Is the Jejunal Permeability in Rats Age-Dependent?

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INTRODUCTION

Age-dependent changes in the transport of drugs across the epithelial membranes of the gastrointestinal tract has not been the subject of extensive study. However, during development and aging several physiological and biochemical changes occur which may affect such transport of drugs. For example, the surface area and the weight of the rat gut are increased in direct proportion to the increase in body weight (1). Splanchnic blood flow and gut motility in the rat decrease with age, while the intestinal transit time increases (2,3). The enterocyte membrane itself undergoes several alterations in lipid composition during postnatal development. The ratios of cholesterol to proteins and phospholipids to proteins have been shown to decrease, while the ratio of cholesterol to phospholipids increases with age (4). These changes lead to a decrease in membrane fluidity (5), and according to the solubility-diffusion model, the effective permeability (P_{eff}) for passively transported compounds is dependent on both partitioning between the aqueous phase and the enterocyte membrane and diffusion within the membrane (6).

Carrier-mediated transport across the intestinal barrier has shown age-dependent changes. The uptake of amino acids per mg of intestine, for example, has been reported to decline about 50% from birth to adolescence (1). For D-glucose, however, both higher and lower rates of absorption in aged compared to young rats have been reported (7).

The aim of this study was to investigate whether the rat jejunal P_{eff} was changed within the age interval of 5 to 30 weeks. This is an important aspect when permeability data should be classified according to the proposed Biopharmaceutical Classification System (BCS) (8). This system classifies drugs according to their solubility and P_{eff}. We studied passive diffusion for three compounds, antipyrine, atenolol and metoprolol, representing a wide range of physicochemical properties (Table I). D-glucose was chosen as a model compound for carrier-mediated transport.

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MATERIALS AND METHODS

Single Pass Perfusion Experiments

Male Sprague-Dawley rats (CDBR, Charles River, Uppsala, Sweden) were housed at controlled conditions (22.4°C, 50% air humidity, 12 hours light cycle) at the Biomedical Center, Uppsala. The age of the rats at the time of the experiments were 5, 7, 9, 12, 15, 18 and 30 weeks old (±2 days), respectively, with 6 animals in each group. The animals had free access to tap water and regular rat food (R36, Lactamin AB, Sweden) until 14–20 hours prior the experiment, when the food was withdrawn. The anesthesia and surgery were performed as described elsewhere (10). Briefly, anesthesia was induced by an i.p. injection of 150 mg/kg body weight of Inactin®-Byk (thiobutabarbital sodium) and the rats were placed on a heating pad to maintain the body temperature at 37 ± 1°C. The abdomen was opened by a midline longitudinal incision and an approximately 10 cm long segment of the proximal jejunum was isolated and cannulated with plastic tubing (4 mm o.d). The jejunal segment was rinsed with 10–20 ml of saline (37°C) until a clear perfusate was obtained, and then the inlet tube was filled with the perfusion solution. The perfusion rate was 0.2 ml/minute (Model 22, Harvard Apparatus Company, USA). An approximately 10 cm long loop of the inlet tube was placed inside the abdominal cavity, in order to keep the perfusion solution at body temperature. The surgical area was covered with a thin plastic sheet and aluminum foil. Each perfusion experiment lasted for 105 min and the perfusate was quantitatively collected on ice at 45, 60, 75, 90 and 105 min. At the end of the perfusion experiment, the segment was rinsed through with 20 ml of saline. All samples were weighed and frozen immediately (−20°C) pending analysis. Approval of this study was given by the Animal Ethics Committee in Uppsala, Sweden (C254/96).

Perfusion Solution

The perfusion solution consisted of 5.4 mM KCl, 48 mM NaCl, 35 mM mannitol, 10 mM D-glucose, and 1 g/l PEG 4000, all in a 70 mM phosphate buffer. The pH and the osmolality were 6.5 and approximately 290 mOsm/kg, respectively. Traces of ^{14}C-labeled PEG 4000 and ^{3}H-labeled D-glucose (2.5 μCi/l and 10 μCi/l, Amersham Labs., England) was added to the solution. The concentrations of antipyrine (Sigma Chemical Co., USA), metoprolol (Astra Hässle AB, Sweden) and atenolol (Diamalt GmbH, Germany) were 1.1, 0.58 and 0.83 mM, respectively. Physicochemical parameters for the four study compounds are given in Table I. The used compounds have earlier been shown to be stable in the perfusion solution (9,10).

Analytical Methods

Antipyrine was assayed by a previously used and validated HPLC method (11,12). The limit of detection was 1.0 μg/ml and the inter-assay variability, expressed as the coefficients of variation at the concentrations of the quality controls, were 6.6, 1.0 and 1.3% at the concentrations 0.194, 3.88 and 9.70 μg/ml, respectively.

