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Comparative study of active absorption by the intestine and disposition of anomers of sugar-conjugated compounds

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Abstract—Active absorption in the intestine and metabolism of the β- and α-anomers of the glucoside and galactoside of *p*-nitrophenol (*p*-NP) were studied to find a more suitable prodrug for poorly absorbed drugs. The everted sac technique was used to investigate the intestinal absorption of these glycosides at 250 μM from the mucosal to the serosal side in the rat jejunum. The absorption clearance of *p*-nitrophenyl α-D-glucopyranoside (*p*-NPαglc) (0.271 ± 0.089 μL/min/cm, mean ± SE, N = 8) was much lower than that of *p*-nitrophenyl β-D-glucopyranoside (*p*-NPβglc) (4.45 ± 0.34 μL/min/cm, mean ± SE, N = 4) which is actively absorbed by a glucose transport carrier [Mizuma *et al.*, *Biochem Pharmacol* **43**: 2037–2039, 1992]. However, the major constituent appearing on the serosal side was *p*-NP (aglycone) after absorption of *p*NPαglc, whereas it was *p*-NPβglc itself after absorption of *p*-NPβglc. The total amount transported to the serosal side after 20 min of *p*-NPαglc absorption, which was similar to that of *p*-NPβglc, was significantly decreased in the absence of Na^+, indicating the active absorption of *p*-NPαglc by a Na^+-dependent glucose transport carrier. Perfusion with a mucosal solution of *p*-NPαglc showed that the *p*-NP concentration on the serosal side (15.8 ± 1.56 μM, mean ± SE, N = 3) was significantly ($P < 0.05$) higher than that on the mucosal side (5.84 ± 1.24 μM, mean ± SE, N = 3) at 20 min. This indicated that the *p*-NP appearing on the serosal side was derived not from absorption of *p*-NP but from hydrolysis of *p*-NPαglc through the intestinal membrane during absorption. On the other hand, after absorption of *p*-nitrophenyl β-D-galactopyranoside (*p*-NPβgal), which is actively absorbed by glucose transport carrier, *p*-NPβgal itself appeared mostly on the serosal side. However, *p*-nitrophenyl α-D-galactopyranoside (*p*-NPαgal) absorption, which resulted in appearance on the serosal side, was not significantly decreased in the presence of 1 mM phloridzin or in the absence of Na^+, indicating that the contribution of the glucose transport carrier to *p*-NPαgal absorption was minimal. The order of the Na^+-dependent intestinal absorption was *p*-NPβglc > *p*-NPαglc > *p*-NPβgal > *p*-NPαgal.

Active absorption by the intestine of β-glucosides (β-D-glucopyranosides) and β-galactosides (β-D-galactopyranosides) of *p*-nitrophenol (*p*-NP*) and β-naphthol from the mucosal to the serosal side by a glucose transport system has been reported in a previous paper [1]. These findings indicated that the glucose and galactose moieties provided these compounds with a new route by way of the glucose transport carrier in the intestine and suggested that conjugating glucose and galactose to poorly absorbable drugs can improve their intestinal absorption since absorption clearance (4.45 μL/min/cm) of *p*-nitrophenyl β-D-glucopyranoside (*p*-NPβglc) (one of these glucosides), for example, was as large as that of 20 mM D-glucose [1]. In this study, based on the prodrug concept, we studied the hydrolysis of the β-glucoside and β-galactoside of *p*-NP which appeared as an aglycone after hydrolysis through the intestinal membrane. The α-anomers of the glucoside and the galactoside were also studied to determine a preference for α or β-anomers as a prodrug.

Materials and Methods

Materials. *p*-Nitrophenyl α-D-glucopyranoside (*p*-NPαglc), *p*-NPβglc, *p*-nitrophenyl α-D-galactopyranoside (*p*-NPαgal), *p*-nitrophenyl β-D-galactopyranoside (*p*-NPβgal), *p*-nitrophenyl β-D-glucuronide (*p*-NP glucuronide) and phloridzin were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). *p*-NP and methanol (HPLC grade) were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Other chemicals were of analytical grade or better.

Absorption experiment. The modified everted sac technique was used to study intestinal absorption from the mucosal to the serosal side as reported previously [1]. Briefly, male Wistar rats (180–230 g, Shizuoka Animal Laboratory) fasted overnight were anesthetized with ether. After the intestinal blood was removed by saline perfusion, the jejunum was obtained. The everted small intestine was placed in 30 mL of incubation medium (modified Krebs–

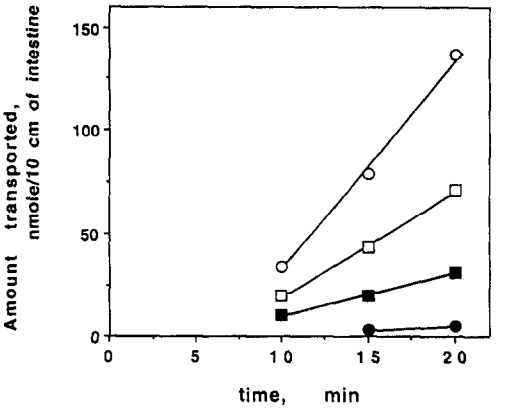

Fig. 1. Time course of absorption of *p*-NP β- and α-glucosides and *p*-NP β- and α-galactosides. Data represent a typical experiment. (○) *p*-NPβglc, (●) *p*-NPαglc, (□) *p*-NPβgal, (■) *p*-NPαgal.

