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Comparative Assessment of D-Xylose Absorption between Small Intestine and Large Intestine

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Abstract

The present study aimed to evaluate the absorption of D-xylose, a passively absorbed five-carbon monosaccharide, from the large intestine compared with the small intestine, in order to explore the absorption potential of the large intestine.

D-Xylose absorption was evaluated in the intestinal loop and everted sacs in rats and comparisons were made between small intestine (mid-gut) and large intestine (colon). The absorption of D-xylose was smaller, by an order of magnitude or more, after administration into the loop of large intestine than after administration into that of small intestine, based on appearance in plasma and disappearance from the intestinal loop. D-Xylose absorption was practically insignificant (nominal 4·9%) in 60 min in the large intestine, whereas it was moderate (57·0%) in the small intestine. Consistently, the uptake of D-xylose in everted sacs was about 20 times larger in the small intestine than in the large intestine.

Thus the passive membrane permeability of D-xylose was demonstrated to be negligible in the large intestine, even though the small intestine was fairly permeable. This result helps rationalize kinetic modelling strategies assuming the small intestine as the sole absorption site for gastrointestinal absorption in-vivo. It also suggests that hydrophilic drugs with molecular size similar to or larger than D-xylose may not be good candidates for colonic drug delivery by controlled release.

Several studies have recently suggested that the membrane permeability of the large intestine (or colon) may be comparable with that of the small intestine, e.g. for dextrans (Hosoya et al 1993), indometacin (Kimura et al 1994) and azetirelin (Sasaki et al 1994), raising increasing interest in colonic drug delivery. If a drug is absorbable in the large intestine, sustained release formulations which can release the drug in the colon as well as in the small intestine would be useful for sustaining efficacy for a longer period than formulations which release drugs only in the small intestine. For peptide drugs, the colon, where peptidase activities are presumed to be lower compared with the small intestine, may be utilized as a potential absorption site in combination with colon-specific controlled release techniques.

It was suggested, however, in our previous study (Yuasa et al 1996b) that D-xylose, which is a passively absorbed five-carbon monosaccharide and clinically used to assess intestinal absorptive functions (Ohkohchi & Himukai 1984; Craig & Atkinson 1988; Rolston & Mathan 1989), is not absorbed at all in the large intestine, even though it is fairly absorbable in the small intestine. This is consistent with the conventional view, though without substantial evidence, that the large intestine has disadvantages as a site for drug absorption compared with the small intestine because of its smaller surface area. Although it helps to rationalize kinetic modelling strategies assuming small intestine as the sole absorption site for gastrointestinal absorption in-vivo as proposed in our preceding studies (Yuasa et al 1995a, b,1996b), the large intestine may not be as promising for drug delivery as recently hoped. Thus it requires more extensive characterization of the membrane permeability of the large intestine to develop successful kinetic modelling strategies and colonic drug delivery strategies. In the present study we focussed on comparing D-xylose absorption from the small intestine and the large intestine.

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Materials and Methods

Materials

D-[U-^{14}C]Xylose (2·7 GBq mmol^{-1}) was purchased from Amersham International (Buckinghamshire, UK). [^{3}H]PEG 4000 (0·041 GBq g^{-1}), [^{3}H(G)]inulin (10·6 GBq g^{-1}) and Biofluor, a scintillation fluid, were purchased from DuPont-NEN Co. (Boston, MA, USA). Scintisol EX-H, a scintillation fluid, was purchased from Dojindo Lab. (Kumamoto, Japan). Soluene-350, a tissue solubilizer, was purchased from Packard Instrument Co., (Meriden, CT, USA). Unlabelled D-xylose was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade and commercially obtained.

