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RESEARCH NOTE

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Effect of Stilbenoids on TNF-α-induced Adipokine Secretion

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Abstract Tumor necrosis factor (TNF)-α is chronically elevated in adipose tissues of obese rodents and humans. Increased levels of TNF-α have been implicated in both the induction of atherogenic adipokines, such as plasminogen activator inhibitor (PAI)-1, and the inhibition of the anti-atherogenic adipokine, adiponectin. In this study, we investigated the effects of *trans*-stilbene, piceatannol, rhaponticin, and piceid on the TNF-α-induced atherogenic changes of adipokines in 3T3-L1 cells. Exposure to TNF-α for 24 hr increased PAI-1 secretion and decreased adiponectin secretion. Among stilbenoids, piceatannol significantly inhibited the increased secretion of PAI-1 induced by TNF-α. Adiponectin secretion decreased by TNF-α was recovered after *trans*-stilbene and rhaponticin treatments. Our results showed that stilbenoids exerted different effects on TNF-α-induced changes in adipokines secretion in 3T3-L1 adipocytes according to their structural characteristics.

Keywords: stilbenoid, 3T3-L1 adipocyte, plasminogen activator inhibitor-1, adiponectin

Introduction

Stilbenoids, a non-flavonoid class of phenolic compounds, are naturally occurring phytochemicals that can be found in various foods, such as grapes, wine, bilberries, cranberries, and peanuts. These molecules act as natural protective agents that defend the plant against viral and microbial attack, excessive ultraviolet exposure, and disease (1). Among the stilbenes, resveratrol has been shown to reduce the synthesis of lipids in rat liver (2) and 3T3-L1 adipocytes (3). In addition, resveratrol was shown to increase the activity of sirtuins, which is a protein deacetylase involved in apoptosis, mitochondrial function, energy homeostasis, and longevity (4).

Based on such therapeutic evidence, structurally similar stilbenes have been shown a great deal of interest in the characterization of their activities and the creation of substituted, modified stilbenes with various functional groups. *Trans*-stilbene is the structurally basic form of stilbene (5). Recent research has shown that piceatannol is a metababolites of resveratrol (6) and has pharmacological activities such as antioxidant (7), anti-cancer (8), and cardioprotective effects (9). Peceid is propably the most abundant form of resveratrol in nature and has protective action against lipid peroxidation (10). Rhaponticin had high antioxidant activity (11) and induced apoptosis of human stomach cancer and that stilbene moiety in the molecule is essential for induction of apoptosis (12).

Obesity is a rapidly growing problem that is reaching epidemic proportions worldwide (13) and is associated with an increased risk of premature death (14). The cellular mechanisms that link obesity and atherosclerosis are complex and have not been fully elucidated. However, increasing evidence suggests that the changes in adipokines due to the presence of excess adipose tissue may be a cause of atherosclerosis (15).

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Recent studies have identified that the adipocyte secretes a variety of bioactive factors (adipokines) involved in energy metabolism, inflammation, and cardiovascular functions (16). Adiponectin, also known as adipoQ and Acrp30, is a complement factor abundantly expressed in adipocytes that increases fatty acid oxidation and insulin sensitivity (17). This adipokine possesses direct anti-diabetic, anti-atherogenic, and anti-inflammatory activities (18,19). Notably, hypo-adiponectinemia is an independent risk factor for the development of type 2 diabetes (20), hypertension, and coronary artery disease (21). In contrast to adiponectin, plasma levels of plasminogen activator inhibitor (PAI)-1 are markedly elevated in obese individuals as well as in patients with insulin resistance, type 2 diabetes, and cardiovascular diseases (CVDs) (22,23). PAI-1 is thought to be the link between obesity, insulin resistance, and an increased risk for CVD (24). Tumor necrosis factor (TNF)-α is chronically elevated in adipose tissue of obese rodents and humans (25) and several studies indicate that TNF-α may be a direct contributor to elevated PAI-1 (26) and reduced adiponectin in obesity (27).

Therefore, we examined whether stilbenoids had anti-atherogenic activity via the modulation of adipokines. To test this hypothesis, the effects of various stilbenoids on the TNF-α-induced secretion of adipokines in 3T3-L1 adipocytes were we investigated.

Materials and Methods

Materials *Trans*-stilbene was obtained from ChemService (Westchester, PA, USA). Piceatannol and rhaponticin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Piceid was supplied from Sequoia Research Products Ltd. (Pangbourne, UK).

