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Emodin ameliorates high-fat-diet induced insulin resistance in rats by reducing lipid accumulation in skeletal muscle

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ABSTRACT

Emodin, an anthraquinone derivative isolated from root and rhizome of Rheum palmatum, has been reported to have promising anti-diabetic activity. The present study was to explore the possible mechanism of emodin to ameliorate insulin resistance. Insulin resistance was induced by feeding a high fat diet to Sprague-Dawley rats. The blood glucose and lipid profiles in serum were measured by an enzymatic method, and a hyperinsulinaemic-euglycaemic clamp was used to evaluate insulin resistance. L6 cells were cultured and treated with palmitic acid and emodin. The lipid content was assayed in the soleus muscle and L6 cells by Oil Red O staining. Western blot, qRT-PCR, and immunohistochemical staining were used to detect the following in the rat soleus muscle and L6 cells: protein levels, mRNA levels of FATP1, FATP4, transporter fatty acid translocase (FAT/CD36), and plasma membrane-associated fatty acid protein (FABPpm). We found that blood glucose, triglyceride (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were significantly decreased in the emodin group. Oil Red O staining and the level of TG in skeletal muscle and L6 cells confirmed that lipid deposition decreased after treatment with emodin. Furthermore, the protein levels and mRNA levels of FATP1 in skeletal muscle and in L6 cells of rats were significantly decreased, yet the protein levels and mRNA levels of FATP4, FAT/CD36 and FABPpm did not drop off significantly. The study suggest that emodin ameliorates insulin resistance by reducing FATP1-mediated skeletal muscle lipid accumulation in rats fed a high fat diet.

1. Introduction

Insulin resistance (IR) in skeletal muscle plays a major role in the development of type 2 diabetes and the state happens long before the hyperglycemia becomes evident (DeFronzo, 1988). Numerous factors have been implicated in the process, including insulin signaling mechanisms (Lee-Young et al., 2013; Yang et al., 2014) and fatty acid (Holloway et al., 2011). Previous studies have demonstrated a strong inverse correlation between intramuscular lipid content and insulin sensitivity in both animals and humans (Dziewulska et al., 2012; Shepherd et al., 2014; van Hees et al., 2011). Increasing fatty acid influx into skeletal muscle or reducing fatty acid oxidative metabolism both cause insulin resistance. Skeletal muscle uptake of long-chain fatty acid (LCFA) is a key step within fatty acid metabolism, as it is used to synthesize neutral lipids and act as substrate for the participation of β-oxidation. There are four transport proteins that exert transport function in skeletal muscle, including transporter fatty acid translocase (FAT/CD36), plasma membrane-associated fatty acid protein (FABPpm), fatty acid transport proteins (FATP) 1 and 4 (Glatz et al., 2010). It has been reported that insulin-stimulated fatty acid uptake is completely abolished in FATP1-null adipocytes and greatly reduced in the soleus muscle of FATP1-knockout mice (Wu et al., 2006). In addition, lipid accumulation in 3T3-L1 adipocytes was reduced along with reduced FATP1 expression (Choi et al., 2011). A recent study found that FATP1 KO mice were protected from fat-induced accumulation of intramuscular fatty acyl-CoA and insulin resistance in skeletal muscle (Kim et al., 2004). Thus, we have

Abbreviations: ANOVA, one-way analysis of variance; ELISA, enzyme-linked immunosorbent assay; FATP1, fatty acid transport protein 1; FATP4, fatty acid transport protein 4; FAT/CD36, transporter fatty acid translocase; FABPpm, plasma membrane-associated fatty acid protein; FBG, fasting blood glucose; GIR, glucose infusion rate; HFD, high fat diet; HOMA2_IR, homeostasis model assessment insulin resistance index; LCFA, long-chain fatty acid; LDL-C, low density lipoprotein cholesterol; NFD, normal food diet; qRT-PCR, quantitative real-time polymerase chain reaction; PPARγ, peroxisomal proliferator-activated receptor-γ; SD, Sprague–Dawley; S.E.M., standard error of the mean; TC, total cholesterol; TG, triglyceride

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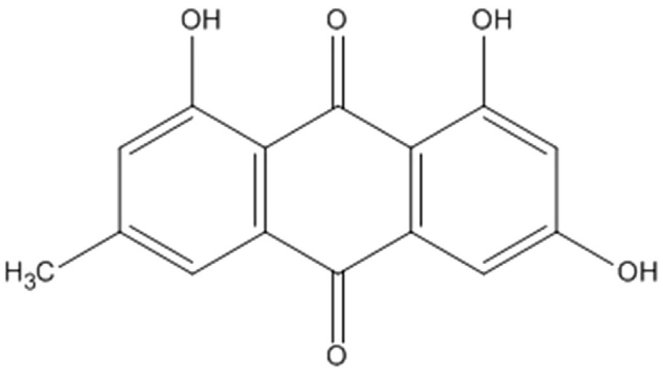

Fig. 1. Schematic structure of emodin.

some reasons for speculating that FATP1 plays a key role in fatty acid transport across cell membrane and lipid accumulation in the skeletal muscle.

