Biochemical and Biophysical Research Communications 473 (2016) 290–295

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Anti-obesity potential of enzymatic fragments of hyaluronan on high-fat diet-induced obesity in C57BL/6 mice

Byong-Gon Park [a], Yoon-Sun Park [b], Joo Woong Park [c], Eunji Shin [c], Woon-Seob Shin [b, *]

[a] Department of Physiology, Catholic Kwandong University College of Medicine, Gangneung, 25601, Republic of Korea
[b] Department of Microbiology, Catholic Kwandong University College of Medicine, Gangneung, 25601, Republic of Korea
[c] Biostream Technologies, Yongin-si, Gyeonggi-do, 17098, Republic of Korea

ARTICLE INFO

Article history:
Received 14 March 2016
Accepted 20 March 2016
Available online 22 March 2016

Keywords:
Antiobesity
Hyaluronan fragments
High-fat diet
PPAR-Alpha
Liver steatosis

ABSTRACT

Hyaluronan has diverse biological activities depending on its molecular size. The hyaluronan fragments (50 kDa) can decrease adipogenic differentiation in vitro. However, in vivo anti-obesitic effects of hyaluronan fragments have not been elucidated. Therefore, we examined the anti-obesity effects of hyaluronan fragments on high-fat diet induced obesity in C57BL/6 mice. Oral administration of hyaluronan fragments (200 mg/kg for 8 weeks) decreased body weight, adipose tissues, serum lipid (low-density lipoprotein cholesterol, triglyceride), and leptin level. Hyaluronan fragments decreased the hypertrophy of adipose tissue and ameliorated liver steatosis. The mRNA expression of leptin was reduced in adipocyte by treatment with hyaluronan fragments. Additionally, hyaluronan fragments enhanced the mRNA expression of *PPAR-α* and its target genes *UCP-2* and decreased mRNA expression of *PPAR- γ* and fatty acid synthase in liver. In conclusions, hyaluronan fragments had marked effects on inhibiting the development of obesity in obese mice fed the high-fat diet. It suggested that enhancing *PPAR-α* and suppressing *PPAR-γ* expression are two possible mechanisms for the anti-obesitic effect of hyaluronan fragments.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Overweight and obesity have become an epidemic and affect over 2.1 billion individuals worldwide [1]. Obesity is caused by overnutrition and decreased physical activity and is characterized by an excess storage of lipids in the adipose tissue, which can be accounted for by both adipocyte hyperplasia and hypertrophy [2]. It is one of the main factors responsible for the increased incidence of cardiovascular disease, type 2 diabetes and several types of cancer [3,4]. Therefore, obesity is an increasing issue in healthcare, and mechanisms to control obesity have been actively studied worldwide [5,6]. Although several drugs have been developed to treat obesity, no ideal compounds or drugs have been developed yet [7]. Because diets containing high-fat foods are becoming common, there is a need to find suitable alternatives to ameliorate the effects of a high-fat diet, thereby decreasing the risk associated with obesity [8,9].

Hyaluronan is a major component of the extracellular matrix (ECM) in multiple tissues [10]. Despite its simple structure, hyaluronan has numerous functions depending on its molecular weight [11]. High molecular weight hyaluronan has been used in diverse clinical applications, including ECM regeneration, wound healing, and osteoarthritis treatment [12]. In contrast, others have found that hyaluronan oligosaccharides increase *MMP-13* expression which subsequently induces the loss of ECM proteoglycan and collagen in human articular cartilage [13,14]. Moreover, hyaluronan downregulation has been reported to have inhibitory effects on adipogenesis in 3T3-L1 cells in vitro and on fatty liver development in high-fat diet-fed C57BL/6J mice in vivo [15]. Furthermore, a direct inhibitory effect of hyaluronan fragment (HF) on adipogenesis has been demonstrated in 3T3-L1 preadipocytes [16]. However, in vivo anti-obesitic effects of hyaluronan fragments had not yet been studied. Therefore, it is need to evaluate in vivo anti-obesitic effect of HF. Based on the above reports, we speculated that the administration of HF could modulate the abnormal lipid metabolism and cause weight loss in a high-fat diet C57BL/6 mice.

* Corresponding author.
E-mail addresses: bgpark@cku.ac.kr (B.-G. Park), pyskth@cku.ac.kr (Y.-S. Park), pjwrnds@empas.com (J.W. Park), eunji.shin0930@gmail.com (E. Shin), shinws@cku.ac.kr (W.-S. Shin).

http://dx.doi.org/10.1016/j.bbrc.2016.03.098
0006-291X/© 2016 Elsevier Inc. All rights reserved.

2. Materials and methods

2.1. Reagents

The hyaluronan (2000 kDa) sample (fermented with *Streptococcus zooepidemicus*) of high purity was obtained from Biostream Technologies (Yongin, Korea). Hyaluronidase was purified from a culture of *Vibrio splendidus* BST398 (KCTC-11899).

2.2. Preparing medium molecular weight of HF

To obtain medium molecular weight HF (50 kDa), high molecular weight hyaluronan was hydrolyzed using hyaluronidase isolated from *V. splendidus* BST398 (KCTC-11899) according to a previous report [16]. Briefly, 2000 kDa of hyaluronan (1 g) was dissolved in 100 mL of 0.1 M Tris buffer (pH 7.0) and incubated with hyaluronidase for 4 h at 30 °C. After enzymatic hydrolysis, the hyaluronan hydrolysates were filtered using an ultrafilter (Sartorius Slice, NMWCO 10 kDa; Sartorius Co., Germany). The ultrafiltrate of HF was freeze-dried and used as the HF sample (50 kDa) after the analysis of molecular weight by size exclusion chromatography.

