



D-Allose is absorbed via sodium-dependent glucose cotransporter 1 (SGLT1) in the rat small intestine

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ABSTRACT

D-Allose is the C3 epimer of D-glucose and has been reported to have beneficial health effects. The transporter mediating intestinal transport of D-allose is unknown. We examined whether D-allose is absorbed via sodium-dependent glucose cotransporter 1 (SGLT1) as well as via glucose transporter type 5 (GLUT5) using rats. For examination of absorption via SGLT1, KGA-2727, an SGLT1-specific inhibitor, and D-allose were orally administered. KGA-2727 blocked the increase of plasma D-allose levels and suppressed them throughout the experiment (0–180 min), whereas without KGA-2727, the plasma D-allose levels peaked at around 60–90 min. For examination of absorption via GLUT5, rats were fed a high-fructose diet for 3 weeks to increase the abundance and activity of GLUT5 in the small intestine. High-fructose diet-fed rats did not exhibit significant changes in the plasma D-allose levels compared to control rats fed a high-glucose diet. These results indicate that SGLT1 but not GLUT5 mediates the intestinal absorption of D-allose.

1. Introduction

D-Allose, one of the rare sugars, is the C3 epimer of D-glucose and has been reported to have beneficial health effects such as inhibitory effects on cancer cell proliferation [1–3] and protective effects against ischemia-reperfusion injury [4,5]. Matsuo et al. reported that 91.2% of orally administered D-allose (8 g/kg body weight [BW]) was recovered in an intact form from the urine within 24 h in rats [6]. In a human study, the urinary excretion rate of D-allose was reported to be 67% [7]. However, the transporter that mediates the intestinal absorption of D-allose is unknown. D-glucose and D-fructose are widely abundant in daily food products and are absorbed across the apical membrane in the small intestine by sodium-dependent glucose cotransporter 1 (SGLT1) and glucose transporter type 5 (GLUT5), respectively [8]. Therefore, we examined whether D-allose is absorbed via SGLT1 using KGA-2727, an SGLT1-specific inhibitor [9] as well as via GLUT5 using rats fed with fructose. In the present study, we show the first data indicating D-allose transport in the small intestine is mediated by SGLT1.

2. Method

2.1. Materials

D-Allose was obtained from Matsutani Chemical Industry Co., Ltd. (Hyogo, Japan). KGA-2727 was obtained from Kissei Pharmaceutical Co., Ltd. (Nagano, Japan).

2.2. Animal experiments

Five-week-old male Sprague–Dawley rats were purchased from Kiwa Laboratory Animals Co. Ltd., (Wakayama, Japan). The rats were housed under the following conditions: 22 °C ± 3 °C, 55% ± 15% humidity, and 12 h light and dark cycles. The rats were allowed free access to a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). This study was approved by the Animal Care Committee of Kindai University (permit number KABT-31-010), and the animals were maintained in accordance with the guidelines.

2.2.1. Examination of D-allose absorption via SGLT1

The experimental design was based on previous studies [9,10]. To examine the absorption via SGLT1, the rats were fasted for 16 h, after

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which D-allose and KGA-2727 (2 g + 0.3 mg/kg BW) or D-allose (2 g/kg BW) alone was orally administered. D-allose and KGA-2727 were dissolved in Milli-Q water and administered at a volume of 10 ml/kg BW. Blood samples were withdrawn from the tail vein and collected into a heparinized tube at 0, 30, 60, 90, 120, and 180 min after administration.

2.2.2. Examination of D-allose absorption via GLUT5

To examine the absorption via GLUT5, the rats were divided into two groups. One group was fed a high-fructose diet for 3 weeks to increase the abundance and activity of GLUT5 in the small intestine [10]. The other group served as a control and was fed a high-glucose diet for 3 weeks. Both the groups of rats were fasted for 16 h and D-allose was orally administered (2 g/kg BW). D-Allose was dissolved in Milli-Q water and administered at a volume of 10 ml/kg BW. Blood samples were withdrawn from the tail vein and collected into a heparinized tube at 0, 30, 60, 90, 120, and 180 min after administration. To confirm the mRNA expression of GLUT5 and SGLT1, the rats were sacrificed by exsanguination from the abdominal aorta under isoflurane anesthesia after the experiment. The small intestine was collected and immersed in RNAlater solution (Thermo Fisher Scientific, Inc. Waltham, MA, USA), and stored at -80°C prior to RNA extraction. RT-qPCR analyses were carried out as described previously [10].