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) (shown in Fig. 1) is an anthraquinone compound extracted from the root and rhizome of *Rheum palmatum*, which has possessed a great potentials in anti-viral, anti-tumor, and anti-inflammatory activities. Many studies have confirmed that emodin could improve the blood glucose levels, and improve lipid profiles at the same time, holding a great promise in anti-diabetic through a variety of mechanisms, such as inhibiting 11β-hydroxysteroid dehydrogenase type 1 (Feng et al., 2010), activating AMP- activated protein kinase (Song et al., 2013) and peroxisomal proliferator-activated receptor-γ (PPARγ) (Xu et al., 2013).

Lipid accumulation in skeletal muscle leads to insulin resistance and emodin can improve blood lipid levels; thus, the effect of emodin could be to ameliorate lipid accumulation in skeletal muscle and to improve insulin resistance, which is worth further confirmation. Therefore, we hypothesized that emodin could improved insulin resistance in high fat diet (HFD) rats by decreasing skeletal muscle lipid accumulation through inhibiting FATP1-mediated fatty acid transport across the cell membrane. In the present study, we explored the effect of emodin on insulin resistance and lipid accumulation in skeletal muscle in HFD rats and L6 cells.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (SD) rats aged 6 weeks were procured from Laboratory Animal Center of ChongQing Medical University (Chongqing, China). They were maintained in temperature-.

controlled environment (25 ± 1 °C) and kept on a 12:12 light-dark cycle (lights on at 08:00 am) in our animal center. The animals received water and their respective diets ad libitum for the entire 15-week period. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments approved by the Ethical Committee for Animal Studies of the First Affiliated Hospital of Chongqing Medical University.

2.2. Drugs and reagents

Emodin (purity 98%) was purchased from Life Science & Service (Shanghai, China). The food elements, such as sodium cholate, egg yolk powder, and cholesterol (all from JingChang Chemical Products Co., Zhengzhou, China), lard, sugar, and normal diet particles (Laboratory Animal Center of ChongQing Medical University, Chongqing, China) were procured from the commercial resource. Oil Red O was purchased from Sigma-Aldrich (St. Louis, Mo). Polyclonal rabbit anti-FATP1, FAT/CD36, FABPpm, FATP4 were purchased from BIOSS (Beijing, China). All products related to RNA reagents were purchased from Takara Bio Inc. Rabbit anti-FATP1 antibody was purchased from Boaosen (Beijing, China). All other reagents were from commercial corporations and of analytical standard (YuNuo Biological Technology Co., Chongqing, China).

2.3. Induction of experimental insulin resistance rat model and emodin treatment

After one week acclimatization period, the rats were randomly divided into two groups: normal food diet (NFD, ~10% energy from fat) or high fat diet (HFD, 45% of energy from fat [lard]). The diets were made in-house as described elsewhere (Turner et al., 2007) for the first 8 weeks. The rats were fed NFD+Vehicle (NFD+V), while the rats were fed HFD were randomly divided into three groups: HFD+Vehicle (HFD+V), HFD+Emodin (25 mg/kg, HFD-EM25), HFD+Emodin (100 mg/kg, HFD-EM100) during the next six weeks. Rats were administered once daily by intragastric gavage.

2.4. Biochemical analysis

At the end of the first, ninth and fifteenth week, all rats were weighted after an overnight fasting (12 h). Meanwhile, a blood sample was collected from the tail vein and the serum was separated by centrifugation (1500 × *g*) for 15 min at 4 °C and stored at −80 °C until analysis. Fasting plasma glucose (FPG), triglycerides (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were assayed using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Immunoreactive insulin (IRI) was tested by a specific RIA kit (Beijing North Institute of Biology, China). Insulin resistance was evaluated by the homeostasis model assessment of insulin resistance (HOMA2-IR) index (Safar et al., 2011).

2.5. Enzyme-linked immunosorbent assay (ELISA)

The content of TG in soleus muscle and palmitic acid in L6 cells were analyzed using commercially available ELISA-based assay systems. Assays were performed using the protocols recommended by the manufacturer.

2.6. Hyperinsulinemic-euglycemic clamp

After being fasted 18 h (Ayala et al., 2006), the rats were anaesthetized with 10% chloraldurate (0.4 ml/100 g body weight), and a tube that was connected to a tee (gift from Professor Zhou, ChongQing Medical University) was used for insulin and glucose infusion was inserted into the femoral vein. The rats were allowed to stand for another 2 h before starting hyperinsulinemic-.

euglycemic clamp and blood samples were collected every 10 min from the tail vein to measure blood glucose by glucometer (One TouchTM; LifeScan, Milpitas, CA). Euglycemia was maintained at a variable infusion of 20% glucose during the clamp and the infusion rate and pattern of insulin are described elsewhere (Safar et al., 2011). Glucose infusion rate (GIR) was calculated at steady state during clamps from 60 min to 120 min.

2.7. Cell culture and palmitic acid treatment

L6 skeletal muscle cells were a kind gift from Professor Qifu Li (the First Affiliated Hospital of Chongqing Medical University). L6 cells were cultured in α-MEM with 10% fetal bovine serum (Hyclone, Logan, UT), and 100 units/ml penicillin and 100 mg/ml streptomycin