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Effects of 1,5-anhydroglucitol on postprandial blood glucose and insulin levels and hydrogen excretion in rats and healthy humans

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Abstract

The inhibition by 1,5-anhydro-D-glucitol (1,5-AG) was determined on disaccharidases of rats and humans. Then, the metabolism and fate of 1,5-AG was investigated in rats and humans. Although 1,5-AG inhibited about 50 % of sucrase activity in rat small intestine, the inhibition was less than half of D-sorbose. 1,5-AG strongly inhibited trehalase and lactase, whereas D-sorbose inhibited them very weakly. 1,5-AG noncompetitively inhibited sucrase. The inhibition of 1,5-AG on sucrase and maltase was similar between humans and rats. 1,5-AG in serum increased 30 min after oral administration of 1,5-AG (600 mg) in rats, and mostly 100 % of 1,5-AG was excreted into the urine 24 h after administration. 1,5-AG in serum showed a peak 30 min after ingestion of 1,5-AG (20 g) by healthy subjects, and decreased gradually over 180 min. About 60 % of 1,5-AG was excreted into the urine for 9 h following ingestion. Hydrogen was scarcely excreted in both rats and humans 24 h after administration of 1,5-AG. Furthermore, 1,5-AG significantly suppressed the blood glucose elevation, and hydrogen excretion was increased following the simultaneous ingestion of sucrose and 1,5-AG in healthy subjects. 1,5-AG also significantly suppressed the blood glucose elevation following the simultaneous ingestion of glucose and 1,5-AG; however, hydrogen excretion was negligible. The available energy of 1,5-AG, which is absorbed readily from the small intestine and excreted quickly into the urine, is 0 kJ/g (0 kcal/g). Furthermore, 1,5-AG might suppress the blood glucose elevation through the inhibition of sucrase, as well as intestinal glucose absorption.

Key words: **1,5-Anhydroglucitol: Postprandial blood glucose: Disaccharidase: Hydrogen excretion: 1,5-Anhydro-D-glucitol**

1,5-Anhydro-D-glucitol (1,5-AG) is a hexose polyol that is made from 1,5-anhydro-D-fructose (1,5-AF). The raw material of 1,5-AF is produced from maltodextrin using glucan lyase from red algae[1,2]. 1,5-AG, which has 60 % of the sweetness of sucrose, is found in low levels in natural plant foods and about 4·38 mg of 1,5-AG is ingested per day in daily life[3]. 1,5-AG is also produced sparingly from glycogen in the liver[3] and is a polyol that is mostly contained in the body[3]. The concentration of 1,5-AG in the blood is about 14 μg/ml in normal adults[4]. 1,5-AG seems to be safe, because the eating experience of 1,5-AG is very long. The safety of 1,5-AG is certified in experiments using animals[5–7].

No specific abnormal observations in the tissues and organs are found, and the concentrations of blood glucose and TAG are not affected in rat experiments of the consecutive feeding of a diet containing 5 or 10 % 1,5-AG[8]. Furthermore, the anti-oxidative and oxidative indices of plasma and the amount of 8-oxo-2'-deoxyguanosine excreted into the urine are not significantly different from that of the control group[8]. These results support that 1,5-AG is a safe ingredient in food.

1,5-AG is used as a marker of blood glucose control in patients with diabetes mellitus[9] and is reabsorbed actively by the sodium glucose cotransporter-4 from the renal tubule[10,11]. The tubular reabsorption of 1,5-AG is competitively inhibited by glucose. Therefore, the urinary excretion of 1,5-AG is increased by the competitive inhibition of glucose in patients with high urinary glucose, and as a result the concentration of 1,5-AG in the blood is lowered[9,12–14]. Also, 1,5-AG is involved in insulin secretion[15]. Furthermore, 1,5-AG inhibited the elevation of blood glucose in a mouse model of diabetes mellitus[16]. However, it is not clear whether 1,5-AG, which is ingested excessively as a food ingredient, has a specific physiological function or expresses harmful effects.

Monosaccharides such as D-sorbose, D-psicose and L-arabinose strongly inhibit the activity of sucrase and maltase in the small intestine[17] and suppress the elevation of postprandial blood glucose and insulin[18]. 1,5-AG is a hexose, as are D-sorbose and D-psicose. Some studies have reported that 1,5-AG inhibits disaccharidase activity and suppresses the elevation of postprandial blood glucose[16–18]. In addition, 1,5-AG may retard the absorption of glucose from the small intestine. Therefore, 1,5-AG seems to inhibit the activity of disaccharidases and to suppress postprandial blood glucose and insulin.

Abbreviations: 1,5-AF, 1,5-anhydro-D-fructose; 1,5-AG, 1,5-anhydro-D-glucitol; BBMV, brush border membrane vesicles; FOS, fructo-oligosaccharide.

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In the present study, the inhibition of 1,5-AG and 1,5-AF, the precursor of 1,5-AG, on small intestinal disaccharidases was determined using brush border membrane vesicles (BBMV) from the small intestines of rats and compared with that of D-sorbose for which the inhibition was already reported[17]. Then, the inhibition of 1,5-AG upon rat small intestinal disaccharidase was compared with that in humans using small intestinal homogenates. Furthermore, the metabolism and fate of 1,5-AG was first investigated using rats before human experiments. 1,5-AG was administered orally to rats, and the effects on blood concentrations and urinary excretion of 1,5-AG and hydrogen excretion were determined. In addition, a sucrose or glucose solution with or without 1,5-AG was administered orally to rats, and the suppressive effects on postprandial blood glucose and insulin were determined. Similar physiological functions were also investigated using healthy human participants. The results obtained from the present study could support the potential application of 1,5-AG as a food ingredient.

Methods

Ethical approval

The protocol for animal studies was approved by the Committee on Animal Experiments of the University of Nagasaki (Nagasaki, Japan). These experiments were conducted according to the Guidelines on the Care and Use of Laboratory Animals (National Institutes of Health) and the standards relating to the Care and Management of Experimental Animals (notification no. 88, from the Prime Minister's office).

Experiments using donated tissues from human intestines were conducted according to guidelines laid down in the Declaration of Helsinki. All procedures involving human subjects were approved by the Ethics Committee of the University of Nagasaki Siebold (received no. 170, approved no. 163) and Juzenkai Hospital in Nagasaki, Japan. All subjects provided written informed consent to participate in the study. All experiments were carried out in the Public Health Nutrition Laboratory of the Graduate School of Human Health Science at the University of Nagasaki Siebold.

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Materials

1,5-AG (purity: >99 %) and 1,5-AF (purity: >98 %) were provided by Nihon Starch Co., Ltd. D-Sorbose (purity: >98 %) was provided by Matsutani Chemical Industry Co., Ltd. Fructo-oligosaccharide (FOS, purity: >98 %) was provided by Meiji Co., Ltd. (formerly Meiji Seika Kaisha Ltd).Glucose was used as a reference to the response of blood glucose and insulin. FOS was used as a reference of a typical oligosaccharide not hydrolysed by enzymes of the small intestine, fermented completely by gut microbiota[19,20] and consists of 1-kestose (39 %), nystose (53 %) and 1^F-*β*-fructofuranosyl-nystose (7 %).

Animals, diets and feeding

For the preparation of the small intestinal homogenates, five male Wistar rats (200 g) were fed a standard solid diet (MF, Oriental Yeast Co.) and distilled water *ad libitum* for 7 d. The animal quarters were maintained at a temperature of 22–24°C and a 12 h light–12 h dark cycle. The humidity was controlled at 50–55 %.