2.3. HPLC determination of D-allose

The plasma was mixed with an equal volume of 0.6 mol/l perchloric

acids, a protein precipitating reagent. The mixture was centrifuged at 8000 g for 10 min at 4°C . The plasma D-allose levels were determined using the HPLC system (JASCO Corporation, Tokyo, Japan) as described previously [10]. The HPLC system was equipped with a Finepak GEL SA-121 anion-exchange column (10 cm \times 6.0 mm [i.d.], JASCO). The column was maintained at 80°C . The injection volume was 20 μl . Elution was performed with a gradient of 0.25 mol/l sodium borate buffer having a pH of 8.0 (solvent A) and 0.6 mol/l sodium borate buffer having a pH of 8.0 (solvent B) as follows: gradient from 80%A/20%B to 60%A/40%B for 17 min; gradient from 60%A/40%B to 0%A/100%B for 1 min; maintenance at 0%A/100%B for 17 min; gradient from 0%A/100%B to 80%A/20%B for 2 min; and maintenance at 80%A/20%B for 20 min. The eluate from the column was mixed with a reagent consisting of 50 mmol/l guanidine hydrochloride, 0.5 mmol/l sodium metaperiodate, and 0.1 mol/l boric acids in 200 ml/l acetonitrile and adjusted to pH 11.0 with 5 mol/l NaOH. This mixture was heated at 170°C in a reaction oven. The guanidine adducts of D-allose were monitored using a fluorescence detector with excitation at 310 nm and emission at 415 nm. The flow rate of both the mobile phase and the guanidine reagent was 0.5 ml/min. The maximum plasma concentration (C_{max}) and time taken to reach C_{max} (T_{max}) were estimated directly from the observed plasma concentration-time data.

