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Ginseng Saponin-Re and *Coix lachrymajobi var. mayuen* Regulate Obesity Related Genes Expressions, TNF-alpha, Leptin, Lipoprotein Lipase and Resistin in 3T3-L1 Adipocytes

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In order to determine if the mRNA and protein expression levels of 3T3-L1 adipocytes are influenced by oriental medicines, adipocytes were treated with 100 μg/ml of G-Re and aqueous extract of a *Coix lachrymajobi* var. *mayuen* (AEC) every other day for 12 days, respectively. The tumor necrosis factor alpha (TNF-α). mRNA and protein expressions were suppressed markedly in treated mature adipocytes. Those of lipoprotein lipase (LPL) levels were found to increase gradually in preadipocytes differentiating into mature adipocytes. Those were higher than that of the untreated mature adipocytes. The treated adipocytes showed reduction of leptin expression levels, while in untreated mature adipocytes cell, those of levels were significantly higher after the conversion of preadipocytes into mature adipocytes. The resistin levels in the treated adipocytes were significantly decreased comparing to that of the untreated mature adipocytes. In conclusion, the expression levels of LPL, TNF-α, leptin and resistin mRNA and proteins are shown to be regulated by G-Re and AEC, making them potential candidates for controlling fat mass related obesity.

Key words : 3T3-L1 adipocyte, TNF-α, lipoprotein lipase, leptin, resistin

Introduction

Recently, obesity rates have increased noticeably in many Asian countries as well as Korea, particularly during the last 10-15 years, and have become a major public health issue in most countries [5]. Obesity is one of the main risk factors for chronic diseases. Being overweight carries an increased risk of health problems such as cardiovascular disease, insulin resistance and type II diabetes mellitus, hyperlipoproteinaemia, hypertension, gallbladder disease, cancer of the endometrium and breast cancer, arthrosis and psychological stress in postmenopausal women. Many chronic diseases are mostly related to an increased intra-abdominal fat mass [7,25,29,43]. Adipose tissues reserve not only energy but also secrete a variety of factors such as leptin, IL-6, TNF-α, resistin, LPL and fatty acid [14,17]. These factors are believed to be involved in regulating adipose mass and obesity. One of the molecules produced in adipose tissue is the TNF-α which has been shown to regulate lipid metabolism of adipose biology and also TNF-α level is elevated in cases of obesity and decrease the expression levels of adiponetin involved in lipogenesis [14]. It should be noted that leptin secretion from the adipose tissue is closely influenced by the secretion of TNF-α [40]. A 167-amino acid protein, leptin is involved in adipose signaling, which decreases the appetite and reduces the body weight through its actions on the hypothalamus and neuropeptiedes [12]. LPL plays a key role in the plasma lipoprotein metabolism, which is involved in the clearance of triacylglycerols from circulation and regulates the energy metabolism and mainly found in the adipose tissue, cardiac muscle and skeletal muscle [8,30]. In addition, TNF-α regulates the inhibition of LPL gene transcription. Resistin, a 94-amino acid polypeptide that contains 11 Cys residues, was recently identified as a hormone secreted by adipocytes [4]. Although some controversial discussion has been continued, the level of resistin secretion is known to be increased by obesity and upregulated significantly during the differentiation of preadipocytes to mature adipocytes [38].

Many studies have examined the applications of using oriental medicine, particularly acupuncture, herbal acupuncture, oriental herbal medicines, and reported excellent results in treating [1,2,31]. The purpose of this study was to determine if the fat mass could be reduced by a treat-

