Pharmaceutical Research, Vol. 15, No. 8, 1998

Assessment of Regional Differences in Intestinal Fluid Movement in the Rat Using a Modified *In Situ* Single Pass Perfusion Model

Araz A. Raoof,[1,2] Jackie Butler,[1] and John G. Devane[1]

Received February 17, 1998; accepted April 21, 1998

KEY WORDS: net water flux; regional fluid movement; single pass perfusion; antipyrine; rat.

INTRODUCTION

In order to establish successful strategies for optimising drug delivery, prediction of oral drug absorption is necessary in the early stages of drug development. Several in vitro and in situ experimental models are now available and are used to predict the absorption of drugs in vivo (1,2). The in situ perfusion model has the obvious advantage over in vitro models of providing an intact lymphatic and blood flow circulation. However, from a pharmaceutical industry perspective the model is time consuming and unsuitable for high throughput screening. Within the rat in situ model, the single pass perfusion technique showed the most constant absorption rate and also a good correlation with human data using a similar perfusion technique (2,3). Regional variations in transmucosal fluid movement were previously reported in laboratory animals including rabbits and dogs (4,5). Such variations in rabbits were suggested to contribute towards the regional intestinal absorption of aspirin and metoclopramide (4). The effects of artificially induced fluid movement on the intestinal absorption of drugs have also been studied in animals (6,7). The results of these studies, although they contrast with those relating to humans, indicate that fluid absorption increases the absorption of drugs through various mechanisms.

In the present study, transmucosal fluid movement was investigated in three different regions of the rat intestine [upper small intestine (USI), lower small intestine (LSI) and the large intestine (LI)] using a developed in situ single pass perfusion model automated to perfuse 12 rats simultaneously. ^{14}C-polyethylene glycol 4000 (^{14}C-PEG 4000) was used as an impermeable marker for measuring net water flux with antipyrine as a transcellular passively absorbed marker (2). Antipyrine is totally absorbed from the gut following oral administration and its intestinal absorption, using in situ single pass perfusion in rats, was shown to increase and decrease considerably with fluid absorption and secretion respectively (7,8).

[1] IVIVR Cooperative Working Group, Elan Pharmaceutical Technologies, Athlone, Ireland.

[2] To whom correspondence should be addressed. (e-mail: raooaraz@iol.ie)

MATERIALS AND METHODS

In Situ Single Pass Perfusion

Male Sprague-Dawley rats (Charles River, UK) weighing between 200 and 300 g were used. The rats were housed in an environmentally controlled room (20–22°C, 12 hour light/dark cycle) and were provided with food and water *ad libitum.* For each experiment, 12 rats were prepared consecutively as follows: rats were fasted (15–21 h) prior to use and anesthetized with halothane followed by an i.v. (Penal vein) injection of sodium pentobarbitone (60 mg/kg). To maintain body temperature, the rats were placed on a heating pad and under a surgical lamp. The trachea and the right jugular vein were cannulated with polyethylene tubing to facilitate respiration and to maintain anesthesia respectively. The abdomen was opened by midline incision and the common bile duct was cannulated to divert the bile from the gut. A segment from the USI (10 cm starting 1 cm below the stomach), LSI (15 cm proximal to caecum) or the LI (9 cm starting below the caecum to the rectum) was measured and cannulated from both ends with plastic tubing. Care was taken to avoid circulatory system damage and the segments were kept moist using 37°C saline. In order to clear the intestine of solid particles, the segments were rinsed with saline until the outlet perfusate was clean. After surgery, the rats were placed in a thermostatically controlled incubator set at 37°C. The average preparation time, from initial anesthesia to transfer of the animal to the cabinet, was 12 min. Once the 12 rats had been prepared all were dosed from the same central reservoir (equilibrated at 37°C) using a 12 channel pump (Watson Marlow Multichannel Pump 202U) set at a flow rate of 0.2 ml/min. To determine binding of the markers to the tubing two perfusate samples were taken, one from the central reservoir and a pooled eluant sample from each of the 12 perfusion lines. The 12 outlets of the pump were then attached to the 12 inlet cannuli (i.e. 4 into the USI, 4 to the LSI and 4 to the LI). The gut preparations were all perfused at the same time with a buffer solution (pH 6.4, 290 mOsm/l) containing the following: NaCl (48 mM), KCl (5.4 mM), NaH_2PO_4 (43 mM), Na_2HPO_4 (28 mM), mannitol (35 mM), D-glucose (10 mM), PEG 4000 (8.4g/l), ^{14}C-PEG 4000 (5 μCi/l) (Amersham Labs, Buckinghamshire, UK) and antipyrine (2.5 mM). To check for marker stability inlet perfusate samples were taken from the single central reservoir at 50, 100 and 150 min after the onset of perfusion. Outlet perfusate samples, however, were quantitatively collected between 0–30, 30–50, 50–70, 70–90, 90–110, 110–130 and 130–150 min and all samples were stored at −20°C until analysis. Intestinal segments, at the end of each experiment, were excised, measured and weighed.