To prepare BBMV of the small intestine, another fifteen male Wistar rats (300 g) were fed a standard solid diet (MF) and distilled water *ad libitum* for 5 d in the same conditions described above. To observe the blood concentration and urinary excretion of 1,5-AG and hydrogen excretion following oral administration of 1,5-AG, another ten male Wistar rats (270–280 g) were fed an experimental diet for 7 d and housed under the same conditions described above. Furthermore, to observe the suppressive effects of 1,5-AG on the elevation of blood glucose and insulin levels following sucrose or glucose administration, another twenty male Wistar rats (270–280 g) were housed for 7 d under the same conditions as mentioned above. All rats were purchased from Clea Japan Inc.

In vitro experiments for small intestinal disaccharidases

Preparation of rat small intestinal homogenates and brush border membrane vesicles. After overnight fasting, five rats (250 g) were killed by decapitation and the small intestines were immediately removed, slit opened and washed with ice-cold 0·9 % NaCl. The mucosa was scraped off with slide glasses on ice-cold glass plates, weighed and homogenised in ice-cold 0·9 % NaCl (10 % wet w/v) using a homogeniser (Polytron; Kinematica Inc.). All of the homogenates were stored at –80°C until analysis. Before analysis, the samples were re-homogenised and diluted to adequate concentrations.

BBMV were prepared using the modified method of Kessler *et al.* from the small intestines of fifteen male Wistar rats (350 g)[21]. The BBMV obtained were suspended in an adequate volume of 0·9 % NaCl and used for the analysis of the inhibitory effects by 1,5-AG and other inhibitors on disaccharidases. Samples were stored at –80°C until analysis.

Preparation of human small intestinal homogenates. Fragments of human small intestines without malignant tissues were donated from five patients from Juzenkai Hospital. Patients provided written informed consent to donate their fragments of small intestine after resection of tumourous tissue in the small intestine. Immediately after the resection, the tissue fragments were soaked in ice-cold 0·9 % NaCl and transferred to our laboratory. After washing with 0·9 % NaCl, the mucosa was gently scraped off with two slide glasses onto an ice-cold glass plate and homogenised in a 19-fold volume of ice-cold 0·9 % NaCl using a homogeniser[22]. Samples were stored at –80°C until analysis.

Assay for inhibition of 1,5-anhydro-D-glucitol and 1,5-anhydro-D-fructose on disaccharidases of small intestine. The analysis of the hydrolysing activity on disaccharides in BBMV from rats and homogenates from human (*n* 5) and rat (*n* 5) small intestinal mucosa was carried out following Oku's method[23], which is modified partially from the Dahlqvist method using glucose oxidase[24]. Glucose was used as a standard. Sucrose, maltose, trehalose and lactose were used as substrates and prepared at 112 mM in 0·1 M maleate-Na buffer (pH 6·0). D-Sorbose was used as an inhibitor to compare the degrees of inhibition of 1,5-AG and 1,5-AF on disaccharidases and was prepared at concentrations of 10 and 100 mg/ml using 0·1 M maleate buffer (pH 6·0) to measure the inhibitory effects.

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In the regular assay[17,18], after the adequately diluted suspension of BBMV (0·1 ml) was pipetted into a small glass test tube, 20 µl of the inhibitor solution of various concentrations (0, 10, 100 mg/ml) was added to the test tube and incubated for 5 min at 37°C. Then, 0·1 ml of 112 mM substrate in 0·1 M maleate-Na buffer (pH 6·0) was added to the tube (total volume was 220 µl, final concentration of the substrate was 50·9 mM and final concentration of the inhibitor was 5 mM for 10 mg/ml and 50 mM for 100 mg/ml). As a control, 0·1 M maleate-Na buffer (pH 6·0) was used instead of an inhibitor solution. After incubation for 10–30 min at 37°C, 2·4 ml of TGO reagent, which contained 10 U/ml glucose oxidase, 5 U/ml peroxidase, 0·05 mM 4-aminoantipyrine and 0·4 mM *p*-phenolsulfonic acid sodium salt in 0·5 M TRIS-HCl (pH 7·0), was added to the tube to stop the hydrolysing reaction and to start the reactions of glucose oxidase and peroxidase; the reaction mixture was further incubated for 10 min at 37°C. Then, two drops of 4N NaOH was added to the tube to stop the reaction, and the optical absorbance was read at 505 nm using a spectrophotometer (UVmini-1240; Shimadzu Corp.). Protein concentrations were determined using the Bradford method[25] and a bovine serum albumin was used as a standard.

In vivo experiments using healthy rats

Determination of blood concentration and urinary excretion of 1,5-anhydro-D-glucitol and hydrogen excretion. To compare the effects of 1,5-AG on blood concentrations and urinary excretion of 1,5-AG and hydrogen excretion, FOS, which is non-digestible and fermentable, was used as a test substance and glucose, which is not present in the large intestine, was used as a reference. FOS (400 mg), 1,5-AG (600 mg) or glucose (600 mg) was dissolved in 2·5 ml of distilled water. After overnight fasting, rats (270–280 g) were administered with 2·5 ml of test solutions using a stomach tube (*n* 5/group). Immediately after administration of the test substance, rats were moved to a Metabolica apparatus (5-gang; Sugiyamagen Co., Ltd) to collect the circulating air for hydrogen determination, urine and faeces[22]. 1,5-AG in blood was measured by the colorimetric method using a 1,5-AG determination kit (Rana1,5-AGautokit; Kainos Co., Ltd), given that it could not be measured accurately using HPLC.

Analysis of 1,5-anhydro-D-glucitol by HPLC in urine. After the test solution (2·5 ml) was orally administered to rats, the urine and faeces were collected separately using the Metabolica apparatus for 24 h. The collected urine was measured by volume and then filtered through a membrane filter (0·22 µm × 13 mm; Millipore Corp.) to remove bacteria and small pellets for HPLC analysis. The analysis of 1,5-AG was carried out using an HPLC system (LC-20AD; Shimadzu Corp.) using a refractive index detector (RID-10A; Shimadzu Corp.) and a Shodex NH_2P-50 4E column (4· φ × 250 mm; Showa Denko Co., Ltd)[8]. Analysis was performed at a column temperature of 40°C. The sample (10 µl) was injected into the HPLC system and eluted with CH_3CN–water (75:25) solution at a flow rate of 0·5 ml/min[8]. The limit concentration of detection of glucose was 10 µg/ml in HPLC analysis.

Circulating air collection and analysis of hydrogen. The flow rate of the circulating air in the Metabolica apparatus was adjusted to 200 (SD 30) ml/min. To obtain the initial value, 40 ml of circulating air was collected using a plastic syringe (50 ml) 30 min after moving the rat into the apparatus. Then, the circulating air was collected at 1-h intervals for 4 h, 2-h intervals from 4 to 10 h and at 24 h following administration of the test solution[22]. To avoid hunger, the experimental diet was fed to the rats 4 h after administration of the test solution. the circulating air, 5 ml, was loaded into a simple gas chromatograph (Breath Gas Analyzer BGA1000D; Laboratory for Expiration Biochemistry Nourishment Metabolism Co., Ltd) to measure the concentration of hydrogen[22].

Suppressive effect of 1,5-anhydro-D-glucitol on the elevation of blood glucose and insulin levels by single oral administration of sucrose or glucose in rats. After overnight fasting, rats (260–280 g) were administered with 2·5 ml of test solutions using a stomach tube. The test solutions were as follows: sucrose (600 mg) in 2·5 ml, a mixture of sucrose (600 mg) and 1,5-AG (120 mg) in 2·5 ml, glucose (400 mg) in 2·5 ml and a mixture of glucose (400 mg) and 1,5-AG (80 mg) in 2·5 ml. Blood (120 µl) was collected from the tail veins using heparinised haematocrit tubes at 30-min intervals until 180 min following administration of the test substance, and then the plasma was prepared by haematocrit centrifugation (3300; Kubota Co., Ltd) at 2000 ***g*** at 20°C for 5 min.