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ment with the aqueous extracts of the oriental medicines, *C. lachrymajobi* var. *mayuen*. In order to support this hypothesis, ginsenoside-Re is used as control since its efficacy for hypolipidemic metabolism is widely accepted [16,24,27]. Traditionally, these medicines have been prescribed for metabolic disorders referred in traditional literature such as *Huang di nei jing, Ben cao gang mu* and *Dong yi bao jian* in both China and Korea [33]. Ginseng (*Panax ginseng C. A. Meyer*) has long been used as a tonic oriental folk medicine in traditional Chinese and Korean medicines. It has been believed for a long time that ginseng is beneficial to humans. The ginsenosides, which are saponins extracted from ginseng, are responsible for the various physiological and pharmacological activities of ginseng [11,44]. Many clinical and experimental reports have suggested that ginseng might have beneficial effects as an antiatherogenic agent, a suppresser of cholesterogenesis and lipogenesis. It also reduces elevated serum total cholesterol and low density lipoprotein cholesterol levels and acts as an antioxidant enhancer [23,27,32,41]. Adlay (C. *lachrymajobi* var. *mayuen*) is an annual crop that has long been used in traditional Chinese and Korean medicines and as a nourishing food including nutrients; protein 16.2%, lipid 4.65%, carbohydrate 79.17%, and a small quantity of vitamin B_1 330 mg [55]. The seed of adlay has been reported to exhibit anti-inflammatory, stomachic, diuretic and antispastic effects *in vivo* and has been used in Asian countries for treating warts, rheumatism, the female endocrine system and neuralgia. Recent studies demonstrated that adlay seeds could inhibit the allergic diseases and increase the antitumor and anticancer effects in experimental animals. Nagao *et al.* [42] isolated a number of benzoxazinones from the adlay seed, and reported anti-inflammatory activity. Park *et al.* [49] reported that the lipid components in plasma and feces were decreased in rats fed different fat diets containing adlay seed. Hidaka *et al.* [19] showed that the ingestion of adlay seed tablets could increase the activities of cytotoxic T-lymphocytes and natural killer cells. Check and K'Ombut [9] also reported decreased fibrinolytic activities of blood plasma of Wistar rats fed an adlay mixed diet. However, there are few reported of the antiobesity effects in 3T3-L1 adipocytes treated with AEC compared with that of G-Re.

In order to verify this hypothesis, *in vitro*, mouse 3T3-L1 adipocytes were treated with G-Re and AEC for 12 days. And then the levels of TNF-α, leptin, resistin, and LPL mRNA and protein expression were measured by reverse transcription-polymerase chain reaction and western blot analysis. As a result, our study suggests that these compounds can promote obesity marker gene expressions, which affects the regulation of body fat mass in relation to obesity.

Materials and Methods

Materials

Dulbecco's modified Eagle's medium (DMEM), Penicillin-streptomycin, Trizol and fetal bovine-serum (FBS) were obtained from GIBCO / BRL (Invitrogen, Carisbad, CA, USA). Insulin sodium salt, 3-isobutyl-1-methylxanthine, dexamethasone, and gencenoside-Re were purchased from the Sigma Co. (St. Louis, MO, USA). The 3T3-L1 cell line was obtained from the Korean Cell-line Bank, Cancer Research Institute, Seoul National University College of Medicine. The Oriental herbal medicine, *C. lachrymajobi* var. *mayuen* was purchased from the Kyungdong market in Seoul, Korea. Unless otherwise noted, all other chemicals and reagents were obtained from Sigma Co. (St. Louis, MO, USA).

Cell culture

3T3-L1 preadipocytes used passage 12 were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 1% penicillin-streptomycin and 10% FBS (Invitrogen, Carisbad, CA, USA). The cells were cultured in 6 well plates at 37°C in a humidified 5% CO_2 incubator and differentiated into adipocytes. The confluent cells were placed in a differentiation medium (DMEM containing 10% FBS, 10 μg/ml insulin (Sigma Co.), 0.5 mM 3-isobutyl-1-methylxanthine (Sigma Co.), and 1 μM dexamethasone (Sigma Co.)) for 3 days. The medium was then changed to DMEM containing 10% fetal bovine-serum, 10 μg/ml insulin, 100 μg/ml gensenoside-Re and AEC, respectively, every other day. After an additional 12 days, the cells were harvested with Trizol (Invitrogen) in order to isolate the RNA.

Extraction of herbal medicine

The oriental herbal medicine, *C. lachrymajobi* var. *mayuen*, were soaked in cold distilled water for 4hr and then extracted by boiling in a round glass flask. The contents were filtered through gauze. The filtrate was distilled, and coln-centrated in an evaporator (Buchi totavapor R-124, Flawil,

Switzerland). The extracted herbal medicine was dried by freeze drying (Telstar Lioalfa 6-80, Avon, Spain) and stored at -20°C until the experiment.

