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Inhibitory effect of sinigrin on adipocyte differentiation in 3T3-L1 cells: Involvement of AMPK and MAPK pathways☆

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ABSTRACT

Adipocyte differentiation is a critical adaptive response to nutritional overload and affects the metabolic outcome of obesity. Sinigrin (2-propenyl glucosinolate) is a glucosinolate belong to the glucoside contained in broccoli, brussels sprouts, and black mustard seeds. We investigated the effects of sinigrin on adipogenesis in 3T3-L1 preadipocytes and its underlying mechanisms. Sinigrin remarkably inhibited the accumulation of lipid droplets and adipogenesis by downregulating the expression of CCAAT-enhancer-binding protein α (C/EBPα), peroxisome proliferator-activated receptor gamma (PPARγ), leptin and aP2. Sinigrin arrested cells in the G_0/G_1 phase of the cell cycle and increased the expression of p21 and p27. CDK2 expression was suppressed by sinigirn in MDI-induced adipocytes. Sinigrin increased the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK) and acetyl–CoA carboxylase (ACC) in the early stage of adipocyte differentiation, suggesting that sinigrin has anti-adipogenic effects through AMPK, MAPK and ACC activation. Sinigrin also inhibited the production of pro-inflammatory cytokines including tumor necrosis factor –alpha (TNF-α) and interleukin (IL)-6, IL-1β and IL-18. Taken together, these data suggest that sinigrin inhibits early-stage adipogenesis of 3T3-L1 adipocytes through the AMPK and MAPK signaling pathways.

1. Introduction

Obesity is an important risk factor for various pathological diseases including type II diabetes, hypertension and cardiovascular disease. Appropriately, obesity has become the focus of research directed at chronic diseases [1]. The development of obesity is characterized by increased number and size of differentiated adipocytes [2]. Adipocytes play a critical role in the regulation of adipose tissue and lipid homeostasis related to obesity. Differentiation of preadipocytes to adipocytes is a multi-step process accompanied by the regulation of various transcription factors, including CCAAT-enhancer-binding protein (C/EBP)α, C/EBPβ, C/EBPδ and peroxisome proliferator-activated receptor gamma (PPARγ), which are responsible for the expression of adipogenesis-related genes [3]. Lipid droplets in adipocytes are important role in lipid metabolism and regulation, and lipid accumulation regulates triglyceride synthesis. Inhibition of the adipocyte differentiation from preadipocytes and the release of glycerol following the breakdown of triglycerides present in the lipids are important for the prevention and management of obesity.

AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase consisting of a catalytic α subunit and two regulatory subunits β, and γ. AMPK mediates the synthesis and degradation of fatty acids as a regulatory sensor of cellular energy metabolism, and is critical role in maintaining energy homeostasis in the body [4]. When AMPK is activated, AMPK phosphorylates target proteins for the metabolism of carbohydrate and lipids. AMPK inactivates acetyl-CoA carboxylase (ACC), which is a downstream substrate of AMPK and an essential enzyme for lipid biosynthesis [5,6]. Activated AMPK attenuates lipid accumulation during adipogenesis by inhibiting the expression of sterol regulatory element binding protein-1 (SREBP-1), c/EBPα, PPARγ and fatty acid synthase (FAS) [7]. In addition to AMPK, mitogen activated protein kinase (MAPK) also appears to be crucial in adipogenesis [8]. Therefore, AMPK could be considered as a target for treatment of obesity.

There has been increasing interest in the search for bioactive compounds from natural sources that act to prevent various chronic diseases and to improve health. Many pharmacological approaches have been examined to assess whether diverse plants and their components inhibit adipogenesis and lipid accumulation. Sinigrin (2-propenyl glucosinolate) has potent anti-oxidant, anti-tumor and anti-inflammatory