2.4. Statistical analyses

All data were expressed as mean \pm SE for each group. Statistical

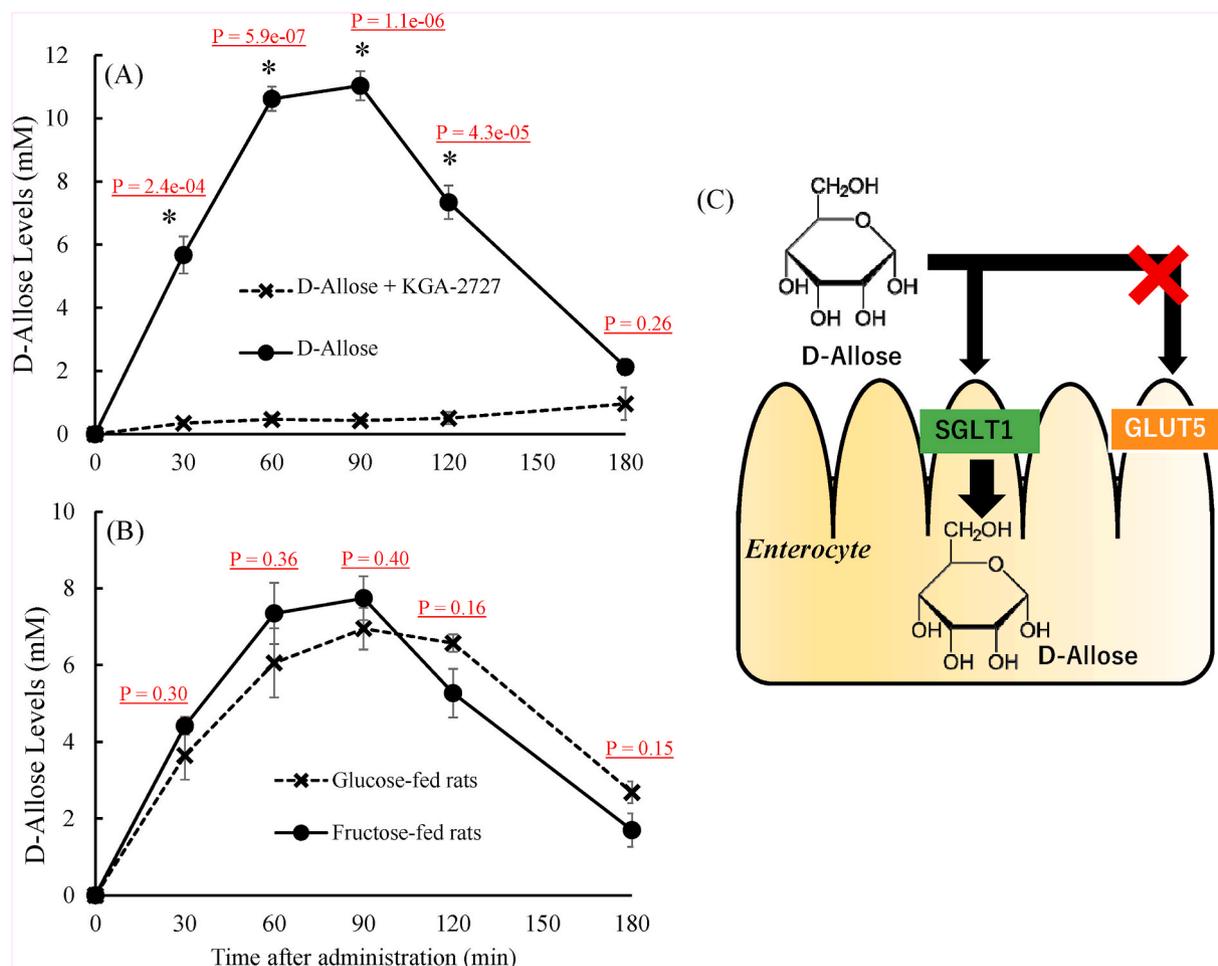


Fig. 1. The plasma D-allose levels in rats orally administered with D-allose and KGA-2727 (2 g + 0.3 mg/kg BW) or D-allose (2 g/kg BW) alone (A), and in rats administered with D-allose (2 g/kg BW) after consuming the high-glucose or high-fructose diet (B), and schematic diagram of intestinal D-allose absorption (C). All the rats were fasted for 16 h before the oral administration. Data are means \pm SE (n = 4). A single asterisk (*) represents statistically significant differences ($P < 0.05$).

analysis was conducted using an unpaired Student's t-test, and the data were analyzed using the IBM SPSS statistics software, version 19.0 (IBM Co., New York, USA). P-values of <0.05 were considered statistically significant and have been indicated by a single asterisk (*).

3. Results and discussion

Fig. 1A shows the plasma D-allose levels in rats that were orally administered with D-allose and KGA-2727 or D-allose alone. KGA-2727 blocked the increase of plasma D-allose levels and suppressed them throughout the experiment (0–180 min), whereas without KGA-2727, the plasma D-allose levels peaked at around 60–90 min in rats. The C_{max} and T_{max} values were 0.983 ± 0.59 mM and 120 ± 34 min, respectively, in rats gavaged with D-allose and KGA-2727, whereas the C_{max} and T_{max} values were 11.1 ± 0.53 mM and 75.0 ± 8.7 min, respectively, in rats gavaged with D-allose alone. These results indicate that the intestinal D-allose absorption is mediated via SGLT1 (Fig. 1C). In rats gavaged with D-allose and KGA-2727, low levels of D-allose were detected in the plasma at a C_{max} value of 0.983 mM. This means that a small amount of D-allose was absorbed by paracellular transport and/or simple diffusion, as SGLT1 knockout mice have been reported to absorb a small amount of glucose [11].