* Abbreviations: *p*-NP, *p*-nitrophenol; *p*-NPβglc, *p*-nitrophenyl β-D-glucopyranoside; *p*-NPαglc, *p*-nitrophenyl α-D-glucopyranoside; *p*-NPβgal, *p*-nitrophenyl β-D-galactopyranoside; *p*-NPαgal, *p*-nitrophenyl α-D-galactopyranoside; *p*-NP glucuronide, *p*-nitrophenyl β-D-glucuronide.

Table 1. Absorption clearances of *p*-NP glycosides

Medium conditions	*p*-NP glucoside (μL/min/cm) β-anomer§	α-anomer	*p*-NP galactoside (μL/min/cm) β-anomer‖	α-anomer
Control	4.45 ± 0.34	0.271 ± 0.089	1.87 ± 0.18	0.652 ± 0.075
Phloridzin (1 mM)	0.476 ± 0.036‡	0.192 ± 0.031	0.905 ± 0.139†	0.713 ± 0.129
Na^+-free	0.424 ± 0.018‡	0.052 ± 0.034*	0.340 ± 0.043‡	0.466 ± 0.090

Absorption clearance was calculated from the amount of *p*-NP glycoside appearing on the serosal side, as noted in Materials and Methods.

Symbols represent significantly different values compared with control (*$P < 0.01$, †$P < 0.005$, ‡$P < 0.001$).

§ Data from previous report [1]. ‖Data combined with that in previous report [1].

Values represent means ± SE (N = 3–11).

Ringer bicarbonate phosphate buffer, pH 7.4) [2] containing the glycoside (*p*-NPβglc, *p*-NPαglc, *p*-NPβgal or *p*-NPαgal) (250 μM) in a beaker (37°). The mucosal side was bubbled with gas (95% O_2, 5% CO_2). For single-pass perfusion with a mucosal solution, 20 mL of the incubation medium as described above were placed in a beaker and the incubation medium was perfused through the beaker at a flow rate of 13 mL/min. When necessary, Na^+-free medium on the mucosal side was prepared by displacing Na^+ with K^+. The serosal side was filled with 5 mL of the incubation medium without glycoside, then the absorption study was started.

HPLC assay. Glycosides (*p*-NPαglc, *p*-NPβglc, *p*-NPαgal, *p*-NPβgal), *p*-NP and *p*-NP glucuronide (metabolite of *p*-NP) were determined by HPLC. The conditions of HPLC assay were the same as in the previous report [1]. The mobile phase was composed of 26–34% methanol and 0.05% phosphoric acid in water.

Data analysis. Statistical analysis was performed by Student's *t*-test. Absorption clearance was obtained by the following equation:

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

where the absorption rate was obtained by

$$\text{Absorption rate} = \frac{X_{t_2} - X_{t_1}}{t_2 - t_1} \quad (2)$$

where t_1 and t_2 represent times after incubation, and X_{t_1} and X_{t_2} represent the amount of glycoside transported onto the serosal side after incubation times t_1 and t_2, respectively.

Results and Discussion

Time course of absorption of p-*NP glycosides from mucosal to serosal side.* The time courses of intestinal absorption of *p*-NPβglc, *p*-NPαglc, *p*-NPβgal and *p*-NPαgal from the mucosal to the serosal side are shown in Fig. 1. Since the amount of *p*-NP glycosides transported to the serosal side, which escaped any metabolism during the transport process, increased linearly with incubation time