Analytical Methods

^{14}PEG 4000 in the perfusate samples was determined by mixing 0.3 g of the samples with 2 ml Ultima Gold XR scintillant (Canberra Packard Ltd., Berkshire, UK) and counting for 5 min using a liquid scintillation counter (Canberra Packard 1800 TR).

Antipyrine was assayed as previously described with some modifications (9). The system consisted of an autosampler injector (Jasco 851-AS), an HPLC pump (Jasco PU-980) and a UV detector (Jasco UV-975, UV/VIS detector) set up at 256 nm. 10

0724-8741/98/0800-1314$15.00/0 © 1998 Plenum Publishing Corporation

microlitres of the sample were injected directly on the column (Supelco LC-ABZ C18, 150 × 4.6 mm, 5 μ particle size, Sigma-Aldrich Chemical Co.). The mobile phase (50 mM sodium acetate:acetonitrile, 92:8 v/v) was delivered through the system at a flow rate of 1.5 ml/min. Under these conditions, antipyrine had a retention time of 8.5 min. and linear calibration curves were obtained between 40 and 400 μg/ml.

Data Analysis

Net water flux (NWF, μl/min/cm) was calculated at steady state using the following equations (2):

$$NWF = \frac{(1 - PEG_{out}/PEG_{in}) \cdot Q}{L} \quad (1)$$

where PEG_{in} and PEG_{out} were the mean inlet (n = 4 and outlet (n = 5 last samples) ^{14}C-PEG 4000 concentrations respectively. Q was the perfusion flow rate (0.2 ml/min) and L was the length (cm) of the intestinal segments.

The recovery of ^{14}C-PEG 4000 was calculated as follows:

$$\text{PEG recovery} = \Sigma PEG_{out}/\Sigma PEG_{in} \quad (2)$$

where ΣPEG_{in} and ΣPEG_{out} were respectively the accumulated amounts of ^{14}C-PEG 4000 entering and leaving the intestine at steady state.

The fraction absorbed (Fa) estimated as that which disappeared from the intestinal lumen and the intrinsic absorption clearance (Cla, μl/min/g intestine) of antipyrine were calculated as follows: (10).

$$Fa = 1 - \frac{C_{out}}{C_{in}} \cdot \frac{PEG_{in}}{PEG_{out}} \quad (3)$$

$$Cla = -Q \cdot \ln(1 - Fa) \quad (4)$$

where C_{in} and C_{out} were the mean inlet (n = 4) and outlet (fluid transport corrected, n = 5 last samples) antipyrine concentrations respectively.

Results in text and tables were expressed as mean ±SEM. Data were analysed using the one-way ANOVA-Bonferroni test. The p-value of 0.05 or less was considered significant.

RESULTS AND DISCUSSION

The automated perfusion model described in this study permits the simultaneous perfusion of 12 rats and is therefore less time consuming than the traditional in situ single pass model using syringe perfusion techniques. Furthermore, the model has the added advantage of studying large numbers of animals under precisely the same experimental conditions. Parallel to absorption studies, the model allows for full drug stability investigation during perfusion as the inlet perfusate samples from the central reservoir can be taken at the same time points as the outlet samples. This can be important for compounds undergoing degradation in the perfusion media and avoids the need for separate drug stability studies. In this study, ^{14}C-PEG 4000 was used as an impermeable marker for measuring net water transport with antipyrine as a transcellular passively absorbed marker (2). Adsorption of the marker compounds to the polyethylene tubing was negligible and no significant changes in their inlet concentrations were observed with time, thus indicating their stability. Steady state conditions, used for water and antipyrine transport measurements, were achieved after 50 min of the start of perfusion. A complete recovery of ^{14}C-PEG 4000 was observed in all the regions indicating an intact intestinal epithelial barrier during perfusion (Table I). The Fa and the Cla obtained in this study showed no significant differences in antipyrine absorption between the studied regions (Table I). The latter was normalised for intestinal weight arising from variations in the intestinal segment weights between the regions. In general, Cla in this model is estimated by reference to the drug fraction which disappears from the intestinal lumen. This may result in drug dependant overestimation of results for compounds undergoing lumenal degradation, intestinal first pass metabolism and/or having high tissue binding capacity. However, this is unlikely in the case of antipyrine as the drug is stable, rapidly and totally absorbed, exhibits no gut metabolism and has a low binding to plasma and tissue proteins (8,9). In addition, the Fa obtained in this study is similar to that reported for human jejunum using similar perfusion techniques, suggesting similarities in antipyrine absorption between man and rat (9). In our previous rat study, antipyrine permeability was lower in the colon by comparison to the upper and the lower parts of the small intestine (11). This was due to the greater tube surface area calculated for the large intestine by comparison to the other intestinal regions rather than to any decrease in Fa in the colon.