Absorption in intestinal loop

Male Wistar rats, weighing about 300 g, were fasted overnight prior to experiments. Under light ether anaesthesia, each rat was cannulated into the right jugular vein and the abdomen was opened by midline incision to select 5 cm of mid-gut segment about 30 cm below pylorus or colonic segment immediately below ileo-caecal junction. The intestinal segment was internally washed with saline and the distal end was ligated with thread. A needle attached to a syringe for D-xylose administration was inserted through the proximal end and ligated over the tissue. The D-xylose solution (0·3 mM) for administration was prepared in phosphate buffer (20·1 mM $Na_2HPO_4 \cdot 12H_2O$ 47·0 mM KH_2PO_4, 101·0 mM NaCl, pH 6·4) and added with trace amounts of [^{14}C]D-xylose and [^{3}H]PEG

4000 as a nonabsorbable marker. After administering 0·5 mL of the D-xylose solution, the needle was pulled out of the loop and the ligation was secured. The abdominal incision was sutured and the rat, which shortly regained consciousness, was maintained in a metabolic cage at the ambient temperature of 25°C. Blood samples (250 μL) were periodically taken from the right jugular vein through the cannula, placed in a centrifuge tube containing 5 units of heparin and centrifuged for 3 min with a Microfuge E (Beckman Instruments, Palo Alto, CA) to obtain plasma. One hour after administration, the rat was killed by puncturing its heart under ether anaesthesia, the abdomen was opened to isolate the intestinal loop and the luminal solution was drained in a vial. Fifty-microliter aliquots of plasma, luminal solution and dosing solution were taken in counting vials and added with 3 mL of Scintisol EX-H, a scintillation fluid, for radioactivity determination with a liquid scintillation counter (LSC-1000, Aloka Co., Tokyo, Japan).

The fraction remaining (F_r) of D-xylose was estimated by correcting for minor volume changes based on the changes in PEG 4000 concentrations:

$$F_r = \frac{C_f}{C_i} \Big/ \frac{C_f'}{C_i'} \quad (1)$$

where C_i and C_f are the initial and final concentrations of D-xylose, respectively, and C_i' and C_f' are those of PEG 4000. The fraction absorbed (F_a) of D-xylose was obtained as the fraction disappeared from the loop as follows:

$$F_a = 1 - F_r \quad (2)$$

Assuming first-order absorption (disappearance), the absorption rate constant (k_a) was estimated as follows:

$$k_a = -\frac{\ln F_r}{t} = -\frac{\ln(1 - F_a)}{t} \quad (3)$$

where t represents the absorption period. Finally, the apparent membrane permeability clearance (CL_{app}) was estimated as follows:

$$CL_{app} = k_a \cdot V \quad (4)$$

where V is the volume of the intestinal loop (100 μL cm^{-1} as the administered volume).

Uptake in everted sacs

Male Wistar rats, weighing about 300 g, were used without fasting prior to experiments. Two 2 cm everted sacs were prepared from each of mid-gut, caecum and colon as previously described (Yuasa et al 1996a) and stored in Krebs–Ringer-bicarbonate buffer (KRB (mM): 118 NaCl, 4·7 KCl, 2·5 $CaCl_2$, 1·2 KH_2PO_4, 1·2 $MgSO_4$, 25 $NaHCO_3$ and oxygenated with 95% O_2–5% CO_2 gas, pH 7·4) on ice until use. Three everted sacs from different rats were, after preincubating for 5 min in KRB, incubated for 5 min at 37°C and 100 cycles min^{-1} in 20 mL of KRB containing 0·3 mM D-xylose with trace amounts of [^{14}C]D-xylose and [^{3}H]inulin (a nonabsorbable marker). Uptake was terminated by rinsing the everted sacs briefly in ice-cold buffer. About a half of each everted sac (ca 150 mg) was cut and placed in a counting vial for determining wet tissue weight, and solubilized for radioactivity determination as described previously (Yuasa et al 1996a).

The uptake was estimated by subtracting the amount of D-xylose in the adherent fluid from the total amount in the sample and then subtracting the initial adsorption to the tissue at 0 min. The adherent fluid volume was estimated by dividing the amount of tissue-associated inulin by its concentration in the medium. The uptake was divided by time to calculate the uptake rate, and then by concentration to estimate the apparent membrane permeability clearance (CL_{app}). Although D-xylose uptake was not examined for its time course, it can be assumed to be proportional to time up to 5 min as confirmed in the small intestine for L-glucose uptake (Yuasa et al 1986), which is comparable with D-xylose uptake. The CL_{app} values, which were originally obtained in terms of 100 mg wet tissue weight, were converted to be expressed in terms of unit length using the following values of wet tissue weight for unit length: 148, 280 and 211 mg cm^{-1}, respectively, for mid-gut, caecum and colon.