RNA isolation and reverse transcription-PCR

The total RNA was isolated from differentiated adipocytes at day 12 using a Trizol reagent (Invitrogen). 1 μg RNA was reverse-transcribed in a 20 μl reaction mixture using MMLV reverse transcriptase (Invitrogen). The cDNA was amplified in a 20 μl reaction mixture. The PCR conditions are as follows: 0.4 μM each primer, 0.2 mM deoxynucleoside triphosphate mixture (Perkin Elmer, Norwalk, CT, USA), 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, and 1.0 U of Taq DNA polymerase (Perkin Elmer). The reaction mixtures were incubated in a thermal controller (Model PTC-100; MJ Research, Ramsey, Minnesota, USA) for 30 cycles (denaturation at 94°C for 45 s, annealing at 53°C for 45 s, extension at 72°C for 90 s). The PCR products were resolved on 0.1% agarose gels containing ethidum bromide. The intensities of the bands were measured using an image documentation system (ImageMaster VDS; Pharmacia, Uppsala, Sweden) with an image analysis software (ImageMaste TotalLab; Pharmacia). The DNA size marker was run in parallel to validate the predicted sizes of the amplified bands (GeneRuler 1 kb DNA Ladder; MBI, Amherst, NY, USA). GADPH, leptin, lipoprotein lipase, and the resistin primer sequences were designed using the primer selection software offered through the Web site (Primer 3; Center for Genome Research, The whitehead Institute for Biomedical Research, Cambridge, MA, www.genome.wi.mit.edu). The primary sequences specific for the genes examined and the predicted product sizes are as follows.

Table 1. Primers and expected sizes of PCR products with each primer pair

Gene		Primer	Sizer (bp)
GAPDH[a]	sense	5′-atcccatcaccatcttccag-3′	576
	antisense	5′-acctgcttacaccaccttcttg-3′	
TNF-α[b]	sense	5′-ccagaactccaggcggtgtctgtg-3′	414
	antisense	5′-gtggtttgctacgacgtgggctac-3′	
Leptin	sense	5′-acatttcacacacgcagtcg-3′	318
	antisense	5′-ctcaaagccaccacctctg-3′	
LPL[c]	sense	5′-gtcgcctttctcctgatgac-3′	410
	antisense	5′-cttgctgcttctcttggctc-3′	
Resistin	sense	5′-cttcaactccctgtttccaa-3′	395
	antisense	5′-aagtatgtgtgcttgtgtgtgg-3′	

a : Glyceraldehydes-3-phosphate dehydrogenase, b : Tumor necrosis factor alpha,
c : Lipoprotein lipase

Western Blotting

Immunoblots were prepared to analyze the protein levels of TNF-α, LPL, leptin and resistin (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and β-actin (Sigma). After treatment with G-Re and AEC for the indicated amounts of time, the cells were harvested, and proteins were then extracted with protein lysis buffer and heated at 95℃ for 10 min. The protein concentrations were quantified using the BioRad protein assay (BioRad Lab., Hercules, CA, USA) by following the procedure described by the manufacturer. Aliquots containing 40 μg of total cell proteins were resolved on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and transferred onto PVDF membranes (Sigma Co., St. Louis, MO, USA). Membranes were blocked in 5% nonfat milk powder (w/v) in PBS containing 0.01% Tween-20 for 1 h at room temperature and then probed using the above-mentioned primary antibodies. After incubation overnight at 4℃, membranes were washed in PBST and incubated with the appropriate peroxidase-conjugated secondary antibody (Amersham Co., Arlington Heights, IL, USA). Specific complexes were revealed by chemiluminescence according to the enhanced chemiluminescence Western blotting detection reagent (Pierce, Rockford, IL, USA).

Results

Effects of the herbal medicines on the gene expression of TNF-α in 3T3-L1 mouse adipocytes

The study shows that G-Re and the AEC regulate mRNA and protein expression levels of TNF-α in 3T3-L1 adipocytes (Fig. 1A, B). During the normal maturation process from the preadipocytes to adipcytes, those expression levels of TNF-α gradually increased. As the preadipocytes matured to adipocytes, those expression levels of TNF-α were significantly higher. When the fully mature adipocytes were treated with either G-Re or AEC, their TNF-α expression levels were markedly lower than the untreated mature adipocytes. There were no significant differences between the adipocytes treated with G-Re and AEC which indicates that AEC influences the expression of TNF-α as much as the G-Re.

