Inhibitory Effect of Vitamin U (S-Methylmethionine Sulfonium Chloride) on Differentiation in 3T3-L1 Pre-adipocyte Cell Lines

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Background: S-methylmethionine sulfonium chloride was originally called vitamin U because of its inhibition of ulceration in the digestive system. Vitamin U is ubiquitously expressed in the tissues of flowering plants, and while there have been reports on its hypolipidemic effect, its precise function remains unknown. Objective: This study was designed to evaluate the anti-obesity effect of vitamin U in 3T3-L1 pre-adipocyte cell lines. Methods: We cultured the pre-adipocyte cell line 3T3L1 to overconfluency and then added fat differentiation-inducing media (dexamethasone, IBMX [isobutylmethylxanthine], insulin, indomethacin) and different concentrations (10, 50, 70, 90, 100 mM) of vitamin U. Then, we evaluated changes in the levels of triglycerides (TGs), glycerol-3-phosphate dehydrogenase (G3PDH), AMP-activated protein kinase (AMPK), adipocyte-specific markers (peroxisome proliferator-activated receptor γ [PPAR-γ], CCAAT/enhancer-binding protein α [C/EBP-α]), adipocyte differentiation and determination factor 1 [ADD-1], adipin, fatty acid synthase, lipoprotein lipase) and apoptosis-related signals (Bcl-2, Bax). Results: There was a gradual decrease in the level of TGs, C/EBP-α, PPAR-γ, adipin, ADD-1 and GPDH activity with increasing concentrations of vitamin U. In contrast, we observed a significant increase in AMPK activity with increasing levels of vitamin U. The decrease in bcl-2 and increase in Bax observed with increasing concentrations of vitamin U in the media were not statistically significant. Conclusion: This study suggests that vitamin U inhibits adipocyte differentiation via down-regulation of adipogenic factors and up-regulation of AMPK activity. (Ann Dermatol 24(1) 39 ∼ 44, 2012)

Keywords: Adipocyte differentiation, S-methylmethionine sulfonium chloride, Vitamin U

INTRODUCTION

Adipose tissue serves as an energy storage depot to maintain lipid homeostasis, thereby promoting the survival of the human body. Obesity occurs when the adipose tissue is overloaded with high-energy nutrients without subsequent expenditure1. Because adipocytes play a critical role in energy balance through the storage of triglycerides (TGs) or the release of free fatty acids in response to changes in energy demands, understanding the molecular mechanisms of adipocyte differentiation may provide clues for the development of strategies for the prevention and treatment of obesity. The mechanisms of adipocyte differentiation have been extensively studied in preadipocyte culture systems. Characterization of the regulatory regions of adipose-specific genes has led to the identification of key transcription factors in the complex transcriptional cascade that occurs during adipocyte differentiation2. These factors include peroxisome proliferator-activated receptor γ (PPAR-γ)3, CCAAT/enhancer binding protein (C/EBP)4,5, adipocyte
differentiation and determination factor 1 (ADD-1), fatty acid synthase (FAS) and lipoprotein lipase (LPL). In addition to playing a central role in the storage of lipids, adipose tissue secretes numerous bioactive substances called adipokines that contribute to cell proliferation as well as the differentiation of preadipocytes into adipocytes. These substances include tumor necrosis factor-α, adiponectin, adipin, and interleukin-6 (IL-6), which also regulate the inflammatory response.

Recently, a number of natural compounds, such as herbal drugs and flavonoids, have received considerable attention for their abilities to regulate adipogenesis. S-methylmethionine sulphonium chloride is abundant in the tissues of flowering plants, especially in raw cabbage, and has also been called vitamin U (from the Latin ‘ulcus’ meaning sore or ulcer), although its classification as a vitamin has not yet been accepted. Gessler et al. reported that vitamin U can decrease the level of lipid peroxidation and inhibit monoamine oxidase activity. Also, they reported that vitamin U has a hypolipidemic effect.

