1,2-Vinyldithiin from Garlic Inhibits Differentiation and Inflammation of Human Preadipocytes1–3

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Abstract

Obesity is a state of chronic low-grade inflammation. Limiting white adipose tissue (WAT) expansion and therefore reducing inflammation could be effective in preventing the progression of obesity and the development of associated complications. We investigated the effects of 1,2-vinyldithiin (1,2-DT), a garlic-derived organosulfur, on the differentiation and inflammatory state of human preadipocytes. Preadipocytes were prepared from subcutaneous adipose tissue of nonobese young women and differentiated in the presence of 1,2-DT. Inflammatory preadipocytes were obtained following treatment with human macrophage-secreted factors. 1,2-DT (100 μmol/L) significantly reduced gene expression of PPARγ (−40%), CCAAT/enhancer binding protein-α (−25%), lipoprotein lipase (−22%), leptin (−30%), and adiponectin (−15%). Lipid accumulation was also significantly diminished in preadipocytes differentiated in the presence of 100 μmol/L 1,2-DT (−37%) compared with controls. Furthermore, 100 μmol/L 1,2-DT treatment for 10 d significantly reduced PPARγ activity (−27%). The protein expression of perilipin and the secretion levels for 2 adipokines, leptin and adiponectin, were significantly diminished in 1,2-DT-cultured preadipocytes (−37, −51, and −43%, respectively). Moreover, the secretion of inflammatory molecules (interleukin-6 and monocyte chemoattractant protein-1) induced by macrophage-secreted factors was partially abolished in 100 μmol/L 1,2-DT-treated preadipocytes (−28 and −25%, respectively). In conclusion, we demonstrated that 1,2-DT, a garlic-derived organosulfur, has antiadipogenic and antiinflammatory actions on human preadipocytes and may be a novel, antiobesity nutraceutical. J. Nutr. doi: 10.3945/ jn.109.105452.

Introduction

Garlic (Allium sativum) has been used for >5000 y, not only as a culinary spice, but also as a medicinal herb due to its antibacterial and antifungalic properties. Recently, there has been an increasing interest in the active constituents of garlic. Several studies have shown that garlic constituents have the potential to reduce the risk of cardiovascular diseases (1). Benefits associated with garlic extracts have been described in both lipid (2–4) and carbohydrate metabolism (5). Garlic extracts reduce hypertension (6,7) and have antithrombotic (8,9) and antiatherosclerotic properties (10).

Garlic contains numerous compounds, including organosulfurs. This includes 1,2-vinyldithiin (1,2-DT),9 a lipophilic component mainly found in oily macerates of crushed garlic, arising from the degradation of allicin (11). In contrast to the numerous studies on other garlic components, such as sulfides, very few studies have examined the biological properties of 1,2-DT. In addition to antioxidant properties (12), it was suggested that the vinyldithiin component could reduce cholesterolemia (13).

Obesity prevalence is increasing and is one of the main reasons for premature death in both industrialized and emerging societies. Obesity is a usual risk factor in the development of metabolic and cardiovascular disorders, including dyslipidemia, insulin resistance/type 2 diabetes, and atherosclerosis (14). Modulating fat mass expansion is thus an important challenge. In this context, adipocyte differentiation/adipogenesis is a

9 Abbreviations used: 1,2-DT, 1,2-vinyldithiin; C/EBP, CCAAT/enhancer binding protein; FBS, fetal bovine serum; IL, interleukin; LPL, lipoprotein lipase; MCP-1, monocyte chemoattractant protein-1; PLIN, perilipin; RPMI, Roswell Park Memorial Institute medium; WAT, white adipose tissue.
crucial process in the expansion of adipose tissue during the human life span and, consequently, in the development of obesity. Adipogenesis is the cellular transition of a fibroblastic cell, the preadipocyte, to a highly specialized cell that accumulates triglycerides, the adipocyte. Preadipocyte differentiation is under the control of 2 critical families of transcription factors, CCAAT/enhancer binding protein (C/EBP) and PPAR, specifically PPARγ (15). C/EBPβ is induced early during preadipocyte differentiation and is required for the expression of downstream adipogenic transcription factors PPARγ2 and C/EBPα. PPARγ2 is predominantly expressed in adipose tissue and constitutes the major regulator of adipogenesis by inducing anabolic processes such as triacylglycerol synthesis/lipid metabolism through the transcriptional induction of genes encoding proteins such as the lipoprotein lipase (LPL) or perilipin (PLIN). PPARγ2 and C/EBPα are thought to act synergistically to promote preadipocyte differentiation (16). During terminal differentiation, adipocytes secrete adipose-specific molecules, such as the adipokines leptin and adiponectin (17).

