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Effects of Ageing on the Oral Absorption of D-Xylose in Rats: Analysis of Gastrointestinal Disposition

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Abstract

The effects of ageing on the oral (gastrointestinal) absorption of D-xylose were investigated by analysing the gastrointestinal disposition after oral administration to young (9 weeks) and old (53 weeks) rats. A linear model assuming first-order gastric emptying followed by first-order intestinal absorption was fitted to remaining fraction vs time profiles for the stomach and small intestine to estimate the gastric emptying rate constant (k_g) and the intestinal absorption rate constant (k_a).

In young and old rats, k_g values were $0{\cdot}087 \pm 0{\cdot}008$ and $0{\cdot}070 \pm 0{\cdot}007\ min^{-1}$, respectively, and k_a values were $0{\cdot}020 \pm 0{\cdot}002$ and $0{\cdot}018 \pm 0{\cdot}002\ min^{-1}$, suggesting an insignificant effect on ageing on the rate of oral absorption. The average intestinal lumen volume (V_{av}) was unchanged with ageing, and so was the apparent intestinal membrane permeability clearance (CL_{app}) as the product of k_a and V_{av}. However, the small intestinal transit time (T_{si}) was suggested to be twice that in older rats (171 min) than in young rats (78 min) by the analysis of gastrointestinal disposition of inulin, a non-absorbable marker. It was also shown that our preceding finding of an increase in the fraction absorbed of D-xylose with ageing can be solely ascribable to the delay in intestinal transit.

Thus, among various determinants of oral absorption, only T_{si} was found to be altered with ageing. The $CL_{a,app}$ and k_a of passively absorbed drugs such as D-xylose may be generally unchanged, and the fraction absorbed may increase with ageing by the delay in intestinal transit.

Age-dependent changes in gastrointestinal drug absorption have been left largely inconclusive, as reviewed by Schmucker (1985) and in our preceding report (Yuasa et al 1995b) on the pharmacokinetic analysis of age-dependent oral absorption of D-xylose. Although a number of gastrointestinal functions have been suggested to change with age, its implications in oral absorption, as well as age-dependent changes in oral absorption, have been little understood.

D-Xylose has been clinically used to assess intestinal absorptive functions and can also be a marker for the effects of ageing, and has been subjected to exceptionally extensive evaluation of age-dependent changes in its oral absorption (Schmucker 1985; Craig & Atkinson 1988). However, although its fraction absorbed has been suggested to be unchanged with ageing in man (Weiner et al 1984; Johnson et al 1985, 1986) as has its apparent rate of absorption (Johnson et al 1985, 1986), those results do not appear to be easily correlated with general suggestions of changes (or decline) in gastrointestinal functions. Unfortunately almost all those studies were clinical studies with limited data, and have not been subjected to verification using in-situ or in-vitro techniques. There is also a report of decreased fractions absorbed in the elderly (Weiner et al 1984), inconsistent with the findings by Johnson et al (1985, 1986).

We therefore conducted a pharmacokinetic study in rats for more extensive evaluation of age-dependent changes in the oral absorption of D-xylose, and found that the orally-absorbed fraction of D-xylose was significantly increased with ageing but the apparent absorption rate constant was unchanged (Yuasa et al 1995b). In the present study, we further examined the mechanism of the age-dependent change in the oral absorption of D-xylose in rats, evaluating the rate constants of gastric emptying and intestinal absorption, and intestinal transit by using our recently proposed method of gastrointestinal disposition analysis (Yuasa et al 1995a).

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Materials and Methods

Materials

D-[U-^{14}C]Xylose ($1{\cdot}7\ GBq\ mmol^{-1}$) was purchased from Amersham International plc (Buckinghamshire, UK). [^{3}H(G)]Inulin ($15{\cdot}8\ GBq^{-1}\ g$) 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., Inc. (Meriden, CT, USA). D-Xylose was purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were of analytical grade and commercially obtained.

Animals

Male Wistar rats were used after fasting overnight. Their ages and weights were as follows: young, 9 weeks and 268 ± 3 g; old, 53 weeks and 477 ± 9 g.

