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Intestinal active absorption of sugar-conjugated compounds by glucose transport system: implication of improvement of poorly absorbable drugs

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Abstract—The intestinal absorption of glucose- and galactose-conjugated compounds was studied in the everted sac of the rat small intestine. The absorption clearance of *p*-nitrophenyl β-D-glucopyranoside (p-NPglc) at 250 μM in the mucosal side (4.45 ± 0.34 μL/min/cm, mean ± SE, N = 4), calculated by dividing the absorption rate by the drug concentration, was significantly decreased (0.476 ± 0.036 μL/min/cm) in the presence of 1 mM phloridzin, an inhibitor of glucose transport, and in the absence of Na^+, a cosubstrate of the glucose transport carrier (0.424 ± 0.018 μL/min/cm). The absorption clearance of *p*-NPglc was decreased as its concentration increased. In the same experiment, the absorption clearance of *p*-nitrophenyl β-D-galactopyranoside (1.99 ± 0.23 μL/min/cm) was also significantly decreased in the presence of phloridzin and in the absence of Na^+. However, the absorption clearance of *p*-nitrophenyl β-D-mannopyranoside (0.811 ± 0.013 μL/min/cm) was low and not significantly decreased in the presence of phloridzin ($P > 0.1$). Furthermore, the absorption clearance of β-naphthyl β-D-glucopyranoside and β-naphthyl β-D-galactopyranoside was also significantly decreased in the presence of phloridzin ($P < 0.001$). These results indicated that the glucose and galactose moieties provided these compounds with a new route by way of the glucose transport carrier for intestinal absorption.

A number of drugs which are pharmacologically active are poorly absorbed from the intestine and it would be valuable to find ways of overcoming this poor absorption. Glucose and galactose are well known to be actively absorbed by glucose transport carriers in the small intestine [1–3]. In this report, we investigated whether conjugation of a glucose or galactose moiety to a compound resulted in active absorption of that compound from the mucosal side to the serosal side by the glucose transport system. *p*-Nitrophenol (*p*-NP*) and β-naphthol (β-NA) were used as the model compounds for conjugation with sugar.

Materials and Methods

Materials. *p*-Nitrophenyl β-D-glucopyranoside (*p*-NPglc), *p*-nitrophenyl β-D-galactopyranoside (*p*-NPgal), *p*-nitrophenyl β-D-mannoside (*p*-NPman), *p*-nitrophenyl β-D-glucuronide, β-naphthyl β-D-glucopyranoside (β-NAglc), β-naphthyl β-D-galactopyranoside (β-NAgal) and phloridzin were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Methanol (HPLC grade) was purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Other chemicals were of analytical grade or better.

Absorption experiment. The everted sac technique was applied to study intestinal absorption from the mucosal to the serosal side. Male Wistar rats (180–230 g; Shizuoka Animal Laboratory) fasted overnight were anesthetized with ether. After the injection of 0.2 mL of heparin solution (1000 U/mL) into the femoral vein, glass canulae were inserted into the portal vein for the perfusion of cold physiological saline on ice. Then a ligature was placed around the inferior vena cava just anterior to the entrance of the right renal vein, and a small incision was made just inferior to the ligature. Blood in the intestine was removed by perfusion. The jejunum was obtained by cutting the intestine at 1 cm below the Treitz ligament. After being everted in cold saline on ice, the intestine was canulated with glass canulae at both ends. The positions were the site 2 or 12 cm below the Treitz ligament, respectively. After being connected to a disposable 10 mL plastic syringe in a manner similar to the method reported by Doluisio *et al.* [4], everted small intestine was placed in 30 mL of incubation medium (modified Krebs–Ringer–bicarbonate phosphate buffer, pH 7.4) [5] containing glycoside in a beaker through which gas (95% O_2, 5% CO_2) was bubbled. The serosal side was filled with 5 mL of incubation medium without glycoside through the syringe. For the preparation of Na^+-free medium, Na^+ was displaced with K^+. After this procedure, the absorption experiment was started. Incubation media (100 μL) were sampled from both the serosal and the mucosal sides at the times until 30 min. For the assay of *p*-nitrophenyl glycosides and β-naphthyl glycosides, the samples were mixed with 100 μL of acetonitrile containing 100 μM 2,4-dihydroxy benzoic acid or with 200 μL of 75% acetonitrile in water containing 10 mM sodium 2-naphthalenesulfonate, respectively, as internal standards for analysis by HPLC.