Atenolol and metoprolol were determined by a HPLC system consisted of a pump (Shimadzu LC-10AD), an autosam-

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Table I. Physicochemical Properties of the Four Study Compounds

Compound	MW	pKa	log P (n-oct./water)	log D pred. (n-oct./water, pH 6.5)	N
Antipyrine	188	1.5 (base)	0.38	0.38	3
Atenolol	266	9.6 (base)	0.16	−2.94	7
Metoprolol	267	9.7 (base)	1.88	−1.32	4
D-glucose	180	—	−3.0 (pH 7.4)	−3.0	10

Note: MW, molecular weight; log P, partition coefficient between n-octanol and water for the unionised compound; log D pred, the predicted partition coefficient at pH 6.5; N, the hydrogen bond number, calculated according to Stein (16). Data adopted from references (9) and (10).

pler (CMA/200), a μBondapak C_{18} column (Waters), a fluorescence detector (Jasco FP-920) and an integrator (Shimadzu C-R5A). Both atenolol and metoprolol were detected at 225 nm excitation wavelength and 300 nm emission wavelength. Separation was achieved using water: acetonitrile: glacial acetic acid: 1-heptane sulfonic acid (80: 19: 1: 0.625 μM) as mobile phase, with a flow rate of 2 ml/min. The perfusate samples were diluted 101 times with water, and 10 μl was then injected on the column. Atenolol and metoprolol were eluted at 2.1 and 8.1 min, respectively. The limit of quantitation (LOQ) for metoprolol was 0.3 μg/ml (6.7% CV) and the inter-assay variations were 3.9, 2.3 and 1.6% at the concentrations 0.693, 1.39 and 2.77 μg/ml, respectively. The LOQ for atenolol was 0.4 μg/ml (5.3% CV) and the inter-assay variations were 2.9, 2.8, 1.3% at 0.896, 1.79, 3.58 μg/ml, respectively.

The concentrations (dpm/ml) of ^{14}C-labeled PEG 4000 and 3H-labeled D-glucose were determined by liquid scintillation counting for 2*10 min (Mark III, Searle Analythical Inc., USA) after the addition of 8 ml Beckman Ready Safe®. The pH and the osmolality were measured by a pH-meter (Metrohm 632) and an osmometer (Vescor 5500), respectively.

Data Analysis

Calculations were made from steady-state concentrations in the outlet perfusate, i.e. 45–105 min after the initiation of the perfusion (10).

The net water flux, NWF, per cm of the jejunal segment was calculated from equation 1:

$$NWF = \frac{(1 - [PEG_{out}]/[PEG_{in}]) \times Q_{in}}{L} \quad (1)$$

where $[PEG_{out}]$ and $[PEG_{in}]$ are the inlet and outlet concentrations of ^{14}C-PEG 4000, respectively, and Q_{in} is the inlet flow rate (0.2 ml/min).

The volume, V, of the intestinal segment during each sampling interval at steady state was estimated using the following equation (12):

$$V = \frac{\Sigma PEG_{in} - \Sigma PEG_{out}}{[PEG_{out}]} - \text{tube volume} \quad (2)$$

The total amount of ^{14}C-PEG 4000 that had entered the system at a certain time point, Σ PEG in, minus the total amount that had left the system at the same time point, Σ PEG out, represents the amount in the system. This amount is then divided by the outlet concentration of ^{14}C-PEG 4000 in the actual sampling interval to estimate the volume of the whole perfusion system. Since the inlet tube is filled with the perfusion solution at the start of the perfusion, only the volume of the outlet tube is subtracted from the volume of the whole system to estimate the segment volume. Knowing the length, L (measured at 45 min), of the perfused segment and assuming it to have the shape of a cylinder, the inner radius, r, was readily estimated:

$$r = \sqrt{\frac{V}{\pi \times L}} \quad (3)$$

The effective permeability, P_{eff}, across the jejunal mucosa was calculated according to a parallel tube model (13):

$$P_{eff} = \frac{Q_{in} \times \ln (C_{in}/C_{out})}{2\ \pi r L} \quad (4)$$

where C_{in} and C_{out} are the inlet and outlet concentrations, respectively. The outlet concentrations were corrected for the fluid flux (12).

The possible influence of age on the jejunal P_{eff} for the four study compounds, NWF, radius and volume was tested with one-way analysis of variance (StatView, Abacus Concepts, Inc., USA). A probability-value less than 0.05 was considered to be significant. All data are presented as the mean and the 95% confidence interval (n = 6).