Oral absorption experiments

Young and old rats were given an oral dose (100 mg/$5\ mL\ kg^{-1}$) of D-xylose with a trace amount of [^{14}C]D-

xylose and a trace amount of [^{3}H]inulin as a nonabsorbable marker, which were dissolved in saline, using a gastric tube, and were left free in a metabolic cage at an ambient temperature of 25°C. The rats were killed at specified times under light ether anaesthesia for sampling the gastrointestinal contents and tissues of duodenum and three equal lengths of segments (jejunum, mid-gut and ileum) as described in our previous report (Yuasa et al 1995a). After adding the appropriate amount of saline, the gastrointestinal contents and tissues were homogenized, and a portion of each homogenized sample was solubilized with Soluene-350 for the determination of radioactivity by liquid scintillation counting.

The remaining fraction (FR) of D-xylose in the gastrointestinal tract of each segment was estimated as the sum of that in the contents sample and that in the fluid adhering to the tissue, and corrected for the total fraction of inulin recovered from the gastrointestinal tract. The volume of adherent fluid was estimated from the amount of inulin associated with the tissue.

Gastrointestinal disposition analysis

As discussed in our previous report (Yuasa et al 1995a), assuming that the apparent intestinal membrane permeability clearance (CL_{app}) and the intestinal lumen volume for unit length (V) are constant along the small intestine, the intestinal absorption can be described as a first-order process with a rate constant of k_a, regardless of the distribution pattern of a given solute in the intestinal tract. Therefore, further assuming that the gastric emptying is described by a first-order rate constant of k_g, and that the gastric absorption and the transfer from the small intestine to the large intestine are negligible, the same model equations as those for a linear compartment model consisting of the stomach and small intestine compartment can be used to describe the remaining fraction of dose in the stomach (FR_s) and small intestine (FR_{si}) as follows:

$$FR_s = e^{-k_g \cdot t} \quad (1)$$

$$FR_{si} = \frac{e^{-k_a \cdot t} - e^{-k_g \cdot t}}{1 - k_a/k_g} \quad (2)$$

Equations 1 and 2 were simultaneously fitted to FR_s and FR_{si} data for D-xylose to estimate k_a and k_g, using a nonlinear regression program, PCNONLIN (Statistical Consultants, Inc., Lexington, KY), and weighted according to the reciprocal of the variance.

The gastrointestinal disposition data for a nonabsorbable marker can be used to characterize gastrointestinal transit. The distribution centre in the small intestine (DC) and the mean residence time (MRT) were estimated as functions of the experimental period, T, using the following equations, and DC vs MRT profiles were examined to assess the intestinal transit:

$$DC = \sum \frac{FR'_i}{FR'_{si}} \cdot x_i \quad (3)$$

$$MRT = \frac{\int_0^T FR'_{si}\,dt}{FR'_{si}} = \frac{T}{1 - e^{-k'_g \cdot T}} - \frac{1}{k'_g} \quad (4)$$

where:

$$FR'_{si} = 1 - e^{-k'_g \cdot t} \quad (5)$$

FR'_i is the fraction remaining in the ith segment, and x_i is the dimensionless distance, normalized for the length of the small intestine, of the centre of the ith segment from the pylorus. The primed (′) factors are for the nonabsorbable marker. The k'_g for inulin was assumed to be the same as the k_g for L-xylose. Since the residence time in the small intestine varies for solute molecules because of continuing gastric emptying, the MRT should be more appropriate as a variable than the experimental period (T) in the analysis of intestinal transit. The term DC is defined as the sum of the dimensionless distance of the midpoint of each segment weighted by the distribution of the nonabsorbable marker.

Measurement of intestinal lumen volume

As in the gastrointestinal absorption experiments, the small intestine was isolated after administering 5 mL kg^{-1} saline, using a gastric tube, or without administering saline. Ligations were made to separate the duodenum, jejunum, mid-gut and ileum. After injecting saline containing a trace amount of [^{3}H]inulin into each segment, 0·5 mL for duodenum and 2 mL for the other segments, the contents were immediately collected for the determination of radioactivity. The intestinal lumen volume was estimated from the dilution of inulin.