Glucose concentrations were measured by the glucose oxidase method[26]. After plasma (10 µl) was pipetted into a small tube, glucose oxidase reagent (1·5 ml) was added and the tube was incubated at 37°C for 15 min. Then, the tube was heated in boiling water for 150 s to stop the reaction. The absorbance was read immediately using a spectrophotometer (UVmini-1240) at a wavelength of 505 nm. Insulin concentrations were measured immunologically using an ELISA kit (Morinaga Biochemical Institute Co.) with a guinea-pig-derived antibody[27].

Human experiments using healthy subjects

Characteristics of the participants. The present study involved human participants and was carried out using a within-subjects, repeated-measures design. Participants randomly ingested each substance repeatedly at intervals of 1 week or more. In all, ten healthy participants (four male, 25·7 (SD 1·9) years, 60·7 (SD 3·5) kg, BMI 21·0 (SD 1·3) kg/m^2; six female, 28·1 (SD 3·9) years, 51·3 (SD 4·3) kg, BMI 20·9 (SD 1·3) kg/m^2) voluntarily participated in this study. The exclusion criteria were a history of gastrointestinal diseases, carbohydrate malabsorption, diabetes, obesity or pulmonary disease[28–32]. The average value of the fasting blood glucose concentration of the subjects was 4·5 ± 0·23 mmol/l and was within the normal range. Before the experiments, we ensured that all participants were hydrogen producers and not methane producers. The participants had not taken any antibiotics or laxatives during the 2 weeks before the experiments.

Preparation of test solutions for human subjects. To investigate the effects of 1,5-AG on blood concentrations and urinary excretion of 1,5-AG and breath hydrogen excretion, 10 or 20 g of 1,5-AG was dissolved in 120 ml of warm

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sterilised water. The test solutions were stored at 5°C until consumption. Furthermore, sucrose (30 g), glucose (30 g), sucrose (30 g) with 1,5-AG (6 g) or glucose (30 g) with 1,5-AG (6 g) was dissolved in 150 ml of warm sterilised water to observe the effects on blood glucose and insulin levels. The tolerance level of FOS, because of its osmotic effects in the large bowel, is 0·3 g/kg of body weight for the normal Japanese population[33]. Therefore, 5 g of FOS was dissolved in 120 ml of warm sterilised water in accordance with our proposed method[30–32].

Special meals for human subjects. The period during which the food intake of the subjects was restricted was very long. Hence, the experimental meals, from which hydrogen was not produced, were given to the subjects after the final blood collection. Meals consisted of combinations of canned tuna fish (Sea-chicken mild, 80 g; Hagoromo Food Co. Ltd), boiled eggs (50 g) and tea. The energy supplied to the subjects was 214–232 kJ (895–970 kcal), protein was 58–65 g and carbohydrate was about 1 g on experimental days. If needed, more tea or boiled eggs were given[28–30]. In addition, each subject was given a multivitamin tablet (Nature Made; Otsuka Pharmaceutical Co. Ltd) as a supplement every day during the experimental periods.

Experimental protocol for human subjects. All test substances were given in a random order at intervals of 1 w or more. Experiments were carried out under the direction of a physician and with the methods used in our previous studies[28–32,34]. After overnight fasting, the subjects came to our laboratory in the morning, their health status was examined and their blood pressure and pulse rate were measured. Then, 750 ml of end-expiratory gas was collected using a collection bag (Quintron Instrument Co. Inc.) and blood (120 µl) was collected from the fingertip using a heparinised haematocrit tube. After ingesting the test substance, end-expiratory gas was collected at 1-h intervals for 14 h[28–30], and blood (120 µl) was collected at 30-min intervals for 180 min. After 1,5-AG (10 or 20 g) was ingested by healthy subjects (n 10), urine was collected between 0 and 3, 3–6 and 6–9 h following ingestion. 1,5-AG concentrations in serum were measured by the colorimetric method using a 1,5-AGautoKit and urinary 1,5-AG was measured by HPLC as described above.

Participants were prohibited from ingesting foods containing non-digestible carbohydrates such as dietary fibre, oligosaccharide and sugar alcohol for 3 d before each experimental day. During the experiment, they were also prohibited from ingesting foods or beverages except for warmed water or tea, as well as from sleeping and smoking. Participants were required to sit on a chair and were prohibited from exercising with hyperventilation until the final collection of end-respiratory gas. Participants ingested an experimental meal that did not produce breath hydrogen so that they would not feel hungry.

After ingesting the test substance, the type and onset time of abdominal symptoms, the frequency of defecation and the macroscopic observation of stool shape were recorded by the participants themselves using a standardised format. The research supervisor explained to all participants the method for self-recording and classifying the stool shape and abdominal symptoms, and then confirmed the recordings when received. The examination of stools used the following descriptors: stage 1, very hard; stage 2, hard; stage 3, normal; stage 4, soft; stage 5, very soft; and stage 6, watery[34]. The questionnaire on abdominal symptoms included items about the experience of upper and lower abdominal pain, vomiting, nausea, thirst, flatus, distension and borborygmus for 14 h following ingestion of the test substance.

Assay of plasma glucose and insulin and analysis of breath hydrogen. Inhibition of 1,5-AG on disaccharidases of the small intestine, the plasma glucose and insulin concentrations and hydrogen excretion in the end-expiratory gas were measured by the methods described above.

Statistical analyses

In *in vitro* experiments, the activity of disaccharidase using rat small intestinal BBMV was calculated as specific activity (micromoles of substrate hydrolysed/mg protein per h) and then expressed as the percentage of activity by the addition of 1,5-AG *v.* control. In the inhibition experiment using rat and human homogenate, data were shown as mean and standard deviations, and compared using unpaired Student's t test.

In *in vivo* experiments in rat, the responses of blood glucose and insulin by the administration of sucrose *v.* (1,5-AG and sucrose) or glucose *v.* (1,5-AG and glucose) were compared using paired Student's t test at each time point, respectively, and hydrogen excretion by the administration of glucose, 1,5-AG and FOS was compared using ANOVA and Dunnett's *post hoc* test, at each time point after normal distribution test, respectively.

In human experiments, the responses of blood glucose, insulin and hydrogen excretion, serum concentration and urinary excretion of 1,5-AG were calculated as the mean and standard deviations, and then the normal distribution test was performed. Thereafter, Student's t test was performed for normal distribution data, except hydrogen excretion, and Mann–Whitney U test was performed for the hydrogen excretion at each time point. A P value <0.05 was considered significant. Analyses were conducted using SPSS for Windows 23.0 (SPSS Inc.).

Results

In vitro experiments of inhibition on hydrolysing activity of disaccharidases

Inhibition by 1,5-anhydro-D-glucitol and 1,5-anhydro-D-fructose on hydrolysing activity of disaccharidase in brush border membrane vesicles of rat small intestine. Inhibition of the activity of disaccharidases such as maltase, sucrase, trehalase and lactase by 1,5-AG and 1,5-AF was determined using BBMV of rat small intestine and was compared with that of D-sorbose (Fig. 1). Although inhibition of sucrase by 1,5-AG was about 10 % of control at a concentration of 10 mg/ml, it increased markedly to about 50 % at a concentration of 100 mg/ml (Fig. 1(a)). However, inhibition of sucrase by 1,5-AG was less than half of that of D-sorbose. Inhibition of sucrase by 1,5-AF was similar to that of 1,5-AG.