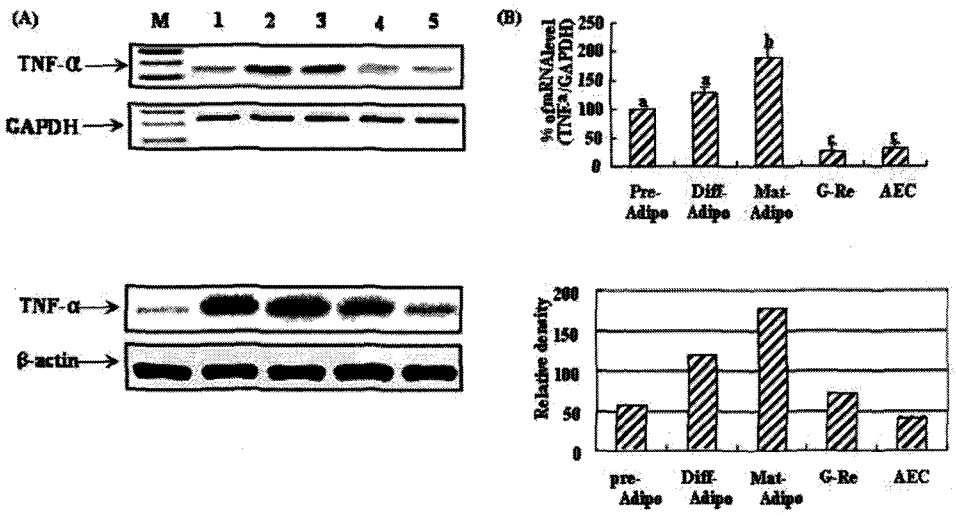

Fig. 1. Effects of G-Re and AEC on TNF-α mRNA and protein expression levels in 3T3-L1 adipocytes. (A), The cultures were treated with 100 μg/ml G-Re and AEC for 12 days. In upper part, the total RNA was extracted and analyzed by RT-PCR for TNF-α and GAPDH mRNA; the lower part, 40 μg proteins were loaded for protein expressions, M, molecular size standards; lane 1, untreated preadipocyte (Pre-Adipo); lane 2, untreated differentiation adipocyte (Diff-Adipo); lane 3, untreated control adipocyte (Mat-Adipo); lane 4, treated with 100 μg/ml G-Re; lane 5, treated with 100 μg/ml AEC; (B), The intensities of the amplified bands were determined from the gel shown in (A). The amounts of the target cDNA were then normalized against GAPDH cDNA in the corresponding sample and are shown in the upper graph. Those of protein bands were normalized to β-actin in corresponding samples in lower part. The values for the control were set to 100%. The data represents the mean ± s.d. of three independent experiments expressed as a percentage of the control value. The data were evaluated for statistic significance with a one-way ANOVA followed by Duncan's multiple range test. The means with the same letter were not significantly different. Therefore, means with a different letter, eg, 'a' or 'b' were statistically different. A p value<0.05 was considered significant.

Effects of the herbal medicines on the gene expressions of LPL in 3T3-L1 mouse adipocytes.

The mRNA and protein expressions of LPL, a typical adipocyte marker, were shown in Fig. 2A, B. The LPL expression levels were found to increase gradually while the preadipocytes differentiated into mature adipocytes. Treating the 3T3-L1 adipocytes with 100 μg/ml of G-Re resulted in an increase in the LPL expression levels compared with that of the mature adipocytes. Those of AEC were also showed a similar tendency to G-Re treatment adipocytes that is known to increase those expression levels of LPL. Consequently, treatments of the oriental herbal medicine, AEC, can improve the plasma triglyceride-rich lipoprotein metabolism by enhancing LPL expression in adipocytes. Therefore, it is expected that AEC might be used to control obesity induced by a high fat diet compared with G-Re known to play a significant role in lipolysis.

Effects of herbal medicines on gene expressions of leptin in 3T3-L1 mouse adipocytes

The leptin mRNA and protein expression levels were significantly higher after the conversion of the 3T3-L1 mouse preadipocytes to mature adipocytes (Fig. 3A, B). However, the leptin gene expression levels in the adipocytes treated with 100 μg/ml of either G-Re or AEC were markedly suppressed compared with that of that of Mat-adipo but there were not significantly differences in leptin expression levels between G-Re and AEC groups. Therefore, this result suggest that the changes in leptin gene expression with G-Re and AEC administrations may be involved in reduced fat cell size and / or number associated with triglyceride accumulation.

Effects of the herbal medicines on the gene expression of resistin in 3T3-L1 mouse adipocytes

Resistin expressions were detected in the differentiated adipocytes by RT-PCR and reversely, Western blot barely detected in the preadipocytes. This result is shown in Fig. 4A, B. Resistin mRNA in the fully mature adipocytes was 1.6 times higher than that of the differentiated adipocytes. In contrast, when the 3T3-L1 mature adipocytes were treated with 100 μg/ml G-Re and AEC, the resistin gene expression levels were significantly lower than that of

