Preventive Effects of Acidic Xylooligosaccharide on Contact Hypersensitivity in Mice

Kyoji Yoshino,*[,a] Naoki Higashi,[b] and Kunimasa Koga[c]

[a]Department of Chemistry and Biochemistry, Numazu College of Technology, 3600 Ooka, Numazu, Shizuoka 410–8501, Japan, [b]Graduate Schools of Human Health Science, Tokyo Metropolitan University, 3–6–33 Azuma-cho, Akishima, Tokyo 196–8540, Japan, and [c]Department of Biological Science and Technology, Tokai University, 317 Nishino, Numazu, Shizuoka 410–0395, Japan

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The preventive effects of acidic xylooligosaccharide against contact hypersensitivity were investigated in mice. Percutaneous administration of acidic xylooligosaccharide at a dose of 0.10 mg/ear exhibited suppressive activity against contact hypersensitivity in mice. Oral administration at a dose of 200 mg/kg body weight also showed the same effects. Xylan, xylobiose, and glucuronic acid, sugars related to acidic xylooligosaccharide, exhibited suppressive effects on contact hypersensitivity only following oral administration at a dose of 200 mg/kg body weight. These results suggest that daily intake of acidic xylooligosaccharide may be advantageous for the prevention of contact hypersensitivity.

Key words —— acidic xylooligosaccharide, mouse, contact hypersensitivity

INTRODUCTION

Xylan is a major constituent of plant hemicellulose and one of the main components of dietary fiber. Corncobs, bagasse, cereals, bamboo shoots, mushrooms, and cotton hull bran contain large amounts of xylan. The structure of xylan is variable, ranging from a linear 1,4-β-linked polyxylose chain to highly branched heteropolysaccharides. The main chain of xylan is composed of D-xylose and 1,3-α-linked or 2,1-α-linked branches consisting of l-arabinofuranose at the *O*-3 positions of D-xylose residues, and of D-glucuronic or *O*-2-methyl-D-glucuronic acid at the *O*-2 positions of D-xylose residues. In several materials, xylan is acetylated at the same positions of D-xylose residues.[1)] The partial chemical structure of xylan is shown in Fig. 1. Xylan from *Hericium erinaceum*[2)] and acidic xylans, including glucuronic acid and 4-*O*-methylglucuronoxylan, from wood hemicellulose,[3)] show strong suppressive activities against various types of tumor. Anti-inflammatory activities of 4-*O*-methylglucuronoxylan from *Chamomilla recutita*[4)] and acidic, highly branched heteroxylan from *Plantago species*[5,6)] have also been reported. These findings suggest that polysaccharides consisting of xylose and glucuronic acid residues and their degradation products can ameliorate some inflammatory diseases. Xylooligosaccharide, a linear polymer of 1,4-β-linked xylose, is produced industrially by enzymatic degradation of solubilized xylan. It has also been reported that xylooligosaccharide exhibits various beneficial effects, such as improving intestinal condition,[7)] decreasing putrefaction products in the intestine,[8)] reducing blood cholesterol level, and suppressing blood glucose level.[9)] On the other hand, acidic xylooligosaccharide consisting of a linear polymer of 1,4-β-linked xylose bound with glucuronic acid is a byproduct of the production of xylooligosaccharide and there have been few reports about its beneficial effects for animal health. This oligosaccharide is a water-soluble white powder and has a fresh acidic taste. The chemical structures of these oligosaccharides are also shown in Fig. 1. Previously, we reported that the oral administration of maltooligosaccharide and isomaltooligosaccharide could prevent contact hypersensitivity in mice.[10)] In the present study, we investigated the preventive effects of acidic xylooligosaccharide and related sugars on contact hypersensitivity in mice.

