OLEACEIN INHIBITS ADIPOCYTE DIFFERENTIATION IN 3T3-L1 CELLS

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Abstract: Obesity is becoming a public health threat worldwide, especially in developed countries, because it raises the risk of various diseases. Overweight and obesity are mainly caused by excessive caloric intake that exceeds the daily needs of the body, as well as low physical activity. Since in highly developed countries there is an increasing prevalence of overweight and obesity, which may lead to diseases of the cardiovascular system, the substances, including substances of plant origin, that can prevent the occurrence of such diseases are sought. Oleacein, which is a secoiridoid found in olive leaves and oil, has been reported to have some beneficial biochemical and pharmacological effects. The present study shows how that compound affects adipocyte differentiation in 3T3-L1. To perform this study, 3T3-L1 preadipocytes were treated with oleacein in variable concentrations. Viability was analyzed through the neutral red and the MTT assays, and triglycerides were stained with Oil Red O. Cells differentiation was analyzed under an inverted microscope. Oleacein at a concentration range of 6.25-50 µmol/L suppressed intracellular triglyceride accumulation during adipocyte differentiation in a concentration-dependent manner without effect on cell viability. These findings indicate that oleacein is capable of preventing 3T3-L1 preadipocytes differentiation and adipogenesis.

Keywords: oleacein, adipocytes, adipogenesis, obesity

Obesity, which is an imbalance between energy intake and expenditure, is becoming a public health threat worldwide, especially in developed countries, and the obese are at increased risk of other diseases and complications, such as hypertension, type 2 diabetes, respiratory diseases, cardiovascular diseases, metabolic syndrome, polycystic ovary syndrome, orthopedic injuries and diseases, and cancer (1, 2). Adipose tissue is a source of energy because it stores energy in the form of fat. When the energy demand increases, it releases the fat into circulation. In addition, it protects the internal organs, secretes hormones and adipokines that control / regulate appetite, blood pressure, insulin sensitivity, fibrinolysis, the blood clotting system, and metabolizes sex hormones. Adipocytes are capable of producing pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNFα). Obesity has been demonstrated to be associated with chronic, low-grade inflammation in adipose tissue (3, 4).

The main factor responsible for the increase in adipose tissue, and consequently overweight and obesity, is increased adipocyte proliferation and differentiation. In case of an excessive supply of energy, the cells store the energy in the form of fat. Hence, it is believed that the ability to regulate adipocyte differentiation may result in a pharmacological contribution to obesity prevention (5). The process of adipocyte differentiation is regulated by a number of transcription factors, the most important of which are peroxisome proliferator-activated receptor gamma (PPARγ), Krüppel-like factors (KLFs), and sterol regulatory element-binding protein 1c (SREBP1c). These factors regulate adipocyte differentiation and also control the expression of genes such as ACC and FAS (6).

There is considerable research interest in potential anti-obesity compounds, particularly in such that can affect fat storage. Although several drugs have been developed to treat obesity, no ideal compounds or drugs have been discovered or developed yet and

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there is an urgent need for novel materials when the treatment of obesity is concerned (7). Currently, new substances with properties regulating the differentiation of adipocytes, and thus preventing obesity, are found in natural products. Searching for new natural compounds with such properties and studying their mechanisms of action may contribute to obtaining new effective drugs that prevent obesity and related diseases. (8).

Oleacein, also known as the dialdehydic form of elenolic acid conjugated with 3,4-(dihydroxyphenyl)ethanol (3,4-DHPEA-EDA), is a secoiridoid found in extra virgin olive oil. Oleacein exhibited hypotensive, antioxidative, and anti-inflammatory activity (9). Although previously reported data revealed that oleuropein and hydroxytyrosol occurring in olive (Olea europaea, Oleaceae) leaves and olive fruits inhibit adipocyte differentiation in 3T3-L1 cells (10), a number of published works demonstrated that oleacein, which is a dialdehyde derivative of oleuropein, has similar, but much stronger activity than oleuropein (11, 12). Therefore, the aim of the present study is to assess if oleacein can affect early adipogenesis through inhibition of 3T3-L1 pre-adipocytes differentiation in vitro.

EXPERIMENTAL

Chemicals

Oleacein for experiments was isolated from Ligustrum vulgare leaves as described previously (11). Dulbecco's modified Eagle's medium (DMEM high-glucose), newborn calf serum (NCS), fetal bovine serum (FBS), and phosphate-buffered saline (PBS) were purchased from Thermo Scientific. Insulin, dexamethasone (DEX), isobutylmethylxanthine (IBMX), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), neutral red, and Oil Red O were purchased from Sigma-Aldrich. Dimethylsulfoxide (DMSO), ethanol, isopropanol were purchased from Purchem.