In the present study, ^{14}C-PEG 4000 was perfused in an isotonic solution (289 ± 1.1 mOsm/l) in order to minimise fluid movement across the intestinal segments. The concentration ratio (^{14}C-PEG 4000 out/in) of the marker compound at steady state, however, was statistically different at each time point between the regions, thus causing regional variation in NWF (Table I, Figure 1). Net fluid secretion was observed in the USI, while net fluid absorption was observed in the LSI and the LI and although the difference was not statistically significant net fluid absorption was greater in the LSI than in the LI (Figure 2). Similar results, in rabbits and dogs have been reported for the USI (duodenum) while more variation in fluid transport was observed for other parts of the small intestine and the LI (4,5). In rabbits, for instance, net fluid absorption was greater in the ileum than in the jejunum while in dogs it was similar in the jejunum and the ileum and 5 times higher

Table I. Summary of Absorption Parameters (Fraction Absorbed [Fa], Intrinsic Absorption Clearance [Cla], ^{14}C-PEG4000 Recovery [PEG Recovery], Net Water Flux [NWF]) Obtained in the Upper Small Intestine [USI], Lower Small Intestine [LSI] and the Large Intestine [LI] Using the *In Situ* Single Pass Perfusion Model in Rats. (n = 36 for Each Region)

Parameters	USI	LSI	LI
antipyrine			
Fa (%)	38.62 ± 1.89	47.44 ± 2.17	40.04 ± 3.77
Cla (μl/min/g intestine)	115.25 ± 6.74	111.96 ± 5.76	90.89 ± 8.37
^{14}C-PEG 4000			
PEG recovery (%)	105 ± 2	108 ± 1	108 ± 2
NWF[a] (μl/min/cm)	0.21 ± 0.11	−1.10 ± 0.10	−0.81 ± 0.10

[a] USI *vs* LSI ($p < 0.001$), USI *vs* LI ($p < 0.001$), LSI *vs* LI ($p > 0.05$). Positive values indicate net water secretion, negative values indicate net water absorption.

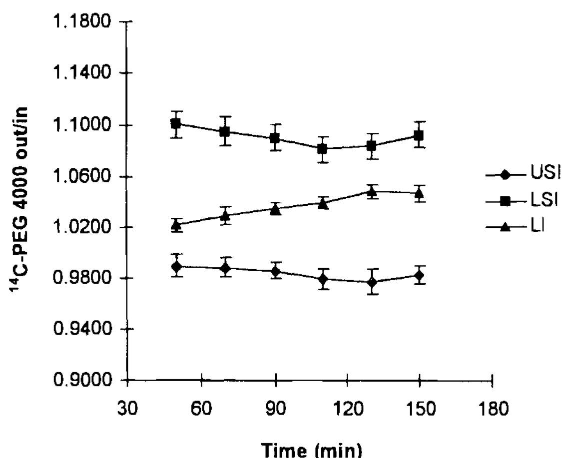

Fig. 1. ^{14}C-PEG 4000 concentration ratio at steady state in the studied regions of the rat intestine (n = 36 for each region). USI *vs* LSI $P < 0.001$ (at each time point), USI *vs* LI and LSI *vs* LI were also statistically significant at each time point with p values ranging between (<0.001 $-<0.05$) and (<0.001 $-<0.01$) respectively.

in the colon than in either of the former (4,5). In rats, net water absorption, under similar experimental conditions, was reported in jejunum, ileum and colon with no significant differences being noted between the regions but as with our results the mean net water absorption was lower in the colon than in the ileum (12). The mechanism underlying water absorption across the intestine is unclear although it is known to be passive; using transcellular and paracellular routes or aquaporin channels. Water absorption and secretion are two distinct processes and are believed to occur in different regions of the mucosa e.g. crypt cells are described as secretory while villus is an absorption site (13). In rats, however, net secretion in the small intestine is not confined to the crypts and may also occur from villous epithelium (14). Regional differences in fluid movement can arise from regional variation in electrolyte transport which is not homogenous throughout the intestine e.g. Na^+ and Cl^- are absorbed by different region-specific transport mechanisms in the gut and in different amounts (13). Active amines and polypeptide hormones such as serotonin, gastrin, somatostatin, etc. are also reported to play an important role in the modulation of water and electrolyte transport and their distribution is markedly different throughout the gut (13).