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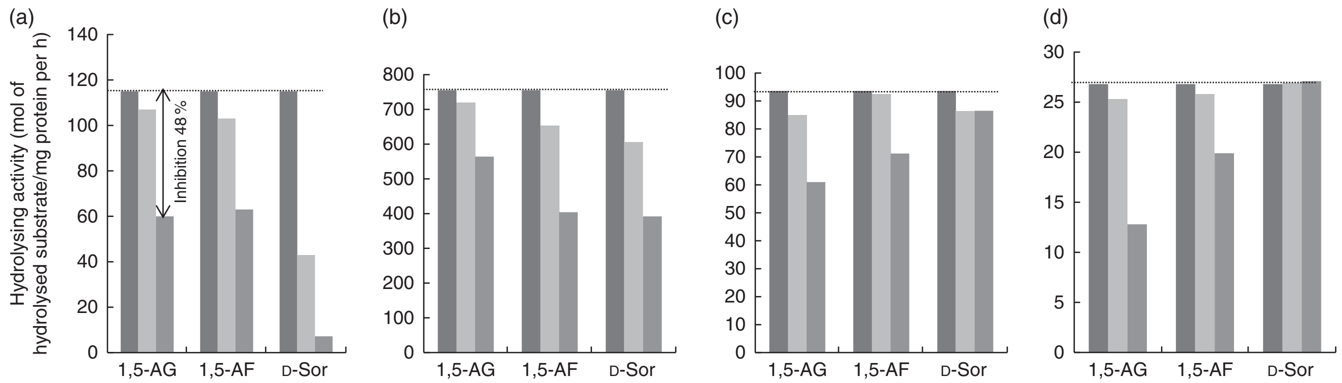

Fig. 1. Inhibitory effects of 1,5-anhydro-D-glucitol (1,5-AG,) 1,5-anhydro-D-fructose (1,5-AF) and D-sorbose (D-Sor) in brush border membrane vesicles of rat small intestine. (a) Sucrase, (b) maltase, (c) trehalase, (d) lactase. A 0·1-ml aliquot of brush border membrane vesicles and 0·02 ml of 10 mg/ml or 100 mg/ml inhibitor in 0·1 M maleate-Na buffer (pH 6·0) were incubated for 5 min at 37°C. Then, 0·1 ml of 112 mM substrate was added to the medium and further incubated for 10–30 min at 37°C. Values are the means of the hydrolysing activity using duplicate determinations. ■, 0 mg/ml; ■, 10 mg/ml; ■, 100 mg/ml.

Inhibition of maltase by 1,5-AG increased in a concentration-dependent manner and was 25 % of control at a concentration of 100 mg/ml (Fig. 1(b)). The inhibition of maltase by 1,5-AF was slightly stronger than that of 1,5-AG and was about 47 % of control at a concentration of 100 mg/ml. The inhibition of trehalase by 1,5-AG was strongest among three inhibitors at a concentration of 100 mg/ml, although the degree of inhibition was distinctly lower than that for sucrase (Fig. 1(c)). Also, 1,5-AF strongly inhibited trehalase in comparison with D-sorbose; however, the inhibition was weaker than that by 1,5-AG. Furthermore, 1,5-AG strongly inhibited lactase and showed about 55 % inhibition of controls at a concentration of 100 mg/ml. However, D-sorbose showed very weak inhibition of lactase at the same concentration (Fig. 1(d)). The inhibition of lactase by 1,5-AF was also stronger than that of D-sorbose, but about half that of 1,5-AG.

D-Sorbose showed the strongest inhibition of sucrase among four disaccharidases; the inhibition of maltase was very weak compared with that of sucrase. Furthermore, inhibition of trehalase and lactase by D-sorbose was very low or negligible. However, 1,5-AG inhibited clearly every sucrase, maltase, trehalase and lactase at a concentration of 100 mg/ml. Namely, the degree of inhibition by 1,5-AG was roughly similar for all disaccharidases, although that of inhibition by D-sorbose was markedly different among four disaccharidases.

Inhibitory mode of action of sucrase by 1,5-anhydro-D-glucitol in brush border membrane vesicles of rat small intestine. 1,5-AG inhibited about 50 % of sucrase activity and about 25 % of maltase activity at a concentration of 100 mg/ml (final concentration: 61 mM). Therefore, 1,5-AG was dissolved at 61 mM or 122 mM in a 112 mM sucrose solution to investigate the inhibitory mode of action of 1,5-AG on sucrase. Two inhibition lines crossed to the non-inhibition line on the *x*-axis of Lineweaver–Burk plot (Fig. 2). Thus, 1,5-AG non-competitively inhibited sucrase in the rat small intestine. The *K*m of sucrase from BBMV of rat small intestine was 21·6 mM and V_{max} was 169·5 moles of substrate hydrolysed/mg protein/h. The K_i of 1,5-AG on sucrase was 62 mM. Also, the inhibitory mode of action of 1,5-AG on maltase was investigated, as well as sucrase, but reasonable results could not be obtained.

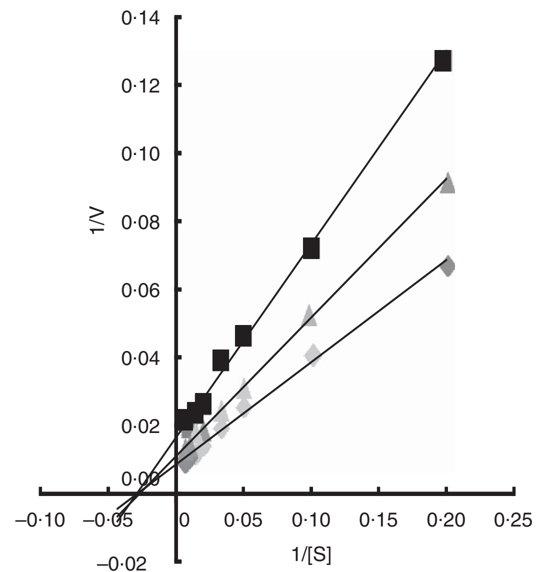

Fig. 2. Lineweaver–Burk plot of 1,5-anhydro-D-glucitol (1,5-AG) on sucrase from rat small intestinal brush border membrane vesicles. [S], concentration of sucrose in 0·1 M maleate-Na buffer (pH 6·0); V, micromol of substrate hydrolysed/mg protein per h; ◆, sucrose alone; ▲, +1,5-AG 10 mg/ml; ■, +1,5-AG 20 mg/ml.

Comparison of inhibition of sucrase and maltase by 1,5-anhydro-D-glucitol using homogenates of small intestine of humans and rats. 1,5-AG and 1,5-AF showed the strongest inhibition of sucrase among four disaccharidases in the rat small intestine, followed by maltase at a concentration of 100 mg/ml. However, inhibition of sucrase and maltase by 1,5-AG and 1,5-AF in human small intestine has not been described previously. To compare the inhibition of sucrase and maltase by 1,5-AG on humans and rats, the activity of sucrase and maltase was measured in the presence of 1,5-AG or 1,5-AF using homogenates of small intestine from humans and rats (Fig. 3).

No significant difference was observed in the sucrase or maltase activity inhibited by 1,5-AG between humans and rats.