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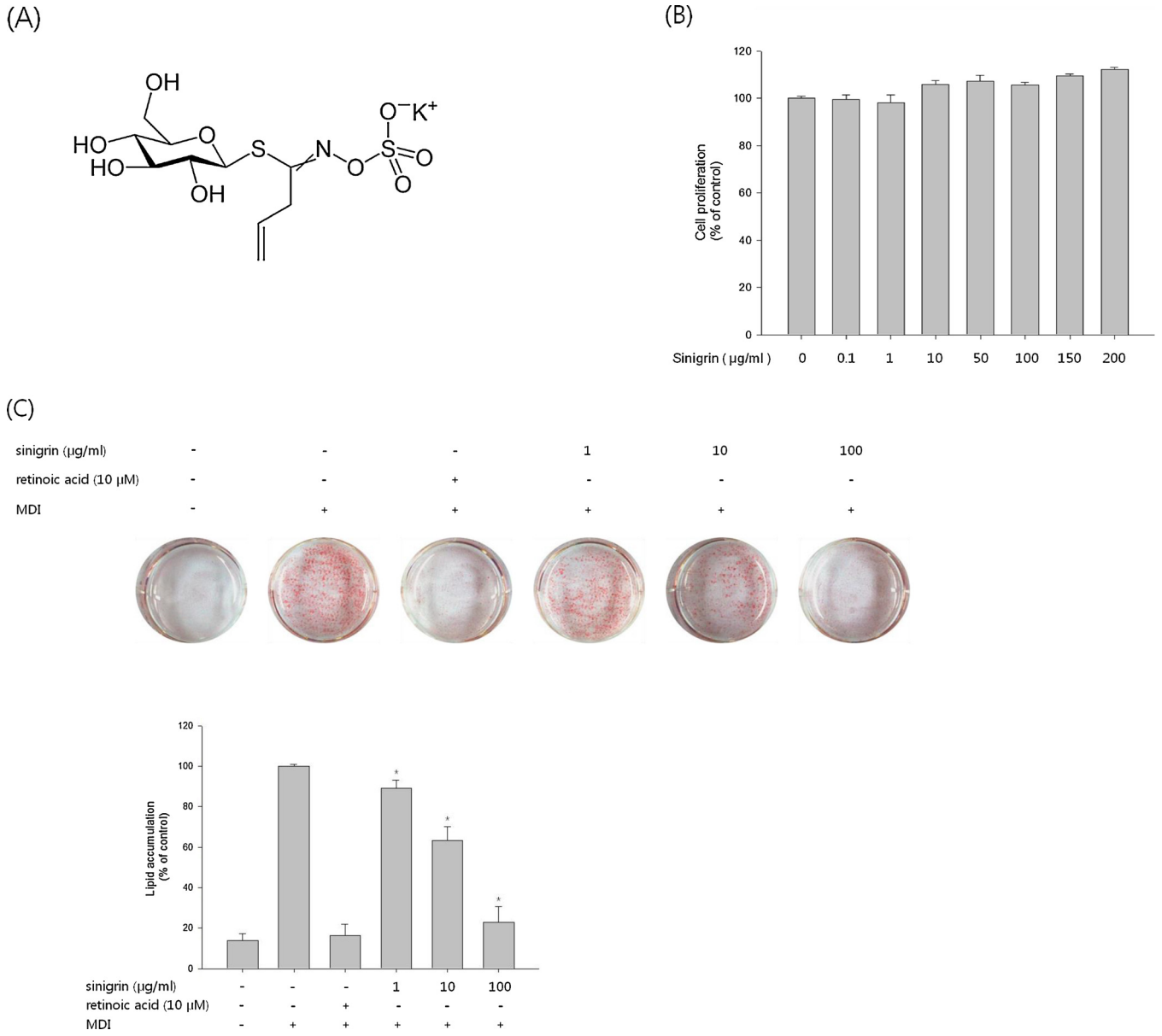

Fig. 1. Effect of sinigrin on cell proliferation and lipid accumulation in 3T3-L1 cells.
(A) Chemical structure of sinigrin hydrate. (B) 3T3-L1 cells were incubated in the absence and presence (1, 10, and 100 μg/mL) of sinigrin for 48 h. Cell proliferation was assessed by the MTT assay. (C) Confluent 3T3-L1cells were differentiated in the absence or presence of the indicated concentrations of sinigrin for 8 days. Lipid droplets were measured by Oil Red O staining. The results are the mean ± SEM of five replications from a representative experiment. *$p < 0.05$, significantly different from the MDI-treated group.

effects. It is a phytochemical found in the Brassicaceae family including brussels sprouts, broccoli and the seeds of black mustard [9–11]. In preliminary experiments, we showed that exposure of preadipocytes to sinigrin can induce significant changes in the expression of adipogenesis-related genes. However, the inhibitory effects of sinigrin on the function of adipocytes and related molecular mechanisms remained unclear.

The aim of this study was to investigate the effects of sinigrin on adipogenesis using mouse 3T3-L1 cells and the underlying mechanism. The data indicate that sinigrin inhibits adipogenesis-related gene expression and the production of pro-inflammatory cytokines by suppressing the MAPK and AMPK signaling pathways.

2. Materials and methods

2.1. Materials

All chemicals including isobutyl-3-methylxanthine (IBMX), were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise stated. Sinigrin hydrate was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Lipofectamine Plus, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and bovine calf serum (BCS) were purchased from Life Technologies, Inc. (Carlsbad, CA, USA). Antibodies against extracellular signal-regulated kinase (ERK) 1/2, phospho-ERK 1/2, p38, phospho-p38, C-Jun N-terminal kinase (JNK), phospho-JNK, p21, p27, cyclin-dependent kinases (CDK) 2, interleukin (IL)-1β, IL-18 and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). AMPK, phospho-AMPK, ACC, phospho-ACC, FAS, aP2, tumor necrosis factor-alpha (TNF-α) and IL-6