Obesity is also considered a chronic and low-grade inflammatory state (14,18). White adipose tissue (WAT) from obese subjects produces large quantities of inflammatory factors through the cells in the stroma vascular fraction, which includes macrophages (19,20). To elucidate the consequences of macrophage accumulation and inflammation on the biology of adipose tissue in obesity, our laboratory developed an experimental approach using primary culture of human preadipocytes and macrophages. We previously demonstrated that macrophage-secreted factors induce an inflammatory state in human preadipocytes characterized by an increased secretion of cytokines and chemokines [interleukin (IL)-6, IL-8, monocyte chemotactic protein-1 (MCP-1)] (21,22). We suggested that, in the context of obesity, the factors secreted by accumulated macrophages induced an inflammatory state in preadipocytes that could perpetuate inflammation in obese adipose tissue.

In this study, we investigated the effects of 1,2-DT on preadipocyte differentiation through the evaluation of lipid accumulation, gene and protein expression of adipose markers, PPARγ-DNA-binding activity, and adipokine secretion in human preadipocytes. We then evaluated the potential antiinflammatory effect of 1,2-DT by measuring the secretion of the cytokine IL-6 and the chemokine MCP-1 in preadipocytes treated with macrophage-secreted factors.

Materials and Methods

Compounds. 1,2-DT dissolved in dimethyl sulfoxide was supplied by BioXtract SA. Resveratrol dissolved in dimethyl sulfoxide was purchased from Sigma.

Isolation of human preadipocytes. Subcutaneous adipose tissue was obtained from nonobese (BMI <30 kg/m²), young women undergoing elective surgery. None had diabetes or metabolic disorders or were taking medication. Human preadipocytes were isolated and cultured as described (21,23,24).

Differentiation of human preadipocytes. Preadipocytes were seeded in DMEM-10% fetal bovine serum (FBS) during a 24-h plating. During the first 4 d, preadipocytes were cultured in serum-free media containing 50 mmol/L insulin, 100 mmol/L dexamethasone, 0.25 mmol/L isobutylmethylxanthine, and 100 mmol/L rosiglitazone. After 4 d, this medium was replaced with a serum-free medium that contained 50 mmol/L insulin, 100 mmol/L rosiglitazone, and 100 mmol/L dexamethasone. On d 10 (corresponding to complete in vitro preadipocyte differentiation), the majority of cells contained large lipid droplets (21).

Preparation of human macrophage-conditioned medium and inflammatory preadipocytes. We performed the isolation of plasma blood mononuclear cells from healthy women’s blood and induced their differentiation in macrophages cells (21,22). Macrophages were incubated in Roswell Park Memorial Institute medium (RPMI)-1% FBS for 5 h with 100 µg/L lipopolysaccharide (from Escherichia coli 0127:B8, Sigma) and then in fresh RPMI-1% FBS for 18 h prior to collecting the media (activated conditioned media). Control media consisted of RPMI-1% FBS kept at 37°C for 24 h in the absence of macrophages.

Inflammatory (activated conditioned media) preadipocytes and their secretion were prepared by treating cells with AcCM medium (21,22). The concentrations of 2 inflammatory cytokines, IL-6 and MCP-1, were markedly increased in AcCM preadipocytes compared with control (RPMI-1% FBS) cells (IL-6, 129.1 ± 47.2 vs. 8.4 ± 1.3 ng/mL; MCP-1, 144.1 ± 27.4 vs. 24.0 ± 7.5 ng/mL; n = 7) as expected.

