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Anti-diabetic effects of chondroitin sulfate on normal and type 2 diabetic mice

Mihoko Moto[a,b,*], Naoko Takamizawa[c], Tetsuhei Shibuya[d], Asae Nakamura[b], Kaname Katsuraya[a], Kazuyasu Iwasaki[e], Katsuyuki Tanaka[e], Akihiko Murota[d]

[a] Faculty of Human Ecology, Wayo Women's University Graduate School, Konodai, Ichikawa, Chiba 272-8533, Japan
[b] Department of Health and Nutrition, Wayo Women's University, Konodai, Ichikawa, Chiba 272-8533, Japan
[c] Center for Advanced Biochemical Sciences, Waseda University, Wakamatsu-cho, Shinjuku-ku, Tokyo 162-8480, Japan
[d] School of Science and Technology, Meiji University, Higashimita, Tama-ku, Kawasaki, Kanagawa 214-8571, Japan
[e] General Head Research, Food, Okuno Chemical Industries Co., Ltd., Hanaten-higashi, Tsurumi-ku, Osaka 538-0044, Japan

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ABSTRACT

We evaluated the inhibitory effects of CS with different molecular weights from 170,000 to 10,000 Da on a-glucosidase activity *in vitro*. Low-molecular-weight CS (< 16,000 Da) showed a higher inhibition of a-glucosidase activity than high-molecular-weight CS. In addition, we examined the effects of a single administration of low-molecular-weight CS on postprandial blood glucose in ICR mice. Low-molecular-weight CS inhibited the elevation of postprandial blood glucose in mice loaded with sucrose or starch as the source of carbohydrates. Finally, we investigated the anti-diabetic effects of daily administration of low- molecular-weight CS using type 2 diabetes model KK-Ay mice. The elevation of fasting blood glucose was inhibited in the CS group. These results suggest that the administration of low-molecular-weight CS prevents the elevation in the postprandial blood glucose level by reducing the digestion of carbohydrates in the gastrointestinal tract, leading to reductions of hyperglycemia in KK-Ay mice.

1. Introduction

The International Diabetes Federation estimated the number of diabetes mellitus cases in 2015 to be more than 400 million individuals worldwide, and expected that this number will increase to 600 million by the year 2040. Some 90% of diabetic individuals have type 2 diabetes mellitus. Correcting postprandial hyperglycemia has been demonstrated to be clinically effective in preventing the development and progression of type 2 diabetes (Standl, Schnell, & Ceriello, 2011). This led to an approach to suppress the digestion and absorption of carbohydrates by inhibiting carbohydrate digestive enzymes such as α-amylase and α-glucosidase to mitigate the elevation of postprandial blood glucose levels (Deguchi et al., 1998; Hosaka et al., 2011; Shan et al., 2016; Uchida et al., 2013).

Chondroitin sulfate (CS) present in the connective tissue of animals, is an acidic polysaccharide (Nomura, 2008). Acidic polysaccharide containing sulfate and/or carboxyl groups includes alginate, fucoidan and heparin other than CS (Toida, Chaideddgumjorn, & Linhardt, 2003). Acidic polysaccharides were shown to have biological activities, anticoagulant (Qi et al., 2012), antitumor (Zhang et al., 2016), antivirus (Yang, Jia, Zhou, Pan, & Mei, 2012) and immunomodulation (Han et al., 2012) effects. Many reports (Cho, Lee, & You, 2011; Sun, Wang, Shi, & Ma, 2009; Sun, Wang, & Zhou, 2012) have indicated that the biological activities of acidic polysaccharides depend on their structural features such as the degree of sulfation, molecular weight, and monosaccharide composition. As to alginate and fucoidan, a certain relationship has reportedly been found between their inhibitory effect of α-glucosidase activity and their molecular weight (Kim, Rioux, & Turgeon, 2014; Nakamura, Aki, Hashiguchi-Ishiguro, Ueda, & Oku, 2008; Vinoth et al., 2015), but such a relationship with the molecular-weight of CS has not been reported. Additionally, the anti-diabetic effects of CS have not yet been reported *in vivo*.

