Effect of Aging on the Intestinal Transport of Hydrophilic Drugs in the Rat Small Intestine

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The effect of aging on the intestinal transport of hydrophilic drugs (and probe compounds) was investigated in the rat small intestine.

Passive transport was suggested to be unchanged with aging from 8 (young) to 54 (old) and further to 101 (very old) weeks old, as shown for D-xylose and urea in single-pass intestinal perfusion (under urethane anesthesia), where steady-state transport across the intestinal membrane into the blood stream was evaluated. The passive transports of cephradine, 5-fluorouracil (5-FU) and L-glucose were also unchanged, though they were compared only between the young and the old. Consistently, the passive uptake in the intestinal everted sacs, where the entry process into the membrane was evaluated for 5-FU, D-xylose, urea and polyethylene glycol (PEG) 900, was unchanged with aging from the young to the very old.

The carrier-mediated transport of cephradine was also unchanged with aging from the young to the old in perfusion under anesthesia, though that of D-glucose was declined by about 50% with aging from the young to the old and thereafter remained constant in the very old.

In perfusion in unanesthetized rats, age independency in passive transport (examined for cephradine, L-glucose and D-xylose) and an age-dependent decline in D-glucose transport were also observed, suggesting that the findings under anesthesia are not qualitatively distorted.

These results suggest that, although carrier-mediated transport may moderately decline with aging, the barrier function of the intestinal membrane to passive permeation of hydrophilic drugs (with molecular weight below 1000) may be unaffected by aging, supporting the suggestion from our previous *in vivo* studies that age-dependent increases in the orally absorbed fraction may be predicted for incompletely absorbed drugs because of delayed intestinal transit rather than increased intestinal transport (membrane permeability).

Key words intestinal absorption; aging; rat; passive transport; carrier-mediated transport; hydrophilic drug

We previously reported in rats that the fraction of oral D-xylose dose absorbed ($F_{a,oral}$) was increased with aging, while the apparent rate constant of absorption (k_a') from the gastrointestinal tract to the systemic circulation was unchanged.[1] Subsequently, by analyzing the gastrointestinal disposition of D-xylose, we revealed that the rate constants of gastric emptying (k_g) and intestinal absorption (k_a) were unchanged with aging, consistent with the unchanged k_a', and the increased $F_{a,oral}$ of D-xylose was attributable to prolonged small intestinal transit time (T_{si}).[2] We also found in the same study that the average intestinal lumen volume (V_{av}) was unchanged with aging, suggesting that the unchanged k_a means unchanged apparent membrane permeability clearance (CL_{app}) according to the relation of $k_a = CL_{app}/V_{av}$. D-Xylose, a five-carbon monosaccharide with a hydrophilic nature, has been suggested to be transported passively. Therefore, provided that the passive intestinal transport (membrane permeability) is unchanged with aging, increases in the $F_{a,oral}$ of some other passively transported hydrophilic probes (mannitol, polyethylene glycol (PEG) 400 and PEG 900)[3,4] may be also attributable to an increase in T_{si}. However, the effect of aging on passive intestinal transport has been little investigated *in vitro* and *in situ*, and the increases in $F_{a,oral}$ found in the literature have been *a priori* assumed by the authors to be an indication of increased membrane permeability.[3,4]

We therefore examined the effect of aging on the passive intestinal transport of several hydrophilic drugs (and probes) in the rat small intestine, using a perfusion technique (*in situ*) and everted sacs (*in vitro*), to clarify the mechanism of reported increases in $F_{a,oral}$. D-Glucose and cephradine, which are partially transported by carriers, were also included in the perfusion experiments to examine the suggestion that carrier-mediated nutrient transport may likely decline with aging,[5–11] which is mostly based on clinical observations and *in vitro* studies without substantial verification *in situ*. The present study provides basic information for adjusting oral dosage regimens for the elderly to meet the ever increasing demands associated with a rapid growth of the aged population.

