Fitoterapia 81 (2010) 358–366

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Berberine inhibits adipogenesis in high-fat diet-induced obesity mice

Yueshan Hu [a], Gareth E. Davies [a,b,c,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University, Brookings, SD 57007, USA
[b] Avera Institute for Human Behavioral Genetics, Avera Behavioral Health Center, Sioux Falls, SD 57108, USA
[c] Department of Psychiatry, Sanford School of Medicine, University of South Dakota, Sioux Falls, SD 57108, USA

ARTICLE INFO

Article history:
Received 15 September 2009
Accepted in revised form 14 October 2009
Available online 25 October 2009

Keywords:
Berberine
Adipogenesis
Fat diet mice
PPARγ
C/EBPα
GATA

ABSTRACT

Our previous studies illustrated that berberine inhibited adipogenesis in murine-derived 3T3-L1 preadipocytes and human white preadipocytes. In this study, the effects of berberine on the adipogenesis of high-fat diet-induced obesity (FD) or normal diet (ND) mice and possible transcriptional impact are investigated. The results demonstrated that in FD mice, berberine reduced mouse weight gain and food intake and serum glucose, triglyceride, and total cholesterol levels accompanied with a down-regulation of PPARγ expression and an up-regulation of GATA-3 expression. Berberine had no adverse effects on ND mice. These encouraging findings suggest that berberine has excellent pharmacological potential to prevent obesity.

Published by Elsevier B.V.

1. Introduction

From a metabolic perspective, obesity is characterized by increased fat mass arising from the prolonged imbalance between energy intake and energy expenditure. Potential strategies for therapeutic intervention for treating obesity include altering neural signals in the brain to regulate appetite, altering nutrient absorption in the gut, and modifying fat storage and promoting fat oxidation in adipose tissue [1]. The control of adipogenesis is a strong candidate as a potential strategy for the prevention of obesity [2] and adipocyte differentiation plays a major role during the process of fat mass growth [3,4]. A number of key transcription factors have been identified in the complex transcriptional cascade that occurs during adipocyte differentiation including peroxisome proliferator activated receptor γ (PPARγ) [5], CCAAT/enhancer binding protein α (C/EBPα) [6] and more recently reported GATA binding proteins 2 and 3 (GATA-2, GATA-3) [7,8]. Moreover, in vitro models such as 3T3-L1 mouse preadipocyte [9], human primary preadipocyte [10] and in vivo animal models such as high-fat diet-induced obesity mouse [11] have been developed and used extensively in the investigation of adipogenesis.

Berberine is a botanic alkaloid isolated from many herbs including Coptis Rhizome and Hydrastis Canadensis. Berberine has been demonstrated as having potential as a treatment for diarrhea [12], cancer [13,14], diabetes [15] and obesity. Studies on the anti-obesity effects of berberine have been reported by several groups showing inhibition of adipogenesis during the differentiation of 3T3-L1 preadipocytes [16], via targeting of IkB kinase beta (IKKβ) [17], reduction of adipogenic enzyme expression [18], and decreasing PPARγ expression [19]. In addition, recent studies have indicated that berberine prevents obesity in vivo by inducing glycolysis [20] and activating AMP-activated protein kinase [21,22]. However, the effect of berberine on adipogenic control transcription factors GATA-2 and 3 in a high-fat diet-induced obesity mouse model has not been reported.

Our previous studies showed that berberine inhibited adipogenesis both in 3T3-L1 mouse preadipocytes and human white preadipocytes (HWP) [23,24] and was accompanied by increased expression of GATA-2 and GATA-3, with decreased expression of PPARγ and C/EBPα (3T3-L1). In this present study, our results indicate that berberine inhibits adipogenesis in fat diet (FD) mice accompanied with up-

* Corresponding author. Department of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University, Brookings, SD 57007, USA. Tel.: +1 605 688 4090; fax: +1 605 688 6232.
E-mail address: Gareth.Davies@sdstate.edu (G.E. Davies).

0367-326X/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.fitote.2009.10.010

regulated GATA-2 (mRNA) and GATA-3 (mRNA and protein) expression without any obvious toxicity in FD and normal diet (ND) mice.

2. Materials and methods

2.1. Mouse experiments

All experiments were approved by the Institutional Animal Care and Use Committee of South Dakota State University. Nine-week-old C57BL/6J male mice were purchased from The Jackson Laboratory. Group 1 mice were fed with normal diet (ND, D12450Bi, Research Diets Inc. New Brunswick, NJ, USA) for 9 weeks, group 2 mice were fed with ND for 6 weeks and then high-fat diet (FD, D12451i, Research Diets Inc. New Brunswick, NJ, USA) for 3 weeks. The mice were maintained according to The Jackson Laboratory guidelines for animal care and housed at 22 ± 2 °C temperature, $55 \pm 5\%$ relative humidity, with a light/dark cycle of 12 h. Food (group 1 with normal fat diet and group 2 with high-fat diet until sacrifice) and water were provided ad libitum. After stabilizing for 2 weeks and balancing the difference in body weight between the subgroups, the mice were intraperitoneally injected with various concentrations of berberine (Sigma-Aldrich, Saint Louis, Missouri, USA) or vehicle (PBS) solution for 36 days (group 1, subgroup 1: normal diet mice treated with vehicle, subgroup 2: normal diet mice treated with 3 mg/kg/day berberine). In group 2, subgroup 1: high-fat diet mice treated with vehicle, subgroup 2–4: high-fat diet mice treated with 0.75, 1.5, 3 mg/kg/day berberine respectively). Six mice per subgroup were obtained finally. Mouse weight and food intake were recorded every 3 days with the day of treatment with berberine or control as day 0. Blood samples were collected from the hearts of anesthetized mice after feeding and treatment for 36 days following fast for 16 h. When sacrificed, mouse liver, kidney, spleen and epididymal fat tissues were dissected and weighed. Epididymal fat tissues were immediately frozen in liquid nitrogen and stored at −80 °C.

