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Emodin improves lipid and glucose metabolism in high fat diet-induced obese mice through regulating SREBP pathway

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ABSTRACT

Currently, obesity has become a worldwide epidemic associated with Type 2 diabetes, dyslipidemia, cardiovascular disease and chronic metabolic diseases. Emodin is one of the active anthraquinone derivatives from Rheum palmatum and some other Chinese herbs with anti-inflammatory, anticancer and hepatoprotective properties. In the present study, we investigated the anti-obesity effects of emodin in obese mice and explore its potential pharmacological mechanisms. Male C57BL/6 mice were fed with high-fat diet for 12 weeks to induce obesity. Then the obese mice were divided into four groups randomly, HFD or emodin (40 mg/kg/day and 80 mg/kg/day) or lovastatin (30 mg/kg/ day) for another 6 weeks. Body weight and food intake were recorded every week. At the end of the treatment, the fasting blood glucose, glucose and insulin tolerance test, serum and hepatic lipid levels were assayed. The gene expressions of liver and adipose tissues were analyzed with a quantitative PCR assay. Here, we found that emodin inhibited sterol regulatory element-binding proteins (SREBPs) transactivity in huh7 cell line. Furthermore, emodin (80 mg/kg/day) treatment blocked body weight gain, decreased blood lipids, hepatic cholesterol and triglyceride content, ameliorated insulin sensitivity, and reduced the size of white and brown adipocytes. Consistently, SREBP-1 and SREBP-2 mRNA levels were significantly reduced in the liver and adipose tissue after emodin treatment. These data demonstrated that emodin could improve high-fat diet-induced obesity and associated metabolic disturbances. The underlying mechanism is probably associated with regulating SREBP pathway.

1. Introduction

Obesity has become a worldwide epidemic with the increasing westernization of food habits and lifestyle. The World Health Organization estimates that up to 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese (WHO, 2013). There is compelling evidence to suggest that obesity is associated with Type 2 diabetes (Haslam and James, 2005), dyslipidemia (Fried et al., 2008), cardiovascular disease and chronic metabolic diseases (Haffner, 2007); James et al., 2004). The

Abbreviations: ACC, acetyl-Coenzyme A carboxylase; ACL, ATP citrate lyase; ACS, acyl-CoA synthetase; AMPK, AMP-activated protein kinase; BAT, brown adipose tissue; WAT, white adipose tissue; DHCR7, 7-dehydrocholesterol reductase; DHCR24, 24-dehydrocholesterol reductase; ERS, endoplasmic reticulum stress; FADS, fatty acid desaturase; FAS, fatty acid synthase; FDPS, farnesyl diphosphate synthetase; FDFT, farnesyl diphosphate farnesyl transferase; GPAT, glycerol-3- phosphate acyltransferase; HDL-c, high-density lipoprotein cholesterol; LDL-c, serum low-density cholesterol; TC, total cholesterol; TG, triglyceride; HFD, high-fat diet; HL, hepatic lipase; LPL, lipoprotein lipase; ApoE, Apolipoprotein E; ApoB, Apolipoprotein B; UCP, uncoupling protein; SR-BI, scavenger receptor class B, member 1; MVK, mevalonate kinase; LPDS, lipoprotein deficient serum; PCSK9, proprotein convertase subtilisin/kexin type 9; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGCR, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase; HMGCS, 3-hydroxy-3- methylglutaryl-Coenzyme A synthase; LSS, lanosterol synthase; SREBPs, sterol regulatory element-binding proteins; SCD, stearoyl-Coenzyme A desaturase; Sc4mol, sterol-C4-methyl oxidase-like; SE, squalene epoxidase

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current methods for the treatment of overweight and obese people include diet, exercise and drug therapy (Kremers et al., 2010). However, a lot of anti-obesity drugs have been withdrawn from the market because of various serious adverse effects throughout the past few years (Greenway and Caruso, 2005). Nowadays, orlistat is the only anti-obesity drug approved by the US Food and Drug Administration (FDA) for long-term use (Giuseppe and Pamela, 2012). Therefore, new therapeutic strategies with less significant side effects are urgently needed.

Sterol regulatory element-binding proteins(SREBPs) is a well-known nuclear transcription factors involved in the biosynthesis of cholesterol, fatty acid, and triglyceride in mammals (Goldstein et al., 2006). In particular, SREBP has been shown to play a critical role in synthesizing of lipids, suggesting that inhibition of SREBP pathway might be a potential approach to treat obesity and related metabolic diseases, such as Type 2 diabetes and insulin resistance (Taskinen, 2003; Xiao and Song, 2013).

Emodin (6-methyl-1,3,8-trihydroxyanthraquinone) is a major anthraquinone derivative that obtained from *Rheum palmatum* and some other Chinese herbs (Shang and Yuan, 2002), which possess anti-inflammatory (Lin et al., 2012), anticancer and hepatoprotective activities (Chang et al., 1996; Lin et al., 1996). Previous studies have shown that emodin decreased serum activities of ALT and blood lipids in high caloric laboratory chaw-induced nonalcoholic fatty liver (Dong et al., 2005), and significantly reduced blood glucose in streptozotocin (STZ)-induced diabetic cardiomyopathy (Wu et al., 2014). It has also been reported that emodin attenuated lipid accumulation in white adipose tissue through AMP-activated protein kinase activation (Tzeng et al., 2012). However, the anti-obesity effect and the underlying mechanism of emodin on high-fat diet induced obesity mice have not been fully explored.

