Note

Effects of Salmon Nasal Cartilage Proteoglycan on Plasma Glucose Concentration and Active Glucose Transport in the Small Intestine

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Summary Recently, proteoglycan was purified from the nasal cartilage of salmon. Although several physiological effects have been reported, the effect of salmon nasal cartilage proteoglycan (salmon PG) on glucose metabolism remains unclear. We studied the effect of salmon PG on rat plasma glucose levels. Oral administration of 1% salmon PG significantly attenuated the increase in portal plasma glucose levels following an oral glucose tolerance test (OGTT). Additionally 1% salmon PG delayed the increase in peripheral glucose concentration induced by the OGTT. Mucosal administration of 1% salmon PG significantly decreased active glucose transport using the everted jejunal sac method. Furthermore, transmural potential difference (Δ PD) measurements using the everted jejunum revealed that 1% salmon PG significantly decreased glucose-dependent and phlorhizin (inhibitor of sodium-glucose co-transporter 1; SGLT1)-sensitive Δ PD. These results suggest that salmon PG decreases glucose absorption via SGLT1 in the jejunum, thereby attenuating the increase in portal and peripheral plasma glucose levels in rats.

Key Words proteoglycan, plasma glucose level, jejunum, SGLT1

Proteoglycan (PG) is a major non-collagenous component of the extracellular matrix, particularly in the cartilage and skin. In these tissues, PG interacts physically and functionally with substances, including collagen and fibulin (1). PG is composed of a protein core from which negatively charged glycosaminoglycans branch out. Glycosaminoglycans enable PG to swell by holding water, thereby absorbing large pressure changes in the cartilage and skin. PG also plays an important role in cell growth and differentiation, and in the innate immune system (2).

Recently, PG was purified from the nasal cartilage of the salmon as aggrecan, a cartilage-specific and high molecular weight PG (3, 4). Large quantities of salmon PG are now available for detailed investigation of its physiological function. Previous reports indicate that salmon PG attenuates the progression of colitis and promotes the generation of progenitor cells for granulocyte-macrophages, erythrocytes, and megakaryocytes (5, 6).

Although salmon PG may be a useful therapeutic agent, the mechanism of its intestinal absorption remains unclear. Recently, our group found that salmon PG was partially absorbed via clathrin-mediated endo-

cytosis in the rat jejunum (7). These data also suggested that approximately 90% of salmon PG remains in the intestine, where it may be excreted or exert physiological effects in the intestine. Because salmon PG contains glycosaminoglycan (GAG) chains in its polysaccharide structure, it has a large molecular weight (344 kDa) and may be poorly absorbed. Cellulose and pectin are known to be poorly absorbable polysaccharides. It has been reported that they suppress increases in plasma glucose concentration and glucose absorption in the small intestine (8, 9). We hypothesized that salmon PG may have effects similar to those of cellulose and pectin.

In this study, we investigated the effect of salmon PG on rat plasma glucose concentrations and glucose absorption in the small intestine. Our results indicated that salmon PG suppressed the increase in plasma glucose concentration following an oral glucose tolerance test (OGTT), and suggested that its suppressive effects occur via decreased active glucose absorption in the jejunum.

Experimental Procedures

Salmon PG was kindly supplied by Kakuhiro Co., Ltd. (Aomori, Japan). The animals were treated in accordance with the institutional and national guidelines for

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Fig. 1. Effect of salmon PG on plasma glucose levels. After oral administration of a glucose solution (2 g/kg, at 0 min), with (dashed line) or without (solid line) 1% salmon PG, the portal (A) and peripheral (B) plasma glucose levels were measured every 30 min. The data are expressed as the mean $\pm$ SE, $n=4$. * $p<0.05$ compared to the plasma glucose level in control rats at the same time.

the care and use of laboratory animals, and the study was approved by the Animal Usage Ethics Committee of Tohoku Women's College. Male Sprague Dawley rats (CLEA Japan, Inc., Shizuoka, Japan) were anesthetized with urethane (1.0 g/kg ip) containing α -chloralose (50 mg/kg). The animals were fasted for 12 h prior to the experiments.

For OGTT, glucose solution (2 g/kg) with or without 1% salmon PG was orally administered via a gastric tube in a single treatment. To perform portal blood sampling, we inserted a polyethylene tube in the rat portal vein. To perform peripheral venous blood sampling, we drew blood from the rat tail vein. To measure the concentration of portal and peripheral plasma glucose, we used the Blood Glucose Monitoring System (Glucose Pilot, Aventiir Biotech, LLC, Carlsbad, CA).

To measure the value of the active glucose transport, everted sacs (4.0 cm in length) were prepared from rat jejunum. The sacs were filled with Tyrode's solution (119 mM NaCl, 2.4 mM K_2HPO_4 , 0.6 mM KH_2PO_4 , 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 21 mM $NaHCO_3$, and 10 mM glucose, and oxygenated with 95% O_2 /5% CO_2 gas, pH 7.4). This solution was also used as the incubation solution. The glucose-free solution contained no glucose. The everted sac was incubated for 1 h in 40 mL

Fig. 2. The effect of salmon PG on active glucose transport in the rat jejunum. Everted jejunal sacs were incubated in solution, with 1.5, 5.0, or 10 mg/mL salmon PG (hatched bar) or without PG (white bar), for 1 h, and active glucose transport was measured. The data are expressed as mean $\pm$ SE, $n=4$. * $p<0.05$ compared to the glucose transport in control rats at the same concentration.

incubating solution at 37°C in all experiments. After incubation, one end of the everted sac was cut, and all the serosal liquid was collected. The volume of the serosal liquid and the weight of the sac were then measured. We measured the serosal and mucosal glucose concentrations through reductive sugar estimation, using the glucose oxidase method (Glucose CII-Test, Wako Pure Chemical Industries, Ltd., Osaka, Japan). The final glucose concentration was determined by absorbance at 505 nm. The glucose transport value was assessed by the amount of glucose transported from the mucosal to the serosal side per gram of wet tissue weight per hour ($\mu\text{mol}/\text{wet g}\cdot\text{h}$).

