol containing 4% Nonidet P-40 and quantified by ELISA at 510nm.

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

Quantitative Real-Time Polymerase Chain Reaction (PCR) Total RNA was extracted using total RNA extraction kit (Quiagen, Hilden, Germany). One microgram of RNA was used as a template for each reverse transcription (RT)-PCR using SuperScriptTM III One-Step RT-PCR System (Invitrogen, Carlsbad, CA, U.S.A.). Newly-synthesized cDNA from 3T3-L1 control cells and HOA 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 then collected by centrifugation at 13000rpm for 1 min at 4°C. To obtain the cellular lysate, the cells were lysed on ice for 30min in RIPA buffer (50mM Tris-HCl, pH 7.5, 0.15M NaCl, 1% NP-40, 0.1% sodium dodecyl sulfate (SDS), 1mM dithiothreitol (DTT), and 1mM phenylmethylsulfonyl fluoride (PMSF)), which contained a mixture of protease inhibitors (Roche, Mannheim, Germany). Insoluble materials were removed by centrifugation at 13000rpm for 10min at 4°C. A total of 50 μ g of the supernatants were separated using a 10% polyacrylamide gel containing 10% SDS, 1.5M Tris-HCl, 0.035% *N,N,N,N*-tetra-methylenediamine, and 7mg ammonium persulfate. The separated proteins were

transferred to a nitrocellulose membrane (Whatman, Dassel, Germany) at 36mA in a transfer buffer containing 39mM glycine, 48mM of Tris base, 0.037% SDS, and 20% methanol. The membranes were sequentially incubated with anti-PPAR γ , anti-CEBP α , and rabbit polyclonal immunoglobulin (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) at 1:1000 dilutions, respectively, and anti-actin, mouse monoclonal immunoglobulin (Sigma, St. Louis, MO, U.S.A.) at 1:2000 dilutions. Horseradish peroxidase-conjugated anti-rabbit and anti-mouse immunoglobulin (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) were used as secondary antibodies at a dilution of 1:1500, respectively. All detections were performed using an electrochemiluminescence detection reagent (Amersham Biosciences, Uppsala, Sweden).

Statistics Analysis Data are expressed as the mean $\pm$ S.D. Experimental results were analyzed for statistical significance (Student's *t*-test and one way analysis of variance (ANOVA)). Values of $p < 0.05$ were considered statistically significant (* $p < 0.05$).

RESULTS

Effects of HOA on Cytotoxicity and Adipogenesis

Inhibition in 3T3-L1 Cells The chemical structure of HOA is shown in (Fig. 1A). To determine the cytotoxicity of HOA, 3T3-L1 cells were treated with various concentrations (6.25–100 μ M) of HOA and cell viability was measured by the MTT assay. Results showed that HOA did not cause cytotoxicity at a concentration of 6.25–100 μ M in 3T3-L1 cells (Fig. 1B). We next measured the adipogenic effect of HOA on adipocyte differentiation. We used a differentiation mixture (insulin, dexamethasone, 3-isobutyl-1-methylxanthine) to induce differentiation of 3T3-L1 cells. 3T3-L1 cells were treated with 25, 50, and 100 μ M of HOA during differentiation, and after 8d, were stained with Oil Red O. As shown in Figs. 1C and D, HOA induced a dose-dependent decrease of triglyceride accumulation. We observed that cells treated with 100 μ M of HOA showed a significant reduction of lipid accumulation through inhibited

differentiation of 3T3-L1 preadipocytes.

Effects of HOA on PPAR γ Transcription Activity and Gene Expression of PPAR γ and CEBP α To identify the inhibitory mechanism of HOA in 3T3-L1 cells, gene expression of PPAR γ and CEBP α , important transcription factors in regulation of adipogenesis, were evaluated by the quantitative

real-time PCR method after treatment of fully-differentiated (day 8) cells with 50 μ M and 100 μ M of HOA. As shown in Fig. 2A, HOA treatment (100 μ M) resulted in effectively suppressed expression of PPAR γ and CEBP α mRNA levels. Using a reporter assay system, we also performed an analysis of PPAR γ transcriptional activity after HOA treatment. HEK 293T cells were transfected with the pGL3-PPRE-Luc and PPAR γ plasmids after treatment with HOA or troglitazone, an agent for enhancement of adipocyte differentiation. As shown in Fig. 2B, HOA treatment (100 μ M) inhibited not only PPAR γ transcriptional activity but also troglitazone-induced PPAR γ transcriptional activity. These findings indicated that HOA inhibits both PPAR γ transcriptional activity and mRNA level. To find out whether HOA has an inhibitory effect on the protein expression of PPAR γ and CEBP α in 3T3-L1 cells, were evaluated by Western blot analysis after treatment of fully-differentiated (day 8) cells with HOA or troglitazone. As shown in Fig. 3A, HOA treatment (100 μ M) significantly suppressed expression of PPAR γ and CEBP α protein levels. Furthermore, we showed that HOA treatment (100 μ M) significantly inhibited troglitazone-stimulated PPAR γ and CEBP α protein levels (Fig. 3B). These results indicated that HOA reduced the expression of CEBP α directly *via* inhibition of PPAR γ activity.