RESULTS AND DISCUSSION

The mean recovery of the non-absorbable water flux marker ^{14}C PEG 4000 during the *in situ* jejunal perfusion experiments was more than 95% at all ages (Table II). The NWF was

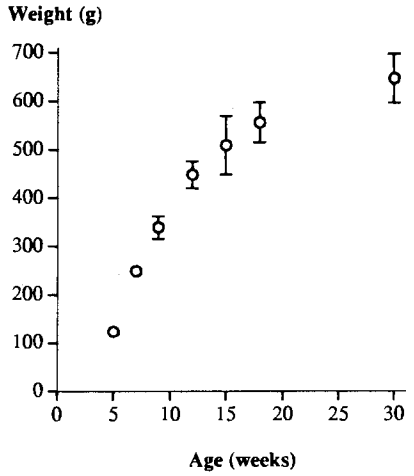

Fig. 1. The body weight of the rats in the different age-groups (mean and 95% C.I., n = 6).

Table II. Recovery of the Non-Absorbable PEG 4000 (PEG recovery), Net Water Flux (NWF), Inner Radius and Volume of the Perfused Jejunal Segments at Different Ages

	Age (weeks)						
Parameter	5	7	9	12	15	18	30
PEG recovery (%)	97 ± 3	95 ± 2	96 ± 2	96 ± 3	95 ± 3	97 ± 2	97 ± 2
NWF* (ml/h/cm)	0.008 ± 0.031	0.018 ± 0.037	0.022 ± 0.037	−0.011 ± 0.024	−0.009 ± 0.012	0.017 ± 0.023	0.010 ± 0.022
Inner radius (cm)	0.23 ± 0.04	0.25 ± 0.06	0.24 ± 0.05	0.24 ± 0.06	0.23 ± 0.03	0.23 ± 0.05	0.21 ± 0.04
Volume (μl/cm)	163 ± 62	199 ± 111	181 ± 71	184 ± 94	164 ± 42	179 ± 76	149 ± 56

Note: All parameters were obtained under steady state conditions and the results are presented as the mean values and the 95% confidence intervals (n = 6). * A negative NWF indicates a fluid flux from the mucosal side (lumen) to the serosal side (blood).

low, and the overall mean was 0.008 ± 0.010 ml/h/cm, with no statistically significant differences over age (Table II). The almost complete recovery of ^{14}C PEG 4000, stable NWF and rapid transport of D-glucose, indicated that the viability of the intestinal mucosa was maintained in all experiments (Table II and III).

The surface area and the weight of the gut have been reported to increase in direct proportion to the increase in body weight of the rat (1). In the present report, the study period was 25 weeks, from 5 to 30 weeks of age, and the weight of the rats increased more than five times (Figure 1). The average weight of the rats in the youngest and oldest age groups was 123 ± 8 and 647 ± 51 g, respectively. Although this increase in weight suggests a proportional increase in intestinal surface area, the P_{eff} for the four study compounds were not found to be significantly changed with increasing age (Table III). The overall mean values of the P_{eff} for the seven age groups of rats were 7.1 ± 1.6, 0.1 ± 0.2, 2.3 ± 0.7 and 7.3 ± 1.8 × 10^{-5} cm/s for antipyrine, atenolol, metoprolol and D-glucose, respectively. These P_{eff}-values are in the same range as the data that we have reported earlier, except from antipyrine, which is significantly ($p < 0.05$) lower (44%) (10). One plausible reason for this discrepancy might be that these Sprague-Dawley rats came from another breeder. Whether rats from various breeders show different intestinal permeability has not been well-addressed in the scientific literature. Furthermore, atenolol is still a low permeability drug, and metoprolol and antipyrine are still classified as high permeable compounds, according to the BCS (8).

The estimated mean inner radius and volume of the perfused jejunal segments in the various age-groups are shown in Table II. There were no significant differences in the segmental radius or volume at the different ages of the rats. This observation is in good agreement with previously findings reported by Yuasa and coworkers (3). They estimated the small intestinal volume to be 25–30 μl/cm, and not dependent on age. This was approximately a six times smaller volume compared to our results (Table II). The relatively stable inner radius and volume/cm of the isolated segments, and also the stable P_{eff}-values between the different age groups, suggest that the surface area available for absorption per cm is relatively constant over this age interval in rats.

As discussed above, the P_{eff} for passively absorbed compounds might be dependent on the chemical composition of the membrane itself, according to the solubility-diffusion model (6). The differences in the lipid composition of the membrane between newborn and adult intestinal mucosa has been suggested to be an adaptive response of the intestine to different nutritional conditions, although a possible age dependent effect can not be excluded (14). In the same age interval as in the present study, the ratios of cholesterol to phospholipids increased, and cholesterol to proteins decreased as a function of age, resulting in a decreased membrane fluidity (4,5). However, these potential age-dependent changes in membrane composition in the rats did not result in any significant age-dependent P_{eff} for none of the compounds in the present study (Table III).