Results

The plasma concentrations of D-xylose after administration into the loop of large intestine were lower, by an order of magnitude or more, than those after administration into the loop of small intestine, and practically undetectable at several sampling times (Fig. 1). Consistently, the disappearance of D-xylose was insignificant from the large intestine (nominal 4·9%) in 60 min, whereas it was moderate (57·0%) in the small intestine (Table 1). Assuming first-order absorption (disappearance) from the intestinal loop, the apparent membrane permeability clearances (CL_{app}) were 0·085 and 1·440 μL min^{-1} cm^{-1}, respectively, for large intestine and small intestine. These CL_{app} values were comparable with those in everted sacs in both large intestine and small intestine. All these results strongly support the findings of our previous study (Yuasa et al 1996b), that D-xylose absorption in the large intestine is negligible. In that study, the fraction of D-xylose recovered was examined along the gastrointestinal tract relative to that of PEG 4000 as a nonabsorbable marker. The relative recovery of D-xylose decreased as sampling progressed

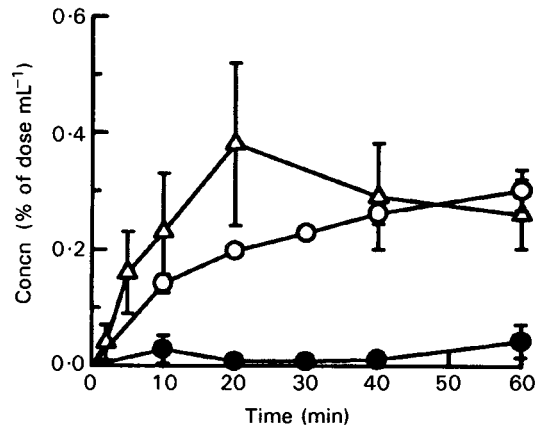

FIG. 1. Comparison of plasma concentrations of D-xylose in rats following various administration routes. The data are represented as the mean ± s.e. (n = 3). △, oral-route data from our preceding study (Yuasa et al 1996b); ○, small intestinal loop (for administration details, see Methods); ●, large intestinal loop (for administration details, see Methods).

Table 1. Parameters of intestinal D-xylose transport in rats.

Method	Site	F_a (%)	CL_{app} ($\mu L\ min^{-1} cm^{-1}$)
Loop	Small intestine	57·0 ± 5·8	1·440 ± 0·220
	Large intestine	4·9 ± 2·9	0·085 ± 0·051
Everted sacs	Small intestine	—	2·443 ± 0·369
	Large intestine	—	0·164 ± 0·164
	Caecum	—	1·826 ± 0·401

Data are represented as the mean ± s.e. (n = 3). F_a, fraction absorbed; CL_{app}, apparent membrane permeability clearance.

down the small intestine, suggesting the disappearance of D-xylose by absorption. However, it remained unchanged from caecum to faeces, suggesting that D-xylose is not absorbed at all in the large intestine (colon and rectum).

Although the CL_{app} in caecal everted sacs was closer to that in the small intestine rather than that in the large intestine, it is consistent with the preceding in-vivo study (Yuasa et al 1996b), where some potential absorption of D-xylose was suggested from a slight decrease in the relative recovery of D-xylose in the caecum.

It should be noted that the rate of appearance of D-xylose in plasma after administration into the loop of small intestine was comparable with that after oral administration. This is also consistent with our previous suggestion that the intestinal absorption of D-xylose is slow enough, compared with gastric emptying, to be small-intestine-absorption limited (Yuasa et al 1995a,b).