MATERIALS AND METHODS

Materials and Animals —— Oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one), which is commonly used as a sensitizer for contact hypersensitivity, was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Xylobiose (purity > 98%) and D(+)-xylose (reagent grade quality) were also purchased from Wako Pure Chemical Industries, Ltd. Xylan (from oats and spelt) and D-glu-

*To whom correspondence should be addressed: Department of Chemistry and Biochemistry, Numazu College of Technology, 3600 Ooka, Numazu, Shizuoka 410–8501, Japan. Tel. & Fax: +81-55-926-5861; E-mail: k-yoshino@numazu-ct.ac.jp

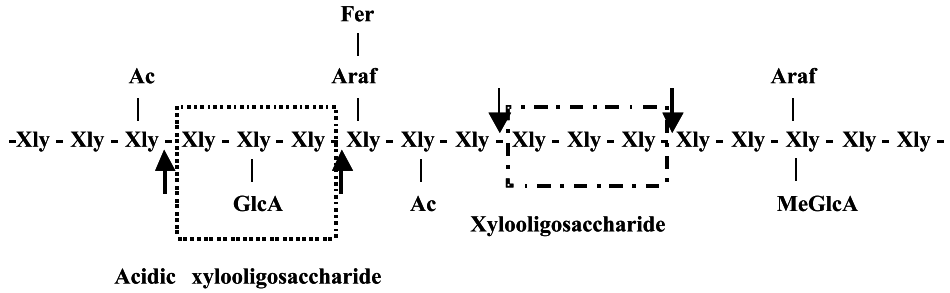

Fig. 1. Partial Chemical Structures of Xylan, Xylooligosaccharide, and Acidic Xylooligosaccharide

Xly, D-xylose; Araf, L-arabinofuranose; Ac, acetyl group; Fer, ferulic acid; GlcA, glucuronic acid; MeGlcA: 4-*O*-methyl-D-glucuronic acid. Arrows show the breaking points by endo-β-1,4-xylanase. The degrees of polymerization of xylose are varied.

curonic acid (purity 97%) were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and Lancaster Synthesis (Morecambe, U.K.), respectively. Acidic xylooligosaccharide was supplied by Suntory Ltd. (Osaka, Japan). Acidic xylooligosaccharide consisted of the following components: 4.3% glucuronoxylose, 17.1% glucuronoxylobiose, 34.0% glucuronoxylotriose, 28.8% glucuronoxylotetraose, 3.8% glucuronoxylooligomers not less than pentamer, and 22.2% unknown. Other chemicals used were of reagent grade. Four-week-old male ICR mice were purchased from Japan SLC Inc. (Shizuoka, Japan). Mice were maintained under conventional conditions throughout the experimental period and given tap water and commercial laboratory chow (5L37; Japan SLC Inc.) *ad libitum*. Throughout the experiment, the animals were handled in accordance with "*The Guide for the Animal Experiments in Numazu National College of Technology*."

Mouse Model of Contact Hypersensitivity —— Contact hypersensitivity was induced by the following procedures.[10,11] Sensitization was performed by carefully shaving off the hair on the abdominal region followed by application of 0.1 ml of 0.5% (w/v) oxazolone solution in ethanol to the skin. Five days after sensitization, the animals were challenged by application of 20 μl of 0.5% (w/v) oxazolone solution in acetone to both sides of the right ear of each animal. The mice were killed under anesthesia with diethyl ether 24 hr after challenge. Circular plugs (5.0 mm in diameter) were removed from both ears using a punching apparatus, and the right ear (WR) and left ear (WL) specimens were weighed.

For percutaneous administration, xylan, acidic xylooligosaccharide, xylobiose, xylose, and glucuronic acid were dissolved or suspended in oxazolone/acetone solution at challenge, and a dose of 0.10 mg/ear was administered. Hydrocortisone, a well-known steroid-type anti-inflammatory agent, was also assayed at the same dose. A control experiment was performed with only the solvents used for dissolution of the samples. Each group consisted of 5 mice.

For oral administration, sugar samples were dissolved in 0.5% tragacanth gum solution, and doses of 100 and 200 mg/kg body weight were administered once a day for 3 days before challenge. The challenge was carried out 1 hour after the last administration. Hydrocortisone was also assayed at the same doses. A control experiment was performed with 0.5% tragacanth gum solution. Each group consisted of 5 mice.