Cell culture and treatment 3T3-L1 cells were supplied from the American Type Culture Collection (Rockville, MD, USA), maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine serum, and differentiated as described previously (25). Briefly,

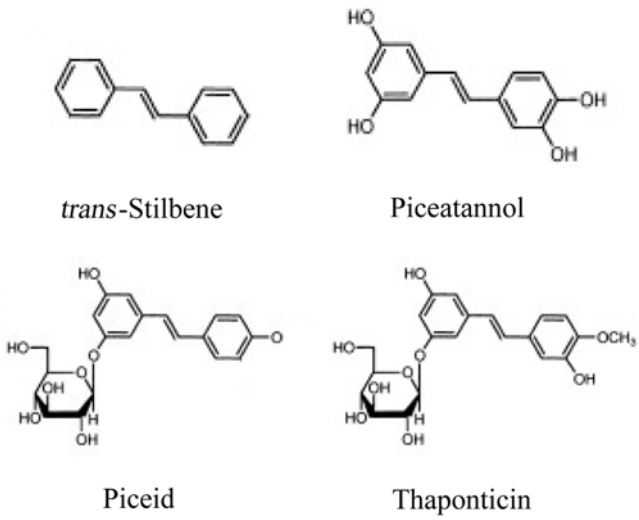

Fig. 1. Structures of stilbenoid compounds.

differentiation was induced by replacing the medium with fetal bovine serum (FBS)-supplemented DMEM containing 0.5 mM 3-isobutyl-methylxanthine, 0.25 μM dexamethasone, and 1 μg/mL insulin for 3 days. The medium was again changed to DMEM containing 10% FBS only. The medium was changed every 2 days for the following 8 days. TNF-α treatment was carried out after a 6 hr pretreatment with 5-100 μM of stilbenoids. The conditioned medium was harvested 24 hr later.

Measurement of PAI-1 and adiponectin by enzyme-linked immunosorbent assay (ELISA) The conditioned culture medium from 3T3-L1 adipocytes was collected from each sample. The concentrations of PAI-1 and adiponectin were assayed using a mouse PAI-1 ELISA kit (Molecular Innovations, Inc., Southfield, MI, USA) and a mouse adiponectin ELISA kit (Adipogen, Inc., Seoul, Korea), respectively. Each concentration was determined from the standard curve and expressed as the percent of control.

Statistical analysis Results are shown as the mean± standard deviation (SD) of triplicate determinations. Statistical analyses were performed using GraphPad Prism 4 software (San Diego, CA, USA). One-way analysis of variance (ANOVA) was used to compare quantitative data among groups. The Bonferroni post-hoc test was used if ANOVA was significant ($p<0.05$).

Results and Discussion

As known from *trans*-3,4',5-trihydroxystilbene (commonly called resveratrol), naturally occurring hydroxystilbenes consists of 2 benzene rings connected through olefin. It was already reported that resveratrol effectively inhibited TNF-induced changes of adkpokines in 3T3-L1 by our group (28). Based on this finding, the aim of this work is to investigate the different effect of stilbenes on TNF-α-induced change of adipokines according to their structure. Four stilbenoids were selected to determine the regulatory effect on TNF-α induced adkpokine secretion in mature adipocytes. The structures of candidate compounds are