MATERIALS AND METHODS

Chemicals D-[^{14}C(U)]Glucose (11.0 GBq/mmol), L-[1-^{14}C]glucose (1.7 GBq/mmol), [^{14}C]urea (2.1 GBq/mmol), [carboxyl-^{14}C]inulin-carboxyl (0.096 GBq/g), [1,2-^{14}C]PEG 4000 (0.67 GBq/g), 5-[6-^{3}H]fluorouracil (5-[6-^{3}H]FU, 555 GBq/mmol), [1,2-^{3}H]PEG 900 (2.05 GBq/g), [^{3}H(G)]inulin (10.6 GBq/g) and [1,2-^{3}H]PEG 4000 (0.088 GBq/g) were purchased from Dupont-NEN Co. (Boston, MA, U.S.A.). D-[^{14}C(U)]Xylose (3.44 GBq/mmol) was purchased from Amersham International, PLC (Buckinghamshire, U.K.). Unlabeled D-glucose, 5-FU, urea and PEG 900 were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Unlabeled L-glucose, D-xylose and cephradine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents were of analytical grade and commercially obtained.

Urea and PEG 900 were selected as probe compounds that are passively transported and generally used to

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evaluate the barrier function of the intestinal membrane. 5-FU, L-glucose and D-xylose, the transports of which have been characterized in our laboratory, were included for the evaluation of passive transport in an effort to further generalize our finding. D-Glucose was selected as a probe transported by an extensively characterized Na^+-dependent carrier system. Cephradine was selected as a probe transported by a H^+-dependent peptide carrier system, which is of pharmaceutical interest with regard to the transport of peptide-type drugs, and it was also tested for age dependency in its passive transport component.

Animals Male Wistar rats were purchased at their age of 7 weeks old from Shizuoka Laboratory Animal Center (Hamamatsu, Japan) and were raised on standard chow at an air-conditioned animal facility. They were used without fasting prior to the experiments. Their ages and weights (mean ± S.E.) were as follows: young, 8 weeks and 275 ± 2 g; old, 54 weeks and 514 ± 6 g; very old, 101 weeks and 509 ± 9 g.

Intestinal Perfusion Experiments Intestinal single-pass perfusion was carried out in rats anesthetized with urethane (1.13 g/4.5 ml/kg, i.p.) or without anesthesia as previously described,[12-15] using a 10-cm midgut segment and a perfusion rate of 0.15 ml/min. In perfusion in unanesthetized rats, a surgical operation to attach inflow and outflow cannulas was carried out under light ether anesthesia and perfusion was initiated right after the rat regained consciousness in a Bollman cage. Perfusion solutions consisted of 20.1 mM Na_2HPO_4, 47.0 mM KH_2PO_4 and 101.0 mM NaCl (pH 6.4), and contained an appropriate amount of an unlabeled drug (or probe compound) and a trace amount of the ^{14}C or ^{3}H-labeled drug (except for cephradine) in combination with a trace amount of ^{3}H or ^{14}C-labeled inulin as a nonabsorbable marker. The outflow solution was collected for 20 min at 5-min intervals, starting 25 min after the initiation of perfusion, by which time the steady state was achieved. The concentrations of the drugs and inulin were measured by radioactivity determination. Cephradine was quantitated with a HPLC system: column, Wakosil 5Ph 4.6 mm × 200 mm (Wako Pure Chemical Industries, Ltd., Osaka, Japan); mobile phase, 12% acetonitrile in 0.02 M NaH_2PO_4 (pH 5.0); flow rate, 1.6 ml/min; and detector, UV 262 nm.

The fraction absorbed (F_a) was estimated as the fraction which disappeared from the intestinal lumen, correcting for minor volume changes based on changes in inulin concentrations. The F_a was determined as the average absorptions for four sampling periods.