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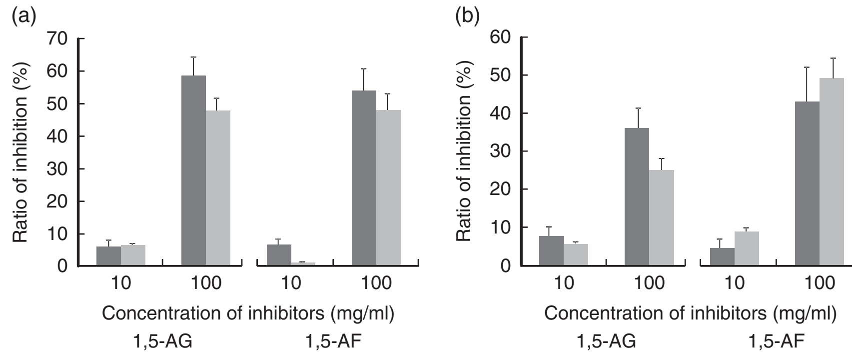

Fig. 3. Comparison of inhibition of sucrase (a) and maltase (b) by 1,5-anhydro-D-glucitol (1,5-AG) and 1,5-anhydro-D-fructose (1,5-AF) in small intestinal homogenates of humans and rats. Numerical numbers in columns show the percentage of inhibition *v.* 0 mg/ml of inhibitor (1,5-AG or 1,5-AF). No significant difference was observed between the activity of humans (■) and rats (□) by Student's *t* test. A 0·1-ml aliquot of small intestinal mucosa homogenate and 0·02 ml of 0·1 M maleate-Na buffer (pH 6·0), and 10 mg/ml or 100 mg/ml inhibitor in 0·1 M maleate-Na buffer (pH 6·0) were incubated for 5 min at 37°C. Then, 0·1 ml of 112 mM substrate was added to the medium (total volume: 0·22 ml) and further incubated for 10–30 min at 37°C. Values are the means of the ratio of inhibition using duplicate determinations (human *n* 5, rat *n* 5). The ratio of inhibition was not significantly different between humans and rats at the same concentrations of the inhibitor.

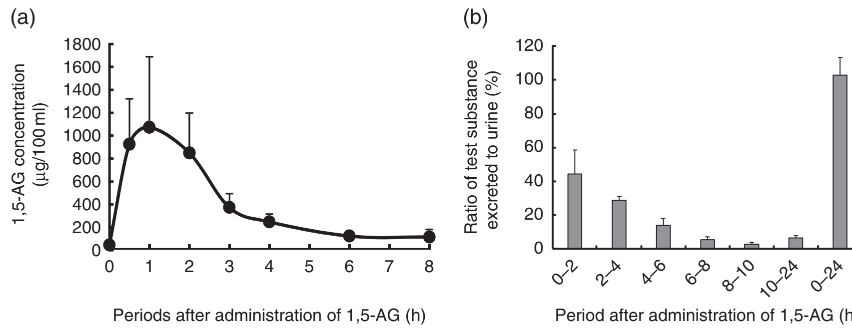

Fig. 4. Changes in 1,5-anhydro-D-glucitol (1,5-AG) blood concentrations (a) and the ratio of 1,5-AG excreted into urine after oral administration of 1,5-AG (b) in healthy rats. After 1,5-AG (600 mg) was administered orally to healthy rats (*n* 5), venous blood (120 μl) was collected from the tail after 0, 30, 60, 120, 240, 360 and 480 min, and urine was collected for 24 h using a Metabolica apparatus. 1,5-AG plasma concentrations were determined by the colorimetric assay using a 1,5-AG determination kit. 1,5-AG urinary concentrations were measured using HPLC.

The inhibition of sucrase by 1,5-AG was slightly stronger in humans than in rats at a concentration of 100 mg/ml, but was very weak at a concentration of 10 mg/ml. The inhibition of maltase was also slightly stronger in humans than in rats, but the degree of inhibition was slightly weaker than that of sucrase. The inhibition of sucrase by 1,5-AF was slightly stronger in humans than in rats and was a little weaker than that by 1,5-AG. The inhibition of maltase by 1,5-AF was slightly stronger in rats than in humans. These results suggest that the inhibitory effects of 1,5-AG on sucrase and maltase in the small intestine is roughly similar between humans and rats, and is stronger for sucrase.

In vivo *experiments using rats*

Effects of 1,5-anhydro-D-glucitol administered orally on blood concentrations and urinary excretion of 1,5-anhydro-D-glucitol and hydrogen excretion in rats. The effects of oral administration of 1,5-AG on blood concentrations and urinary excretion of 1,5-AG and hydrogen excretion were investigated using rats non-adapted to 1,5-AG. After 1,5-AG (600 mg) was administered orally to rats after overnight fasting, 1,5-AG concentrations in blood were measured at 0, 30, 60, 120, 180, 240, 360 and 480 min after administration. As shown in Fig. 4(a), 1,5-AG was detected slightly at 0 min, increased rapidly to 9·5 μg/ml at 30 min and then reached a peak (11 μg/ml) at 60 min following administration. Thereafter, it decreased gradually to 4 μg/ml at 180 min and returned to basal levels 480 min following administration. These results demonstrate that 1,5-AG is absorbed readily from the small intestine and appears rapidly in the blood.

1,5-AG (600 mg) administered orally was excreted rapidly into the urine in rats. The urinary excretion of 1,5-AG was more than 40 % of 1,5-AG administered for 2 h and increased to about 90 % until 6 h following administration (Fig. 4(b)). Thus, mostly 100 % of 1,5-AG administered was excreted to the urine 24 h after administration. These results suggest that 1,5-AG, which is absorbed readily from the small intestine, is not metabolised and is excreted rapidly into the urine. The pattern of urinary excretion reflected the decreasing blood concentrations of 1,5-AG.

Fig. 5 shows the change of hydrogen excretion after oral administration of 1,5-AG, FOS and glucose to rats non-adapted to the diet containing each test substance. When FOS (400 mg) was administered orally to rats, hydrogen started to be excreted at 2 h and reached a peak (about 80 parts per million) at 8–10 h following administration, and the excretion of hydrogen was significantly higher than that of glucose or 1,5-AG ($P < 0.05$). Thereafter, it decreased to basal levels 24 h after administration. Hydrogen was scarcely excreted during the 24 h following administration of 1,5-AG (600 mg). These results demonstrate that almost all of

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1,5-AG is readily absorbed from the small intestine after oral administration and does not reach the large intestine where it could be fermented by gut microbiota.

Suppressive effect of 1,5-anhydro-D-glucitol on the elevation of blood glucose by oral administration of sucrose or glucose in rats. 1,5-AG strongly inhibited sucrose activity. Therefore, when sucrose is administered simultaneously with 1,5-AG to rats, the elevation of blood glucose by sucrose is considered to supress through the inhibitory effect for sucrase. To investigate the suppressive effect of 1,5-AG for the elevation of blood glucose, a mixture of sucrose (600 mg) and 1,5-AG (120 mg) was administered simultaneously to rats (*n* 5/group) after overnight fasting and blood glucose concentrations were measured for 180 min. The elevation of blood glucose by administration of sucrose was significantly suppressed in the presence of 1,5-AG at 30, 60 and 90 min after administration ($P < 0·05$) (Fig. 6(A)). Thus, 1,5-AG might suppress the elevation of blood glucose by sucrose administration through the inhibition of sucrase.

1,5-AG may also inhibit glucose absorption from the small intestine, because the renal tubular reabsorption of glucose is competitively inhibited by 1,5-AG[10,11]. To investigate the suppressive effects of 1,5-AG on the elevation of blood glucose by glucose administration, glucose (400 mg) and 1,5-AG (80 mg) were administered simultaneously to rats (*n* 5/group) after overnight fasting. The elevation of blood glucose by glucose administration also was suppressed significantly in the presence of 1,5-AG at 30 and 60 min after administration ($P < 0·05$) (Fig. 6(B)), suggesting that 1,5-AG disturbs glucose absorption in the small intestine.