In the present study, we evaluated the inhibitory effects of CSs with different molecular weight on α-glucosidase activity *in vitro*. In addition, carbohydrates tolerance tests with normal ICR mice were performed to examine the effect of a single administration of CS on the elevation of postprandial blood glucose levels. Finally, we investigated the anti-diabetic effects of the daily administration of CS using type 2 diabetes model KK-A^{y} mice.

* Corresponding author at: Faculty of Human Ecology, Wayo Women's University Graduate School, Konodai, Ichikawa, Chiba 272-8533, Japan.
E-mail addresses: moto@wayo.ac.jp (M. Moto), takamizawa.n@aoni.waseda.jp (N. Takamizawa), t_shibuya_111@yahoo.co.jp (T. Shibuya), a-nakamura@wayo.ac.jp (A. Nakamura), katsuraya@wayo.ac.jp (K. Katsuraya), k-iwasaki01@okuno.co.jp (K. Iwasaki), k-tanaka01@okuno.co.jp (K. Tanaka), amurota@oligodesign.jp (A. Murota).

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Table 1
Hydrolysis condition, molecular weight and sulfate of hydrolyzed chondroitin sulfate.

Hydrolysis condition	Molecular weight (Da)	Degree of sulfation (%)
Not hydrolyzed	170,000	19.1
80 °C, 20 h	130,000	19.1
90 °C, 20 h	82,000	19.1
100 °C, 14 h	50,000	19.0
100 °C, 24 h	33,000	18.8
110 °C, 24 h	16,000	18.2
120 °C, 16 h	10,000	17.1

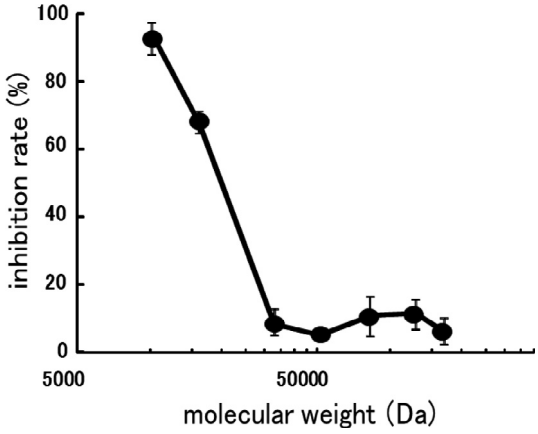

Fig. 1. Inhibitory effect of different-molecular-weight chondroitin sulfate on the activity of α-glucosidase. Results are expressed as means ± SE (n = 3).

2. Materials and methods

2.1. Preparation of CS with different molecular weights by hydrolysis

Chondroitin sulfate ZS tablets (Zeria Pharmaceutical Co., Ltd.), a pharmaceutical, were used as a raw material for CS. Separation of CS from these tablets was carried out by the following method. 80 g of the tablets was dissolved in 400 ml of water, the insoluble portion was removed by filtration, 1600 ml of ethanol was added, and the precipitate was centrifuged. The molecular weight of CS was controlled by using a high-pressure reactor (TEM-V1000, Taiatsu Glass Co., Ltd.) at 35 °C and 0.30 MPa of CO_2, and then following the standard procedures (Murota, Yamanoi, Ohsumi, Katsuraya, & Inoue, 2006). The conditions of hydrolysis are shown in Table 1. Purification of unhydrolyzed CS and hydrolyzed CS was performed with an ultrafiltration membrane (Spectrum/Por 7 MWCO 1000, manufactured by Spectrum Lab. Inc.). Weight-average molecular weight of the purified CS was evaluated by GPC column chromatography (column: SHODEX KS-804 and KS-802, eluent: 0.10 mol/L NaCl). In addition, degree of sulfation of CS was calculated by elemental analysis (varioEL III; Elementar Analysensysteme GmbH). Degree of sulfation (Chevolot et al., 1999) was calculated by the following equation.