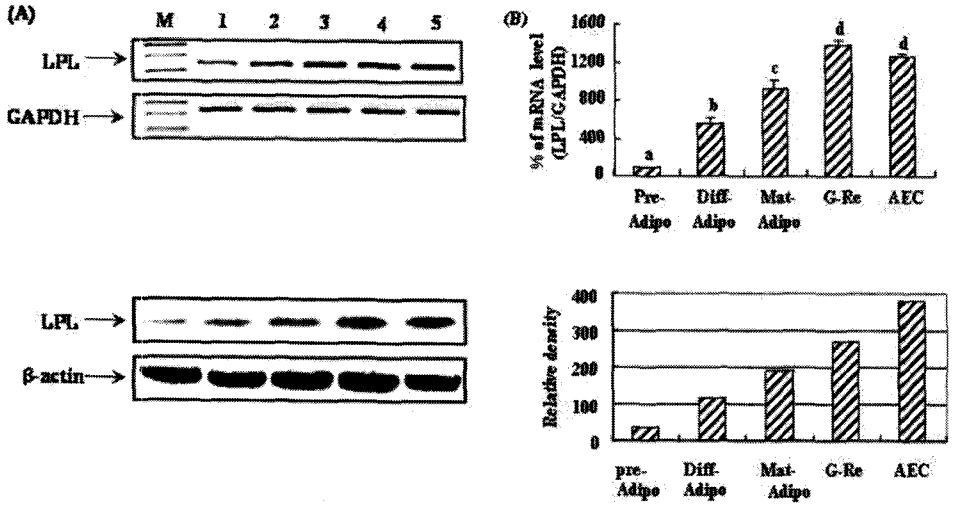

Fig. 2. Effects of G-Re and AEC on LPL mRNA and protein expressions levels in 3T3-L1 adipocytes. (A), The cultures were treated with 100 μg/ml G-Re and AEC for 12 days. Total RNA was extracted and analyzed for LPL and GAPDH mRNA expression in upper part and 40 μg proteins were loaded for Western blot in lower part, M, molecular size standards; lane 1, untreated preadipocyte (Pre-Adipo); lane 2, untreated differentiation adipocyte (Diff-Adipo); lane 3, untreated control adipocyte (Mat-Adipo); lane 4, treated with 100 μg/ml G-Re; lane 5, treated with 100 μg/ml AEC; (B), The intensities of the amplified bands were determined from the gel shown in (A). The amounts of the target cDNA were then normalized against GAPDH cDNA in the corresponding sample and are shown in the upper graph. Those of protein bands were normalized to β-actin in corresponding samples in lower part. The values for control were set to 100%. The data represents the mean ± s.d. of three independent experiments expressed as a percentage of the control value. The data were evaluated for statistic significance with a one-way ANOVA followed by Duncan's multiple range test. The means with the same letter were not significantly different. Therefore, the means with different letter, eg, 'a' or 'b' were statistically different. A p value<0.05 was considered significant.