D-glucose, a carrier-mediated transported compound, showed no significant age-dependent permeability (Table III). This functional data is consistent with an earlier report of Miyamoto et al., who demonstrated that the jejunal level of mRNA for the Na^+-dependent cotransporter (SGLT1), located in the brush border membrane of intestinal epithelial cells, did not change significantly in the age interval of 1–13 weeks in rats (15). It is however not in agreement with the data reported by Toloza and Diamond, who reported that the *in vitro* uptake of D-glucose, D-fructose, D-galactose and L-proline per cm of intestine increased up to the age of 15 weeks, and thereafter decreased (1). The age-related uptake-patterns for D-glucose

Table III. Effective Jejunal Permeability for the Studied Compounds at Different Ages of the Rats

	Age (weeks)						
Compound	5	7	9	12	15	18	30
Antipyrine	7.4 ± 2.2	6.4 ± 0.9	6.9 ± 1.1	7.2 ± 2.8	7.4 ± 2.1	7.2 ± 2.6	6.8 ± 2.2
Atenolol	0.16 ± 0.24	0.19 ± 0.22	0.06 ± 1.0	0.11 ± 0.25	0.22 ± 0.45	0.08 ± 0.14	0.06 ± 0.08
Metoprolol	2.3 ± 0.9	2.5 ± 1.1	2.3 ± 0.6	2.2 ± 0.9	2.3 ± 1.1	2.3 ± 1.1	2.1 ± 0.7
D-glucose	7.6 ± 2.0	5.7 ± 1.0	7.3 ± 1.1	8.1 ± 3.3	8.3 ± 2.5	7.2 ± 2.6	6.6 ± 2.3

Note: The results are presented as mean values (cm/sec*10^{-5}) and the 95% confidence intervals (n = 6).

and L-proline in the colon were also relatively consistent in that study (1).

In the age interval 5–30 weeks, there was no influence of age on the jejunal P_{eff} in the rat, neither for passive diffusion of drugs nor carrier-mediated transport by the D-glucose carrier. However, an age-dependent intestinal P_{eff} might still be valid for very young and very old rats.

REFERENCES

1. E. M. Toloza and J. Diamond. *Am. J. Physiol.* **263**:G593–G604 (1992).
2. F. Varga and T. Csaky. *Pfluegers Arch. Eur. J. Physiol.* **364**:129–133 (1977).
3. H. Yuasa, K. Kawanishi, and J. Watanabe. *J. Pharm. Pharmacol.* **47**:576–580 (1995).
4. C. Hübner, S. G. Lindner, M. Stern, M. Claussen, and A. Kohlschütter. *Biochim. Biophys. Acta.* **939**:145–150 (1988).
5. T. A. Brasitus, K.-Y. Yeh, P. R. Holt, and D. Schachter. *Biochim. Biophys. Acta.* **778**:341–348 (1984).
6. W. D. Stein. *Transport and Diffusion Across Cell Membranes.* Orlando: Academic Press, 1986, pp. 69–112.
7. R. P. Ferraris. *Physiology of the Gastrointestinal Tract.* New York: Raven Press, 1994, Vol 2, pp. 1821–1844.
8. G. L. Amidon, H. Lennernäs, V. P. Shah, and J. Crison. *Pharm. Res.* **12**:413–420 (1995).
9. A. Lindahl, R. Sandström, A.-L. Ungell, B. Abrahansson, T. W. Knutson, L. Knutson, and H. Lennernäs. *Ther.* **60**:493–503 (1996).
10. U. Fagerholm, M. Johansson, and H. Lennernäs. *Pharm. Res.* **13**:1336–1341 (1996).
11. H. Lennernäs, Ö. Ahrenstedt, and A.-L. Ungell. *Br. J. Clin. Pharmacol.* **37**:589–596 (1994).
12. H. Lennernäs, Ö. Ahrenstedt, R. Hällgren, L. Knutson, M. Ryde, and L. K. Paalzow. *Pharm. Res.* **9**:1243–1251 (1992).
13. I. Komiya, J. Y. Park, A. Kamani, N. F. H. Ho, and W. I. Higuchi. *Int. J. Pharm.* **4**:249–262 (1980).
14. S.-H.W. Chu and W. A. Walker. *Pediatr. Res.* **23**:439–442 (1988).
15. K. Miyamoto, K. Hase, Y. Teketani, H. Minami, T. Oka, Y. Nakabou, and H. Hagihira. *Biochem. Biophys. Res. Commun.* **183**:626–631 (1992).
16. W. D. Stein. *The Movement of Molecules Across Cell Membranes.* New York: Academic Press, 1967, pp. 65–125.