ARTICLE IN PRESS
+ MODEL
Journal of Traditional Chinese Medical Sciences (2017) xx, 1–10

Available online at www.sciencedirect.com
ScienceDirect
journal homepage: http://www.elsevier.com/locate/jtcms

Salvianolic acid B improves glucolipid metabolism by regulating adipogenic transcription factors in mice with diet-induced obesity

Dandan Zhao [a,1], Jiacheng Zuo [a,1], Na Yu [a], Xin Fang [b], Fangfang Mo [a], Rui Wu [c], Tian Tian [a], Rufeng Ma [a], Yushan Gao [a], Dongwei Zhang [a], Guangjian Jiang [a], Sihua Gao [a,*]

[a] *Beijing University of Chinese Medicine, Beijing 100029, China*
[b] *Department of Endocrinology, Beijing University of Chinese Medicine, Third Affiliated Hospital, Beijing 100029, China*
[c] *Department of Endocrinology, South Area of Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing 102618, China*

Received 24 May 2017; received in revised form 21 July 2017; accepted 24 July 2017
Available online ■ ■ ■

KEYWORDS
Salvianolic acid B;
Obesity;
Blood glucose;
Adipose tissue;
Adipogenic transcription factors

Abstract *Objective:* To determine the effect of Salvianolic acid B (Sal B) on glucose and lipid metabolism in mice with high-fat diet (HFD)-induced obesity, and to investigate the underlying mechanisms by measuring the expression levels of key adipogenic transcription factors.
Methods: Six-week-old C57BL/6J male mice were fed for 12 weeks with a HFD to induce obesity or a standard diet to serve as normal controls. A mean body weight increase of more than 20% after these 12 weeks was used as the criteria for obesity. HFD-fed obese mice then received a supplement of Sal B (100 mg/kg body weight/day), metformin (75 mg/kg body weight/day) or water (an equivalent volume; served as model controls) by oral gavage for an additional 8 weeks, and the normal controls received water (an equivalent volume) by oral gavage for the same period.
Results: Sal B significantly reduced body weight gain ($P < .05$) without influencing food intake in HFD-fed obese mice relative to model controls. Sal B also reduced the body fat mass of the obese mice relative to model controls in a time-dependent manner ($P < .05$). Sal B significantly decreased the serum concentrations of low-density lipoprotein cholesterol, total cholesterol, triglyceride and free fatty acids by 25.5%, 20.2%, 20.6% and 13.4%, respectively, and increased the concentration of high-density lipoprotein cholesterol by 50.1% relative to model controls.

* Corresponding author. Fax: +86 10 6428 6929.
E-mail address: gaosihua1216@163.com (S. Gao).
Peer review under responsibility of Beijing University of Chinese Medicine.
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.jtcms.2017.07.003

Please cite this article in press as: Zhao D, et al., Salvianolic acid B improves glucolipid metabolism by regulating adipogenic transcription factors in mice with diet-induced obesity, Journal of Traditional Chinese Medical Sciences (2017), http://dx.doi.org/10.1016/j.jtcms.2017.07.003

In addition, Sal B significantly lowered fasting glucose concentrations and improved insulin sensitivity relative to model controls ($P < .05$). Sal B acted by ameliorating the histopathological changes in both brown and white adipose tissues of obese mice. Moreover, in brown adipose tissue, Sal B up-regulated the mRNA and protein expression of PPARγ and c/EBPα, and the protein expression of PPARα and SREBP-1 ($P < .05$). In white adipose tissue, Sal B down-regulated the mRNA expression of PPARγ and c/EBPα, and decreased the protein expression of PPARγ and SREBP-1($P < .05$).

Conclusions: The results suggest that Sal B can reduce body weight gain and regulate glucose and lipid metabolism in mice with diet-induced obesity by regulating adipogenic transcription factors in their adipose tissues.