Effect of HOA on Expression of Adipogenic Genes To examine the effects of HOA on expression of adipogenesis related genes in adipocytes (3T3-L1), we measured expression of aP2, adiponectin, ADD1/SREBP1c, Fas, GLUT4, and resistin at the mRNA level according to HOA treatment by dose and time, respectively. Post-differentiated (day 4) and Fully-differentiated (day 8) cells were treated with 50 μ M and 100 μ M of HOA, followed by extraction of total RNA for analysis using quantitative real-time PCR. On day 4, we found that cells treated with 50 μ M and 100 μ M of HOA effectively reduced mRNA expression of adiponectin, ADD1/SREBP1c and

Fig. 2. Effects of HOA on PPAR γ Transcription Activity and Gene Expression of PPAR γ and CEBP α

(A) Post-confluent 3T3-L1 cells were differentiated in the absence or in the presence of HOA for 8 d. PPAR γ and CEBP α mRNA expression were evaluated by the quantitative real-time PCR. (B) After transfection, HEK 293T cells were treated with 50 μ M and 100 μ M of HOA in the absence or presence of troglitazone for 24 h. Luciferase assay was performed by Dual-Luciferase Reporter Assay System kit. Data are expressed as means $\pm$ S.D. where $p < 0.05$ was considered statistically significant.

Fig. 3. Effects of HOA on Expression of PPAR γ and CEBP α at Protein Level in 3T3-L1 Cells

Post-confluent 3T3-L1 cells were differentiated in the absence or in the presence of HOA for 8 d. PPAR γ and CEBP α protein expression were analyzed by Western blotting. (A) Cells were treated with 50 μ M and 100 μ M of HOA. (B) Cells were treated with 100 μ M of HOA with or without troglitazone (10 μ M). Data are expressed as means $\pm$ S.D. where $p < 0.05$ was considered statistically significant.

Fig. 4. Effects of HOA on Expression of Adipogenic Factors in 3T3-L1 Cells

Post-confluent 3T3-L1 cells were differentiated in the absence or in the presence of HOA for 4 and 8 d. GLUT4, adiponectin, resistin, aP2, Fas, and ADD1/SREBP1c mRNA levels on day 4 (A) and on day 8 (B) of differentiation were evaluated by the quantitative real-time PCR.

resistin. And mRNA expression of aP2 and Fas were inhibited by HOA treatment (100 μ M). But GLUT4 mRNA expression was increased by HOA treatment (50, 100 μ M) after 4 d (Fig. 4A) and it was markedly decreased by HOA treatment (50, 100 μ M) after 8 d (Fig. 4B). On day 8, we also observed that cells treated with 50 μ M and 100 μ M of HOA effectively inhibited mRNA expression of ADD1/SREBP1c. And treatment with 100 μ M of HOA resulted in significantly reduced expression of various adipogenic genes, including aP2, adiponectin, Fas and resistin (Fig. 4B). These results suggested that HOA effectively inhibited adipocyte differentiation through down-regulation of adipogenic genes.

DISCUSSION

Obesity is caused by accumulation of lipid through adipogenesis in adipose tissue. Adipogenesis is the process by which undifferentiated precursor cells differentiate into fat cells; inhibition of adipocyte differentiation is one of the strategies for treatment of obesity. Previous studies have reported that natural products and compounds isolated from natural products have inhibitory effects on adipocyte differentiation in 3T3-L1 preadipocytes. Tea catechin and flavonoids suppress adipocyte differentiation by downregulation of PPAR γ and CEBP α and berberine inhibits 3T3-L1 adipocyte differentiation through the PPAR γ pathway in 3T3-L1 cells.¹⁶⁻¹⁸⁾

Agrimonia pilosa L. has been used in traditional Chinese

Medicine and is known to have biological effects, including anti-viral, anti-inflammatory, and anti-oxidant.^{14,19,20} In our preliminary study, we demonstrated that treatment with 6.25–500 µg/mL of ethanolic extract of *Agrimonia pilosa* L. did not cause significant cytotoxic effects in 3T3-L1 cells (data not shown). HOA, a type of triterpenoid, is a compound isolated from *Agrimonia pilosa* L. It was isolated from the CHCl₃-soluble fraction of *R. woodsii* for the first time and the molecular structure is shown in Fig. 1A.²¹ However, few previous studies have reported any bio-ability effects by HOA.