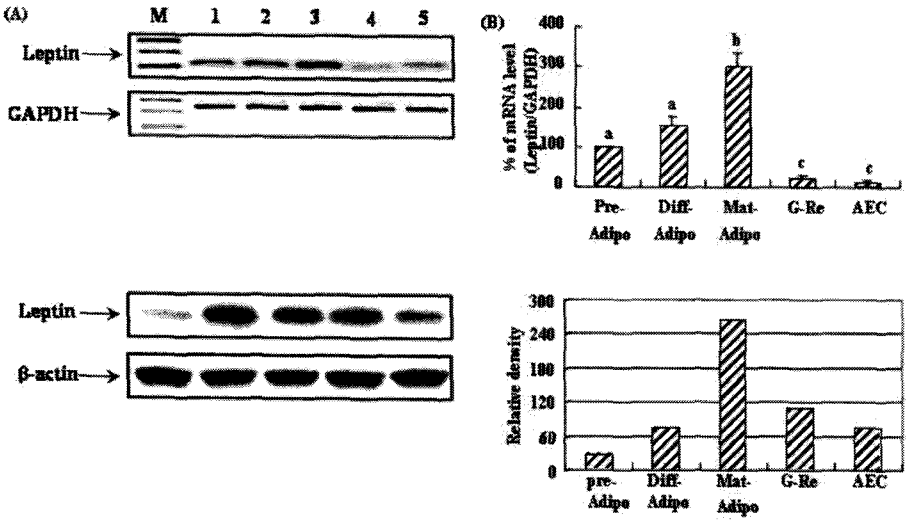

Fig. 3. Effects of G-Re and AEC on leptin mRNA and protein expression levels in 3T3-L1 adipocytes. (A), The cultures were treated with 100 μg/ml G-Re and AEC for 12 days. The total RNA was extracted and analyzed for leptin and GAPDH mRNA expression in upper part and 40 μg proteins were loaded for leptein expressions in lower part, M, molecular size standards; lane 1, untreated preadipocyte (Pre-Adipo); lane 2, untreated differentiation adipocyte (Diff-Adipo); lane 3, untreated control adipocyte (Mat-Adipo); lane 4, treated with 100 μg/ml G-Re; lane 5, treated with 100 μg/ml AEC; (B), The intensities of the amplified bands were determined from the gel shown in (A). The amounts of the target cDNA were then normalized against GAPDH cDNA in the corresponding sample and are shown in the upper graph. Those of protein bands were also normalized to β-actin in corresponding samples in lower part. The values for the control were set to 100%. The data represents the mean ± s.d. of three independent experiments expressed as a percentage of the control value. The data were evaluated for statistic significance with a one-way ANOVA followed by Duncan's multiple range test. The means with the same letter were not significantly different. Therefore, means with different letter, eg, 'a' or 'b' were statistically different. A p value<0.05 was considered significant.

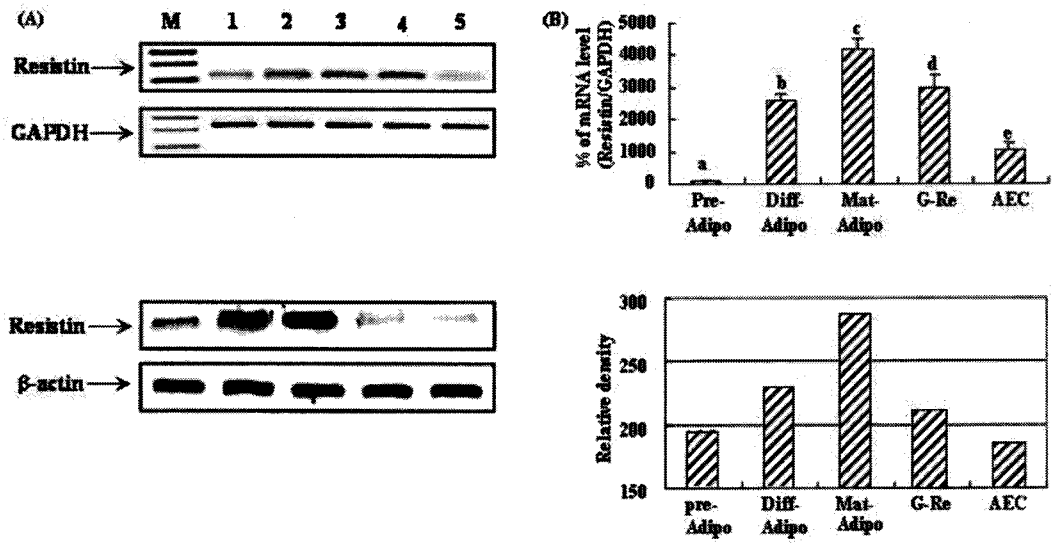

Fig. 4. Effects of G-Re and AEC on resistin mRNA and protein expression levels in 3T3-L1 adipocytes. (A), The cultures were treated with 100 μg/ml G-Re and AEC for 12 days. The total RNA was extracted and analyzed for resistin and GAPDH mRNA expression in upper part and 40 μg proteins were loaded for leptein expressions in lower part, M, molecular size standards; lane 1, untreated preadipocyte (Pre-Adipo); lane 2, untreated differentiation adipocyte (Diff-Adipo); lane 3, untreated control adipocyte (Mat-Adipo); lane 4, treated with 100 μg/ml G-Re; lane 5, treated with 100 μg/ml AEC; (B), The intensities of the amplified bands were determined from the gel shown in (A). The amounts of the target cDNA were then normalized against GAPDH cDNA in corresponding samples and are shown in the upper graph. Those of protein bands were also normalized to β-actin in lower part. The values for the control were set to 100%. The data represents the mean ± s.d. of three independent experiments expressed as a percentage of the control value. The data were evaluated for statistic significance with a one-way ANOVA followed by Duncan's multiple range test. The means with the same letter were not significantly different. Therefore, means with different letter, eg, 'a' or 'b' were statistically different. A p value<0.05 was considered significant.