Estimation of time- and longitudinally-averaged intestinal lumen volume

The time- and longitudinally-averaged intestinal lumen volume for unit length (V_{av}) was estimated using an approximation method reported previously (Yuasa et al 1995a). Briefly, the longitudinally-averaged extra volume (V_e) originating from the dosing volume was calculated by equation 6, and approximated by equation 7:

$$Ve = \sum \frac{FR'_i}{FR'_{si}} \cdot (V_i - V_{i,0}) \quad (6)$$

$$V_e = V_{e,0} \cdot e^{-k_v \cdot t} \quad (7)$$

where V_i is the intestinal lumen volume for unit length for the ith segment, and $V_{i,0}$ is the longitudinally-averaged physiological volume of the ith segment. In equation 7, the small intestine is assumed to absorb the extra water, with a first-order rate constant of k_v, to bring the luminal volume back to its physiological state. The $V_{e,0}$ is the extrapolated initial volume at time 0. In equation 7, the mean residence time (MRT) was used instead of the experimental period (T) for time as explained in the derivation of MRT (eqn 4). The terms $V_{e,0}$ and k_v were estimated by nonlinear regression analysis of the V_e vs MRT profile, using equation 7. The V_{av} was obtained as the sum of the time-averaged value of V_e for the experimental period of 60 min and the longitudinally-averaged physiological volume (V_p) measured without dosing by the following equation:

$$V_{av} = \frac{\int_0^{MRT} V_e\,dt}{MRT} + V_p \quad (8)$$

Results and Discussion

Gastrointestinal distribution profiles

Fig. 1 shows the gastrointestinal distribution profiles of D-xylose and co-administered inulin, nonabsorbable marker, after oral administration in young and old rats. While most of the inulin reached the ileum 60 min after administration in young rats, the majority of the inulin remained at mid-gut in older rats, suggesting a slower intestinal transit in the latter. The total recovery of inulin from the stomach and small intestine was about 100% throughout the experimental period of 60 min in both young and old rats, assuring that its distribution was restricted within the region of the gastrointestinal tract. The remaining fractions of D-xylose were smaller than those of inulin in the small intestine, while they were comparable in the stomach, suggesting that it is absorbed in the small intestine but not in the stomach. The result that the intestinal distribution profiles of D-xylose were similar in shape to those of inulin suggests that the intestinal transit of inulin represents administered materials including D-xylose. The negligible gastric absorption of D-xylose was confirmed in the closed stomach loop in young rats, where the fraction absorbed of D-xylose was about 3%, 60 min after administration. We also confirmed that the biliary excretion of D-xylose is negligible in young rats, being about 0·3%, 60 min after administration to the closed mid-gut loop. Although we did not examine the gastric absorption and biliary excretion in old rats, they can be assumed to be negligible as in young rats, because, as in young rats, the gastrointestinal distribution profiles of D-xylose in older rats showed no sign of gastric absorption or biliary excretion.

Analysis of gastrointestinal disposition

The remaining fractions of D-xylose from all intestinal segments were summed for each time to obtain the total fractions of D-xylose remaining in the small intestine for model analysis. As shown in Fig. 2, the remaining D-xylose vs time profiles for the stomach and small intestine were comparable between young and old rats, and successfully analysed by the model described by equations 1 and 2. The gastric emptying rate constants (k_g) and the intestinal absorption rate constants (k_a) are summarized in Table 1. In both young and old rats, the k_a values were about one-fourth of the k_g values and comparable with the apparent absorption rate constants for the overall gastrointestinal absorption process (0·016 and 0·014 min^{-1}, respectively, for young and old rats) reported previously (Yuasa et al 1995b). This suggests that the oral absorption of D-xylose is intestinal absorption-limited and supports the preceding finding (Yuasa et al 1995b) of an unchanged rate of oral absorption with ageing. Because the gastric emptying was also shown to be unchanged with ageing (Varga 1976; Lin & Hayton 1983), the rate of absorption of highly absorbable drugs which are gastric emptying-limited also would not change with ageing. Gastric emptying has recently been reported to be unchanged with ageing in man (Madsen 1992;