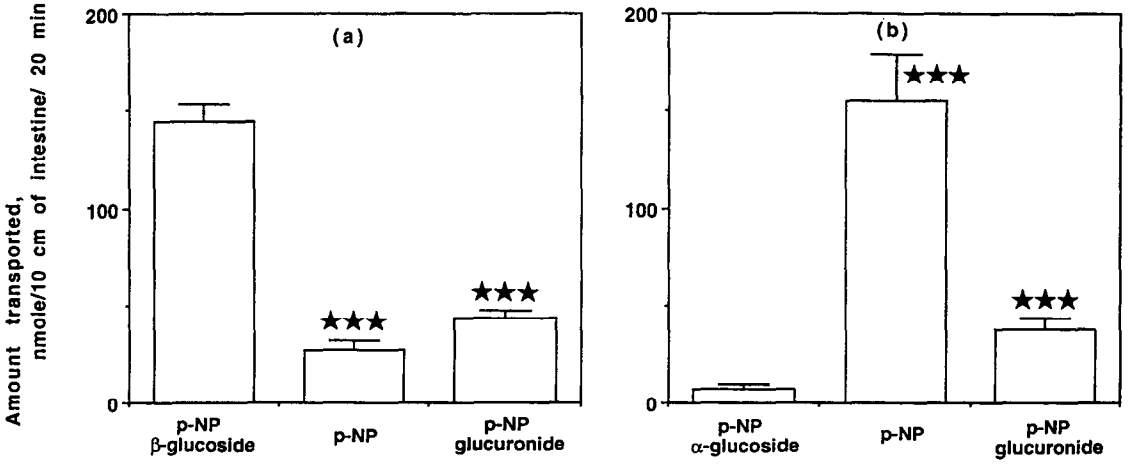

Fig. 2. Serosal appearance profile after absorption of *p*-NPβglc (a) and *p*-NPαglc (b). Data represent means ± SE, N = 4 (a), 5 (b). Asterisks represent significant values compared with that of *p*-NP glucoside (***$P < 0.001$).

Table 2. Effect of transport inhibitors on the total amount transported after absorption of *p*-NPαglc

Medium conditions	Total amount (nmol/10 cm/20 min)
Control	200.4 ± 27.5
Phloridzin (1 mM)	180.0 ± 11.4
Na^+-free	109.9 ± 16.7*

* Represents a significantly different value compared with control ($P < 0.05$).
Values represent means ± SE (N = 3–7).

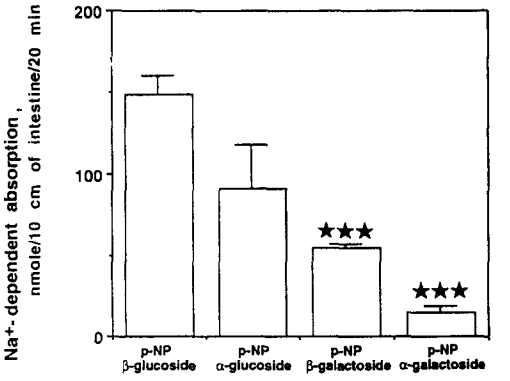

Fig. 3. Active absorption of *p*-NP glycosides by Na^+-dependent glucose transport carrier. The data were obtained by subtracting the mean value of total amount transported to the serosal side after glycoside absorption in the absence of Na^+ from total amount in the presence of Na^+, representing means ± SE (N = 3–5). Asterisks represent significant values compared with *p*-NP β-glucoside (***$P < 0.001$).

after 10 min lag time, the absorption clearances were calculated from the amount of glycoside transported onto the serosal side between 10 (or 15) and 20 min of incubation according to Eqn 1, and are shown in Table 1.

Absorption clearance of p-*NP glucosides*. The effects of phloridzin, a glucose transport carrier inhibitor [3], and Na^+, a co-substrate of the glucose transport carrier [3], on the absorption clearance of *p*-NPαglc as well as *p*-NPβglc are shown in Table 1. The absorption clearance of control *p*-NPαglc was much lower than that of control *p*-NPβglc. The absorption clearance of *p*-NPαglc was decreased in the presence of 1 mM phloridzin, though not significantly. However, it was significantly ($P < 0.05$) decreased in the absence of Na^+.

Serosal appearance profile after absorption of p-*NP glucosides*. Compounds appearing on the serosal side after absorption of *p*-NPβglc and *p*-NPαglc were determined. *p*-NP (aglycone) and its glucuronide (metabolite of *p*-NP) were detected besides *p*-NPβglc or *p*-NPαglc. The major constituent appearing on the serosal side after *p*-NPβglc absorption was the glucoside itself (Fig. 2a). After absorption of *p*-NPαglc, however, *p*-NP was the major constituent appearing on the serosal side (Fig. 2b). Although the absorption clearance, which was obtained from the amount of transported glucoside that escaped hydrolysis during transport through the intestinal membrane, was considerably lower for *p*-NPαglc than for *p*-NPβglc (Table 1), the total amount transported (glucoside + aglycone + glucuronide) to the serosal side after absorption of *p*-NPαglc was approximately comparable to that of *p*-NPβglc (Fig. 2a and b), suggesting that *p*-NPαglc was primarily hydrolysed through the intestinal membrane during absorption. On the mucosal side, on the other hand, 35.4 ± 2.14 μM of *p*-NP were detected after 20 min of *p*-NPαglc absorption in contrast to 5.23 ± 0.70 μM of *p*-NP for *p*-NPβglc (data not shown).