Determination of glycosides. All glycosides were determined by HPLC under the following conditions. The HPLC system (Japan Spectroscopic Co. Ltd, Tokyo, Japan) consisted of a pump, a UV detector (at 302 nm for the assay of *p*-nitrophenyl glycosides), a fluorescence detector (Ex 274 nm, Em 343 nm for β-naphthyl glycosides) and an integrator (D-2500, Hitachi Ltd, Tokyo, Japan). For the assay of *p*-nitrophenyl glycosides, the mobile phase was composed of 34% methanol and 0.05% phosphoric acid in water, and an ODS column (80TM, 6 mm i.d. × 15 cm length, Tosoh Corp., Tokyo, Japan) was used. For the assay of β-naphthyl glycosides, the mobile phase was composed of 44% methanol, 0.05% phosphoric acid and 0.1 g/L KNO_3 in water and the same ODS column was used. The flow rate of the mobile phase was 1.5 mL/min.

Data analysis. Data were analysed with Student's *t*-test. Absorption clearance was obtained by using the following equation:

$$\text{Absorption clearance} = \frac{\text{Absorption rate}}{\text{Glycoside concentration in mucosal side}} \quad (1)$$

* Abbreviations: *p*-NPglc, p-nitrophenyl β-D-glucopyranoside; *p*-NPgal, *p*-nitrophenyl β-D-galactopyranoside; *p*-NPman, *p*-nitrophenyl β-D-mannopyranoside; β-NAglc, β-naphthyl β-D-glucopyranoside; β-NAgal, β-naphthyl β-D-galactopyranoside.

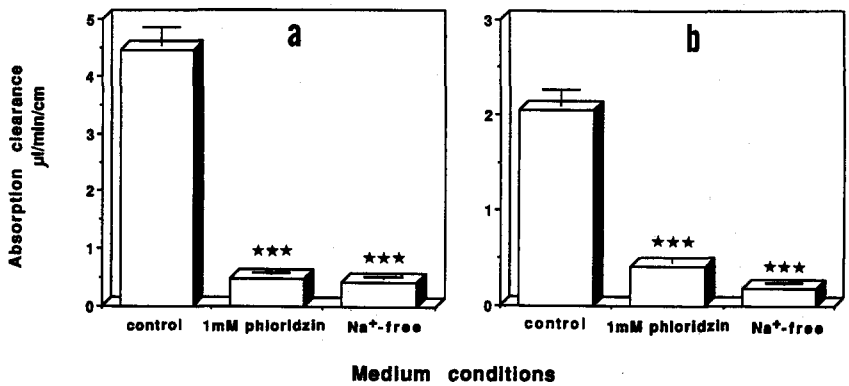

Fig. 1. Intestinal absorption of *p*-nitrophenyl β-D-glucopyranoside (a) and β-naphthyl β-D-glucopyranoside (b) at 250 μM. Values represent mean ± SE (N = 3–7). Asterisks represent significantly different values compared with that of control (*** $P < 0.001$).

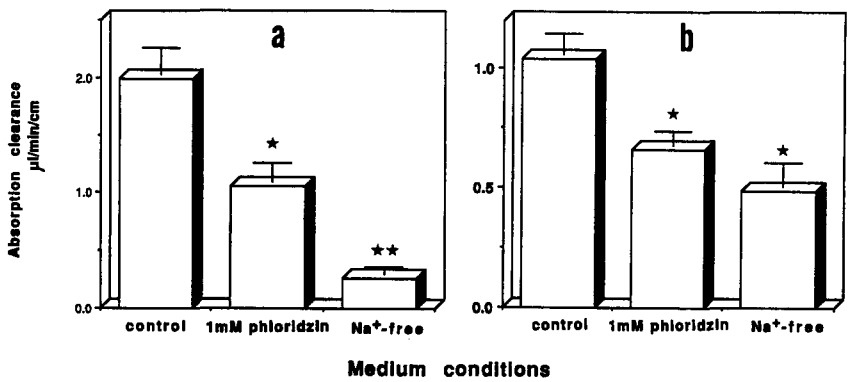

Fig. 2. Intestinal absorption of *p*-nitrophenyl β-D-galactopyranoside (a) and β-naphthyl β-D-galactopyranoside (b) at 250 μM. Values represent mean ± SE (N = 3–8). Asterisks represent significantly different values compared with that of control (* $P < 0.05$, ** $P < 0.01$).

