Evaluation of the Fractional Absorption of D-Xylose by Analysis of Gastrointestinal Disposition after Oral Administration in Rats

Hiroaki YUASA,* Chiaki KUNO, and Jun WATANABE

Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467, Japan.

Received, October 3, 1995; accepted November 28, 1995

As an efficient method for estimating the amount orally absorbed, the fraction of D-xylose absorbed in rats was estimated from the ratio of the fecally excreted proportion of D-xylose dose to that of polyethylene glycol (PEG) 4000 as a nonabsorbable marker (D-xylose/PEG 4000 ratio). The D-xylose/PEG 4000 ratio was demonstrated to be independent of the fecal excretion of D-xylose and PEG 4000 (or sampling period), suggesting that it can represent the fraction of the total dose remaining. This result was consistent with the theoretical prediction based on the assumptions that every small fraction of dose behaves in the same manner with regard to absorption and transit through the absorption site (small intestine), and that the gastrointestinal transit of PEG 4000 is identical with that of D-xylose. The fraction of D-xylose absorbed was estimated to be 95.9% by subtracting the remaining fraction (D-xylose/PEG 4000 ratio) from unity (100%). The D-xylose/PEG 4000 ratio, or drug/marker ratio in general, can be determined before fecal excretion is completed. Thus it can provide an efficient way for estimating the fraction of a drug absorbed that is stable in the gastrointestinal tract, such as D-xylose. Although this proposed method of estimating a fraction is generally not applicable unless a given drug is proved to be stable in the gastrointestinal tract, it can be an efficient screening method to identify poorly absorbable drugs. It is especially useful in basic studies and preclinical tests in laboratory animals.

Key words gastrointestinal absorption; D-xylose; rat; fractional absorption; gastrointestinal transit

The purpose of the present study was to find and propose an efficient method for evaluating the orally absorbed fraction of a drug or the fraction disappearing from the gastrointestinal tract. Orally absorbed fraction can be estimated as the bioavailability on the basis of plasma concentration data or urinary excretion data, if first-pass metabolism is negligible. If a given drug is stable in the gastrointestinal tract, fecal excretion data can provide a more direct estimate of fraction absorbed as the difference between unity and fraction remaining in feces, regardless of the involvement of first-pass metabolism. It can at least provide information about drug disappearance (potential absorption), even if the drug has not been proved to be stable. However, it takes a long time, usually 24 h or more, to assure that fecal excretion is completed and that the fraction remaining can be reliably estimated. To overcome this, we devised and demonstrated a new method of administering a nonabsorbable marker with a drug and determining the ratio of fecally excreted fraction of the drug to that of the nonabsorbable marker (drug/marker ratio) as an estimate of the fraction remaining. The drug/marker ratio can be determined before fecal excretion is completed, providing an efficient way to estimate the orally absorbed fraction. In the present study, D-xylose, which is a passively absorbed five-carbon monosaccharide and has been used to assess intestinal absorptive function,¹⁾ was used as a model compound and polyethylene glycol (PEG) 4000 was used as a nonabsorbable marker.

D-Xylose is reportedly absorbed by passive transport alone in rats, as suggested by concentration and Na⁺ independence in its absorption,²⁾ though it has been suggested that active transport is also involved in guinea pigs²⁾ and hamsters.^{3,4)} Although the absorption mechanism of D-xylose in humans is yet to be fully elucidated, it

has been suggested that passive transport is predominant at least at clinical concentrations.⁵⁾ The orally absorbed fraction of about 70% in humans^{1,6)} is comparable with that in rats in our previous study (about 80%)⁷⁾ and in the present study (about 95%) as described later. Thus the rat can be a good animal model for D-xylose absorption in humans.

MATERIALS AND METHODS

Chemicals D-[U-¹⁴C]Xylose (2.7 GBq/mmol) was purchased from Amersham International plc (Buckinghamshire, U.K.). [³H]PEG 4000 (0.041 GBq/g) and Biofluor, a scintillation cocktail, were purchased from DuPont-NEN Co. (Boston, MA, U.S.A.). Scintisol EX-H, also a scintillation cocktail, was purchased from Dojindo Lab. (Kumamoto, Japan). Soluene-350, a tissue solubilizer, was purchased from Packard Instrument Co., Inc. (Meriden, CT, U.S.A.). Unlabeled D-xylose was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals were of analytical grade and commercially obtained.

