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Original article

Osthole attenuates lipid accumulation, regulates the expression of inflammatory mediators, and increases antioxidants in FL83B cells

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ABSTRACT

Osthole is found in *Cnidium monnieri* (L.) and has anti-inflammatory and anti-oxidative properties. It also inhibits the proliferation of hepatocellular carcinoma cells. This study aimed to evaluate the osthole suppressive nonalcoholic fatty liver disease effects in oleic acid (OA)-induced hepatic steatosis and if it can modulate inflammatory responses and oxidative stress. FL83B cells were pretreated with OA (250 μM) for 24 h, and then added different concentrations of osthole (3–100 μM) for 24 h. Subsequently, lipolysis and transcription factors of adipogenesis and phosphorylation of AMP-activated protein kinase proteins were measured. In addition, cells with OA-induced steatosis were H_2O_2–stimulated, and then incubated with osthole to evaluated if it could suppress its progression to steatohepatitis. Osthole significantly enhanced glycerol release and lipolysis protein expression. Osthole also promoted phosphorylation of AMP-activated protein kinases and increased the activity of triglyceride lipase and hormone- sensitive lipase. Osthole suppressed the nuclear transcription factor kappa-B and the p38 mitogen-activated protein kinase pathway, and decreased the malondialdehyde concentration in FL83B cells with OA-induced steatosis that were treated with H_2O_2. These results suggest that osthole might suppress nonalcoholic fatty liver disease by decreasing lipid accumulation, and through its anti-oxidative and anti-inflammatory effects via blocked NF-κB and MAPK signaling pathways.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a serious and widespread metabolic disorder and encompasses a wide spectrum of liver conditions ranging from simple steatosis, to steatohepatitis and fibrosis, and end-stage liver diseases, including cirrhosis and hepatocellular carcinoma [1,2]. Many recent studies have found that NAFLD is associated with serious cardiometabolic abnormalities, including type 2 diabetes mellitus, metabolic syndrome, obesity, and dysregulated insulin action in the liver [3].

The hallmark of NAFLD is excessive lipid accumulation in the liver, mainly triacylglycerol, in the absence of significant ethanol consumption. This accumulation interferes with the signaling pathways involved in the normal metabolism of hepatocytes, causes insulin resistance, and may even lead to metabolic syndrome abnormalities [4]. Hepatic lipid accumulation results from an imbalance between lipid availability and lipid disposal, and eventually triggers lipid peroxidative stress and hepatic injury [5]. Current treatments for patients with fatty liver disease include change of lifestyle and improvement of insulin sensitivity to alleviate the associated metabolic syndrome [6]. The AMP-activated protein kinase (AMPK) pathway is important in lipid metabolism. Studies indicated that phosphorylated acetyl-CoA carboxylase-1 (ACC-1) activated by phosphorylation of AMPK,

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which can suppress acetyl-CoA catalyze to malonyl-CoA during fatty acid (FA) and triglyceride synthesis [7–9].

Osthole, 7-methoxy-8-(3-methyl-2-butenyl) coumarin, is an active constituent of *Cnidium monnieri* (L.) that has been extracted from many medicinal plants. Osthole has long been used in traditional Chinese medicine for the treatment of eczema, cutaneous pruritus, *Trichomonas vaginalis* infection, and sexual dysfunction [10]. Recent studies have revealed that osthole may have anti-proliferative, vasorelaxant, anti-inflammatory, anti-microbacterial, anti-allergic, and prophylactic effects in hepatitis [11]. Furthermore, anti-cancer effects have been reported for osthole [12–14]. However, the effects of osthole on lipid metabolism and anti-oxidation remain unclear. A few experiments indicated that osthole treatment attenuated liver steatosis by decreasing triglyceride synthesis and had nominal effects on insulin resistance and liver inflammation in histological sections and an animal model [15–17]. The aims of the present study were to examine osthole-induced AMPK signaling, the potential mechanisms for controlling hepatocellular lipid metabolism, and to test the effects of osthole on intrahepatic fatty acid synthesis and inflammation.

2. Materials and methods

2.1. Materials

Osthole (Fig. 1A) purchased from the ChromaDex (Irvine, CA, USA). The purity was >97.9% as determined by HPLC. Stock solution (100 mM) prepared by dissolving osthole in DMSO and stored at −20 °C, as previously described [10]. The final concentration in culture medium of DMSO was ≤0.1%.