SO_3Na (%) = S (%)/(atomic weight of S/molecular weight of SO_3 Na)

2.2. Suppressing a-glucosidase activity of CS with different molecular weight

CS was dissolved in 67 mM phosphate buffer adjusted to pH 6.9, and then 3 mM glutathione solution and 0.6 unit a-glucosidase solution were added. For the control, only buffer solution was used instead of CS solution. Each prepared solution was preheated at 37 °C for 10 min, then 250 µl of 10 mM p-nitrophenyl α-glucoside solution was added, and the mixture was stirred with a vortex mixer and incubated at 37 °C for 20 min. The reaction was terminated by addition of 8.0 ml of a sodium carbonate, and the p-nitrophenol isolated from α-glucoside was evaluated by absorbance at a wavelength of 400 nm, and the inhibition rate was obtained by the following formula. In addition, the control (100% active) was evaluated without addition of acidic polysaccharide, and the blank value was determined by using only buffer instead of enzyme solution.

Inhibition rate (%) = (Control-CS/Control × 100)

2.3. Animals

Seven-week-old male ICR mice and four-week-old male KK-A^y mice were purchased from CLEA Japan, Inc. All mice were kept at 22 ± 2 °C and 55 ± 5% R.H. under a 12 h light-dark cycle with an individual cage and were provided allowed with food (CE-2, CLEA Japan) and water *ad libitum* for at least one week before experimental use. All animal studies were performed in accordance with the guidelines of Wayo Women's University for the care and use of laboratory animals, and were approved by the Animal Experiment Committee of Wayo Women's University (No. 1209).

2.4. Single administration of low-molecular-weight CS in carbohydrate tolerance test on ICR mice

After 18 h of fasting, ICR mice were assigned separately on the basis

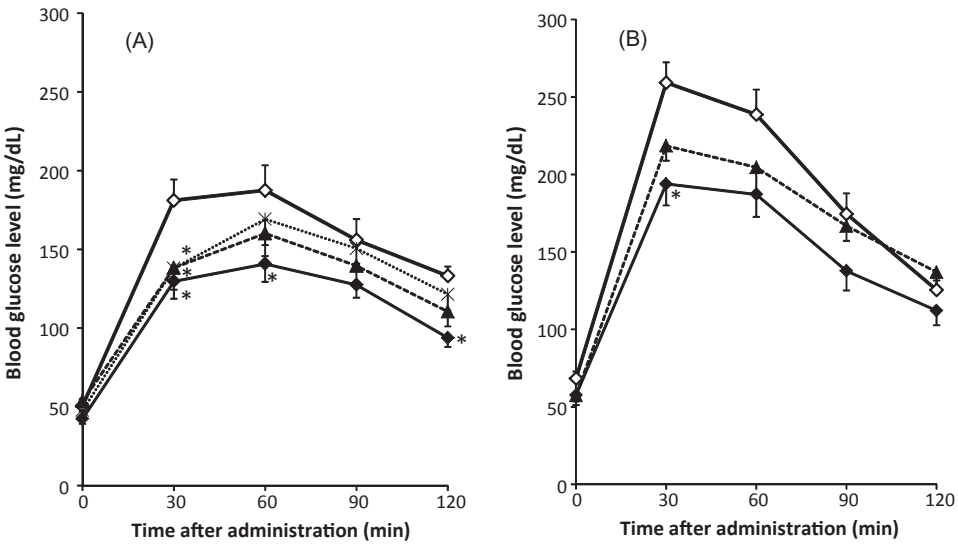

Fig. 2. Effect of low-molecular-weight chondroitin sulfate on blood glucose level in sucrose (A) or starch (B) loaded ICR mice. Results are expressed as means ± SE of 6–7 mice. ◇, control group; ×, CS 25 mg/kg B.W group; ▲, CS 50 mg/kg B.W group; ◆, CS 200 mg/kg B.W group. *Significantly different from the control group, $p < .05$.