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Celastrol suppresses obesity process via increasing antioxidant capacity and improving lipid metabolism

Chaoyun Wang, Chunfeng Shi, Xiaoping Yang, Ming Yang, Hongliu Sun, Chunhua Wang*

School of Pharmaceutical Sciences, Binzhou Medical University, Yantai, Shandong 264003, PR China

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ABSTRACT

High fat diet, as an important risk factor, plays a pivotal role in atherosclerotic process. Celastrol is one of the active triterpenoid compounds with antioxidative and anti-inflammatory characters. The aims of this study were to evaluate the effect of celastrol on weight, blood lipid and oxidative injury induced by high fat emulsion, and investigate its potential pharmacological mechanisms. Male Sprague–Dawley rats were fed with high fat emulsion for 6 wk to mimic high fat mediated oxidative injury. The effects of celastrol on weight and blood lipid were evaluated, and its mechanisms were disclosed by applying western blot, ELISA and assay kits. Long-term consumption of high fat emulsion could significantly increase weight by enhancing total cholesterol (TC), triacylglycerol (TG), apolipoprotein B (Apo B), low-density lipoprotein cholesterol (LDL-c) levels, attenuating ATP-binding cassette transporter A1 (ABCA1) expression, and decreasing the levels of high-density lipoprotein cholesterol (HDL-c) and apolipoprotein A-I (Apo A-I), and inhibit antioxidant enzymes activities, improve nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity. Comparing with model group, celastrol was able to effectively suppress weight and attenuate high fat mediated oxidative injury by improving ABCA1 expression, reducing the levels of TC, TG, LDL-c and Apo B in plasma, and increasing antioxidant enzymes activities and inhibiting NADPH oxidase activity, and decreasing the serum levels of Malondialdehyde (MDA) and reactive oxygen species in dose-dependent way. These data demonstrated that celastrol was able to effectively suppress weight and alleviate high-fat mediated cardiovascular injury via mitigating oxidative stress and improving lipid metabolism.

1. Introduction

Daily dietary types had been proven to closely associate to the production of reactive oxygen (Conforti et al., 2009). Some literatures had been found that long-term high-fat diet treatment could increase the production of reactive oxygen species, promote lipid peroxidation, and then lead to oxidative injury (Nolan et al., 2005; Xu et al., 2009). Dyslipidemia was involved in the development of atherosclerosis, cardiovascular disease and renal ailments. Low-density lipoprotein (LDL), cholesterol, and high fat diet, as important risk factors, can change health status and accelerate obesity process through disturbing lipid metabolism and promoting the formation of lipid-rich plaques in the wall of arterial blood vessels. Oxidative stress plays a key role in the process, low-density lipoprotein (LDL) is oxidized to ox-LDL, and the latter bound with Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and promote the obesity process (Yan et al., 2011). In addition, accumulating evidences indicated that obesity was also accompanied with a state of chronic inflammation, so it may be a very effective method to suppress obesity and cure obese-related disease by using antioxidants or applying anti-inflammatory agents (Kang, 2013).

Thunder God Vine (Tripterygium wilfordii Hook F.) is a perennial vine of the Celastraceae family. The plant contains several therapeutically active compounds, including terpenoids, alkaloids, and steroids, which has been used as a traditional Chinese medicine to promote blood circulation, suppress rheumatism and relieve pain for hundreds of years. Celastrol [3-hydroxy-24-nor-2-oxo-1(10),3,5,7-friedelatetraen-29-oic acid] is one of the active triterpenoid compound isolated from Tripterygium wilfordii Hook F. Previous studies have shown that celastrol was widely used to treat rheumatoid arthritis, allergic asthma, and systemic lupus erythematosus due to its potential anti-inflammatory and antioxidant effects (Tao et al., 2002; Qiu and Kao, 2003; Kim et al., 2009). In this study, we investigate the effect of celastrol on oxidative stress and blood lipid profiles after long term consumption of high fat emulsion, reveal its primary mechanisms, and then extend our opinion.

* Correspondence to: School of Pharmaceutical Sciences, Binzhou Medical University No. 346, Guanhai Road, Laishan District Yantai, Shandong 264003, P.R. China. Tel.: +86 535 6913216; fax: +86 535 6913718.
E-mail address: chunhuawang2012@163.com (C. Wang).

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2. Materials and methods

2.1. Drug and chemicals

Celastrol (Fig. 1), extracted from root pulp of Tripterygium wilfordii Hook F., is the red amorphous powder and unsoluble compound with purity of more than 98%, whose molecular formula is $C_{29}H_{38}O_4$ with a molecular weight of 450.61 Da, which was purchased from KMST Pharmaceutical Technology Development Co., Ltd.(Tianjing,P.R. China). Simvastatin was purchased from Shandong Lukang Pharmaceutical Group Co., Ltd. (Jining, P.R. China). All regents were AR degree grand, Assay kits of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), apolipoprotein A-I (Apo A-I), apolipoprotein B (Apo B), Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were provided from the Jiancheng Institute of Biotechnology (Nanjing, P.R. China). The specific antibodies analyzed target proteins were supplied by Santa Cruz Biotechnologies, USA