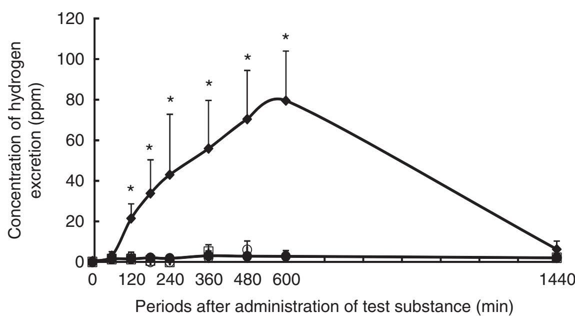

Fig. 5. Hydrogen excretion after oral administration of 1,5-anhydro-D-glucitol (1,5-AG) in healthy rats. Immediately after 1,5-AG (600 mg), fructo-oligosaccharide (FOS, 400 mg) or D-glucose (600 mg) was administered orally to healthy rats (*n* 5), and the rats were transferred to a Metabolica apparatus. FOS, which is non-digestible and fermented completely, was used as a control of fermentation. Glucose was used as a reference, given that it is not fermented in the large intestine. ppm, parts per million; ◆, 400 mg/2·5 ml FOS; ●, 600 mg/2·5 ml 1,5-AG; ○, 600 mg/2·5 ml glucose. *Excretion of hydrogen by the administration of FOS was significantly higher than that by glucose and 1,5-AG, at $P < 0·05$ by ANOVA and Dunnett's test.

Human experiments using healthy subjects

Subject participation. No participants dropped out of the study or experienced side effects. Their health status throughout the entire study period was normal.

Effects of 1,5-anhydro-D-glucitol on blood concentrations and urinary excretion of 1,5-anhydro-D-glucitol and breath hydrogen excretion in healthy human subjects. Fig. 7(A) shows the 1,5-AG concentrations in blood after ingestion of 1,5-AG (10 or 20 g) alone in healthy subjects. 1,5-AG concentrations in serum showed a peak (330 μg/ml) 30 min after ingestion of 10 g of 1,5-AG and a bigger peak (500 μg/ml) 60 and 90 min after ingestion of 20 g ($P < 0·05$). These concentrations decreased slowly over the 180 min following ingestion but did not return to basal levels within 3 h after ingestion. However, blood glucose concentrations did not increase by ingestion of 1,5-AG (10 or 20 g) (data not shown). The ratio of urinary excretion of 1,5-AG was 30–35 % 3 h after ingestion (10 or 20 g) and significantly increased to 55 % for 10 g ingestion and 73 % for 20 g ingestion after 9 h ($P < 0·05$, Fig. 7(B)). The recovery of 1,5-AG from the urine was slightly higher following 20 g ingestion than 10 g. Hydrogen excretion could not be detected from the respiratory gas after ingestion of 1,5-AG (10 or 20 g) (data not shown). These results support the hypothesis that 1,5-AG ingested is readily absorbed from the small intestine, excreted rapidly into the urine and does not arrive at the large intestine to be fermented by gut microbiota.

Effects of 1,5-anhydro-D-glucitol in simultaneous ingestion of sucrose or glucose on blood glucose and insulin levels and hydrogen excretion in healthy human subjects. 1,5-AG significantly inhibited the activity of sucrase in the small intestine of humans and rats. Therefore, 1,5-AG may suppress the elevation of

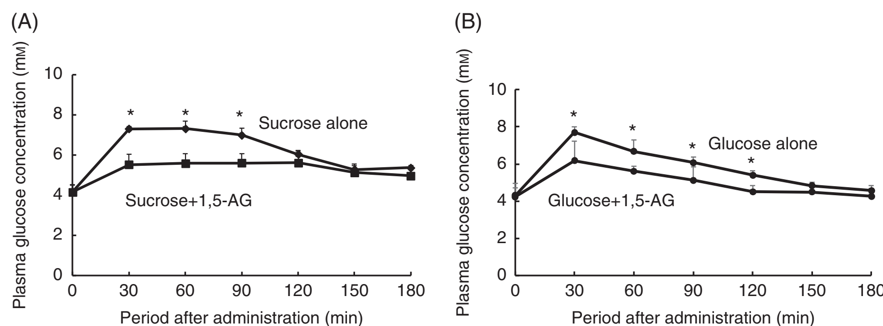

Fig. 6. Suppressive effects of 1,5-anhydro-D-glucitol (1,5-AG) on the elevation of blood glucose by simultaneous administration of sucrose solution (A) or glucose solution (B) with 1,5-AG in healthy rats fed a control diet. Test solutions (2·5 ml) containing sucrose (600 mg), sucrose (600 mg) + 1,5-AG (120 mg), glucose (400 mg) or glucose (400 mg) + 1,5-AG (80 mg) were administered orally to healthy rats (*n* 5) fed a control diet. Serum glucose concentrations were measured by the colorimetric assay using glucose oxidase. *There were significant differences between groups at the same time point at $P < 0·05$ using Student's *t* test.

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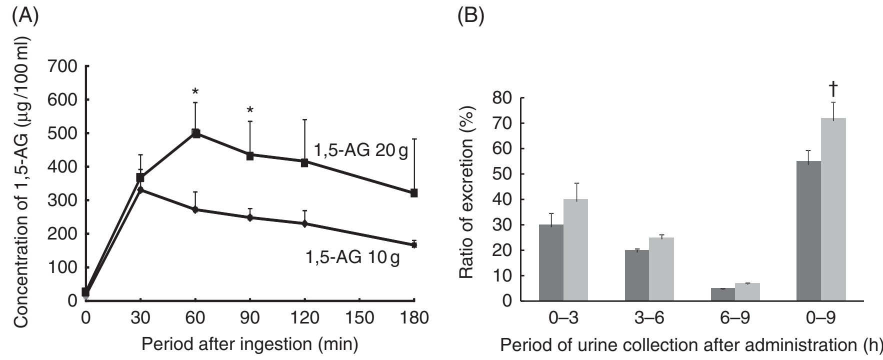

Fig. 7. Serum concentrations (A) and urinary excretion (B) of 1,5-anhydro-D-glucitol (1,5-AG) after ingestion in healthy subjects. After 1,5-AG (10 or 20 g) was ingested by healthy subjects (*n* 10), blood was collected from the fingertip at 0, 30, 60, 90, 120 and 180 min, and urine was collected at 0–3, 3–6 and 6–9 h. 1,5-AG in the serum was measured by the colorimetric assay using a 1,5-AG determination kit and 1,5-AG in urine was measured using HPLC. ■, 1,5-AG 10 g ingestion; □, 1,5-AG 20 g ingestion. *The concentration of 1,5-AG in serum after ingestion of 20 g of 1,5-AG was significantly increased from 10 g of ingestion at 60 and 90 min at $P<0{\cdot}05$ by Student's *t* test. †A total urinary excretion of 1,5-AG after 20 g of ingestion was significantly higher than that in the ingestion of 10 g at $P<0{\cdot}05$ by Student's *t* test.