RNA preparation and real-time PCR. RNA extraction and RT and real-time PCR were performed (21,22). Primers for the tested genes are listed in Supplemental Table 1. All values were normalized to 18S expression.

Nuclear extract preparation and measurement of PPARγ DNA-binding activity. Nuclear extracts were prepared as described previously (25). Protein concentration was measured using a modified Lowry assay (Pierce). DNA-binding activity of PPARγ in the nuclear extract was assessed using a TransAM PPARγ kit (Active Motif Europe).

Western blot analysis. Cell extracts were prepared in a buffer containing a cocktail of protease and phosphatase inhibitors. The membranes were probed with PLIN antibody (Abcam) or tubulin (Sigma-Aldrich) as an internal control. Specific signals were detected with enhanced chemiluminescence detection solution (GE Healthcare) and immediately exposed to X-ray film. Signals were quantified using densitometry.

Adipokine and inflammatory molecule secretion in culture media of preadipocytes. Adipokine (leptin and adiponectin) secretion was measured in culture media from preadipocytes treated with 100 µmol/L 1,2-DT for 10 d (Quantikine immunoassay human leptin and adiponectin, R&D Systems). Inflammatory molecule (IL-6 and MCP-1) secretions were measured in culture media from inflammatory (AcCM) preadipocytes treated with 100 µmol/L 1,2-DT for 10 d (Quantikine immunoassay human IL-6 and MCP-1, R&D Systems). The secretions were normalized to protein concentration determined as described above.

Ethical treatment. Informed personal consent from participants was obtained. This study was approved by the Ethical Committees of Hôtel-Dieu Hospital (Paris, France).

Statistical analysis. Data are presented as means ± SEM. The cell culture experiments were performed at least 2 times using preadipocytes from different people (n = 2) with 3 replicates for each treatment in each experiment. Statistical analysis was performed using Student’s t test for 2-group comparisons (P-values are provided). We found similar results using a nonparametric Wilcoxon’s ranked test. Comparisons between more than 2 groups were carried out using ANOVA with post hoc Student’s t tests. Differences were considered significant at P < 0.05.

Results

Alteration of adipose marker expression by 1,2-DT in human preadipocytes. 1,2-DT at 100 µmol/L (the maximal concentration used in this study) did not have a toxic effect on human preadipocytes and did not affect their proliferation (data not shown). First, we evaluated the effect of 1,2-DT, both at 10 µmol/L and 100 µmol/L concentrations, on gene expression of specific adipogenic markers in human preadipocytes. Gene expression for key transcription factors, PPARγ2 and C/EBPα, was significantly reduced in human preadipocytes differentiated...
for 10 d with 100 μmol/L 1,2-DT (−40 ± 10% and −25 ± 7%, respectively; P < 0.01) compared with control cells. 1,2-DT did not affect C/EBPβ expression (Fig. 1A). Similarly, leptin (−30 ± 11%) and adiponectin (−15 ± 6%) gene expression was also reduced (P < 0.05) with 100 μmol/L 1,2-DT treatment (Fig. 1B). In addition, we observed a reduction of LPL gene expression (−22 ± 5%; P < 0.001) in preadipocytes cultured in the presence of 100 μmol/L 1,2-DT (Fig. 1B). At a concentration of 10 μmol/L, 1,2-DT did not alter gene expression of these adipogenic markers. Thus, we decided to focus the study on the effect of 100 μmol/L 1,2-DT in human preadipocytes. Resveratrol (50 μmol/L) was shown to inhibit differentiation of human preadipocytes and thus we used it as a control (26). In our experimental conditions, a 10-d treatment with resveratrol (50 μmol/L) was shown to inhibit differentiation of human preadipocytes (26). In another set of experiments on new and different preadipocytes (n = 6), we found reduced gene expression of PPARγ2 (Fig. 2A) and LPL (Fig. 2B) when cells were exposed to 1,2-DT during the first 4 d (the early period of preadipocyte differentiation) (−17 ± 7%, P < 0.05; and −28 ± 6%, P < 0.01; respectively) and also during the last 5 d (the late period of preadipocyte differentiation) (−35 ± 4%; and −32 ± 7%, P < 0.001; respectively) of the culture period. A similar result was seen after 10 d treatment with 100 μmol/L 1,2-DT on PPARγ2 (−28 ± 7%) and LPL (−33 ± 9%) gene expression (P < 0.01), which confirms results of the first experiments.