Discussion

Even though colonic drug delivery has been of increasing interest, information about colonic membrane permeability is still scarce. According to a series of studies by Sawada et al (1991) and Tomita et al (1992), the colonic membrane permeability of hydrophilic compounds is comparable with that of the jejunal membrane. This contrasts with our finding of a large difference in the permeability of the small intestine and large intestine to D-xylose. Although they did not test D-xylose, compounds tested in their studies include erythritol and mannitol, which possess physicochemical properties similar to those of D-xylose. The difference between their results and our finding may originate from technical problems. They measured permeability across the mucosal tissue in-vitro, which would represent the permeability of tissue layers rather than epithelial permeability to the blood stream. Because we measured epithelial permeability in the intestinal loop in-situ, our results should be more relevant to in-vivo absorption. Hosoya et al (1993) reported that the colon was 2–3 times more permeable to dextrans than was the jejunum, according to measurements of transport across mucosal tissue; Sasaki et al (1994) found permeability of azetirelin, a TRH analogue, to be similar in the colon and jejunum by measuring transport across everted intestinal tissue. Although these findings, suggesting that the membrane permeability in the large intestine (or colon) may be comparable to or larger than that in the small intestine, appear to be encouraging for colonic drug delivery, they may need to be re-examined for their relevance to in-vivo absorption.

According to a study by Kimura et al (1994), the membrane permeability of decyl alcohol, a lipophilic solute, is about 2 times larger in the small intestine than in the large intestine in tissue uptake experiments similar to ours using everted sacs. In the same study, the permeability of PEGs 600 and 1000, hydrophilic solutes, were also suggested to be 2 to 4 times larger in the small intestine than in the large intestine in the intestinal loop in-situ. Although those reports are consistent with ours in that the membrane permeability is larger in the small intestine than in the large intestine, the small intestine to large intestine ratios of membrane permeability (SI/LI ratios) are quite different from that for D-xylose (about 20 times).

Because lipophilic solutes are generally assumed to be transported by transcellular diffusion through the lipoidal membrane, of which the surface area should be almost equivalent to the total absorptive surface area, the SI/LI ratio of 2, observed for decyl alcohol, may represent the difference in the surface area as stated by Kimura et al (1994) in their report. For hydrophilic solutes, which are generally assumed to be transported by paracellular diffusion, or diffusion through some kind of water-filled pores, SI/LI ratios can depend on the molecular size and the potential difference in the size distribution of paracellular or water-filled pores between small intestine and large intestine, and hence they are not necessarily consistent with those for lipophilic solutes or among different solutes. However, comparing the hydrophilic solutes of D-xylose and the PEGs (Kimura et al 1994), it is surprising that, in the large intestine, the permeability of D-xylose (F_a of 4·9% in 60 min) appears to be lower than that of the PEGs (reported F_a of 10 to 15%), even though the molecular weight is smaller for D-xylose (MW, 150) and, accordingly, in the small intestine the F_a of 57% for D-xylose is larger than those of 15 to 40% reported for the PEGs.

The lower permeability of D-xylose compared with that of the PEGs in the large intestine may be partly explained by the mechanism suggested by Hollander et al (1988) that the paracellular permeability of monosaccharides could be lower than that of the PEGs because of the larger smallest cross-sectional diameter of the molecule. However, it does not explain the result that D-xylose permeates the mucosa more readily than the PEGs in the small intestine, contrarily to the result in the large intestine. The potential contribution of carrier-mediated transport may need to be examined to explain the high permeability of D-xylose in the small intestine relative to that in the large intestine. Although D-xylose has been suggested to be transported passively in the small intestine in rats and humans (Ohkohchi & Himukai 1984; Craig & Atkinson 1988; Rolston & Mathan 1989), the possibility that it may have weak affinity to D-glucose carriers cannot be excluded (Rolston & Mathan 1989). The potential contribution of transcellular diffusion may also need to be investigated for those hydrophilic compounds, also taking into account the oil-to-water partition coefficient and the molecular weight (or molecular diameter or volume).

In conclusion, the passive membrane permeability of D-xylose was demonstrated to be negligible in the large intestine (colon), even though it is fairly permeable in the small intestine. Although the permeation mechanism is yet to be fully clarified and more drugs should be examined for the permeability in the large intestine in comparison with that in the small intestine, this result helps to rationalize kinetic modelling

strategies assuming small intestine as the sole absorption site for gastrointestinal absorption in-vivo as proposed in our preceding studies (Yuasa et al 1995a,b, 1996b). However, it also suggests that hydrophilic drugs with molecular size similar to, or larger than, D-xylose may not be good candidates for colonic drug delivery by controlled release.

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