Gastrointestinal Disposition Experiments The dosing solution containing 1 mM D-xylose with a trace amount of [¹⁴C]D-xylose (125 kBq/ml) and 1.5 mM of [³H]PEG 4000 (250 kBq/ml) was prepared in saline (0.9% NaCl solution). Male Wistar rats, weighing about 300 g and fasted overnight, were given an oral dose of D-xylose (0.5 μmol/0.5 ml/kg) with PEG 4000 (0.75 μmol/0.5 ml/kg) through a gastric tube and kept in a metabolic cage at the ambient temperature of 25 °C. The rats were sacrificed at 0.5, 3, 10 or 24 h after administration by puncturing the heart under light ether anesthesia to sample the gastrointestinal contents and tissues of stomach, duodenum, three equal lengths of intestinal segments (jejunum,

* To whom correspondence should be addressed.

midgut and ileum), cecum and large intestine (colon and rectum) as described.⁸⁾ After adding an appropriate amount of saline, the gastrointestinal contents and tissues were homogenized, and a portion (*ca.* 100 mg) of each homogenized sample was solubilized for the determination of radioactivity as also described in our previous report,⁸⁾ using Scintisol EX-H (5 ml) as a scintillation cocktail and Soluene-350 (1 ml) as a tissue solubilizer.

Feces were weighed in a tared counting vial, and homogenized in saline with a spatula to make 20% homogenate. Roughly 100 mg of the homogenized sample was placed in the same type of vial and was solubilized to determine radioactivity in the same manner as tissue samples.

The fraction recovered (*FR*) of D-xylose in each segment of gastrointestinal tract was estimated as the sum of that in the contents sample and that in the fluid adherent to the tissue. The volume of adherent fluid was estimated by dividing the amount of tissue-associated PEG 4000 by concentration.

Theory for Evaluating Fraction Absorbed It is assumed that a given drug is stable in the gastrointestinal tract and, without being absorbed in the stomach, continuously enters small intestine (or absorption site) by gastric emptying. For each small fraction of dose (Δf) entering the absorption site, the fraction of dose leaving can be defined by multiplying Δf by the remaining fraction (F_r) after the passage (Fig. 1). Assuming that every Δf undergoes absorption in the same manner during the passage through the absorption site as a bolus with a constant transit time, F_r would be constant and, for any fraction of dose entering (F_{in}) as the sum of Δf , the fraction leaving (F_{out}) can be defined as follows:

$$F_{out} = \sum F_r \cdot \Delta f = F_r \sum \Delta f = F_r \cdot F_{in} \quad (1)$$

For a nonabsorbable marker, the fraction of dose leaving (F'_{out}) is equal to the fraction entering (F'_{in}). It can be assumed that both the drug and nonabsorbable marker are carried by the flow of gastrointestinal contents and, hence, the gastrointestinal transit of nonabsorbable marker is identical with that of the drug. Thus, F_{in} can be assumed to be equal to F'_{in} and also to F'_{out} . Substituting F_{in} by F'_{out} , Eq. 1 can be rearranged to give F_r as follows:

$$F_r = \frac{F_{out}}{F'_{out}} \quad (2)$$

Both F_{out} and F'_{out} can be determined as the fraction of dose excreted in feces and F_r should, by assumption, be

Fig. 1. Schematic Representation of Intestinal Absorption
 Δf , small fraction of dose; F_r , fraction remaining.

independent of F_{out} and F'_{out} (or sampling period), representing the remaining fraction of total dose. The fraction absorbed (F_a) can be given as follows:

$$F_a = 1 - F_r \quad (3)$$

Further, assuming that the intestinal membrane permeability clearance for unit length (CL_{app}) and the intestinal lumen volume for unit length (V) are constant along the absorption site or can be assigned as average values, the intestinal absorption can be characterized as a first-order process with the absorption rate constant of k_a ($= CL_{app}/V$) regardless of the distribution of the drug in the absorption site, as discussed previously.⁸⁾ Thus every Δf can be assumed to undergo first-order absorption with the rate constant of k_a during the passage of the absorption site and F_r is described as follows:

$$F_r = e^{-k_a \cdot T_a} \quad (4)$$

where T_a is the transit time in the absorption site.