As expected, control preadipocytes developed numerous large lipid droplets (Supplemental Fig. 2A) whereas preadipocytes differentiated for 10 d in the presence of 100 μmol/L 1,2-DT had fewer and smaller lipid droplets (Supplemental Fig. 2B). When quantifying the Oil red O staining, lipid accumulation decreased (−37 ± 18%; P < 0.05; n = 8) in preadipocytes cultured with 100 μmol/L 1,2-DT.

Reduction of PPARγ DNA-binding activity in nuclear extracts of 1,2-DT–treated preadipocytes. We observed reduced activity of PPARγ when cells were exposed to 100 μmol/L 1,2-DT during the first 4 d (−36 ± 11%; P < 0.05), the last 5 d (−40 ± 9%; P < 0.01), and the entire 10 d (−27 ± 4%; P < 0.001) of the culture period (Fig. 3).

Decrease of PLIN protein expression by 1,2-DT in human preadipocytes. Western blot analysis revealed decreased PLIN protein expression (−37 ± 5%; P < 0.001; n = 4) in human preadipocytes cultured for 10 d with 100 μmol/L 1,2-DT (Fig. 4). We also observed reduced immunoreactivity for PLIN and neutral lipid staining in 1,2-DT–treated preadipocytes.
without a common letter differ, 

Less IL-6 (P decreased secretion of leptin compared with control cells (2). AM PPAR control and 1,2-DT–treated preadipocytes was assessed using TransAM PPARy kit. Values are means + SEM, n = 4. Labeled means without a common letter differ, P < 0.05.

(Supplemental Fig. 3B) compared with control cells (Supplemental Fig. 3A).

**Diminished secretion of adipokines by human preadipocytes with 1,2-DT.** In agreement with the gene expression findings, preadipocytes treated with 100 μmol/L 1,2-DT had decreased secretion of leptin compared with control cells (−51 ± 9%; P < 0.001; n = 5) and adiponectin (−43 ± 8%; P < 0.001; n = 5).

**Reduction of inflammation by 1,2-DT in preadipocytes treated with macrophage-secreted factors.** We have previously demonstrated that when cultured with macrophage-secreted factors, human preadipocytes developed an inflammatory state through a notably higher secretion of cytokines and chemokines (21). Here, we found that when we treated preadipocytes simultaneously with macrophage-conditioned media and 100 μmol/L 1,2-DT for 10 d, these cells secreted less IL-6 (−28 ± 4%; P < 0.01) and MCP-1 (−25 ± 5%; P < 0.01) than inflammatory preadipocytes untreated with the garlic compound (n = 7).

**Accumulation of 1,2-DT only in WAT.** Considering the hydrophobic property of 1,2-DT, we hypothesized that this molecule concentrates in lipid droplets of adipocytes. To examine this, human WAT was incubated with 1,2-DT (100 μmol/L) for a 24-h period. 1,2-DT was mainly found in the lipid fraction of human WAT (40.58 ± 5.48 μg of 1,2-DT/g of WAT; n = 5; P < 0.0001) compared with control WAT cultured in the absence of 1,2-DT. The 1,2-DT accumulated in the lipid fraction of WAT represents 28.12 ± 3.8% of the initial dose incubated with human WAT explants. In contrast, 1,2-DT did not accumulate in human preadipocytes cultured for short (24 h) and long (10 d) periods, indicating the importance of lipid for 1,2-DT storage (data not shown).