2.2. Cell line and treatment

FL83B cells purchased from the Bioresource Collection and Research Center (BCRC, Taiwan). Cells were cultured in F12 medium (Invitrogen-Gibco™, Paisley, Scotland) added with 10% FBS, 2 mM glutamine, 1% penicillin and streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Cells exposed to oleic acid (OA) 250 μM for 24 h, then treated with osthole (3–100 μM) in OA microenvironment for 24 h.

2.3. Cell viability assay

Using MTT assay performed to evaluate the cytotoxicity of osthole. Cells were plated in 96-well plates cultured overnight and treated with osthole for 24 h. The MTT solution (5 mg/ml) was added to each well and incubated for 4 h at 37 °C, followed by isopropanol dissolution of formazan crystals. The OD absorbance was spectrophotometrically measured at 570 nm using a microplate reader (Gene5, Synergy HT, BioTek, USA).

2.4. Oil red O staining

FL83B cells treated drug in 6-well plates for 24 h and fixed using 10% formalin for 30 min. Cells stained with oil red O (Sigma Chemical, St. Louis, MO, USA) for 1 h at room temperature. To remove excess dye cells were washed three times with 1 mL of PBS and dipped in 100% isopropanol. The OD value was measured at 490 nm using a microplate reader (Gene5, Synergy HT, BioTek, USA) to quantify the lipid accumulation.

2.5. Measurement of glycerol production

FL83B cells treated with osthole in OA microenvironment for 24 h, and glycerol levels of culture medium quantified using glycerol quantification kit (GPO-Trinder) according to the manufacturer's instructions (Sigma-Aldrich). The results quantified at 570 nm using a microplate reader (Gene5, Synergy HT, BioTek, USA).

2.6. Antioxidant assay: malondialdehyde concentration and superoxide dismutase activity

FL83B cells treated with OA for 24 h and H_2O_2 (500 μM) for 2 h; then, the cells treated with osthole for 24 h. Superoxide dismutase (SOD) and malondialdehyde (MDA) measured according to the commercial kit instructions (Sigma-Aldrich), and the absorbance at 450 and 532 nm with a spectrophotometer, respectively.

2.7. Preparation of total and nuclear proteins

FL83B cells treated with OA for 24 h and stimulated with H_2O_2 for 2 h. Then, cells treated with osthole in 6 well plates. Cells were harvested with 300 μL protein lysis buffer (150 mM NaCl, 1 mM EDTA, 1 mM DTT, 50 mM Tris–HCl, pH 7.4, 0.5% NP40, and 0.1% sodium dodecyl sulfate [SDS]) containing protease inhibitor cocktail and phosphatase inhibitors (Sigma). Cytoplasm and nuclear proteins were using the NE-PER® extraction reagent kits (Pierce, Rockford, IL, USA). Proteins quantitated using BCA assay kit (Pierce)[18].

2.8. Western blot analysis

An equal amount of protein was separated on polyacrylamide gels and transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). Membranes were incubated with different primary antibodies for phosphorylated acetyl-CoA

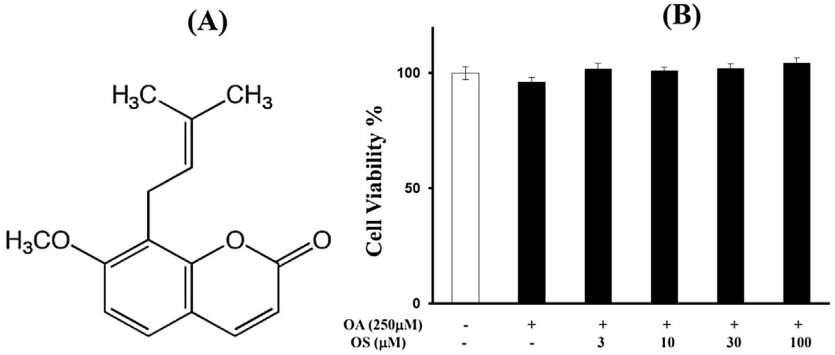

Fig. 1. Structures of osthole (A) and the cytotoxicity of osthole in FL83B cells (B).