The formulation of oleacein

Oleacein was dissolved in DMSO and then the culture medium was added to obtain the appropriate compound concentration in the sample. DMSO in the concentration used (<0.1%) did not influence the performed assays.

Neutral red uptake (NRU) assay

Cytotoxicity was assessed using the neutral red uptake assay as described previously (13). Cells were incubated in 96-well plates for 24 h with DMEM medium supplemented with 10% newborn calf serum (NCS) containing varying concentrations of oleacein in DMSO (1 μM, 5 μM, 10 μM, 50 μM, 100 μM). Cell viability was calculated as percentage versus control (cells incubated with 0.1% DMSO).

MTT assay

Mitochondrial function was assessed using the MTT assay as described previously (13). Cells were incubated in 96-well plates for 24 h with DMEM medium supplemented with 10% newborn calf serum (NCS) containing varying concentrations of oleacein in DMSO (1 μM, 5 μM, 10 μM, 50 μM, 100 μM). Cell viability was calculated as percentage versus control (cells incubated with 0.1% DMSO).

Cell culture and 3T3-L1 pre-adipocyte differentiation

Mouse 3T3-L1 (ATCC® CL-173™) preadipocytes were obtained from ATCC (American Type Culture Collection, Manassas, VA, USA) and maintained in high-glucose DMEM medium supplemented with 10% newborn calf serum (NCS). For the experiments, the cells were plated on 12-well plates. 3T3-L1 preadipocyte differentiation was induced according to the protocol described previously (14) in the presence or absence of oleacein at a concentration range of 6.25-50 µmol/L.

Oil Red O staining and semi-quantitative measurement of triglycerides in 3T3-L1 preadipocytes

3T3-L1 preadipocytes were induced to differentiate into adipocytes. Staining was performed using the protocol described by Kraus et al. (15). The absorbance was measured at the wavelength of 420 nm, using a microplate reader (Biotek Synergy4). The relative content of triglycerides (%) was displayed in differentiation medium containing DMSO (0.1%) - treated cells as a standard.

Statistical Analysis

Quantitative data are presented as average ± SEM. Statistic differences were determined by a one-way ANOVA followed by a Tukey post hoc test. All values with P<0.05 were considered statistically significant.

RESULTS

Effect of oleacein on 3T3-L1 differentiation

3T3-L1 cells were differentiated in the presence of oleacein for 8 days. Fully differentiated adipocytes were stained and the accumulation of total lipids was quantified by Oil Red O. As shown in Figure 1,
Oleacein inhibits adipocyte differentiation in 3T3-L1 cells

Figure 1. Effect of oleacein on adipocyte differentiation (A and B). 3T3-L1 cells were harvested and differentiated in the presence of several concentrations of oleacein (0, 25, 50, and 100 μmol/L) for 8 days. The cells were stained with Oil Red O. (A) Stained intracellular oil droplets were eluted with isopropanol and quantified spectrophotometrically at 420 nm. Error bars represent a standard error (± SEM); results are representative of 3 independent experiments with triplicate for each concentration used. #p < 0.05 vs. control (undifferentiated cells); *p < 0.05 vs. control (differentiated cells incubated without oleacein). (B) Representative photomicrographs of the stained cells.

Figure 2. Effect of oleacein on cell viability. 3T3-L1 cells were incubated in the presence of oleacein at various concentrations for 24 h. The percentage of viability was determined by the MTT assay (A) and the Neutral Red Uptake assay (B).

DISCUSSION AND CONCLUSION

Oleacein is the most active phenolic constituent of extra virgin olive oil. In accordance
with our expectations, oleacein significantly inhibited 3T3-L1 adipocytes differentiation in a concentration-dependent manner. Moreover, the observed effect was more potent, than previously reported for oleuropein (10, 16). That observation is consistent with other studies on the biological activity of oleacein and oleuropein, which indicated that it is oleacein that has stronger antioxidant and protective properties, as well as the ability to activate the Nrf2 pathway (11, 12). Previous studies showed that oleuropein decreased the expression of PPARγ and C/EBPα and their downstream target genes CD36 and GLUT4 (10, 16). The fact that oleacein is similar to oleuropein but shows stronger inhibition of pre-adipocytes differentiation allows us to suppose that the mechanism of observed activity is the same, but it needs further elucidation. What is of importance here is that our findings suggest that oleacein reduces preadipocyte differentiation and lipid accumulation in 3T3-L1 cells, which in turn may have beneficial effects on white adipose tissue formation and accelerate progress in obesity prevention.

Conflict of interest

The authors declare no conflict of interest.

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