Our study was conducted to determine whether vitamin U can modulate differentiation in 3T3-L1 adipocytes. The involvement of glycerol-3-phosphate dehydrogenase (G3PDH), AMP-activated protein kinase (AMPK) and adipocyte-specific markers (PPAR-γ, C/EBP-α, ADD-1, adipsin, FAS, and LPL) were evaluated.

MATERIALS AND METHODS

Culture and differentiation of 3T3-L1

3T3-L1 cells and vitamin U were purchased from the Korea Cell Line Bank (Seoul, Korea) and TCI Corp. (Tokyo, Japan), respectively. 3T3-L1 preadipocytes were incubated at a density of 8,000 cells/cm² (10 cm² dish) and grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at 37°C in 5% CO₂ in air. Confluent preadipocytes were induced to differentiate with inducing medium containing 500 μM IBMX (isobutylmethylxanthine), 1 μM dexamethasone, 100 μM indomethacin and 10 μg/ml insulin. To test the effect of vitamin U on inhibition of adipocyte differentiation, we treated cells with vitamin U (10, 50, 70, or 100 mM) at day 0. To test the effect of vitamin U on fat in adipocytes, we changed the medium to DMEM (with 10% FBS) on day 6 after the induction of adipocyte differentiation and then treated the cells with vitamin U at different concentrations for seven days. Cultures were re-fed every 2-3 days to allow 90% of the cells to reach full differentiation before they were chemically treated. Chemicals were freshly diluted in adipocyte medium before treatment. Cells were washed with fresh adipocyte medium, re-fed with medium containing vitamin U, and then incubated at 37°C in 5% CO₂ in air before analysis.

Cell viability assay (MTT assay)

3T3-L1 cells were treated with lipopolysaccharide (100 ng), interferon-γ (10 ng) and different concentrations of S-methylmethionine sulfoxonium chloride (50, 70, 90, 100 mM). After cells were incubated for 24 hr, MTT (5 mg/ml) was added. Then, the supernatant was discarded, dimethyl sulfoxide was added to the cells and formazan was formed via MTT reduction. Cell viability was measured using an ELISA at 570 nm.

Oil Red O staining

The formation of oil droplets in cells was analyzed using Oil Red O staining as follows. After removal of culture medium, cells were washed twice with phosphate-buffered saline (PBS) and then fixed for at least one hour with pre-chilled 10% formaldehyde in PBS. Cells were stained with 0.7% Oil Red O solution for two hours, followed by three washes with PBS. Cells were maintained in water and photographed. Fat droplets in the adipocytes were stained red, and the Oil Red O stain was eluted with isopropanol and was quantified by measuring the optical density at 515 nm.

TG assay

3T3-L1 adipocytes that were treated as described above were washed with PBS, harvested into 25 mM Tris buffer (pH 7.5) containing 1 mM ethylenediaminetetraacetic acid (EDTA), and lysed via brief sonication. We quantified TG levels in the lysates using the LabAssay™ Triglyceride kit (Wako Pure Chemical Industries. Ltd., Osaka, Japan).

G3PDH activity assay

After removal of the culture medium, cells were washed twice with PBS and lysed in enzyme extraction buffer. Immediately after the extraction, we added an equal volume of dilution buffer supplemented with 2-mercaptoethanol to the sample to prepare a two-fold diluent. We dispensed the substrate solution into each 96-well plate and preincubated the plate at 30°C. We then added 25 μl of a diluted sample and agitated the plate and determined the decrease in absorbance at 340 nm at 30°C.

Reverse transcriptase polymerase chain reaction

Mouse adipocyte-specific markers (PPAR-γ, C/EBP-α, ADD-1, adipsin, FAS, LPL) were quantitatively measured using an RNeasy Lipid mini kit (Qiagen, Austin, TX, USA). The primers and probe sets were ordered from Bioneer Corp. (Daejeon, Korea) and were used according to the manu-