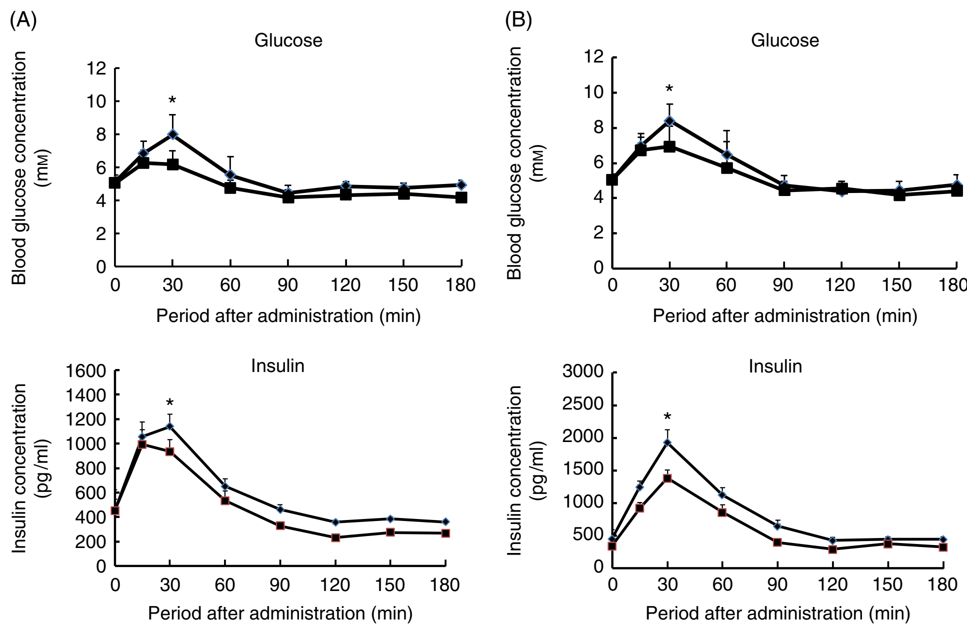

Fig. 8. Suppressive effects of 1,5-anhydro-D-glucitol (1,5-AG) on the elevation of blood glucose and insulin by simultaneous ingestion of sucrose (A) or glucose (B) with 1,5-AG in healthy subjects. To investigate the suppressive effects of 1,5-AG, test solutions containing sucrose (30 g), sucrose (30 g) + 1,5-AG (6 g), glucose (30 g) or glucose (30 g) + 1,5-AG (6 g) were ingested by healthy subjects (*n* 10). Blood (120 µl) was collected from the fingertip using a heparinised haematocrit tube at indicated time periods after ingestion of test substances. Serum glucose was measured by the colorimetric assay using glucose oxidase, and insulin was measured immunologically using an ELISA with guinea-pig-derived antibody. *There were significant differences between groups at the same time point at $P<0{\cdot}05$ using paired Student's *t* test.

blood glucose by sucrose ingestion through the inhibition of sucrase. To investigate the effects of 1,5-AG on the elevation of blood glucose and insulin, sucrose (30 g) and 1,5-AG (6 g) were ingested simultaneously by healthy subjects (*n* 10) (Fig. 8(A)). 1,5-AG significantly suppressed the elevation of blood glucose 30 min after ingestion ($P<0{\cdot}05$) (Fig. 8(A). Also, insulin secretion was significantly suppressed by 1,5-AG 15 and 30 min after ingestion ($P<0{\cdot}05$) (Fig. 8(A)). Thus, 1,5-AG showed suppressive effects on the elevation of blood glucose and insulin levels by sucrose ingestion in healthy human subjects, as well as in rats.

Furthermore, when glucose (30 g) and 1,5-AG (6 g) were ingested simultaneously by healthy subjects (*n* 10), 1,5-AG significantly suppressed the elevation of blood glucose and insulin 30 min after ingestion ($P<0{\cdot}05$) (Fig. 8(B)). These results demonstrate that 1,5-AG disturbs the absorption of glucose in the small intestine in healthy human subjects, as well as in rats, and suppresses the elevation of blood glucose and insulin levels.

When sucrose (30 g) and 1,5-AG (6 g) were ingested simultaneously by healthy subjects, the breath hydrogen excretion increased slightly, but positively, between 2 and 5 h following ingestion, and significantly at 4 h after ingestion ($P<0{\cdot}05$, Fig. 9). These results demonstrate that sucrose, which is not digested in the small intestine, arrives at the large intestine where it is fermented by gut microbiota. However, the breath hydrogen excretion scarcely increased following the simultaneous ingestion of glucose (30 g) and 1,5-AG (6 g) by healthy subjects (Fig. 9).

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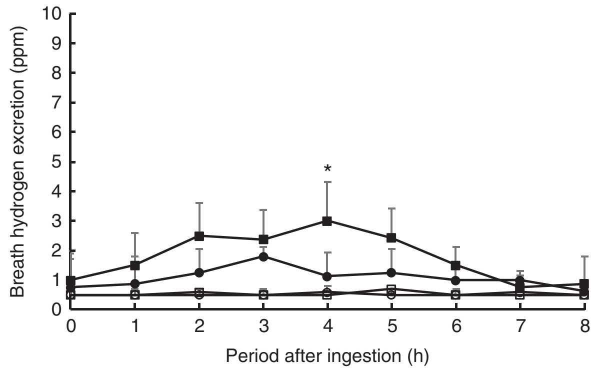

Fig. 9. Hydrogen excretion after ingestion of glucose or sucrose solution containing 1,5-AG in healthy subjects. To investigate the effects of 1,5-AG on breath hydrogen excretion, sucrose (30 g) or glucose (30 g) was ingested simultaneously with 1,5-AG (6 g) by healthy subjects (*n* 10). After ingestion, the end-expiratory gas was collected at 1-h intervals for 8 h and hydrogen concentrations were measured using simple gas chromatography. ppm, Parts per million; ■, Sucrose 30 g + 1,5-AG 6 g; □, sucrose 30 g alone; ●, glucose 30 g + 1,5-AG 6 g; ○, glucose 30 g alone. *Excretion of hydrogen by the ingestion of sucrose and 1,5-AG was significantly higher than that by the ingestion of sucrose alone at 4 h after ingestion, at $P < 0.05$ by Mann–Whitney *U* test.

Thus, although the intestinal absorption of glucose was retarded by 1,5-AG, the sufficient glucose did not arrive at the large intestine where gut microbiota inhabits.

Discussion

In the present study, the inhibition of sucrase, maltase, trehalase and lactase by 1,5-AG was determined using BBMV from rat small intestine and the inhibition of disaccharidases by 1,5-AG in rats was compared with that in humans. Before the human study, the effects of 1,5-AG administered orally on blood concentrations and urinary excretion of 1,5-AG and hydrogen excretion were investigated using rats. In addition, the suppressive effects of 1,5-AG on the elevation of blood glucose by sucrose or glucose, which was administered simultaneously, were investigated in rats. The same physiological functions of 1,5-AG were investigated using healthy human participants.

1,5-AG strongly inhibited sucrase (more than 40 %) at a higher concentration (100 mg/ml), whereas D-sorbose inhibited sucrase 80–90 % at the same concentration. Also, 1,5-AG inhibited the activity of sucrase in human small intestine at a high concentration (100 mg/ml) similar to that observed in rats, although the hydrolysing activity was different between rats and humans. These results suggest that 1,5-AG may suppress the elevation of blood glucose by sucrose ingested simultaneously through the inhibition of sucrase when the ratio of 1,5-AG to sucrose is relatively high.

1,5-AG similarly inhibited maltase in rat BBMV, as well as sucrase, whereas inhibition of maltase by D-sorbose was greatly weaker than that of sucrase. 1,5-AF more strongly inhibited maltase compared with sucrase at a 100 mg/ml concentration. The inhibition of trehalase and lactase by 1,5-AG was strongest among three inhibitors, whereas D-sorbose inhibited trehalase and lactase to a small extent. 1,5-AG non-competitively inhibited sucrase, whereas D-sorbose and D-psicose un-competitively inhibited sucrase[18]. The different degrees of inhibition between 1,5-AG and D-sorbose may be related to different modes of inhibition.

Consecutive feeding with a diet containing 10 % FOS, which is not digested by small intestinal enzymes, causes high osmotic diarrhoea and enlarges the caecum of rats[20,35]. The enlargement of rat caecum is induced by the ingestion of non-digestible and/or non-absorbable carbohydrates such as dietary fibre and resistant monosaccharides and oligosaccharides, which avoid digestion and absorption in the small intestine[35]. However, consecutive feeding with a diet containing 5 or 10 % 1,5-AG did not cause high osmotic diarrhoea and caecal enlargement in rats[8]. These results indirectly demonstrate that 1,5-AG is readily absorbed in the small intestine and does not reach the caecum in rats. In the present study, 1,5-AG administered orally appeared rapidly in the blood 30 min and reached a peak 60 min following administration, and then decreased readily up to 4 h after administration in rats. Concurrently, 1,5-AG was excreted readily into the urine and almost 100 % was excreted 24 h after administration.