The pre-epithelial diffusional resistance, which generates a concentration gradient dropping toward the intestinal surface in the water phase adjacent to the intestinal surface and may affect the permeability estimates, was taken into account using a model incorporated with an unstirred water layer (UWL),[13-15] and the intestinal membrane permeability clearance (CL_m) was estimated on the basis of the concentration at the intestinal surface, using the following equations:

$$\frac{1}{CL_{app}} = \frac{1}{CL_m} + \frac{1}{CL_{aq}} \quad (1)$$

$$CL_{app} = -\frac{Q}{L}\ln(1-F_a) \quad (2)$$

$$CL_{aq} = 2\pi R \cdot P_{aq} = 2\pi R \cdot \frac{D}{\delta} \quad (3)$$

where CL_{app} and CL_{aq} are the apparent membrane permeability clearance and the permeability clearance of UWL, respectively; Q is the flow rate; and L and R are the length and radius of the perfused segment, respectively. The P_{aq} is the permeability coefficient of UWL and is equal to D/δ, where D and δ are the diffusion coefficient and the effective thickness of UWL, respectively. Equation 1 describes that the apparent (or total) resistance ($1/CL_{app}$) consists of the resistance of both the intestinal membrane ($1/CL_m$) and UWL ($1/CL_{aq}$) in series. Equation 2 gives, assuming linearity in absorption, CL_{app} on the basis of the bulk concentration, which is an average of inflow and outflow concentrations based on a tube model. For the UWL-limited absorption of D-glucose at 1 mM,[13] CL_{aq} was approximated by CL_{app}, as $1/CL_m$ is negligible in Eq. 1, and δ was calculated by Eq. 3 using D of 7.04×10^{-6} cm^2/sec for D-glucose.[12] The D of each compound was, assuming it was inversely proportional to the square root of the molecular weight,[16] calculated from that of D-glucose. Using thus estimated D and δ values, the CL_{aq} of each compound was calculated by Eq. 3, and, with the estimate of CL_{app} by Eq. 2, CL_m was calculated by Eq. 1.

Uptake Experiments in Everted Sacs Everted sacs (2 cm in length) were prepared from the rat small intestine (jejunum to midgut) and uptake experiments were conducted as described previously.[12,17] Briefly, the initial uptake into the tissue was measured by radioactivity determination after incubating the everted sacs at 37 °C, and the shaking rate of 100 strokes/min was used in Krebs-Ringer-bicarbonate buffer (pH 7.4) containing an appropriate amount of an unlabeled drug (or probe compound) and a trace amount of the ^{14}C or ^{3}H-labeled drug in combination with a trace amount of ^{3}H or ^{14}C-labeled PEG 4000 as a nonabsorbable marker.

The uptake was estimated by subtracting the amount in adherent fluid and the initial adsorption to the everted sacs. The uptake rate was calculated by dividing the uptake by the time in the initial uptake phase (1 min for 5-FU and urea and 5 min for D-xylose and PEG 900), where uptake was proportional to time, and then divided by the concentration in the medium to obtain the uptake clearance (CL_{up}). The values (mean ± S.E.) of wet tissue weight for unit length were 114 ± 1, 136 ± 2 and 159 ± 5 mg/cm, respectively, in young, old and very old rats.

The CL_{up} values are presented without correcting for the resistance of UWL for the following reasons. In young rats, the resistance of UWL estimated from the reported δ of 104 μm[18] accounted for less than 10% of $1/CL_{up}$ values, suggesting an insignificant contribution to the total resistance. Although δ values in older rats are not available, it could be even smaller, as suggested later in this study in the perfused intestine, and, therefore, it is unlikely that the resistance of UWL would affect CL_{up} estimates.

Statistical Analysis Levels of statistical significance were assessed by analysis of variance.

RESULTS

Effect of Aging on Intestinal Transport in Anesthetized Rats The major part of the present study was conducted by a standard single-pass perfusion method in anesthetized rats to evaluate the effect of aging on steady-state transport across the intestinal membrane into the blood stream.