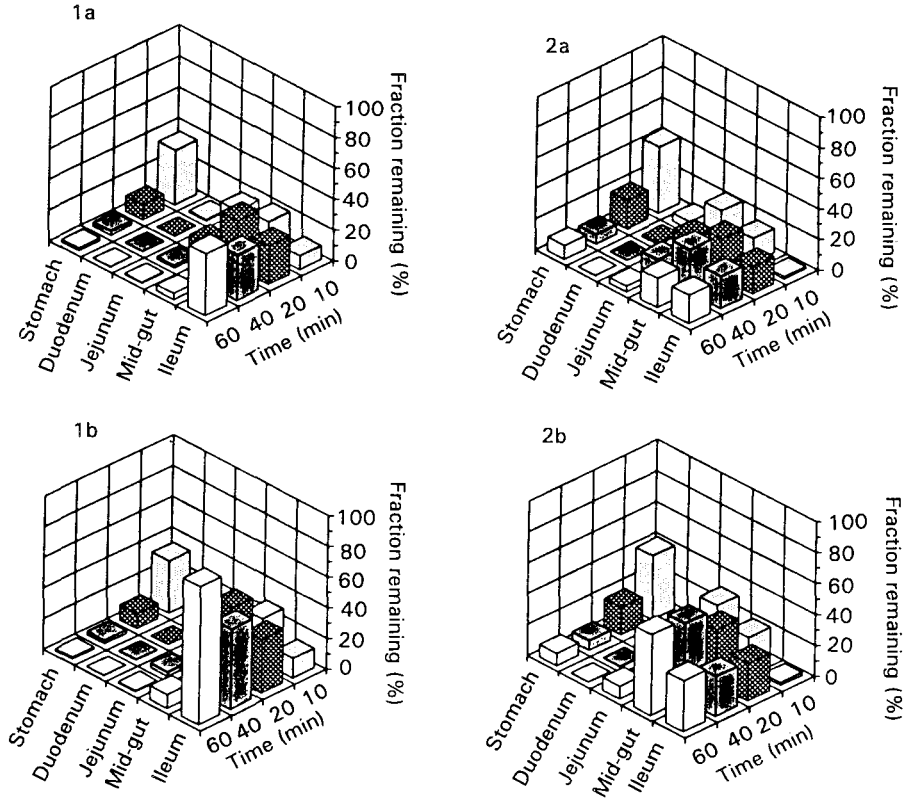

FIG. 1. Gastrointestinal distribution of D-xylose and inulin after oral administration in young and old rats. Panels: 1a, D-xylose for young; 1b, inulin for young; 2a, D-xylose for old; 2b, inulin for old. The data represent average values from five experiments. The s.e. values were mostly between 10 and 20% of the average values.

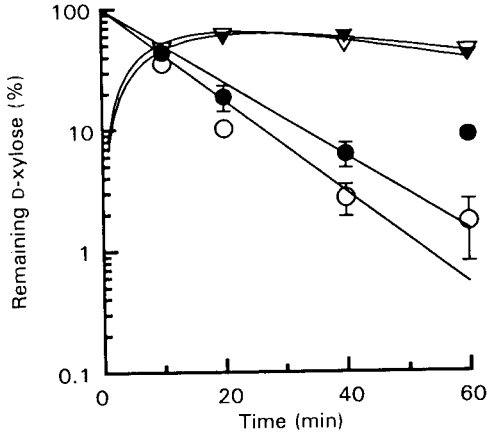

FIG. 2. Remaining D-xylose vs time profiles for the stomach (○,●) and small intense (▽,▼) in young (○,▽) and old (●,▼) rats. The data represent the mean ± s.e. (n = 5). The solid lines represent the computer-fitted profiles.