Fluid absorption, using the in situ perfusion model is widely reported to increase the intestinal absorption of drugs in animals (4,6,7). Regional differences in intestinal fluid movement in rabbits is thought to contribute partly to regional variation in the intestinal absorption of aspirin and metoclopramide while artificially induced fluid absorption and secretion in rats, caused by osmolarity modulation of the perfused solution, has been shown to enhance and diminish jejunal absorption of antipyrine by 49% and 36% respectively (4,7). In contrast to such studies, no variation in antipyrine absorption with fluid absorption and secretion was shown here. Similar results were observed in humans where changes in NWF from secretion to absorption did not enhance the jejunal permeability of antipyrine (6).

In conclusion, the variation in the intestinal fluid movement observed in this study did not affect regional absorption of the passively absorbed compound antipyrine. The effect of such variation on the regional absorption of carrier mediated compounds, nevertheless, remains unclear.

REFERENCES

1. K. M. Hillgren, A. Kato, and R. T. Borchardt. In vitro systems for studying intestinal drug absorption. *Med. Res. Rev.* **15**:83–109 (1995).
2. U. Fagerholm, M. Johansson, and H. Lennernas. Comparison between permeability coefficients in rat and human jejunum. *Pharm. Res.* **13**:1336–1342 (1996).
3. N. Schurgers, J. Bijdendijk, J. J. Tukker, and D. J. A. Crommelin. Comparison of four experimental techniques for studying drug absorption kinetics in the anesthetized rat in situ. *J. Pharm. Sci.* **75**:117–119 (1986).
4. I. Johno, K. Kawakatsu, H. Kuwata, and S. Kitazawa. Segmental differences in transmucosal fluid movement and its effect on gastrointestinal drug absorption in rabbits. *Int. J. Pharm.* **25**:255–263 (1985).
5. E. Grim. Water and electrolyte flux rates in the duodenum, jejunum, ileum and colon, and effects of osmolarity. *Am. J. Dig. Dis.* **7**:17–27 (1962).
6. H. Lennernas. Does fluid flow across the intestinal mucosa affect quantitative oral drug absorption? Is it time for a reevaluation?. *Pharm. Res.* **12**:1573–1582 (1995).
7. H. Ochsenfahrt and D. Winne. The contribution of solvent drag to the intestinal absorption of the basic drugs amidopyrine and antipyrine from the jejunum of the rat. *N-Schmied. Arch. Pharmacol.* **281**:175–196 (1974).
8. M. Danhof, A. Van Zuilen, J. K. Boeijinga, and D. D. Breimer. Studies on the different metabolic pathways of antipyrine in man. Oral versus i.v. administration and the influence of urinary collection time. *Eur. J. Clin. Pharmacol.* **21**:433–441 (1982).
9. H. Lennernas, O. Ahrensedt, R. Hallgren, L. Knuston, M. Ryde, and L. K. Paalzow. Regional jejunal perfusion, a new in vivo approach to study oral drug absorption in man. *Pharm. Res.* **9**:1243–1251 (1992).
10. H. Yuasa, K. Matsuda, and J. Watanabe. Influence of anesthetic regimens on intestinal absorption in rats. *Pharm. Res.* **10**:884–888 (1993).
11. A. Raoof, D. Moriarty, D. Brayden, O. I. Corrigan, I. Cumming, J. Butler, and J. Devane. Comparison of methodologies for evaluating regional intestinal permeability. *Adv. Exp. Med. Biol.* **423**:181–189 (1997).
12. U. Fagerholm, A. Lindahl, and H. Lennernas. Regional intestinal permeability in rats of compounds with different physicochemical properties and transport mechanisms. *J. Pharm. Pharmacol.* **49**:687–690 (1997).
13. E. B. Chang and M. C. Rao. Intestinal water and electrolyte transport: Mechanisms of physiological and adaptive responses. In L. R. Johnson (ed.), *Physiology of the gastrointestinal tract*, Raven Press, New York, 1994, pp. 2027–2081.
14. C. P. Stewart and L. A. Turnberg. A microelectrode study of responses to secretagogues by epithelial cells on villus and crypt of rat small intestine. *Am. J. Physiol.* **257**:G334–G343 (1989).