The effect of aging on the resistance of UWL (or pre-epithelial diffusional resistance) was evaluated by using D-glucose (1 mM) as a probe, of which the intestinal transport is UWL-limited.[13] In anesthetized rats, both F_a and CL_{app} were unchanged in old rats, compared with young rats, but were increased by about 50% in very old rats, suggesting a reduction in the resistance of UWL as represented by about a 50% reduction in δ (Table 1).

The values of CL_m as a measure of intestinal membrane permeability were calculated from observed F_a values (Table 2) using Eqs. 1—3 and the δ values in Table 1.

The CL_m of D-glucose at 100 mM, where carrier-mediated D-glucose transport is saturated and the CL_m mainly reflects the maximum transport capacity,[13] was decreased by about 50% in both old and very old rats, compared with young rats, though the significance level was $p<0.1$ in the old, and slightly above $p=0.1$ in the very old. Cephradine is transported predominantly by peptide carriers at a low concentration of 0.1 mM.[19] However, different from the results for D-glucose, the CL_m for carrier-mediated cephradine transport was independent of age.

Passively transported probe compounds showed no age dependency in CL_m up to the very old (D-xylose and urea) or the old (L-glucose). Although carrier-mediated transport is involved in the intestinal transport of cephradine and 5-FU, they are transported predominantly by passive transport at high concentrations of 40 mM for cephradine[19] and 10 mM for 5-FU.[15] The CL_m's for the passive transport of cephradine and 5-FU were consistently age-independent, comparing the young with the old.

Effect of Aging on Intestinal Transport in Unanesthetized Rats Anesthetic regimens were previously found to reduce intestinal transport.[14,15] Therefore, to examine whether the results in anesthetized rats might be affected by anesthesia, the effect of aging was examined in perfusion in unanesthetized rats for the transport of passively transported cephradine (40 mM), L-glucose and D-xylose (selected arbitrarily from those used in anesthetized rats) and D-glucose, for which an age-dependent change was observed.

The resistance of UWL was unchanged in the old (Table 1), carrier-mediated D-glucose transport was significantly declined (Table 3), and the passive transports of cephradine, L-glucose and D-xylose were unchanged (Table 3). These results are in agreement with those in anesthetized rats, though in unanesthetized rats the resistance of UWL was lower, presumably due to better luminal mixing,[13] and CL_m values were larger, presumably because intestinal transport is lowered by anesthesia in anesthetized rats.[14,15]

Effect of Aging on Intestinal Uptake in Everted Sacs The intestinal uptake in everted sacs was examined to gain information about the effect of aging, specifically on the entry process into the intestinal membrane. Passively transported 5-FU, D-xylose and urea were selected arbitrarily from those used in anesthetized rats, and PEG 900 was included since it was previously reported to show a modest increase (about 40%) with aging, from 9 to 102

Table 1. Effect of Aging on the UWL-Limited D-Glucose Absorption at 1 mM in the Perfused Rat Small Intestine

Age	With anesthesia			Without anesthesia		
	F_a	CL_{app} (μl/min/cm)	δ (μm)	F_a	CL_{app} (μl/min/cm)	δ (μm)
Young	0.377±0.031[a]	7.19±0.73[a]	918±109[a]	0.704±0.055	18.78±2.90	347±52
Old	0.334±0.031	6.12±0.68	1043±128	0.604±0.052	14.18±2.10	456±63
Very old	0.531±0.027[b]	11.43±0.89[b]	550±41[c]	ND	ND	ND

Results are represented as the mean±S.E. ($n=3$ unless otherwise indicated). F_a, CL_{app} and δ represent the fraction absorbed, apparent membrane permeability clearance and effective thickness of UWL, respectively. *a*) $n=6$. *b*) $p<0.01$ compared with the young and the old. *c*) $p<0.05$ compared with the young and the old. ND, not determined.