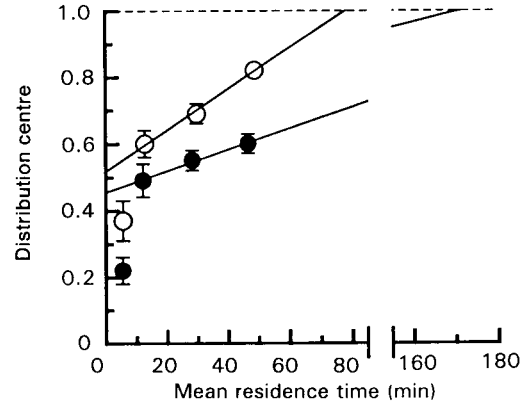

FIG. 3. Effect of ageing on intestinal transit in rats. The distribution centre (DC) and the mean residence time (MRT) were estimated from the distribution of inulin shown in Fig 1 for young (○) and old (●) rats. The data represent the mean ± s.e. (n = 5). The solid lines show the results of the linear regression of the last three data points.

Gainsborough et al 1993), although a few earlier studies suggested delays with ageing (Evans et al 1981; Wegener et al 1988). As for intestinal absorption, this study is the first to evaluate the effect of ageing in-vivo. The effects of ageing on intestinal passive absorption has little been reported in-situ or in-vitro.

Analysis of intestinal transit

The distribution centre (DC) of inulin was plotted against the mean residence time (MRT) for estimating intestinal transit (Fig. 3). The DC values were smaller in old rats than in young rats at every experimental period, suggesting slower intestinal transit in old rats. In both young and old rats, the intestinal transit was faster in the upper small intestine than in the lower small intestine, and DC was in a linear relation with MRT in the lower small intestine, suggesting a constant transit rate. Therefore, we estimated the transit time for the entire small intestine (T_{si}) as the MRT for the intercept of DC = 1 by linear regression of the last three data points, namely, those for the experimental periods of 20, 40 and 60 min. The values of T_{si} were 78 and 171 min, respectively, in young and old rats. Because the length of the small intestine was only about 10% longer in old rats (79·7 ± 1·7 cm) than in young rats (70·7 ± 0·6 cm), the longer T_{si} in old rats means a slower transit velocity.

Haboubi et al (1988) reported a similar delay in intestinal transit with ageing in man, with T_{si} values of 96 and 151 min, respectively, in the young (average age 31 years) and the elderly (81). Lin & Hayton (1983) also observed a delay in intestinal transit with ageing in the lower small intestine, although intestinal transit in the upper small intestine was faster in the older rats and the effects of ageing on the transit of the entire small intestine was unclear. Although some studies suggested unchanged intestinal transit with ageing in rats and man (Varga 1976; Wegener et al 1988; Madsen 1992), the contractile activity of intestinal smooth muscle and intestinal motor activity have been reported to decrease with ageing (Farrar & Zfass 1967; Anuras & Sutherland 1984; Nelson et al 1986). Therefore, it is quite likely that intestinal transit delays with ageing as demonstrated in this study. Meanwhile, gastric emptying, another motility-related factor, was not affected by ageing as described in the preceding section, although the reason has so far been unexplained.

Table 1. Effects of ageing on the in-vivo intestinal membrane permeability of D-xylose in rats.

	k_g (min^{-1})	k_a (min^{-1})	V_{av} ($\mu L\,cm^{-1}$)	CL_{app} ($\mu L\,min^{-1}\,cm^{-1}$)
Young	0·087 ± 0·008	0·020 ± 0·002	22·8 ± 2·8[a]	0·456
Old	0·070 ± 0·007	0·018 ± 0·002	30·8	0·554

Young, 9 weeks; old, 53 weeks; k_a, intestinal absorption rate constant; V_{av}, time- and longitudinally-averaged intestinal lumen volume; CL_{app}, apparent membrane permeability clearance ($k_a \cdot V_{av}$). The values of k_g and k_a are computer-fitted parameters with their respective s.e. [a] Physiological volume (V_p), mean ± s.e. (n = 3).