Results and Discussion

p-NPglc at 250 μM was absorbed in rat small intestine at a constant rate after 10 min of lag time for 30 min. The absorption clearance of *p*-NPglc at the concentration, 4.45 ± 0.336 μL/min/cm (mean ± SE), was calculated by Eqn. 1; the absorption rate was obtained by dividing the amount absorbed during the period from 10 to 20 min (Fig. 1a). This absorption clearance was similar to the value for D-glucose (20 mM) obtained by the same method in our laboratory [6]. The absorption of *p*-NPglc was significantly inhibited by phloridzin (1 mM), a known glucose transport carrier inhibitor [2] ($P < 0.001$) (Fig. 1a). Furthermore, the absorption of *p*-NPglc was significantly decreased in the absence of Na^+, a cosubstrate of the glucose transport carrier [2] ($P < 0.001$) (Fig. 1a). As *p*-NPglc concentration increased from 250 μM to 10 mM, absorption clearance of *p*-NPglc was decreased (4.45 ± 0.336 to 1.17 ± 0.067 μL/min/cm), indicating saturable transport of *p*-NPglc by the carrier (Table 1).

Table 1. Concentration dependency of absorption clearance of *p*-NPglc

p-NPglc mM	Absorption clearance (μL/min/cm) Mean	SE	N
0.25	4.45	0.336	4
1.0	2.80*	0.289	5
2.5	1.86†	0.137	3
10.0	1.17†	0.067	5

Symbols represent significant values compared with that at 0.25 mM of *p*-NPglc (* $P < 0.05$, † $P < 0.001$).

The absorption clearance of β-NAglc at 250 μM (2.04 ± 0.177 μL/min/cm) was lower than that of *p*-NPglc at 250 μM, but was significantly ($P < 0.001$) decreased both in the presence of 1 mM phloridzin and absence of Na^+ (Fig. 1b). This result indicated that conjugation of D-glucose was effective in enabling the intestinal absorption even of β-NA, which is larger than *p*-NP, by the glucose transport system.

The absorption clearance of 250 μM *p*-NPgal (1.99 ± 0.23 μL/min/cm) was lower than that of 250 μM *p*-NPglc, but was significantly decreased both in the presence of 1 mM phloridzin ($P < 0.05$) and in the absence of Na^+ ($P < 0.01$) (Fig. 2a). The absorption clearance of 250 μM β-NAgal (1.04 ± 0.086 μL/min/cm) was lower than that of

250 μM β-NAglc, but was significantly ($P < 0.05$) decreased both in the presence of 1 mM phloridzin and absence of Na^+ (Fig. 2b). These results indicated that galactose-conjugated compounds as well as glucose-conjugated compounds were absorbed by the glucose transport carrier.

The intestinal absorption of mannose-conjugated compounds and glucuronic acid-conjugated compounds were examined as controls, because mannose (glucose isomer) and glucuronic acid (similar structure to glucose) are known to be unabsorbable by any active transport system. The absorption clearance of 250 μM *p*-NPman was much lower (0.811 ± 0.013 μL/min/cm, mean ± SE, N = 3) than that of 250 μM *p*-NPglc. The clearance of *p*-NPman was not significantly decreased in the presence of 1 mM phloridzin (0.760 ± 0.116 μL/min/cm, mean ± SE, N = 3, $P > 0.1$). The absorption clearance of 250 μM *p*-nitrophenyl β-D-glucuronide (0.708 ± 0.199 μL/min/cm, mean ± SE, N = 3) was also much lower than that of *p*-NPglc and was similar to that of *p*-NPman.

We conclude that conjugation of D-glucose and D-galactose to test compounds resulted in active absorption in the intestine by the glucose transport system, due to availability of the sugars on the glucose transport carrier. Further studies are required to determine the extent of the improvement of intestinal absorption by conjugation of glucose or galactose to non- or poorly absorbable drugs.

* Corresponding author. Tel. (81) 426-76-5111 ext. 321; FAX (81) 426-75-2605.

Department of Biopharmaceutics
Tokyo College of Pharmacy
1432-1 Horinouchi
Hachioji
Tokyo 192-03, Japan

TAKASHI MIZUMA
KUNIHIRO OHTA
MASAHIRO HAYASHI
SHOJI AWAZU*

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