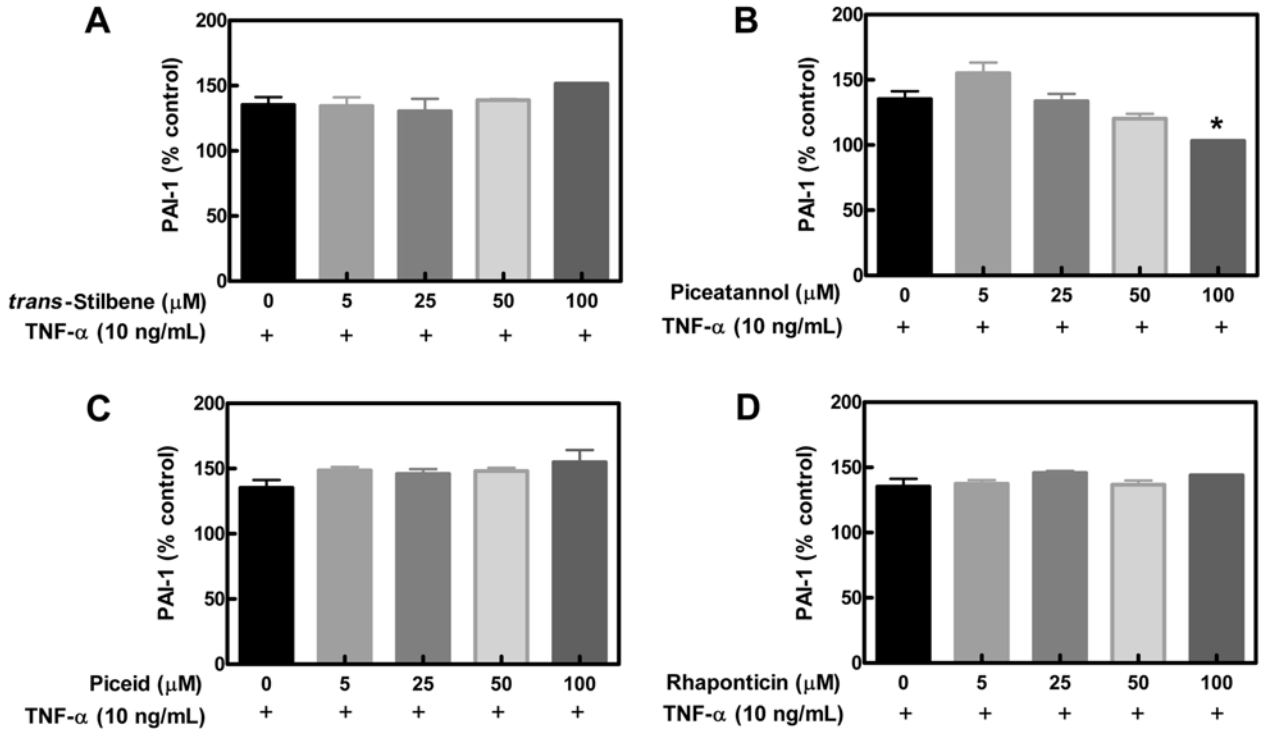

Fig. 2. Effects of stilbenoids treatment on TNF-α-induced PAI-1 secretion in 3T3-L1 adipocytes. After exposure to various concentrations of stilbenoids for 6 hr 3T3-L1 adipocytes were treated with 10 ng/mL TNF-α for 24 hr. (A) *trans*-stilbene, (B) piceatannol, (C) piceid, and ahponticin. Values are expressed relative to untreated control cells. Results are shown as the mean± SD of triplicate determinations. *$p<0.05$ compared with only TNF-α treated groups.