Mat-Adipocytes. The resistin expression levels in the adipocytes treated with AEC were 2.6 times lower than that of the adipocytes treated with G-Re known as the anti-hyperglycemic activity correlated highly with antiobesity. Therefore, these results suggest that the AEC treatment as well as G-Re can improve the insulin resistance and hyperglycemia, and might be a good treatment for obesity and diabetes.

Discussion

The aim of this study was to investigate the effects of the traditional oriental herbal medicines, G-Re and AEC on the mRNA and protein expression levels of the obesity markers: TNF-α, LPL, leptin, resistin in 3T3-L1 differentiated adipocytes.

TNF-α, which has been demonstrated to regulate the biological activity of adipose cells, is produced by adipose tissue in order to regulate the glucose and fatty acid metabolism and hormone receptor signaling [52,56]. After treating the 3T3-L1 adipocytes with the herbal medicines, the TNF-α gene expression levels were markedly suppressed compared with that of the untreated control adipocytes. The TNF-α gene expression level of the adipocytes treated with AEC was decreased more than the adipocytes treated with G-Re but this difference was not significant. Cho *et al.* [11] reported that ginsenoside Rb1 and Rb2 significantly inhibited TNF-α production in RAW 264.7 cells. Attele *et al.* [3] showed that treatment with G-Re significantly reduced plasma cholesterol levels in ob/ob mice. Perrey *et al.*[50] reported that TNF-α diminishes the LPL mRNA level in the 3T3-L1 adipocytes by down-regulating LPL gene transcription. Kern *et al.* [26] showed that the TNF-α mRNA level with increasing adiposity was a significant increase, and a significant inverse relationship between TNF-α expression and the LPL activity was observed. We identified the results obtained in this study with that of previous studies. But, the other side, Hauner *et al.* [18] and Green *et al.* [15] reported that TNF-α increased lipolysis and the release of free fatty acids

in the cultured cells. Accordingly, it is believed that AEC compared with G-Re known to many pharmacological activities as well as antiobesity and antihyperglycemic effects influence TNF-α gene expression and also can regulate the fatty acid metabolism through the increase of lipolysis in adipocytes. However, the pathway of the signaling of the adipocytes metabolism in the 3T3-L1 adipocytes is unknown. For that reason, further studies will be needed to address the potential role of TNF-α as a regulator of lipolysis in 3T3-L1 adipocytes and human obesity.

LPL is synthesized and glycosylated in the endoplasmic reticulum (ER), transported to the cell surface through the Golgi, and finally secreted [36]. LPL is a lipolytic enzyme that catalyzes the hydrolysis of triglycerides in chylomicrons and very low density lipoproteins (VLDL). The LPL activity is lowered, leading to hypertriglyceridaemia. The adipose tissue plays an important role in producing LPL [22]. Masuno *et al.* [37] reported that treating the 3T3-L1 adipocytes with 100 μg/ml ginsenoside Ro, Re, Rg1, and Rh1 increased the secretion of the lipase activity into the medium by 119, 107, 56, and 32%, respectively. And Attele *et al* [3] also showed effects of *Panax ginseng* berry extract, major constituent ginsenoside Re, in obese diadetic mice. As the results, treatment with the extract significantly decreased plasma cholesterol level. Many other papers also reported that the intraperitoneal administration of ginsenoside Rb_2 to rats fed a cholesterol-rich diet decreased both the plasma triacylglycerol and cholesterol levels, as did the other ginsenosides both *in vivo* and *in vitro* [45,48]. However, some ginsenosides had different effects on the plasma lipid level depending on their structures. The results of this study show that the 3T3-L1 adipocytes treated with 100 μg/ml G-Re and AEC showed a higher LPL expression levels than that in the absence of these herbal medicines, which is in agreement with previous reports. Many other reports have indicated that the consumption of adlay seeds is beneficial to humans [28,33,46] have shown that some extracts of the adlay seed possess anti-allergic, antimutagenic, hypolipidemic and prebiotic activity [10, 20,21,47]. However, little is known regarding the effects of AEC in diet-induced obesity on the gene regulation and hypolipidemic activity in obese rats. Therefore, AEC might have similar hypolipidemic effects to G-Re and up regulate LPL protein as well as mRNA expressions, decrease those of leptin expressions and then inhibit lipid accumulation in the adipocytes. Accordingly, it is suggested that the administration of these herbal medicines could change intracellular fatty acid metabolism both *in vitro* and *in vivo* and play a crucial role in regulating leptin level and /or lipoysis via stimulation of LPL activity.