In the present study, we have developed emodin as an inhibitor of SREBP pathway through reporter gene assay and target gene assay in vitro. Then we evaluated whether emodin played a role in treatment of obesity and associated metabolic diseases by using the HFD-induced obese mouse model and explored its molecular mechanism. In addition, the anti-obesity effect of emodin was compared with lovastatin which is the inhibitor of HMGCR and effectively reduces the serum cholesterol level through inhibition of cholesterol synthesis. Here, we reported that emodin might protect against diet-induced obesity and associated metabolic disturbances through the regulation of SREBP pathway.

2. Materials and methods

2.1. Generation of Huh-7/SRE-Luc and reporter gene assays

The generation of Huh-7/SRE-Luc cells and reporter assay was conducted as previously described (Tang et al., 2011). Huh-7/SRE-Luc cells were depleted of sterols by incubatin for 16 h in medium containing lipoprotein deficient serum (LPDS). Thus, the sterol-depletion treatment will induce the maximal activity of SREBP. Then cells were incubated individually with emodin (C15H10O5, MW 270.23, HPLC $\geq$ 98%) which was purchased from Shanghai R&D Center for Standardization of Traditional Chinese Medicine (Shanghai, China) at concentrations of 1, 20, 40 and 80 μM in 96-well plates. The luciferase activity was measured after 16 h treatment and the intensity of EGFP was also measured as the internal control.

2.2. Cell culture and viability assay

Huh-7 cells were purchased from ATCC (Manassas, VA, USA), maintaining in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) with 100 units/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere containing 5% CO_2 at 37 °C. The subsequent experiments were performed when cells were in good condition. Huh-7 cells were plated at a density of 1×10^4 cells per well in 96-well plates, then incubated until 60%–70% confluency. When confluent, cells were treated with emodin at concentrations of 1, 20, 40 and 80 μM. After drug treatment for 24 h, 10 μl of CCK-8 solution was added to each well and incubated at 37 °C for another 1 h. The absorbance of the solutions was detected at 450 nm by a microplate reader (ThermoSci Varioskan Flash, USA). The cell viability rate was calculated as the percentage of CCK-8 absorption as follows: (absorbance of drug-treated sample/absorbance of control sample) × 100%.

2.3. Animals and treatment

The animal protocols used in this study were approved by Shanghai University of Traditional Chinese Medicine. Male C57BL/6 mice were purchased from the SLAC Laboratory (Shanghai,China) and caged individually in a 12:12-hours light/dark cycle, temperature- and humidity-controlled environment.

Six-week-old C57BL/6 mice were fed with high-fat diet (HFD, 60% calories from fat, 20% calories from protein, 20% calories from carbohydrate; Research Diets, D12492) for 12 weeks to induce obesity. The obese animals were then randomly separated into obesity model ($n=8$), low-dose emodin treatment (40 mg/kg, n=8, dissolved in 0.1% (w/v) carboxymethyl cellulose-Na (CMC-Na); purity > 98%, HPLC), high-dose emodin treatment (80 mg/kg, $n=8$) and lovastatin treatment (30 mg/kg, n=8) group. The mice in both normal control and obesity model groups were given the same volume of 0.1% (w/v) CMC-Na. Mice were administered by gavage at the same time in each day for 6 weeks. During the gavage period, mice were continually given free access to water and normal diet (10% calories from fat, 20% calories from protein, 70% calories from carbohydrate; Research Diets, D12450B) for normal control mice or high-fat diet for the mice in model and drug treated groups. Body weight and food intake were recorded every week.

2.4. Biochemical analysis

Serum total cholesterol (TC), triglyceride (TG), HDL cholesterol (HDL-c), and LDL cholesterol (LDL-c) levels were measured with commercially available test kits (Jiancheng, China), fasting glucose level were assayed with the One touch Ultra blood glucose monitoring system (Roche). Liver tissues were homogenized in lysis buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl, 1% Triton) and extracted with an equal volume of chloroform. The chloroform layers were dried and dissolved in isopropyl alcohol to measure lipid levels as described (Zang et al., 2006).

2.5. Intraperitoneal glucose tolerance and insulin tolerance tests

All the mice were fasted overnight after 6 weeks treatment. All animals were intraperitoneally injected with either 1 g/kg glucose or 0.75 U/kg insulin (Sigma) on the third day before they were killed. Glucose levels were measured from tail blood before and 15, 30, 60, or 120 min after the injection with the One touch Ultra blood glucose monitoring system (Roche). All animals were killed 3 days after glucose tolerance or insulin tolerance tests, and blood and liver were harvested. Plasma insulin levels were measured with the Mouse Insulin Elisa kit (Millipore) according to the manufacturer's instructions. The curve of blood glucose concentration and time was plotted and the area under circle (AUC) was calculated according to the formula:

$AUC_{0\text{-}2\ h} = [(G_{0h}+G_{0.25h}) \times 0.25\ h + (G_{0.25h}+G_{0.5h}) \times 0.25\ h + (G_{0.5h}+G_{1h}) \times 0.5\ h + (G_{1h}+G_{2h}) \times 1\ h]/2$where 'G' is the blood