1,5-AG in blood was detected at a low level in rats before administration of 1,5-AG. Although human subjects ingest 1,5-AG from plant foods in daily life, the rats in the present study do not take 1,5-AG from a diet. Therefore, 1,5-AG in blood in rats before administration of 1,5-AG may demonstrate that 1,5-AG is *de novo* synthesised in the liver of rats. 1,5-AG appeared in the blood 30 min after ingestion (20 g) by human subjects, and showed a peak after 60 min; thereafter, it decreased gradually over 180 min. The appearance of 1,5-AG in the blood was similar to that of rats. In addition, 50–60 % of 1,5-AG (10 or 20 g) ingested by human subjects was excreted into the urine 9 h after ingestion, but not 100 %. However, almost all seemed to be excreted into the urine after 24 h, similar to results observed in rats. These results demonstrate that 1,5-AG ingested by human subjects is readily absorbed from the small intestine and excreted rapidly into the urine intact, which is in line with the results of the consecutive feeding with 1,5-AG in rats[8]. In other words, these results suggest that the available energy of 1,5-AG is 0 kcal/g, and 1,5-AG does not appear to have a prebiotic effect because it is not transferred to the large intestine.

The dose level of 1,5-AG was 600 mg/rat in the present study. In the preliminary test, D-sorbose (400 mg) and D-psicose (400 mg) caused more osmotic diarrhoea than 1,5-AG. However, the administration of 1,5-AG (600 mg) did not induce osmotic diarrhoea. The induction of osmotic diarrhoea is affected by the amount of saccharide that arrives in the large intestine. Therefore, these results suggest that 1,5-AG is absorbed readily in the small intestine, whereas D-sorbose and D-psicose are resistant to absorption in the small intestine. The ingestion of 1,5-AG (20 g) by human subjects did not cause any abdominal symptoms or osmotic diarrhoea in the present study, although the ingestion of sorbitol (20 g), which has a molecular weight similar to 1,5-AG, causes these symptoms in healthy participants[36]. This suggests that 1,5-AG ingested by healthy subjects does not arrive at the large intestine but is readily excreted into the urine. In addition, the ingestion of less than 20 g of 1,5-AG does not cause side effects in healthy adult subjects and is safe as a food ingredient.

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When D-sorbose, which strongly inhibited sucrase activity in the rat small intestine, was administered simultaneously with sucrose to rats, it significantly suppressed the elevation of blood glucose[18]. 1,5-AG also distinctly inhibited the activity of sucrase and maltase in human and rat small intestines, although the inhibition by 1,5-AG was weaker than that by D-sorbose. Therefore, the similar suppressive effects of 1,5-AG on blood glucose elevation may be expected. In fact, 1,5-AG significantly suppressed the elevation of blood glucose by sucrose administration in humans and rats ($P < 0{\cdot}05$) (Fig. 6 and 8(A)). These suppressive effects seem to be through the inhibition of sucrase by 1,5-AG. The excretion of breath hydrogen, which is produced by gut microbiota, is dependent on the amount of carbohydrate that is fermented in the large intestine[37]. Therefore, the slight but distinct increase in breath hydrogen excretion following the simultaneous ingestion of sucrose (30 g) and 1,5-AG (6 g) by human subjects demonstrates that sucrose, which is inhibited by 1,5-AG, is fermented by gut microbiota in the large intestine. These results support the hypothesis that the suppressive effects of 1,5-AG are mediated through the inhibition of sucrase.

Kato *et al.*[16] report that the maximum blood glucose concentration is suppressed about 2·78 mmol/l when sucrose (2500 mg/kg of body weight) is administered simultaneously with 1,5-AG (500 mg/kg of body weight) to normal ddy mice. In addition, they found that 1,5-AG suppressed glucose elevation by maltose administration by about 2·78 mmol/l in the blood. They describe the suppressive effects of 1,5-AG on blood glucose elevation as similar between sucrose and maltose, although these suppressive effects depend upon the degree of inhibition of sucrase and maltase. The inhibition of sucrase by 1,5-AG was distinctly stronger than that of maltase in the present study in humans and rats. The suppressive effects of 1,5-AG on blood glucose elevations by sucrose administration were <1·11 mmol/l, and the effect was remarkably weaker than that reported by Kato *et al.*, although the ratio of 1,5-AG to sucrose (1:5) was the same in both experiments. These discrepant results may be the result of the different of animals used in the experiments and the amount of sucrose administered (one-third in the present study).

The renal tubular reabsorption of glucose is competitively inhibited by 1,5-AG[10,11]. Therefore, the intestinal absorption of glucose may be inhibited by 1,5-AG. In the present study, when glucose (400 mg) and 1,5-AG (80 mg) (glucose:1,5-AG = 5:1) were administered simultaneously to healthy rats, the elevation of blood glucose was significantly suppressed in the presence of 1,5-AG ($P < 0{\cdot}05$). Similar suppressive effects of 1,5-AG were also observed in healthy participants who simultaneously ingested 30 g of glucose and 6 g of 1,5-AG, although the significant suppression was detected at only 30 min after ingestion. The suppressive effects of 1,5-AG on the elevation of blood glucose by glucose ingestion must be expressed through the disturbance of glucose absorption in the small intestine. 1,5-AG may inhibit glucose absorption in the small intestine through a GLUT such as SGLT1 or GLUT2. However, breath hydrogen excretion scarcely increased following the simultaneous ingestion of glucose and 1,5-AG, in contrast to the simultaneous ingestion of sucrose and 1,5-AG. Although 1,5-AG inhibits the intestinal absorption of glucose, 1,5-AG itself is absorbed spontaneously from the small intestine. Therefore, the inhibition by 1,5-AG may not be maintained for a long time. As a result, sufficient glucose might not arrive at the large intestine, although the absorption of glucose was retarded by 1,5-AG. If the amount of glucose ingested is increased to 2- or 3-fold and the ratio of 1,5-AG:glucose is raised to more than 1:5, the suppressive effects of 1,5-AG on blood glucose elevation may become clear and breath hydrogen excretion may increase markedly. Further experiments should be carried out to explain this phenomenon.

In summary, a small amount of 1,5-AG is synthesised from glycogen in the body and is consumed in daily life. Therefore, 1,5-AG seems to have a very low toxicity and is safe. 1,5-AG distinctly inhibited the activity of disaccharidases such as sucrase, maltase, trehalase and lactase, whereas D-sorbose strongly inhibited sucrase but barely inhibited trehalase and lactase. 1,5-AG significantly suppressed the elevation of blood glucose and insulin by the ingestion of sucrose or glucose. A large amount of 1,5-AG (20 g) caused no side effects or breath hydrogen excretion in the present study among healthy adult participants. Ingested 1,5-AG was readily absorbed from the small intestine and excreted rapidly into the urine. Thus, the available energy of 1,5-AG is 0 kcal/g. Accordingly, 1,5-AG ingested does not cause osmotic diarrhoea and harmful effects. These results support the use of 1,5-AG as low-energy bulking sweetener in food products.

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T. O. designed the study and had primary responsibility for the final content of the manuscript. T. O. and S. N. conducted the research, analysed data and performed statistical analyses. K. T. and F. S. conducted the research and analysed data. K. Y. contributed to the synthesis of the 1,5-AG and 1,5-AF. All authors read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

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