Pharmacokinetic Analysis of Plasma Concentration

Each rat was cannulated into the right jugular vein under light ether anesthesia and, after regaining consciousness, given an oral or intravenous dose of D-xylose, using the same solution and dose as in gastrointestinal disposition experiments. The intravenous dose was given through the cannula. Blood (250 μ l) was periodically taken 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, U.S.A.) to obtain plasma. The plasma (100 μ l) was placed in a counting vial to which was added 3 ml of Biofluor, a scintillation cocktail, and the radioactivity determined with a liquid scintillation counter (LSC-1000, Aloka Co., Tokyo, Japan). Feces were collected until 24 h after administration and treated for radioactivity determination as described above.

Plasma concentration (C) versus time (t) profiles of D-xylose after intravenous and oral administration were analyzed by a two-compartment model (Eq. 5) and one with first-order absorption (Eq. 6), respectively.

$$C = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \quad (5)$$

$$C = L \cdot e^{-\alpha t} + M \cdot e^{-\beta t} - N \cdot e^{-k_a t} \quad (6)$$

where

$$L = \frac{A \cdot F_a \cdot k_a}{k_a - \alpha} \quad (7)$$

$$M = \frac{B \cdot F_a \cdot k_a}{k_a - \beta} \quad (8)$$

$$N = L + M \quad (9)$$

where A , B , α and β are constants, and k_a and F_a are the absorption rate constant and fraction absorbed, respectively. The A , B , α and β were estimated by fitting Eq. 5 to the concentration versus time profiles after intravenous administration using a nonlinear regression program, PCNONLIN (Statistical Consultants, Inc., Lexington, KY, U.S.A.). The k_a and F_a were estimated by fitting Eq. 6 to the concentration versus time profiles after oral

Fig. 2. Gastrointestinal Distribution Profiles of D-Xylose (A) and PEG 4000 (B) after Oral Administration in Rats

The data are represented as the average values from 3 rats. Keys: S, stomach; D, duodenum; J, jejunum; M, midgut; I, ileum; C, cecum; L, large intestine (colon and rectum); F, feces. FR represents fraction recovered.

administration using PCNONLIN, with A , B , α and β fixed at those estimated for intravenous administration data.

RESULTS AND DISCUSSION

Gastrointestinal Distribution Profiles of D-Xylose and PEG 4000 The distribution profiles of D-xylose and PEG 4000 after oral administration are shown in Fig. 2. The total recovery of PEG 4000 from the gastrointestinal tract and feces was about 100% throughout the experimental periods of 0.5, 3, 10 and 24 h, suggesting negligible PEG 4000 absorption and assuring the feasibility of PEG 4000 as a nonabsorbable marker. The distribution of PEG 4000 indicates that gastric emptying was almost completed by 3 h after administration and that fecal excretion was by 24 h. The recovery of D-xylose from stomach was comparable with that of PEG 4000. Considering that gastric absorption of D-xylose is negligible,⁹⁾ gastric emptying of this monosaccharide can be assumed to be comparable with that of PEG 4000. Although intestinal and fecal recoveries of D-xylose were smaller than those of PEG 4000 and decreased with time, indicating the absorption of the former, the distribution profiles of D-xylose were similar in shape to those of PEG 4000. Thus, the gastrointestinal transit of PEG 4000 seemed to be identical with that of D-xylose, as assumed in the following evaluation of orally absorbed fraction.