Introduction

Obesity is a global-health problem characterized by excessive fat accumulation and abnormal fat distribution, that involves many factors including diet and heredity.[1,2] The prevalence of obesity has grown rapidly, making it a public health concern worldwide.[3] Obesity is an important risk factor for hypertension, dyslipidemia, diabetes, and cardiovascular disease, which are serious threats to human health.[4] Most anti-obesity drugs that are currently available lack any long-term effects and often cause adverse reactions, so the development of new therapeutic strategies and drugs needs to be made a priority.

The pathological changes that occur with obesity are an excessive accumulation of lipid and ectopic lipid deposits in the body as a result of an imbalance between energy intake and consumption.[5] These changes lead to disorders of internal metabolism and function. Adipocyte differentiation, involving the activation of adipogenesis genes, plays an important role in the process of lipid accumulation.[6,7] During the process of adipogenesis, a series of transcription factors play central roles in the regulation pathways.[8–10]

Danshen (*Salvia miltiorrhiza*) is an herb commonly used in Asian countries to treat blood stasis.[11] Salvianolic acid B (Sal B), a water-soluble compound derived from Danshen, has been studied extensively and has been shown to have a wide range of pharmacological effects including anti-oxidation, apoptosis inhibition, coronary artery dilation and aggregation platelet prevention.[11,12] However, the effects of Sal B on metabolism regulation and their underlying mechanisms remain poorly understood.

Here, we studied the effects of Sal B on body mass, glucolipid metabolism, insulin sensitivity and adipogenic transcription factors in a mouse model of high-fat diet (HFD)-induced obesity, to investigate the function and mechanisms of Sal B in obesity and its related metabolic disorders.

Materials and methods

Animal and diets

Fifty male C57BL/6J mice were provided by SPF (Beijing) Biotechnology Co. Ltd. [Certificate NO. SCXK (Jing) 2016-0002] and kept under controlled temperature (23 $\pm$ 2°C) and humidity (55 $\pm$ 10%) conditions on a 12/12-h dark/light cycle. They were randomized by body weight and fed a standard chow diet (10% kcal as fat; normal control group, $n = 10$) or a HFD (60% kcal as fat; $n = 40$) for 12 weeks. The obesity model was considered to be successfully constructed if body weight was 20% greater in HFD-fed mice than chow diet-fed mice at the end of this period. All diets were manufactured by Mediscience Co. Ltd. (Jingsu, China).

Reagents

Sal B was purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China). Metformin was purchased from Sino American Shanghai Squib Pharmaceutical Ltd. (Shanghai, China). Antibodies against peroxisome proliferator-activated receptor gamma (PPARγ, #2435), CCAAT/enhancer-binding protein alpha (c/EBPα #2295), peroxisome proliferator-activated receptor alpha (PPARα, #8934), sterol-regulatory element binding protein 1 (SREBP-1, #3259), and GAPDH (#8245), were purchased from Abcam (Cambridge, MA, USA). All other reagents were obtained from Beijing Sinopharm Chemical Group (Beijing, China).

Animal treatment

Thirty obese mice from the HFD-fed group were selected and randomly subdivided into three groups with ten mice in each. These groups were administered metformin (75 mg/kg body weight/d; metformin group), Sal B (100 mg/kg body weight/d; Sal B group) or vehicle (an equivalent volume of water; model control group) by oral gavage daily for 8 weeks. During the experiment, all mice were fed the previous diet. Food intake, body weight and fasting blood glucose concentrations (FBG) were monitored weekly. At the end of the study, the mice were anesthetized with pentobarbital sodium and sacrificed after an overnight fast. Blood samples were collected and the serum was stored for further analysis. Interscapular brown adipose tissues (iBAT) and epididymal white adipose tissues (eWAT) were removed and the same parts of the iBAT and eWAT tissues from the mice in different groups were fixed in 10% formalin for histological examination. The other parts of the adipose

Please cite this article in press as: Zhao D, et al., Salvianolic acid B improves glucolipid metabolism by regulating adipogenic transcription factors in mice with diet-induced obesity, Journal of Traditional Chinese Medical Sciences (2017), http://dx.doi.org/10.1016/j.jtcms.2017.07.003