Ear swelling ratio was calculated by the following equation:

$$\text{Ear swelling ratio } (\%) = \frac{\{(\text{WR sample} - \text{WL sample})/\text{WL sample}\}}{\{(\text{WR control} - \text{WL control})/\text{WL control}\}} \times 100.$$

Statistics —— Statistical analyses were performed with the nonparametric Mann-Whitney U test for differences between groups, and $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Inhibitory Effect of Percutaneous Administration of Acidic Xylooligosaccharide and Related Sugars on Contact Hypersensitivity in Mice

The effects of percutaneous administration of acidic xylooligosaccharide and related sugars on contact hypersensitivity in mice are shown in Fig. 2. Percutaneous administration of hydrocortisone at a

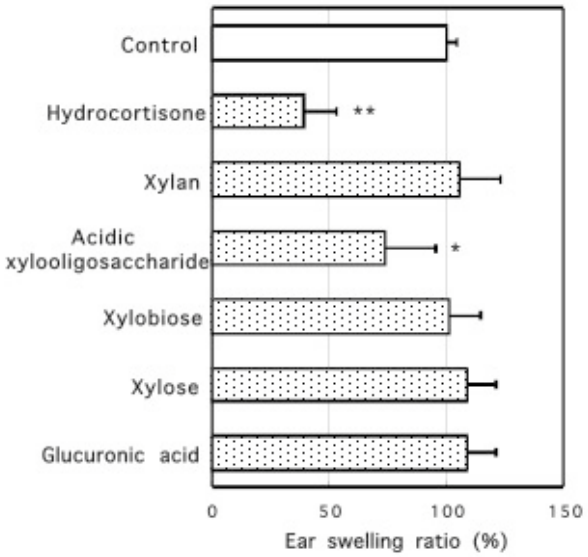

Fig. 2. Inhibitory Effects of Percutaneous Administration of Acidic Xylooligosaccharide and Related Sugars on Mouse Contact Hypersensitivity Induced by Oxazolone

Values are means ± SEM ($n = 5$). Significant difference from the control: $*p < 0.05$, $**p < 0.01$. Dose: 0.10 mg/ear.

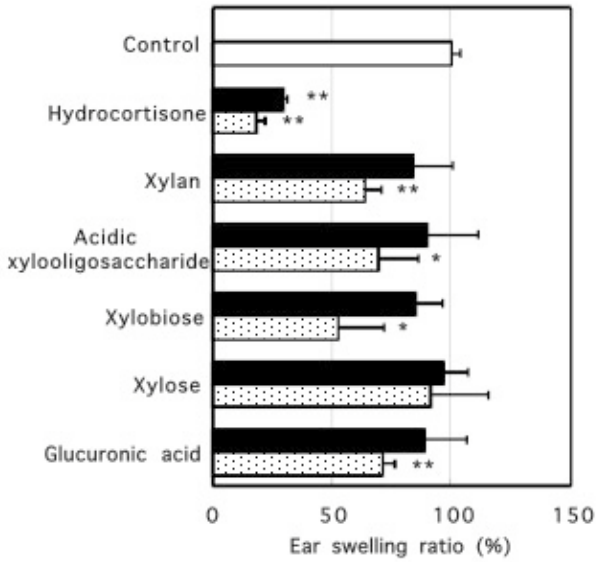

Fig. 3. Inhibitory Effects of Oral Administration of Acidic Xylooligosaccharide and Related Sugars on Mouse Contact Hypersensitivity Induced by Oxazolone

Values are means ± SEM ($n = 5$). Significant difference from the control: $*p < 0.05$, $**p < 0.01$. Dose: ■, 100 mg/kg body weight; ⊡, 200 mg/kg body weight.