Evaluation of Orally Absorbed Fraction As shown in Table 1, the D-xylose/PEG 4000 ratio of fecally excreted fraction of dose was comparable for 10 and 24 h; fecal excretion was about half completed on the basis of the recovery of PEG 4000 at 10 h and almost completed at 24 h. This result is consistent with the theoretical prediction by Eq. 2 that the D-xylose/PEG 4000 ratio (drug/marker ratio) would be independent of the fecally excreted amount of D-xylose and PEG 4000 (or sampling period), representing the remaining fraction for total dose. Because D-xylose, which is biologically inactive, has been suggested to be stable enough,⁷⁾ F_a was estimated using Eq. 3 as 95.5% and 96.2%, respectively, from the D-xylose/PEG 4000 ratio as F_r at 10 and 24 h, and 95.9% as an average.

Although F_a of about 96% from the D-xylose/PEG 4000 ratio was somewhat larger than those of 70 to 80% in rats in our previous study⁴⁾ and in humans in the literature,^{1,6)} it was in agreement with the 99% in the pharmacokinetic

Table 1. Fecal Excretion of D-Xylose and PEG 4000 after Oral Administration in Rats

Time (h)	Fecal excretion (%)		D-Xylose/PEG 4000 (%)	F_a (%)
	D-Xylose	PEG 4000		
10	1.8 ± 0.2	40.4 ± 4.5	4.5 ± 0.2	95.5 ± 0.2
24	3.1 ± 0.4	82.4 ± 3.9	3.8 ± 0.4	96.2 ± 0.4

The data are represented as the mean $\pm$ S.E. ($n=3$).

Fig. 3. Plasma Concentration versus Time Profiles of D-Xylose after Oral (O) and Intravenous (●) Administration in Rats

The data are represented as the mean $\pm$ S.E. ($n=3$). Solid lines represent computer-fitted profiles.

Table 2. Pharmacokinetic Parameters of D-Xylose Disposition in Rats

Parameter	
A (% of dose/ml)	0.96 ± 0.19
B (% of dose/ml)	0.99 ± 0.11
α (h^{-1})	13.51 ± 0.70
β (h^{-1})	1.55 ± 0.09
k_a (h^{-1})	1.31 ± 0.55
F_a (%)	99 ± 1

The data are represented as the mean $\pm$ S.E. ($n=3$).

analysis of plasma concentrations (Fig. 3, Table 2). The F_a estimated from the D-xylose/PEG 4000 ratio in this series of experiments for pharmacokinetic analysis was also in agreement ($95.3 \pm 0.8\%$), suggesting insignificant effects of cannulation and blood sampling.

Thus the proposed method of determining the drug/marker ratio in feces can provide an efficient way for estimating F_a of drugs that are stable in the gastroin-

Fig. 4. D-Xylose/PEG 4000 Ratio along the Gastrointestinal Tract after Oral Administration in Rats

The data are represented as the mean $\pm$ S.E. ($n=3$). Gastrointestinal site is represented as the dimensionless distance from the stomach (normalized by the length of small intestine). Keys; S, stomach; D, duodenum; J, jejunum; M, midgut; I, ileum; C, cecum; L, large intestine (colon and rectum); F, feces. The experimental periods were 0.5 h (Δ), 3 h ($\square$), 10 h (∇) and 24 h ($\circ$).

testinal tract, such as D-xylose. Although the technique can only be used to estimate the fraction that has disappeared unless a given drug is proved to be stable in the gastrointestinal tract, it still can provide useful information for differentiating potential absorption from poor absorption. It is especially useful in basic studies and preclinical tests in laboratory animals.