Fig. 1B shows the plasma D-allose levels in rats gavaged with D-allose (2 g/kg BW) after consuming the high-glucose or high-fructose diet. As we previously reported [10], intestinal GLUT5 mRNA expression in rats fed with the high-fructose diet increased about 10-fold as compared with rats fed with the high-glucose diet, whereas SGLT1 mRNA expression was not affected by different dietary sugars (Fig. 2). Changes in the plasma D-allose levels did not differ between the groups, suggesting that GLUT5 is unlikely to be involved in the intestinal D-allose absorption (Fig. 1C). In our previous study, when D-allulose (also known as D-psicose) was gavaged to rats, plasma D-allulose levels were dramatically higher in the rats fed with the high-fructose diet [10]. In this experiment, C_{max} values were 6.96 and 8.06 mM in the glucose-fed and fructose-fed rats, respectively, both of which were lower than that of rats gavaged with D-allose alone in the previous experiment (11.1 mM, Fig. 1A). The reason for differences in the C_{max} values observed in this study may be the 3-week feeding of a high-glucose or fructose diet before the oral administration of D-allose. The difference in rat age could affect the plasma D-allose levels.

The main strength of the present study is the use of KGA2727. Phlorizin is a well-known and commercially available SGLT1 inhibitor. Phlorizin is hydrolyzed to phloretin on the brush border membrane of the small intestine. The absorbed phloretin inhibits glucose transporter type 2 (GLUT2) which is expressed on the basolateral membrane in the intestinal absorptive cells [12]. Thus, the use of phlorizin can produce both inhibition of SGLT1 and GLUT2, which does not allow to examine whether D-allose is absorbed via SGLT1. This study has some limitations. Although KGA-2727 is a potent, selective, high-affinity inhibitor of SGLT1, another approach using SGLT1-KO mice would strengthen the evidence. For examination of absorption via GLUT5, we induced GLUT5 in the rat small intestine by feeding a high-fructose diet. Since high dose fructose intake is known to induce adverse metabolic effects such as hypertriglyceridemia and insulin resistance, the plasma D-allose levels could be altered. The use of GLUT5-specific inhibitors would be useful to confirm the present finding.

It is conceivable that D-allose is transported via SGLT1 but not GLUT5 in the small intestine because of its chemical structure similarity to D-glucose. This is the first study to reveal the transporter that mediates the intestinal transport of D-allose. D-Allose is expected to potentially compete with glucose for intestinal absorption via SGLT1, which can modulate postprandial blood glucose levels. It has been reported to possess many beneficial physiological functions, including antitumor, anticancer, anti-inflammatory, antioxidant effects, which contributes to human health [13]. D-Allose has about 80% of the sweetness of sucrose with negligible calories [14] and might be an ideal food additive as a

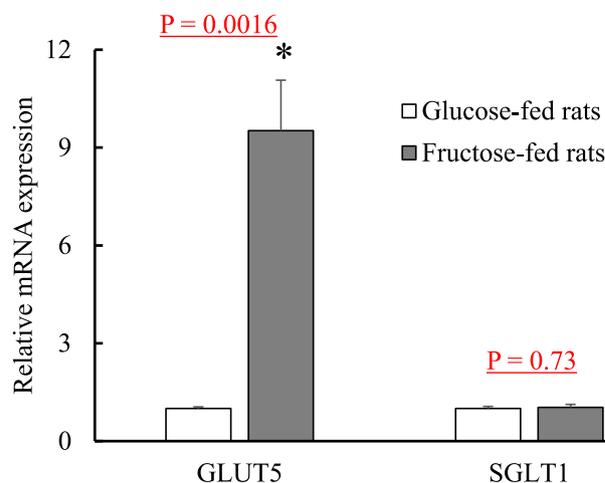


Fig. 2. Relative mRNA expression of intestinal sugar transporters in rats fed the high-glucose or -fructose diet. Data are means \pm SE (n = 4). Expression of each marker was normalized to that in glucose-fed rats. A single asterisk (*) represents statistically significant differences (P < 0.05).

table sugar substitute [13]. This finding would advance our understanding of the beneficial health effects of D-allose.

CRedit authorship contribution statement

Kunihiko Kishida: Conceptualization, Methodology, Software, Validation, Data curation, Formal analysis, Investigation, Validation, Visualization, Writing – original draft, Supervision. **Tetsuo Iida:** Validation, Resources, Writing – review & editing. **Takako Yamada:** Validation, Resources, Writing – review & editing. **Yukiyasu Toyoda:** Methodology, Software, Validation, Data curation, Formal analysis, Investigation, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

T. Iida and T. Yamada are employed by Matsutani Chemical Industry Co., Ltd.

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