In this study, we demonstrate that HOA can inhibit adipocyte differentiation through suppression related transcription factors and adipogenic marker genes. We showed that HOA treatment resulted in decreased hormone mixture MDI induced fat accumulation without cytotoxicity and reduced expression of PPAR γ and CEBP α at both the mRNA and protein levels in 3T3-L1 cells (Figs. 2A, 3A). We also confirmed that HOA treatment resulted in downregulation of both troglitazone-stimulated PPAR γ transcriptional activity and troglitazone-stimulated PPAR γ protein level (Figs. 2B, 3B). These findings demonstrate that HOA suppresses gene expression of PPAR γ through the inhibition of PPAR γ transcriptional activity. In addition, these findings may be explained the direct effect of HOA on inhibition of PPAR γ gene expression.

PPAR γ and CEBP α are critical transcription factors in adipogenesis and play a role in the early stage of adipocyte differentiation.²² Rosen *et al.*³ reported that CEBP α can support adipocyte-specific gene expression in the presence of PPAR γ , at the level of cell morphology and lipid accumulation. This result indicated that PPAR γ and CEBP α participate in pathways of fat cell development, with PPAR γ being the key effector of adipogenesis.

In the present study, we also observed that 3T3-L1 cells treated with HOA showed decreased mRNA expression of several adipogenesis related genes, including aP2, adiponectin, ADD1/SREBP1c, Fas and resistin. On the other hand, post-differentiated (day 4) cells with 50 µM and 100 µM of HOA increased mRNA expression of GLUT4 and fully-differentiated (day 8) cells with 50 µM and 100 µM of HOA decreased mRNA expression of GLUT4 (Figs. 4A,B). These observations may be explained from the effect that GLUT4 is closely associated with insulin. GLUT4 is predominantly expressed in adipocytes and striated muscle and undergoes a dramatic accumulation at the cell surface membrane in response to insulin stimulation.²³ Overexpression of GLUT4 in adipose tissue has been reported to overcome impaired insulin-stimulated glucose uptake in muscle, even in the setting of increased adiposity.²⁴

Adipogenesis genes up-regulated in liver include PPAR γ target genes, aP2 and adiponectin.²⁵ aP2, a member of the cytoplasmic fatty acid binding protein family, was detected in adipose tissue and its expression is highly regulated during differentiation of adipocytes.²⁶ In induction of aP2 expression, PPAR γ activates the promoters of many other fat-cell specific genes.²² Adiponectin is an adipocyte-derived hormone that plays a role in insulin sensitivity and energy homeostasis.²⁷ Previous studies have demonstrated that PPAR γ agonists can induce an increase in adiponectin levels in 3T3-L1 adipocytes, and this effect is associated with adipocyte differentiation via PPAR response element.^{28,29} ADD1/SREBP1c, the most abundant form of SREBPs in liver and adipose tissue, plays a role in adipogenesis.⁸ Overexpression of ADD1/SREBP1c

in the presence of differentiation hormonal inducers resulted in elevated adipocyte marker expression and lipid accumulation in 3T3-L1 cells. ADD1/SREBP1c also controls the genes involved in fatty acid synthesis and overexpression of this factor in adipocyte cell lines stimulates expression of Fas.³⁰ Fas is a major enzyme required for anabolic conversion of dietary carbohydrates to fatty acids, and it functions normally in cells with lipid metabolism.³¹ Resistin, an adipocyte-secreted molecule, is known to be a critical link between obesity and insulin resistance and plays a role in regulation of glucose homeostasis and hepatic glucose production.^{32,33} Banerjee *et al.*³⁴ demonstrated the importance of resistin in regulation of fast blood glucose metabolism in a mouse model and for use in the study of obesity, insulin resistance, and type2 diabetes.

In conclusion, the present study provides direct evidence that the inhibitory effect of HOA on adipocyte differentiation could be mediated through down-regulation of adipogenic transcription factors, including peroxisome proliferator-activated receptor-gamma and CCAAT/enhancer binding protein-alpha, which are related to expression of adipogenic-specific genes. These findings also suggest that HOA has a beneficial effect on prevention of adipogenesis associated obesity.

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