2.2. Animals and treatment

Sixty male Sprague–Dawley rats with weight 200–250 g were purchased from Experimental Animal Department of Shandong Luye-Pharmcautical Co. Ltd., Shandong province, P.R. China. All animals were bred in temperature controlled animal facility with 12 h light–dark cycle. Rats were randomly divided into the following six groups: control group; high fat group (high fat diet); Celastrol treated groups (1 mg/kg, 3 mg/kg and 9 mg/kg); and simvastatin treated group (10 mg/kg). Except for control group, all rats were given oral administration of high fat emulsion (HFE) containing 15% lard oil (W/V), 5% cholesterol (W/V), 2.5% yolk powder (W/V), 0.5% Propylthiouracil (W/V), 1% Tween-80 (V/V) and 76% distilled water (V/V) at a single dosage of 1.0 mL/100 g daily for 6 wk. Rats in celastrol treated group or simvastatin treated group were orally administered with celastrol or simvastatin in the doses indicated above every day, respectively. All rats were weighed at different times. At the end of 6 wk, all rats were briefly anesthetized and killed by bleeding from abdominal aorta. All animals were treated in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Animal care and experimental procedures were approved by the Ethics Committee in Animal and Human Experimentation of Binzhou Medical University.

2.3. Observation of weight

The weights of all rats from different groups were observed at preliminary stage, at the end of 3 wk and 6 wk.

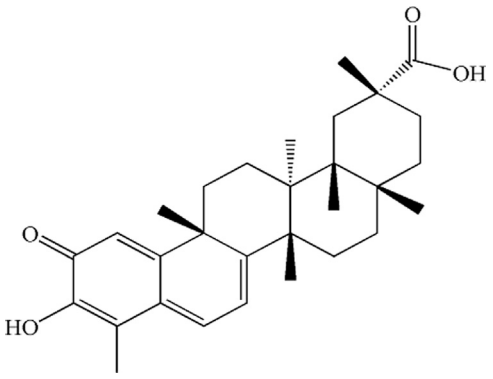

Fig. 1. Molecular structure of celastrol.

2.4. Determination of blood lipids

Immediately after euthanasia, whole blood samples from abdominal aorta were drawn into tubes containing 3.8% trisodium citrate solution at a volume ratio of 9:1, and centrifuged at 1500*g* for 15 min. The levels of TC, TG, HDL-c and LDL-c in the supernatant were quantified using assay kits (Jiancheng Bioengineering Institute, Nanjing, P.R. China)

2.5. Measurement of apolipoprotein A-I, apolipoprotein B

The blood samples were collected from abdominal aorta and centrifuged at 1500*g* for 15 min. The levels of Apo A-I and Apo B in the supernatant were analyzed using assay kits (Jiancheng Bioengineering Institute, Nanjing, P.R. China)

2.6. Assessment of the activities of antioxidant enzyme and the levels of reactive oxygen species and Malondialdehyde

Whole blood samples with 3.8% trisodium citrate solution were centrifuged at 1500*g* for 15 min. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) and the concentration of Malondialdehyde (MDA) in the supernatant were detected by using assay kits according to the manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, P.R., China). The levels of reactive oxygen species in the supernatant were measured using the OxiSelect™ In Vitro Reactive Oxygen Species/Rns Assay Kit (Cell Biolabs Inc., USA).

2.7. Measurement of nicotinamide adenine dinucleotide phosphate oxidase activity

The live tissues were washed, lysed and homogenized, and then were centrifuged at 16,000*g* for 25 min at 4 °C (Wang et al., 2013). The pellets (membrane fractions) were stored at −80 °C until use. An aliquot (50 μg) of the pellet was diluted in 500 μl of phosphate buffer. Dark-adapted lucigenin (20 μmol/L) was added to the sample, and the chemiluminescence measurements were immediately initiated. Chemiluminescence (in arbitrary units) was measured at 30-s intervals for 5 min by using the GloMax-20/20 Luminometer (Turner Biosystems, Inc., USA). NADPH was used as an electron donor substrate. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activities are expressed as the percentage of the control value.

2.8. Analysis of β-actin, ATP-binding cassette transporter A1 and $p22^{phox}$ expression by using western blotting

The rats were killed, and the livers were collected on ice and were kept at −80 °C before use. The liver tissues were homogenized and treated with trizol protein extraction reagent (Invitrogen), and the protein contents of supernatant fluids were determined by the BCA method (BCA protein assay kit, Thermo Scientific). The protein samples were denatured by mixing with sample buffer at 95 °C for 15 min, and 15 μg of equal amounts of protein samples were separated using 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electroblotted to polyvinylidene difluoride (PVDF) membranes (Millipore Corporation, Billerica, MA, USA). The membranes were incubated with blocking buffer containing 1% bovine serum albumin for 2 h at 37 °C, and then were incubated with primary antibodies against β-actin, ABCA1 and $p22^{phox}$ (Santa Cruz Biotechnology, Santa Cruz, CA) diluted in blocking buffer for 2 h at 37 °C or overnight at 4 °C. Membranes were then washed with washing buffer 3 times for 5 min. The membranes were subsequently incubated with a secondary antibody for 2 h at 37 °C,and followed by washing with