Obesity is characterized by increasing the number and size of adipocytes due to hormonal changes and imbalances in the energy metabolism caused by excessive fat intake (1, 2). Obesity is a major factor in the development of heart disease, cancer, hypertension, diabetes, and degenerative arthritis (3-5). 3T3-L1 cell, a fibroblast cell has been widely used in obesity research, and can differentiate into an adipocyte under appropriate conditions (6, 7). Peroxisome proliferator-activated receptor γ (PPAR-γ) is an important factor for developing adipocyte and is a target for insulin sensitizing drugs as glitazones. Activation of PPAR-γ by glitazones leads to fatty acids in the adipocytes. Fat accumulation also leads to an increase in the gene expression of fatty acid synthase (FAS) and fatty acid-binding protein 4 (FABP4), which are responsible for fat synthesis, transport and deposit (8). Therefore, it is of interest to modulate adipocyte differentiation through regulating these transcriptional factors.

In fact, the International Obesity Task Force has estimated that one third of the world’s population will become obese (BMI >30 kg/m²) within the next 20 years or so if the present trend of increasing incidence of obesity continues. However, because the currently used anti-obesity drugs - such as orlistat, sibutramine and sertraline - have several reported side effects (9), research and development of anti-obesity substances to replace these drugs are being actively conducted.

With regard to natural anti-obesity substances, resveratrol and genistein, which are contained in grapes and beans, respectively, have been reported as having anti-obesity effects (10-12). Resveratrol, a kind of stilbene present in nature, is a phytoalexin that is naturally synthesized in some plants against the attacks of pathogens such as bacteria and fungi (13). In this study, the adipogenesis-suppressing activity of 3,5-dimethoxy-(4-methoxyphenyl)benzamide (DMPB), a derivative of resveratrol, was measured in 3T3-L1 cells as part of the search for new anti-obesity substances, and its excellent anti-obesity-related activity was confirmed.

**EXPERIMENTAL**

**Materials**

DMPB was synthesized by the method previously reported (14). Resveratrol was purchased from Sigma-Aldrich (Fig. 1); 3T3-L1 cells were from the American Type Culture Collection (Manassas, VA); FAS and acetyl-CoA carboxylase (ACC) antibodies were from Cell Signaling Technology and a PPAR-γ transcription factor assay kit was from Cayman Chemical.

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Cell culture

The 3T3-L1 fibroblast cells were cultured using Dulbecco’s modified Eagle’s medium (DMEM) culture medium containing 10% FBS, at 37°C, 5% CO₂.

Adipocyte differentiation-suppressing activity

The 3T3-L1 cells were grown and placed in a 48-well plate and treated with a hormone mixture (10 µg/mL insulin, 0.5 µM dexamethasone, and 0.5 mM isobutylmethylxanthine) for 48 h, and then transferred to DMEM medium containing insulin. Each sample was treated for 8 days and observed for adipocyte differentiation. Upon completion of differentiation, the cells were washed with PBS twice and then fixed with 3.7% formaldehyde. After incubating the cells for 1 h using Oil Red O dye, isopropanol was added, and absorbance was measured.

Figure 1. Chemical structure of resveratrol (A) and DMPB (B).

![Chemical structures](image)

Figure 2. Inhibitory effect of DMPB on 3T3-L1 adipocyte differentiation. Induction: hormone mixture treated group. DMPB: hormone mixture and DMPB treated group. Res: hormone mixture and resveratrol treated group. Data are the mean ± SD values of three experiments. *p < 0.05, **p < 0.01, ***p < 0.001 compared with the Induction group.

![Graph showing inhibitory effect](image)

Figure 3. The morphology of 3T3-L1 cells treated with a hormone mixture and DMPB. Induction: hormone mixture treated group. DMPB: hormone mixture and DMPB treated group.

![Morphology images](image)
measured at 510 nm to estimate the amount of triglycerides.

**FAS and ACC expression**

The 3T3-L1 cells were washed twice with ice-cold PBS and then lysed with lysis buffer (50 mM Tris-HCl, 1% Triton X-100, 0.5% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM sodium orthovanadate, 1 mM NaF, and 0.2% protease inhibitor cocktail, pH 7.2). The collected protein was centrifuged at 14,000 rpm for 5 min, whereupon the supernatant was collected for protein quantification. Next, 30 µg of the protein was loaded in 10% SDS-PAGE for electrophoresis, transferred to a nitrocellulose membrane and reacted with ACC antibodies (1 : 500) or FAS antibodies (1 : 1000), and then detected by enhanced chemiluminescence (ECL).

**PPAR-γ transcription activity**

The effect of DMPB on PPAR-γ in 3T3-L1 cells was measured using a PPAR-γ transcription factor assay kit (Cayman Chemical). The 3T3-L1 cells were treated with rosiglitazone 80 µM and each concentration of DMPB, and then the cell extract was added to a dsDNA sequence-coated plate. The PPAR-γ antibodies and the secondary antibodies were reacted in order, and then a detection reagent was added and absorbance was measured at 450 nm.

**Statistical analysis**

Data are the mean ± SD values from three independent experiments. Statistical significance of the obtained data was determined by Student’s *t*-test.

**RESULTS AND DISCUSSION**

**Inhibitory effect on adipocyte differentiation**

After confirming the absence of cytotoxicity in DMPB at concentrations less than 50 µM, the effect of DMPB on adipocyte differentiation was measured and compared with that of resveratrol, as shown in Figure 2. DMPB suppressed the hormone mixture-induced differentiation of adipocytes from 3T3-L1 cells in a concentration-dependent manner, and even strongly suppressed the differentiation at a concentration of 50 µM, as with the non-treated group. In particular, DMPB demonstrated higher adipocyte differentiation suppressing activity at all concentrations compared to resveratrol at the same concentrations. The morphology 3T3-L1 cells treat-
FAS and ACC expression suppressing effect

Fat is composed of fatty acids and glycerol. During the initial stage of fatty acid synthesis in the cell, acetyl-CoA carboxylase (ACC), which is a critical enzyme in the process, induces carboxylation of acetyl-CoA and promotes the production of malonyl-CoA (15-17). Fatty acid is synthesized by the action of FAS, an enzyme with MW 250 kDa, from malonyl-CoA as a substrate (18, 19). Therefore, it is expected that FAS and ACC inhibitors can effectively reduce lipid production and suppress obesity. The effect of DMPB on the expression of fatty acid metabolism-related proteins such as FAS and ACC, the major factors in the suppression of obesity, was measured as shown in Figure 4. DMPB reduced the increase in the production of FAS and ACC in 3T3-L1 cells induced by a hormone mixture in a concentration-dependent manner. Thus, it can be stated that DMPB reduces lipid production by suppressing fatty acid biosynthesis.

PPARγ activity suppressing effect

PPAR-γ is known as a glitazone receptor which controls fatty acid storage and glucose metabolism (20). Because genes activated by PPAR-γ promote the uptake of fatty acid and glucose for its conversion to fatty acids in adipocytes and so deposition of triacylglycerols in these cells to induce obesity, a substance that suppresses PPAR-γ could exhibit an anti-obesity effect (21). The effect of DMPB on PPAR-γ in 3T3-L1 cells was measured, and was observed to suppress the activation of PPAR-γ by rosiglitazone in a concentration-dependent manner (Fig. 5). Thus, it is considered that DMPB reduces fatty acid production by reducing the production of FAS and ACC, while simultaneously suppressing fat absorption by inhibiting PPAR-γ activity.

REFERENCES


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