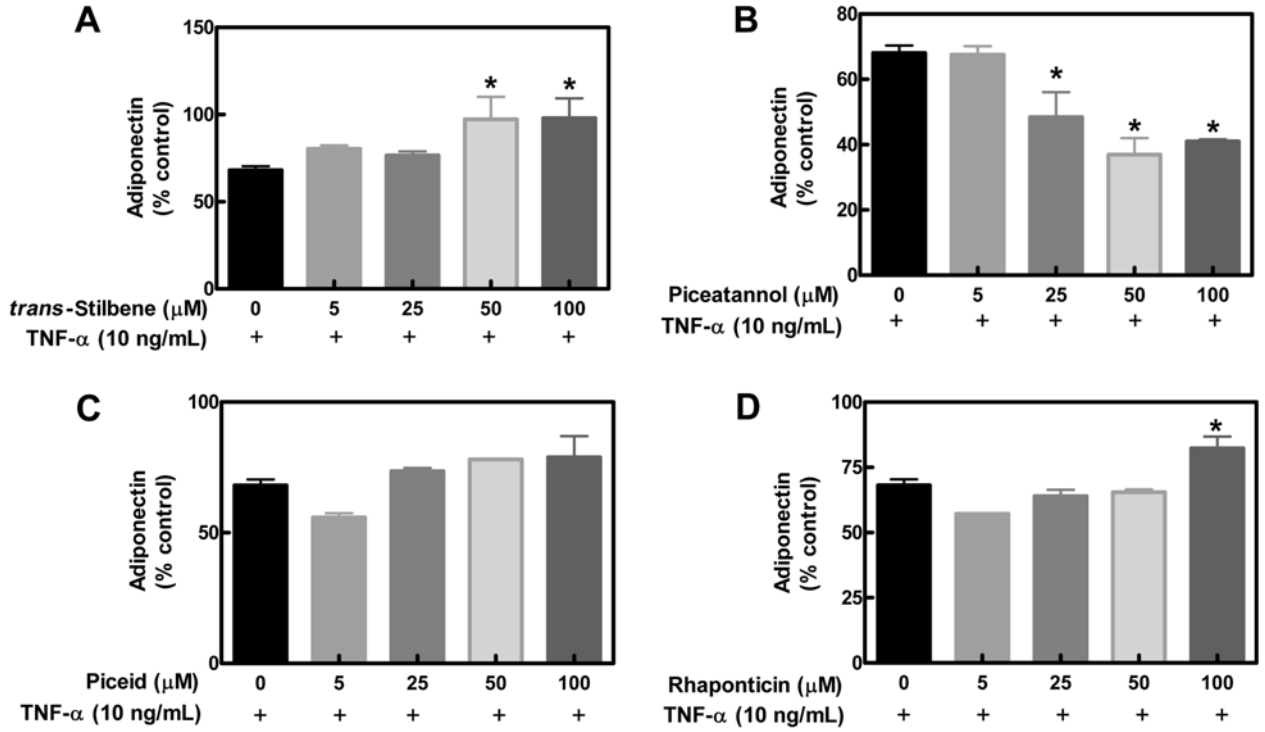

Fig. 3. Effects of stilbenoids treatment on TNF-α-induced adiponectin secretion in 3T3-L1 adipocytes. After exposure to various concentrations of stilbenoids for 6 hr 3T3-L1 adipocytes were treated with 10 ng/mL TNF-α for 24 hr. (A) *trans*-stilbene, (B) piceatannol, (C) piceid, and (D) rahponticin. Values are expressed relative to untreated control cells. Results are shown as the mean±SD of triplicate determinations. *$p<0.05$ compared with only TNF-α treated groups.

shown in Fig. 1.

Piceatannol is a *trans*-3,4',5-tetrahydroxystilbene isolated from the seeds of *Euphobia lagascae* (29). In contrast to piceatannol, rhaponticin, and piceid are dihydroxylated silbenes derived from rhizome of *Theum undalatum* (30) and grape (31), respectively. Expecially, piceid is a glycoside form of resveratrol and the most abundant form of resveratrol in nature (32).

To investigate whether stilbenoids regulated the TNF-α-induced secretion of adipokines, 3T3-L1 adipocytes were pretreated with various concentrations of stilbenoids for 6 hr and then incubated with 10 ng/mL TNF-α for 24 hr. After incubation, conditioned medium was collected for ELISA assay. Treatment with piceatannol attenuated the TNF-α-induced increases in the secretion of PAI-1 in a dose-dependent manner and the effect of 100 μM piceatannol was statistically significant. *Trans*-stilbene, piceid, and rhaponticin showed no significant effects on the TNF-α-induced increase in PAI-1 (Fig. 2A, 2C, 2D). Our results indicated that hydroxyl group is essential to exert inhibitory effect on TNF-α-induced PAI-1 secretion.

In the case of adiponectin secretion, high doses of both *trans*-stilbene (50 and 100 μM) and rhaponticin (100 μM) effectively reversed the decrease in adiponectin secretion induced by 10 ng/mL TNF-α (Fig. 3A and 3D). Piceid increased the secretion of adiponectin; however, this response was not significant (Fig. 3C). For adiponectin secretion, *trans*-stilbene and glycoside form of stilbenes were effective than hydroxylated stilbene. Unlike resveratrol, piceatannol induced a decrease in adiponectin rather than an increase (Fig. 3B). This analog of silbene had only one more hydroxyl group in 3' position compared to resveratrol. However, the effect of piceatannol on adiponectin secretion was opposite of that of resveratrol.

Piceatannol was reported to be a selective inhibitor of signal transducers and activators of transcription (STAT) 3 (33). STAT3 is abundantly expressed in preadipocytes and adipocytes (34) and inhibition of endogenous STAT3 expression significantly decreased preadipocyte proliferation (35). And adipocyte STAT3 is essential for body weight homeostasis, and its deficiency causes higher body weight and increased adiposity (36). We speculated that this STAT3 inhibitory activity of piceatannol was responsible for the decreased adiponectin secretion in adipocytes treated with TNF-α.

Stilbenoids are naturally occurring compounds found in a wide range of plant sources. In the present study, we demonstrated that stilbenoids modulated TNF-α-induced increases in the secretion of PAI-1 as well as a decrease in adiponectin secretion in 3T3-L1 adipocytes. And these responses were shown differently according to their structure. Piceatannol effectively inhibited the increase of PAI-1 secretion by TNF-α, however it also repressed the secretion of adiponectin. Unlike this, *trans*-stilbene and rhaponticin showed modulating activity only for adiponectin secretion.

Increased PAI-1 level is shown in obesity and is associated with thrombotic disease (37). Adiponectin has both an insulin-sensitizing effect and anti-atherogenic effect (19). Given the many findings that adipokines are relevant to the pathology of obesity, much attention has been focused on the mechanism of regulation of these

adipokines' gene expression and secretion. Therefore, further work is still needed to identify the exact mechanism how stilbenoids modulate the adpokine secretion.

In conclusion, our results found stilbenoids showed different effect on TNF-α-induced changes in adipokines secretion according to their structure.

Acknowledgments

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