2.3. Animals and experimental design

C57BL/6 mouse weighing 20 g were purchased from Orient BIO Inc. (Sungnam, Gyeonggi, Korea) and acclimated for 1 week at a temperature of 23 °C ± 1 °C and humidity of 50% ± 5% on a 12-h light/dark cycle. All experiments were performed according to the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health and were approved by the Committee for the Care and Use of Laboratory Animals in Catholic Kwandong University (2013-003, 2015-005).

All mice were randomly divided into three groups (n = 10per group): normal control diet group, high-fat diet control group, and high-fat diet with HF group. The HF-treated group was treated with HF at a dose of 200 mg/kg, and the normal and high-fat diet control groups were given distilled water. HF or water was orally administered to the mice by gavage every day. The two obese groups were continually fed with high-fat diet (65% fat) during this experiment. The control group of normal mice was given water daily and continually fed standard laboratory chow (17% fat). Body weight was measured at least two times a week, and daily food and water intake were recorded once a week.

2.4. Blood lipid analysis

At the end of the experimental period, the mice were anesthetized with ether after withholding food overnight. Before the estimation of lipid profiles, blood samples were collected from the mice by heart puncture using a 1 mL syringe, rested for 15 min at room temperature, and centrifuged at 1,500 g for 10 min. The serum was then decanted and stored at −70 °C. The serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) were measured using clinical chemistry reagent kits (Roche Diagnostics Ltd. Switzerland) and a clinical chemistry analyzer (Cobas 8000 c701; Roche Diagnostics).

2.5. Measurement of leptin and adiponectin

Serum leptin and adiponectin levels were measured in duplicate by ELISA (mouse adiponectin ELISA kit: R&D Systems, Minneapolis, MN, USA and mouse leptin ELISA kit: Abcam, Cambridge, UK) according to the manufacturer's instructions.

2.6. Fat pad weights and histological examination

The adipose tissues (fat pads) and liver from each mouse were removed, weighed, and stored at liquid nitrogen. For histological examination, adipose and liver tissues were fixed in 10% formalin solution and embedded in paraffin. Standard sections of 5 μm thickness were cut, stained with hematoxylin and eosin, viewed under an optical microscope, and photographed at a final magnification of ×200. The average size of adipocytes was measured by using an image analyzer (Image-Pro Plus 3.0: Media Cybernetics, Silver Spring, MD, USA).

2.7. RNA extraction and real-time polymerase chain reaction

Total RNA from 100 mg of adipose tissue was extracted using a lipid tissue mini kit (QIAGEN, Hiden, Germany), total RNA from liver tissues was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) and an aliquot (3 μg) of total RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Warrington, UK) according to the manufacturer's instructions. First-strand cDNAs were amplified by PCR using gene-specific primers. Real-time PCR was conducted using SYBR Green (SYBR Green Master Mix: Applied Biosystems) with the Rotor-Gene system (Corbett Research Co., Mortlake, Australia). The detector was programmed with the following PCR conditions: 40 cycles of 10 s denaturation at 95 °C, 30 s annealing at 53 °C, and 20 s amplification at 72 °C. All reactions were run in triplicate and were normalized to the housekeeping gene *GAPDH*. Primers for genes used in the present study were as follows: mouse *GAPDH*: forward, 5′-TTGTTGCCATCAACGACCCC-3′ and reverse, 5′-GCCGTTGAATTTGCCGTGAG-3′; mouse *PPAR-α*: forward, 5′-TCGGACTCGGTCTTCTTGATG-3′ and reverse, 5′-GGACCTTCGG-CAGCTGGT-3’; mouse *PPAR-β*: forward, 5′-GGTCA-TAGCTCTGCCACCAT-3′ and reverse, 5′-ACTCAGAGGCTCCTGCTCAC-3’; mouse *PPAR-γ*: forward, 5′-CAGTGGGGAGAGAGGACAGA-3′ and reverse, 5′-AGTTCGGGAACAAGACGTTG-3′; mouse adiponectin: forward, 5′-CTTGCCAGT GCTGCCGTCAT-3′ and reverse, 5′-GATGGCAGAGATGGCACTCC; mouse leptin: forward, 5′-AGAGT-GAGGCTTCCAGGACG-3′ and reverse, 5′-GATGGACCA-GACTCTGGCAG-3’; mouse *UCP-2*: forward, 5′-TCCTGCTACCTC CCAGAAGA-3′ and reverse, 5′-TGAGACCTCAAAGCAGCCTC-3’; mouse FAS: forward, 5′-GTTGGCCCAGAACTCCTGTA-3′ and reverse, 5′-GTCGTCTGCCTCCAGAGC-3′.

2.8. Statistical analysis

Results are presented as means ± standard deviations (SD). Statistical differences were analyzed by Mann−Whitney U test (Prism 3.0: Graphpad software, Inc, CA, USA).

3. Results

3.1. Effect of HF on body weight gain and fat pad accumulation

In the present study, obesity was induced in normal mice by feeding a high-fat diet for 8 weeks (Fig. 1A). Compared with mice fed with standard diet, mice fed with high-fat diet had an increased the body weight by 36.4% after 8 weeks of feeding. However, HF-treated mice had significantly decreased body weight by 18.8% compared with the high-fat control group. HF did not affect food or water intake in the high-fat fed mice (data not shown). The body weight changed, and weights of regional adipose pads, including the inguinal subcutaneous, genital, and retroperitoneal adipose pad were 5−7 times higher in obese mice than in normal mice (Fig. 1B−D). However, the weight of inguinal subcutaneous,

Download English Version:

https://daneshyari.com/en/article/1927892

Download Persian Version:

https://daneshyari.com/article/1927892

Daneshyari.com