**Discussion**

In this study, we tested the effects of 1,2-DT, a garlic-derived organosulfur, on human preadipocyte differentiation. The effects of phytochemical components on preadipocyte differentiation have been previously studied in murine models, especially the commonly used 3T3-L1 cell lines; there appear to be marked differences in adipogenesis between human and murine systems. Adipocyte secretion is notably different between human and 3T3-L1 adipocytes. For example, resistin, an adipokine first identified in 3T3-L1 cells, has increased expression during differentiation of murine preadipocytes but a decrease in human cells (27,28). Therefore, a human preadipocyte model would appear to be a more physiologically relevant model to study the biological effects of nutritional compounds on preadipocyte differentiation.

In contrast to other garlic constituents like sulfides, there have been few studies examining the beneficial effects of 1,2-DT. Here, we identify a new effect of this component in reducing the differentiation of human preadipocytes into adipocytes. Preadipocytes cultured in the presence of 100 μmol/L 1,2-DT had a significant decrease in adipose marker gene expression and lipid accumulation. A significant reduction in the expression of the 2 major adipogenic transcription factors, PPARγ2 and C/EBPα, was observed in 1,2-DT–treated preadipocytes, whereas C/EBPβ expression, acting upstream of PPARγ2 and C/EBPα, was unaffected. The 1,2-DT–mediated decrease in PPARγ2 expression is associated with reduced PPARγ activity, suggesting that the negative effect of 1,2-DT on preadipocyte differentiation could be mainly due to an inhibitory effect on PPARγ2, the master regulator of adipogenesis.

PPARγ2 and C/EBPα both play key roles in preadipocyte differentiation by synergistically activating the downstream adipospecific expression of genes involved in glucose and lipid metabolism (16). To examine whether the inhibition of preadipocyte differentiation by 1,2-DT may be mediated through down-regulation of PPARγ2/C/EBPα, we also studied the effects of 1,2-DT on the expression of PPARγ2/C/EBPα gene targets, finding that preadipocyte treatment with 1,2-DT reduced expression of LPL, an enzyme controlling fatty acid storage in adipocytes, and of PLIN, a protein that coats lipid droplets and plays a role in the regulation of lipolysis (29). Leptin and adiponectin expression and secretion were also diminished by 1,2-DT treatment. Therefore, our results indicate that 1,2-DT could alter the in vitro formation of adipocytes through reduced PPARγ2 expression and activity. The precise molecular mechanistic action of 1,2-DT still remains to be elucidated.

The in vitro differentiation of human preadipocytes is estimated to take ~10–12 d. In this study, we have shown that 1,2-DT inhibited gene expression of PPARγ2 and its target gene LPL regardless of the period of exposure.

In a previous set of experiments, we also studied the gene expression of several adipose markers during human preadipocyte differentiation (Supplemental Fig. 4), finding that the gene expression pattern in human preadipocytes during their differentiation is very different from the pattern in established cell lines of preadipocytes. PPARγ gene expression increases progressively during the early period of differentiation and continues to increase during the late period. Gene expression of C/
EBPa also increases during the early period until reaching a peak at d 7 and a plateau from d 7 to 10 (Supplemental Fig. 4A). Gene expression of LPL, one of the PPARγ target genes, also increases during preadipocyte differentiation (Supplemental Fig. 4B). We speculate that because PPARγ2 expression increases during preadipocyte differentiation, gene expression could be altered by active compounds at all times of exposure, as that of its target genes like LPL.