Effect of transport inhibitors on total absorption of p-*NPαglc*. The total transported amount (glycoside + aglycone + glucuronide) of *p*-NPαglc was decreased in the presence of 1 mM phloridzin, though not significantly, but it was significantly decreased in the absence of Na^+ (Table 2), indicating the active absorption of *p*-NPαglc by a Na^+-dependent carrier.

Serosal appearance of aglycone after absorption of p-*NPαglc by mucosal perfusion*. In order to rule out the possibility of a *p*-NP contribution formed on the mucosal side to the appearance of *p*-NP and *p*-NP glucuronide on the serosal side after absorption of *p*-NPαglc, we perfused the medium to the mucosal side to supply intact *p*-NPαglc containing no *p*-NP. Although *p*-NP, which was formed on the mucosal side, was not completely removed, its concentration on the serosal side was significantly ($P < 0.05$) higher than that on the mucosal side at 20 min (Table 3), indicating that the appearance of *p*-NP on the serosal side was derived not from absorption of *p*-NP by passive diffusion, but from hydrolysis of *p*-NPαglc through the intestinal membrane. Under the conditions of mucosal perfusion, the serosal appearance profile after absorption of *p*-NPαglc (data not shown) was similar to that in the non-perfusion study as shown in Fig. 2b where *p*-NP was the major constituent appearing on the serosal side.

p-*NP galactoside absorption: absorption clearance, serosal appearance profile and effect of transport inhibitors*. As shown in Table 1, the absorption clearance of *p*-NPβgal

Table 3. *p*-NP concentration on mucosal and serosal sides after absorption of *p*-NPαglc by mucosal perfusion

	Incubation time (min)		
	10	15	20
Mucosal side	3.68 ± 1.23	4.74 ± 1.74	5.84 ± 1.20
Serosal side	4.67 ± 0.65	10.31 ± 0.96	15.75 ± 1.56*

Data represent means ± SE (N = 3). Units are μM.
An asterisk represents a significantly different value compared with that on the mucosal side ($P < 0.05$).

in the control was significantly decreased in the presence of 1 mM phloridzin and in the absence of Na^+, indicating the active absorption of *p*-NPβgal by the Na^+-dependent glucose transport carrier as reported previously [1]. After *p*-NPβgal absorption, *p*-NPβgal (71.5 ± 1.87%) and *p*-NP glucuronide (28.5 ± 1.87%) (mean ± SE, N = 3) appeared on the serosal side (data not shown), indicating that a part of *p*-NPβgal absorbed through the intestinal membrane was hydrolysed, although no *p*-NP was detected on the serosal side. These results showed a similar characteristic of β-galactoside to that of β-glucoside in terms of transport and disposition in the intestine.

On the other hand, after the absorption of *p*-NPαgal, only *p*-NPαgal appeared on the serosal side (data not shown), indicating the quite different disposition of the α-anomer of the *p*-NP galactoside from that of the α-anomer of the *p*-NP glucoside as shown in Fig. 2a. No significant decrease in the absorption clearance of *p*-NPαgal was observed in the presence of 1 mM phloridzin or in the absence of Na^+ (Table 1). Accordingly, the total amount transported to the serosal side after absorption of *p*-NPαgal was not significantly decreased in the presence of 1 mM phloridzin or in the absence of Na^+. These experiments failed to detect a significant contribution of the Na^+-dependent glucose transport carrier to the absorption of *p*-NPαgal.

Na^+-dependent total absorption of p-*NP glycosides (*p-*NPβglc,* p-*NPαglc,* p-*NPβgal and* p-*NPαgal).* Na^+-dependent total absorption of *p*-NP glycosides by glucose transport carrier was obtained by substracting the total amount transported to the serosal side in the absence of Na^+ from the total amount transported to the serosal side in the presence of Na^+ (Fig. 3). The order of Na^+-dependent total absorption was *p*-NPβglc > *p*-NPαglc > *p*-NPβgal > *p*-NPαgal, indicating that the efficiency of active absorption of glycosides by the Na^+-dependent glucose transport system was glucoside > galactoside and β-anomer > α-anomer.

We conclude that the glucose moiety is more effectively absorbed by the glucose transport system than that of galactose, and that the β-anomer is more effective than the α-anomer. The quite different dispositions of the α and β-anomers of the glucoside through the intestinal membrane is a valuable observation on the design of prodrugs, though the contribution of glycosidase to the hydrolysis of glycoside remains unclear. It would be useful to study the difference between α and β-anomers in disposition in other tissues or organs, such as the plasma and liver. Since these studies are limited to the *p*-NP glucoside and galactoside, further investigations into other compounds and drugs conjugated with glucose and galactose are necessary to obtain insight into sugar-conjugated prodrugs for improved drug absorption.

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