To measure sodium-dependent glucose transport, we measured the transmural potential difference (Δ PD) following our previous method (10). Δ PD was continuously measured by connecting calomel half-cells to the mucosal and serosal solution by means of a 2% agar bridge, and was recorded using a high-sensitivity DC chart recorder (056-1001, Hitachi, Tokyo) (11). The Δ PD value was expressed as positive when cations were transported from the mucosal side to the serosal side of the intestine.

Data are expressed as the mean $\pm$ SE. Statistical comparisons between two means were performed using the paired Student's t -test. More than three mean values were compared by ANOVA, followed by the Bonferroni-Dunn post hoc test using StatView software (SAS Institute, Cary, NC). p -values less than 0.05 were considered significant.

Results and Discussion

First, we determined the effect of salmon PG on plasma glucose concentration using the OGTT. As shown in Fig. 1A, oral administration of 1% salmon PG suppressed the increase in portal glucose concentration induced by the OGTT. In this experiment, portal glucose concentrations 90 and 120 min after oral glucose intake were significantly lower than that in the control group. Figure 1B shows that administration of 1% salmon PG delayed the increase in peripheral glucose concentration following

Fig. 3. The effect of salmon PG on phlorhizin-sensitive Δ PD in the rat jejunum. A and B are typical tracings. Everted jejunal sacs were incubated in glucose-free Tyrode's solution with (B) or without (A) 10 mg/mL salmon PG. Glucose (5 mM) and phlorhizin (0.2 mM) were administered on the mucosal side. C shows the average phlorhizin-sensitive Δ PD (mean $\pm$ SE, $n=4$). * $p<0.05$ compared to Δ PD in control rats.

the OGTT. In the salmon PG-treated group, the plasma glucose concentrations 30 min after oral glucose intake were significantly lower than that in the control group. The maximal plasma glucose level (at 60 min) tended to be lower than that in control group (at 30 min).

To evaluate the mechanism of salmon-PG-induced plasma glucose suppression, we considered the possibility that salmon PG inhibited glucose absorption in the small intestine. Thus, we compared active glucose absorption in everted jejunum preparations, with or without salmon PG. Figure 2 shows that mucosal administration of 1% salmon PG significantly suppressed active glucose transport in the jejunum. It is known that sodium-glucose co-transporter 1 (SGLT1) mediates active glucose transport on the mucosal side of the jejunum (12). To determine if salmon PG suppresses glucose transport via jejunal SGLT1, we compared changes in transmural potential difference (Δ PD)

in the everted jejunum following mucosal addition of glucose, with or without 1% salmon PG. The addition of 1% salmon PG suppressed the glucose-dependent and phlorhizin-sensitive increase in Δ PD (Fig. 3A and B). Furthermore, this value was significantly lower than that in the control group (Fig. 3C). These results suggest that salmon PG suppressed the increase in portal and peripheral plasma glucose concentrations by inhibiting active glucose transport via SGLT1.

In this study, salmon PG attenuated the increase in both portal and peripheral plasma glucose levels. However its effect on peripheral plasma glucose levels was weaker than its effect on portal glucose levels. This may be owing to glucose metabolism in the liver and the different regulators of peripheral plasma glucose level, including insulin and glucagon. Further studies evaluating these factors are necessary. Although we showed in Fig. 3 that salmon PG suppresses active glucose transport via phlorhizin-sensitive SGLT1 in the rat jejunum, the detailed mechanism remains unclear. Salmon PG may directly suppress SGLT1 activity. The protein core of aggrecan, including salmon PG, has three folded globular domains: the G1, G2 and G3 domains. It has been reported that the C-terminal G3 domain physically and physiologically interacts with various extracellular matrix molecules such as sulfated glycolipids on the cell surface, fibulin and tenascin (13). Because interaction between the G3 domain and SGLT1 protein is unknown yet, more study to elucidate involvement of the G3 domain in the suppressive effect of salmon PG on active glucose transport via SGLT1 will be interesting. We also consider the possibility that salmon PG may suppress glucose transport independently via SGLT1 inhibition. The GAG chains of aggrecans, such as salmon PG, have a negative charge and high hydrophilicity (14). It is possible that these chains conjugate the glucose, preventing its absorption. Studies to evaluate the interaction between GAG chains in salmon PG and glucose are needed.

Solutions of poorly absorbable polysaccharides, including pectin and cellulose, have a high viscosity. Flourie et al. found that pectin solutions with a viscosity of 50–350 cP significantly reduced glucose absorption (9). Furthermore, Takahashi et al. found that cellulose containing artificial digesta (880 cP) decreased post-infusion plasma glucose levels in rats. In contrast, control digesta (430 cP) had no effect (8). They suggested that the high viscosity of these polysaccharides in the jejunum restricted the absorption of glucose. In our study, the viscosity of 1% salmon PG solution was 6.5 cP (unpublished data). Because this value is much lower than in pectin and cellulose, we can exclude the possibility that the viscosity of salmon PG mediates this suppression.

Physiologically, our results suggest that salmon PG could be used to improve hyperglycemia. To elucidate this possibility, studies will be performed in hyperglycemic rats.

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