Leptin was recently discovered to play a role in regulating the energy balance in humans and rodents. This 167-amino-acid-containing protein is only produced and secreted by mature adipocytes [6,13]. Leptin mRNA in adipocytes and serum leptin levels are positively related to the adipose tissue mass [34,39]. Sinha and Caro [53] reported that under *in vitro* conditions, insulin stimulates leptin production only after four days in the primary cultures of human adipocytes, which is apparently due to its trophic effects and increased fat-cell size. Maffei *et al.* [34,35] suggested that the leptin mRNA level was higher in the larger adipocytes than in the smaller adipocytes. However, whether or not the leptin expression correlates with the cell size remains as a controversial issue. Nevertheless, the results of this study suggest that treatments with either G-Re or AEC can decrease the number and size of 3T3-L1 mouse adipocytes. In addition, this result is supported by our previous in vivo study, which demonstrated the effect of AEC on the adipocytes in relation to leptin [27]. Consequently, it is expected that these herbal medicines, particularly G-Re and AEC would be a good treatment for obesity in humans.

Resistin, a novel signaling molecule, was most recently identified as a hormone secreted by adipocytes, which leads to adipogenesis and insulin resistance both *in vivo* and *in vitro*. Therefore, resistin might be an important link between obesity and diabetes [51,53]. Steppan *et al.* [54] reported that the resistin levels were significantly increased in both genetic and diet-induced obesity *in vivo*. McTernan *et al.*[38] confirmed that expression of resistin was increased in human adipose tissue and suggested a potential role in linking central obesity to type 2 diabetes and/or cardiovascular disease. We observed that the resistin mRNA and protein expression levels in the fully mature adipocytes were significantly higher than those of differentiation adipocytes. In contrast, when the 3T3-L1 mature adipocytes were treated with 100 μg/ml of either G-Re or AEC, the resistin expression levels were significantly lower than in the absence of these herbal medicines. In addition, the resistin expression levels of the adipocytes treated with AEC were markedly lower than that of the adipocytes treated with G-Re known as antihyperglycemic effector.

Therefore, these results suggest that AEC treatment can improve the insulin resistance level and hyperglycemia and possibly cure obesity and diabetes.

In summary, the administration of the traditional oriental herbal medicines, G-Re and AEC, significantly influenced the mRNA and protein expression levels of the obesity markers: TNF-α, LPL, leptin and resistin in the 3T3-L1 differentiated adipocytes. These results support the overall *in vitro* anti-obese activity of the extract, which may prove to be of clinical importance in improving the management of obesity. In addition, the identification of significant anti-obesity effects in AEC as well as G-Re may provide an opportunity to develop a novel class of antiobesity agents. Further studies will be needed to explore the regulation and function of these herbal medicines in vivo levels in the hope that a better understanding of bioactivity substance, especially, on AEC may lead to new therapies.

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초록 : 지방세포 3T3-L1에 인삼 사포닌 Re와 의이인 추출액 처리시 비만관련 유전자인 TNF-α, lipoprotein lipase, leptin 및 resistin 발현 조절에 미치는 영향

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전통적인 한방약물로 이용되는 인삼(사포닌 Re)과 의이인 추출물을 분화단계별 지방세포에 100 μg/ml 씩 12일간 이틀에 한번씩 각각의 물질을 처리하였다. 비만지표 유전자로 알려져 있는 여러 유전자의 발현양을 mRNA와 단백질 수준에서 조사하였다. 완전성숙 지방세포에서 약물의 처리에 따라 TNF-α 발현이 비처리 세포에 비해 현저히 억제되었고, lipoprotein lipase는 mRNA와 단백질수준에서 그 발현 양이 증가하였다. 약물처리 세포에서 leptin 과 resistin의 유전자 발현은 비처리세포에 비해 유의적인 감소 되었다. 이러한 결과는 인삼 사포닌 뿐 아니라 의이인 추출액에서도 유사한 영향이 관찰되어 특히, 곡물인 의이인 추출액이 인삼 사포닌과 거의 비슷한 항비만 활성을 나타내어 장복으로 인한 세포독성을 최소화하면서 지방세포에 긍정적 영향을 미칠 뿐 아니라 인슐린 저항성 개선 효과도 기대되어진다. 향후 대사성 질환과 밀접한 관련이 있는 비만 관리 향상에 이용 할 수 있을 것으로 사료되어진다. 따라서 이 결과들을 바탕으로 동물실험을 통한 더 자세한 조절기능의 확인과 특히, 의이인 추출액에서의 생리활성물질에 대해서도 많은 체계적인 분석과 관련연구가 필요 할 것으로 사료된다.