Assuming that D-xylose is absorbed only in the small intestine and not in the large intestine, with an absorption constant of k_a, the fraction absorbed (F_a) can be described as:

$$F_a = 1 - e^{-k_a \cdot T_{si}} \quad (9)$$

Using the k_a and T_{si} values obtained in this study, the values of F_a were estimated to be 0·790 and 0·954, respectively, for young and old rats. These values are in good agreement with those estimated from the faecal excretion of D-xylose in our preceding study (Yuasa et al 1995b), 0·817 and 0·956, respectively, for young and old rats. Thus, the increase in the F_a of D-xylose with ageing can be solely ascribable to the delay in intestinal transit.

Estimation of intestinal membrane permeability

Estimating intestinal membrane permeability in-vivo requires estimating the average luminal volume (V_{av}). In young rats, there was no significant increase in the luminal volume, compared with that without administering (physiological volume), after administering 5 mL kg^{-1}, or 1·5 mL per rat, of saline. Therefore, we used the longitudinally-

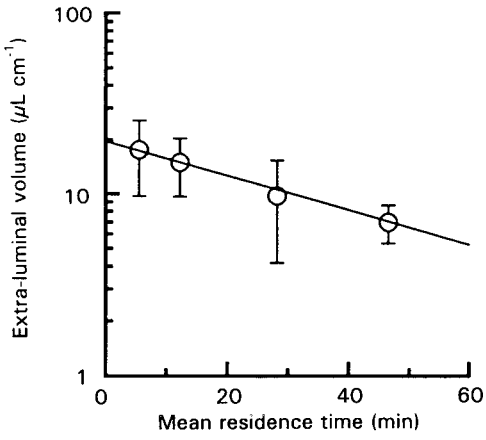

FIG. 4. Longitudinally-averaged extra-luminal volume (V_e) vs mean residence time (MRT) profile in old rats. The data represent the mean ± s.e. (n = 3). The solid line represents the computer-fitted profile.

averaged physiological volume for the entire small intestine (V_p) as V_{av}, and obtained the apparent intestinal membrane permeability clearance (CL_{app}) as the product of k_a and V_{av} (Table 1). In old rats, there was a significant increase in the luminal volume, compared with V_p ($18{\cdot}8 \pm 4{\cdot}0\,\mu L\,cm^{-1}$), after administering saline. Using our proposed approximation method (eqns 6–8), the extra luminal volume originating from the dosing volume (V_e) was successfully described as a mono-exponential function of MRT (Fig. 4), and V_{av} was estimated by equation 8. Although the dosing-associated increase in the luminal volume was found only in old rats, it was moderate. The V_{av} as well as V_p were comparable between young and old rats, as was CL_{app}, suggesting that the intestinal membrane permeability by passive diffusion is not altered by ageing.

The increase, although not profound, in the luminal volume associated with dosing was observed only in old rats. It can be explained by a larger dosing volume for each rat in old rats (2·5 mL per rat) than in young rats (1·5 mL per rat) as a result of setting the dosing volume on the basis of body weight ($5\,mL\,kg^{-1}$). Because the dimension of the gastrointestinal tract would not change significantly between the ages of 8 and 53 weeks, as reported by Meshkinpour et al (1981), dosing-associated changes in the luminal volume can occur regardless of the age according to our previous finding in young rats (Yuasa et al 1995a), where a dosing-associated increase in the luminal volume was observed for the dosing volume of 3 mL per rat but not for that of 1 mL per rat. The former dosing volume is closer to that in old rats in this study and the latter is closer to that in young rats.

Conclusion

The present study demonstrated that, among various factors involved in oral drug absorption by passive transport, only small intestinal transit was altered (delayed) with ageing by the age of 53 weeks in rats, and it can solely account for the increase in the fraction absorbed of D-xylose with ageing. Although scarce information from the literature is not always consistent with the results in this study, similar delay in intestinal transit with ageing and increases in the fractions absorbed of poorly absorbable drugs such as D-xylose can occur also in man.

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