Evaluation of Absorption Site The D-xylose/PEG 4000 ratio of fraction of dose recovered was examined along the gastrointestinal tract to evaluate the absorption sites (Fig. 4), as it represents the fraction remaining at each gastrointestinal site, similarly to that in feces. The D-xylose/PEG 4000 ratios were plotted at the center of each gastrointestinal site, where a significant amount of PEG 4000 was observed in every rat. The D-xylose/PEG 4000 ratio decreased to about 20% before reaching the cecum at 3 h and further decreased to about 5% in the cecum at 10 h, suggesting that D-xylose is mainly absorbed in small intestine and to some extent in the cecum. However, the D-xylose/PEG 4000 ratio in the cecum at 10 h was comparable with that in feces at both 10 and 24 h. On the basis of recovery of PEG 4000, fecal excretion was about half completed at 10 h, with the other half in the cecum, and was almost completed at 24 h (Fig. 1 and Table 1). Therefore, it can be interpreted, at least for about half the dose observed in the cecum at 10 h, that D-xylose and PEG 4000 passed large intestine (colon and rectum) while maintaining their ratio without any significant absorption of D-xylose. This result strongly suggests that D-xylose is not absorbable at all in large intestine, and also that the possibility of its absorption as a bacterial degradation product can be excluded. Considering that the transit time in large intestine can be as long as 8 h¹⁰⁾ and much longer than that in small intestine (1 to 2 h),⁸⁾ the large intestinal membrane

would be practically impermeable to D-xylose or at least far less permeable than small intestinal membrane. The potential incorporation of D-xylose in feces during its formation in large intestine would also be disadvantageous for its absorption.

As it was suggested that small intestine is the major absorption site, using the small intestinal transit time of 74 min⁸⁾ for T_a in Eq. 4, k_a was estimated to be 0.0440 min⁻¹ (2.64 h⁻¹) and, by multiplying the average V of 24 μ l/cm,⁸⁾ CL_{app} was 1.06 μ l/min/cm.

Several studies have recently shown that the permeability of large intestinal membrane can sometimes be comparable to that of small intestinal membrane, e.g., indomethacin,¹¹⁾ dextran¹²⁾ and azetirelin,¹³⁾ raising increasing interest in colonic drug delivery. However, the present study suggested that D-xylose, and hence presumably all monosaccharides, cannot be absorbed in the large intestine, whereas they can be even passively absorbed in the small intestine. This is consistent with the classical view that the large intestine is disadvantageous for absorption because of the smaller surface area than small intestine; thus large intestine may not be as promising as recently expected for drug delivery. More extensive characterization of membrane permeability in relation with physicochemical properties of drugs is required to develop successful colonic drug delivery strategies.

Acknowledgement This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

REFERENCES

- 1) Craig R. M., Atkinson A. J., *Gastroenterology*, **95**, 223—231 (1988).
- 2) Ohkohchi N., Himukai, M., *Jpn. J. Physiol.*, **34**, 669—677 (1984).
- 3) Alvarado F., *Biochim. Biophys. Acta*, **112**, 292—306 (1966).
- 4) Bihler I., Kim N. D., Sawh P. C., *Can. J. Physiol. Pharmacol.*, **47**, 525—532 (1969).
- 5) Rolston D. D. K., Mathan V. I., *Dig. Dis. Sci.*, **34**, 553—558 (1989).
- 6) Johnson S. L., Mayersohn M., Conrad K. A., *Clin. Pharmacol. Ther.*, **38**, 331—335 (1985).
- 7) Yuasa H., Kawanishi K., Watanabe J., *J. Pharm. Pharmacol.*, **47**, 373—378 (1995).
- 8) Yuasa H., Numata W., Ozeki S., Watanabe J., *J. Pharm. Sci.*, **84**, 476—481 (1995).
- 9) Yuasa H., Kawanishi K., Watanabe J., *J. Pharm. Pharmacol.*, **47**, 576—580 (1995).
- 10) Bungay P. M., Dedrick R. L., Matthews H. B., *J. Pharmacokinet. Biopharm.*, **9**, 309—341 (1995).
- 11) Kimura T., Sudo K., Kanzaki Y., Miki K., Takeichi Y., Kurosaki Y., Nakayama T., *Biol. Pharm. Bull.*, **17**, 327—333 (1994).
- 12) Hosoya K., Kubo H., Natsume H., Sugibayashi K., Morimoto Y., Yamashita S., *Biopharm. Drug Disp.*, **14**, 685—696 (1993).
- 13) Sasaki I., Fujita T., Murakami M., Yamamoto A., Nakamura E., Imasaki H., Muranishi S., *Biol. Pharm. Bull.*, **17**, 1256—1261 (1994).