Table 2. Effect of Aging on the Intestinal Transport of Hydrophilic Drugs in the Perfused Small Intestine of Anesthetized Rats

Compound	C_{in} (mM)	F_a			CL_m (μl/min/cm)		
		Young	Old	Very old	Young	Old	Very old
D-Glucose (C)	100	0.246±0.013	0.164±0.017[a]	0.202±0.060	10.67±1.74	5.61±1.61[a]	5.59±1.57
Cephradine (C)	0.1	0.132±0.015	0.107±0.005	ND	3.75±0.80	2.77±0.22	ND
Cephradine (P)	40	0.054±0.003	0.048±0.001	ND	0.99±0.07	0.89±0.03	ND
5-FU (P)	10	0.134±0.013[a]	0.136±0.021	ND	2.96±0.42[a]	3.25±0.71	ND
L-Glucose (P)	1	0.033±0.005[b]	0.040±0.006	ND	0.54±0.08[b]	0.69±0.13	ND
D-Xylose (P)	1	0.029±0.006	0.038±0.006	0.043±0.014	0.48±0.11	0.64±0.11	0.71±0.25
Urea (P)	0.1	0.223±0.031[a]	0.182±0.056	0.204±0.087	6.02±1.28[a]	4.84±1.88	5.05±2.99

Results are represented as the mean±S.E. ($n=3$ unless otherwise indicated). C_{in}, F_a and CL_m represent the inflow concentration, fraction absorbed and membrane permeability clearance, respectively. Predominant transport mechanism is indicated by C (carrier-mediated transport) or P (passive transport) in parenthesis after the name of each compound. *a*) $n=6$. *b*) $n=5$. ND, not determined.

Table 3. Effect of Aging on the Intestinal Transport of Hydrophilic Drugs in the Perfused Small Intestine of Rats without Anesthesia

Compound	F_a		CL_m (μl/min/cm)	
	Young	Old	Young	Old
D-Glucose (C)	0.376±0.025	0.253±0.010[a]	11.58±1.65	6.34±0.43[b]
Cephradine (P)	0.071±0.007	0.067±0.005	1.21±0.14	1.16±0.10
L-Glucose (P)	0.066±0.009	0.076±0.015	1.05±0.15	1.31±0.29
D-Xylose (P)	0.065±0.010	0.052±0.005	1.07±0.19	0.84±0.09

Results are represented as the mean±S.E. ($n=3$). F_a and CL_m represent the fraction absorbed and membrane permeability clearance, respectively. The inflow concentrations (C_{in}) were 100 and 40 mM, respectively, for D-glucose and cephradine, and 1 mM for the others. Predominant transport mechanism is indicated by C (carrier-mediated transport) or P (passive transport) in parenthesis after the name of each compound. *a*) $p<0.02$ compared with the young. *b*) $p<0.05$.

Table 4. Effect of Aging on the Passive Intestinal Uptake of Hydrophilic Drugs in the Everted Sacs of Rat Small Intestine

Compound	C_m (mM)	CL_{up} (μl/min/cm)		
		Young	Old	Very old
5-FU	10	5.95±0.68	5.41±0.87	3.87±1.00
D-Xylose	1	1.44±0.07	1.53±0.18	1.84±0.27
Urea	0.1	4.74±0.37	5.18±1.31	3.92±0.38
PEG 900	3	0.81±0.01	0.78±0.05	0.69±0.18

Results are represented as the mean±S.E. ($n=3$). C_m and CL_{up} represent the concentration in medium and uptake clearance, respectively.

weeks old in rats.[4]

As shown in Table 4, the intestinal uptakes of all tested drugs were unchanged with aging from 8 (young) to 101 weeks old (very old), consistent with the results of the perfusion experiments.

DISCUSSION

Comparisons of transport were mainly made between young and old rats, representing mature adults and those in an early senescent stage, respectively, and it was found that only D-glucose transport was reduced in the old. Transport in very old rats, representing those in the senescent stage, was evaluated as much as the rats were available, and it was found that the transport was not different from that in old rats, though the resistance of UWL was reduced.