dose of 0.10 mg/ear significantly reduced the ear swelling ratios in mice as compared to controls. The rate of inhibition was 60.9%. Xylan, xylobiose, xylose, and glucuronic acid showed no significant effects. Acidic xylooligosaccharide significantly suppressed contact hypersensitivity in this mouse model, with a rate of inhibition of 26.4%. These results suggest that the degree of polymerization of xylose in acidic xylooligosaccharide is important for its preventive effect against contact hypersensitivity in mice. The active components in acidic xylooligosaccharide are thought to be larger than trimers of xylose. Acidic xylooligosaccharide used in this study included 66.6% of such oligomers. Glucuronic acid may not be necessary for the anti-inflammatory activities of acidic xylooligosaccharide. Further investigations are necessary to clarify the mechanisms responsible for the preventive effects of percutaneous administration of acidic xylooligosaccharide on contact hypersensitivity in this mouse model. Many oligosaccharides are not expected to be absorbed into mouse skin, because they are hydrophilic due to the large numbers of hydroxyl groups in their chemical structures and their high molecular weights. Chitosan, which consists of a linear 1,4-β-linked polyglucosamine chain, is known to exhibit binding activities to some dyes[12] and suppressive activities against contact hypersensitivity after percutaneous administration in mice.[10] Acidic xylooligosaccharide may also bind or react with the hapten oxazolone and inhibit its absorption through the skin of mice.

Inhibitory Effect of Oral Administration of Acidic Xylooligosaccharide and Related Sugars on Contact Hypersensitivity in Mice

The effects of oral administration of acidic xylooligosaccharide and related sugars on mouse contact hypersensitivity are shown in Fig. 3. Oral administration of hydrocortisone at doses of 100 and 200 mg/kg body weight significantly reduced the ear swelling ratios in mice as compared to controls with inhibition rates of 70.8 and 81.9%, respectively. None of the sugars used in this study showed significant suppressive activities against contact hypersensitivity at a dose of 100 mg/kg body weight. Following administration at a dose of 200 mg/kg body weight, xylan, acidic xylooligosaccharide, xylobiose, and glucuronic acid showed significant suppressive effects against contact hypersensitivity in mice with rates of inhibition of 36.5, 30.6, 47.4, and 28.9%, respectively. Only xylose showed no significant effects. These results suggest that oral administration of oligomers and polymers of xylose could prevent contact hypersensitivity in mice induced by

oxazolone. The degree of polymerization of xylose is not important for the effect, although monomeric xylose showed no effect. Glucuronic acid could also contribute to the effects of acidic xylooligosaccharide in the case of oral administration. We could obtain only a crude xylooligosaccharide in this study and both percutaneous and oral administration significantly suppressed contact hypersensitivity in this mouse model, similarly to acidic xylooligosaccharide (data not shown). However, its purity was low (43.3%), and further studies are required to re-examine the activities of xylooligosaccharide using a preparation with higher purity.

Some digestion-resistant saccharides, such as xyloglucan and its degradation products from *Tamarindus indica* L.[13] and β-glucan from *Lentinus edodes*[14] were reported to exhibit immunoregulatory activities. The suppressive activities of xyloglucan and its degeneration products from *Ganoderma lucidum*[15] on delayed-type hypersensitivity in mice and the anti-inflammatory activities of β-glucan from *Dictyophora indusiata* Fisch.[16] in some rat models are also known. Contact hypersensitivity is a disease state attributed to type IV allergy, a previously acquired delayed-type reaction. The suppressive activities of oral administration of acidic xylooligosaccharide on contact hypersensitivity in mice may also be due, in part, to its effects on cell-mediated immune responses in mice. Further studies are required to investigate the effects of xylooligosaccharide on the levels of various cytokines, such as interleukin-12 and γ-interferon, in mice.

In conclusion, the daily intake of acidic xylooligosaccharide may prevent contact hypersensitivity in humans. It should be noted that excessive intake of digestion-resistant oligosaccharides, such as acidic xylooligosaccharide, can cause diarrhea.

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