2.2. Serum glucose, triglyceride, and cholesterol assay

Blood samples from each mouse were collected and serum was separated by centrifugation for the measurement of levels of glucose, triglyceride and total cholesterol. Serum glucose concentrations were analyzed using Glucose (HK) Assay Kit (Sigma-Aldrich, Saint Louis, Missouri, USA). Serum triglyceride concentrations were analyzed using Serum Triglyceride Determination Kit (Sigma-Aldrich, Saint Louis, Missouri, USA). Serum cholesterol concentrations were analyzed using Amplex® Red Cholesterol Assay Kit (Invitrogen, Carlsbad, CA, USA). All experimental assays were done according to the manufacturer's instructions.

2.3. RNA and protein purification and quantification from epididymal fat tissues

RNA and protein were isolated from 60 mg of epididymal fat tissues with Trizol (Invitrogen, Carlsbad, CA, USA) following the protocol recommended by the manufacturers. RNA was dissolved in pure water and quantified at 260/280 nm, and sample integrity was checked by 1.5% agarose gel electrophoresis. Protein concentration was determined using the Micro BCA Protein Assay Kit (Fisher Scientific, Pittsburgh, PA, USA) according to the manufacturer's instructions.

2.4. Quantitative Real-Time RT-PCR

Real-Time reverse transcriptase polymerase chain reaction (RT-PCR) analysis was used to measure mRNA expression of genes PPARγ, C/EBPα, GATA-2 and GATA-3 in the control of β-actin. Total RNA (0.8 μg) from each sample was used for a reverse transcription reaction using the TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA) following the manufacturers' instructions. PCR was done using the SYBR green Master Mix (Applied Biosystems, Foster City, CA, USA) following the manufacturers' instructions. The primer (Integrated DNA Technologies, Coralville, IA, USA) sequences are shown in Table 1. The cycling conditions of amplification were as follows: an initial step of denaturation at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s. Real-Time PCR was performed using Mx3000P Real-Time Thermocyclers (Stratagene, La Jolla, CA, USA). The relative mRNA levels of these genes were calculated by $2^{-\text{delta delta CT}}$ method and normalized with control-treated groups.

2.5. Immunoblot analysis

Protein expression of GATA-2, GATA-3, PPARγ and C/EBPα in the control of β-actin was assessed by Western Blot. Protein samples from the same group were homogenized as previously reported [25]. Protein (25 μg) was separated by polyacrylamide gel electrophoresis using the BioRad Electrophoretic System (60 V for 1 h followed by 90 V for 5 h). The proteins were then transferred to nitrocellulose membranes at 35 V overnight. The membrane was blocked for 1 h at room temperature in 5% nonfat milk in Tris-buffered saline (TBS), then washed with Tween-20-TBS (TTBS, 0.1% Tween-20) three times (15 min, 5 min, and 5 min), followed by incubation with primary antibodies mouse GATA-2, GATA-3 (dilution 1:200), PPARγ, C/EBPα and β-actin (dilution 1:500) at room temperature for 2 h (all antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA). The membrane was rinsed three times (15 min, 5 min, and 5 min), then incubated at room temperature for 1 h with horseradish peroxidase-conjugated second antibody (1:10,000). The membrane was then incubated in ECL reagent (GE Healthcare, Piscataway, NJ, USA) for

Table 1
Real-Time RT-PCR primers.

Oligonucleotide	Sequences (5 to 3′)
Mouse PPARγ-F	CGCTGATGCACTGCCTATGA
Mouse PPARγ-R	AGAGGTCCACAGAGCTGATTCC
Mouse C/EBPα-F	AGAAGTCGGTGGACAAGAACAGCA
Mouse C/EBPα-R	GCGTTGTTTGGCTTTATCTCGGCT
Mouse GATA-2-F	GCGCCTGATTTAAGAATGGGTGGT
Mouse GATA-2-R	AGGAGCACTTAACGGCAGGATGAA
Mouse GATA-3-F	TTTACCCTCCGGCTTCATCCTCTT
Mouse GATA-3-R	TGCACCTGATACTTGAGGCACTCT
Mouse β-actin-F	TGTGATGGTGGGAATGGGTCAGAA
Mouse β-actin-R	TGTGGTGCCAGATCTTCTCCATGT