The reduction in the resistance of UWL with aging is consistent with suggestions in several earlier studies reporting increases in the intestinal transport of UWL-limited probes such as linolenic acid.[5,20] The effective thickness of UWL estimated from transiently-induced transmucosal potential difference changes[20] and that of the microclimate pH layer[21] have also been reported to decrease with aging. Meanwhile, the dimension (length, surface area and volume) of the small intestine is reportedly unchanged after maturation, at about 10 weeks old in rats,[22] suggesting that the linear flow velocity which affects the resistance of UWL according to a laminar flow assumption[12,13] may not be changed. Therefore, increased luminal mixing associated with reduced barrier functions of the mucous layer may be responsible for the reduction in the resistance of UWL.

It has long been suggested that the resistance of UWL should be taken into account to obtain unbiased estimates of intestinal membrane permeability in intestinal perfusion experiments.[13] However, luminal mixing would be more efficient *in vivo*, reducing the resistance of UWL, and, as previously demonstrated for 5-FU,[15,23] gastrointestinal absorption after oral administration becomes complete and gastric emptying-limited within the range of membrane permeability where it is controlled more by intrinsic membrane permeability than by pre-epithelial diffusivity in perfusion experiments. Therefore, practically, the effect of UWL and its age-dependent changes may not be very significant in drug absorption *in vivo*.

D-Glucose transport has been reported to decline moderately with aging in the intestinal everted sacs (or tissue) of rabbits,[6] rats[7,8] and mice,[10] in agreement with our findings. The functional reduction of Na^+-dependent D-glucose carriers has also been reported in brush border membrane vesicles of rats.[7-9] Amino acid transport by Na^+-dependent carriers also reportedly declines with aging in rats.[11] Our finding of an age-dependent decline in D-glucose transport is consistent with those earlier reports, and the Na^+-dependent carrier system may also likely decline with aging. On the other hand, 5-methyltetrahydrofolate transport[24] by folate carriers, and valproic acid transport[25] presumably by monocarboxylic acid carriers, are reportedly age-independent up to about 100 weeks old in rats, similarly to our finding for cephradine. Although these carrier-mediated transport systems showing no age dependency have pH dependency in common,[26] the relation between the difference in the transport mechanism, whether primarily coupled with Na^+ or H^+ (or HCO_3^-) and that in age dependency remains unresolved. In this context, potential alterations of Na^+ and H^+ concentrations at the intestinal surface may also need to be examined in more detail.[21]

The transport of all passively transported probes (hydrophilic drugs with a molecular weight below 1000) was unchanged with aging, suggesting that the barrier function of the intestinal membrane to the passive permeation of this class of drugs is unaffected. Because the average luminal volume (V_{av}) is unchanged with aging, as reported in our previous study,[2] the intestinal absorption rate constant (k_a) as the ratio of membrane permeability clearance to V_{av} would also be unaffected. Although we cannot exclude the possibility of slight changes in membrane permeability, as reported for PEG 900,[4] the increase in intestinal transit time by about two-fold in old rats compared with young rats[2] is likely to be more responsible for the increases in the orally absorbed fraction of D-xylose and some other passively absorbed hydrophilic probes (mannitol, PEG 400 and PEG 900).[3,4]

Thus, from the findings of unchanged intestinal transport in this study and unchanged luminal volume and prolonged intestinal transit in our previous study,[2] it is predicted that the fraction absorbed of incompletely absorbed hydrophilic drugs would increase with aging, while that of the completely absorbed ones would be unchanged, as reported for cephradine.[27] Because it was

also shown in our previous study that the gastric emptying rate constant was unchanged,[2] it is predicted that the apparent rate constant of absorption from the gastrointestinal tract would not be affected by aging, as reported for several drugs[5] and D-xylose.[3] Although the clinical relevance of these suggestions remains to be verified, intestinal transit seems to be persistently delayed in humans as well as rats, as discussed previously.[1,2] For hydrophilic drugs absorbed incompletely in humans, we may potentially see age-dependent increases in the orally absorbed fraction and may need to take it into account for dosage adjustments.

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