There is growing interest in the use of phytochemical compounds as medicinal alternatives due to their lack of toxicity and the relative ease and cost of production. However, their molecular basis and mechanism of action remain to be determined. A large number of studies have described the effects of several phytochemical components on fat cell formation. Some of them have demonstrated an inhibitory effect on adipocyte differentiation, similar to what our data has shown with 1,2-DT. For example, pine needle extract, which is traditionally consumed in East Asia, suppressed the differentiation of 3T3-L1 cells in part by downregulating gene expression of PPARγ (30). Flavonoids and, notably, genistein (a soy isoflavone) inhibited adipocyte formation by decreasing the expression and activation of C/EBPβ and downstream adipogenic transcription factors like PPARγ. Moreover, this compound exhibited strong lipolytic properties (31,32). Docosahexaenoic acid, a fatty acid in fish oil, has antiadipogenic effects because it suppresses lipid accumulation and increases basal lipolysis in 3T3-L1 adipocytes (33). Ginsenoside Rb1 extracted from ginseng enhances PPARγ2 and C/EBPα gene expression by acting on their promoter and thus increasing adipogenesis (34). Another constituent derived from garlic, DADS, was also shown to accelerate differentiation of 3T3-L1 cells via PPARγ activation (35). All these studies have been conducted in murine cell lines and their biological effect is thus unknown in human preadipocytes and adipocytes. The originality of our study is the observation that 1,2-DT exerts an antiadipogenic effect in human primary preadipocytes. Recent studies demonstrated the antiadipogenic effects of genistein and combination of genistein, quercetin and resveratrol in primary human cells (26,36). It should be emphasized that unlike other natural components such as flavonoids, 1,2-DT did not influence the lipolytic activity of human mature adipocytes (data not shown).

Previous results from our laboratory have shown that the presence of an inflammatory microenvironment, i.e. human macrophage-secreted factors, causes preadipocytes to exhibit a proinflammatory state (21,22). We verified that 1,2-DT did not induce a proinflammatory phenotype in human preadipocytes (data not shown). Rather, we reveal that 1,2-DT reduces the secretion of IL-6 and MCP-1, 2 molecules highly produced by human preadipocytes treated with macrophage factors. Increased production of inflammatory factors from WAT appears to underlie the link between obesity and its associated metabolic complications. In fact, the cytokine IL-6 and the chemokine MCP-1, whose plasma levels increase in obese individuals, are associated with the metabolic complications of obesity (37–39). In addition, MCP-1 is implicated in macrophage accumulation in WAT from obese rodents (40,41). The results of our study suggest that 1,2-DT could help to limit inflammation and macrophage accumulation in human obese WAT and thus the development of metabolic disorders associated with obesity.

In this study, 1,2-DT exerted antiadipogenic and antiinflammatory actions on human preadipocytes at a concentration of 100 μmol/L. 1,2-DT is only present in garlic oily macerates and not in fresh garlic, but a plasmatic concentration of 100 μmol/L (72 mg of 1,2-DT diluted in 5 L of plasma) can be considered equivalent to 170 g of fresh garlic extracted by oily maceration. Interestingly, WAT is an important site of storage for hydrophobic molecules such as steroid hormones and cholesterol. Here, we observed that 1,2-DT, a lipophilic compound, significantly accumulated in the lipid fraction of human WAT and not in human preadipocytes, thus revealing the importance of lipid droplets in the accumulation of this compound in WAT. This confirms the results of a previous in vivo study showing an accumulation of 1,2-DT in rodent fat tissue after oral ingestion (11).

Thus, we propose that due to its capacity to accumulate in human WAT, the biological action of 1,2-DT on human preadipocytes could be accentuated and the amount required to observe its effects could be reduced, thus decreasing the risk of possible toxicity of the compound. Furthermore, it is possible that the beneficial effect of 1,2-DT in vivo might be observed at lower concentrations than those used in vitro.

However, a release of the 1,2-DT accumulated in the lipid fraction of WAT is necessary. Thus, an association of the 1,2-DT to a phytochemical compound with lipolytic activity, such as the resveratrol or ajoene, another constituent derived from garlic, could be proposed to increase the bioavailability of 1,2-DT within WAT. However, the investigation of an absence of possible interactions between these compounds is required.

In conclusion, we have demonstrated for the first time, to our knowledge, that 1,2-DT, a garlic-derived organosulfur, inhibits human preadipocyte differentiation and has antiinflammatory properties. As a consequence, 1,2-DT could constitute an original dietary supplement in the treatment of obesity and its associated pathologies by limiting the expansion and inflammation of human WAT.

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Literature Cited


