Human Jejunal Permeability of Two Polar Drugs: Cimetidine and Ranitidine

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Purpose. To determine the human jejunal permeability of cimetidine and ranitidine using a regional jejunal perfusion approach, and to integrate such determinations with previous efforts to establish a baseline correlation between permeability and fraction dose absorbed in humans for soluble drugs.

Methods. A sterile multi-channel perfusion tube, Loc-I-Gut®, was inserted orally and positioned in the proximal region of the jejunum. A solution containing cimetidine or ranitidine and phenylalanine, propranolol, PEG 400, and PEG 4000 was perfused through a 10 cm jejunal segment in 6 and 8 subjects, respectively.

Results. The mean P_{eff} (± se) of cimetidine and ranitidine averaged over both phases were 0.30 (0.045) and 0.27 (0.062) $\times 10^{-4}$ cm/s, respectively, and the differences between the two were found to be statistically insignificant. The mean permeabilities for propranolol, phenylalanine, and PEG 400 averaged over both phases and studies were 3.88 (0.72), 3.36 (0.50), and 0.56 (0.08) $\times 10^{-4}$ cm/s, respectively. The differences in permeability for a given marker were not significant between phases or between the two studies.

Conclusions. The 10-fold lower permeabilities found for cimetidine and ranitidine in this study, compared to propranolol and phenylalanine, appear to be consistent with their less than complete absorption in humans.

KEY WORDS: intestinal permeability; drug absorption; cimetidine; ranitidine; biopharmaceutic classification system.

INTRODUCTION

The oral bioavailability of a drug is affected by several factors such as dissolution, transit time, intestinal permeability and first pass metabolism in the gut and/or liver. Of these, the intestinal permeability is a major determinant of extent of drug absorption and quantitatively represents the fundamental membrane transport coefficient of the intestinal mucosa of the drug (1). The primary objective of the present study was to evaluate the relationship between effective human jejunal permeability, P_{eff}, and the pharmacokinetic parameters of rate and extent of absorption of the H_2 receptor antagonists, cimetidine, and ranitidine, using the regional jejunal perfusion methodology (2–4). An important secondary objective of these studies was to integrate such determinations of *in vivo* permeabilities with previous efforts to establish a baseline correlation between permeability and fraction dose absorbed in humans for soluble drugs. Such efforts may also allow the establishment of simple correlations between human jejunal permeabilities and permeabilities from animal and tissue culture and facilitate pre-clinical estimation of human absorption. Such simple, rapid, and inexpensive screening procedures would eventually aid in determining the biopharmaceutic classification of drugs for both the setting and establishment of regulatory bioavailability and bioequivalence requirements.

MATERIALS AND METHODS

Materials

Cimetidine and ranitidine were obtained from Smith-Kline Beecham Pharmaceuticals (Philadelphia, PA) and Glaxo Wellcome Pharmaceuticals (Research Triangle Park, NC), respectively. Propranolol and phenylalanine were purchased from Ayerst Laboratories, Inc. (Philadelphia, PA) and Twin Laboratories, Inc. (Ronkonkoma, NY), respectively. Polyethylene glycol (PEG) 400, 1000, 1500, and 4000 were obtained from Union Carbide Chemicals and Plastic Company, Inc. (Danbury, CT). Mannitol, $NaH_2PO_4 \cdot H_2O$, Na_2HPO_4, glucose, KCl, and NaCl of USP or NF grade were used to prepare the perfusate, and were purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA) and Abbott Laboratories (North Chicago, IL). All solvents used in the assays were of HPLC grade.

The perfusates contained 1 mM cimetidine or 0.5 mM ranitidine, 0.003 mM propranolol, 0.06 mM phenylalanine, 12.5 mM PEG 400, 1.5 mM PEG 4000, 35 mM mannitol, 5 mM KCl, 49 mM $NaH_2PO_4 \cdot H_2O$, 21 mM Na_2HPO_4, 10 mM glucose, and 45 mM NaCl. The final osmolality and pH of the perfusate solutions were adjusted to 290 mOsm/l and 6.5, respectively.

Subjects

All subjects gave written informed consent to participate in the study that followed the tenets of the Declaration of Helsinki promulgated in 1964, and was approved by the Institutional Review Board at the University of Michigan Medical Center. All subjects were between the ages of 18–40 years, and were judged healthy based on medical history, physical examination, and laboratory tests prior to the study period.

Experimental Procedures

Following an overnight fast, the intubation of the perfusion tube and perfusion of the jejunal segment with the drug-

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Table I. Effective Permeabilities of Cimetidine and Ranitidine and Various Reference Compounds Estimated from Regional Perfusion Studies

Perfusion period	Compound	P_{eff} ($\times 10^4$) cm/s	sd ($\times 10^4$) cm/s	CV	n	se ($\times 10^4$) cm/s
1	Cimetidine	0.296	0.139	0.47	7	0.053
2	Cimetidine	0.304	0.198	0.65	5	0.089
1	Propranolol	4.141	2.185	0.53	8	0.773
2	Propranolol	2.747	1.309	0.48	6	0.534
1	Phenylalanine	4.023	2.081	0.52	8	0.736
2	Phenylalanine	3.653	2.282	0.62	6	0.932
1	PEG 400	1.043	0.419	0.40	8	0.148
2	PEG 400	0.818	0.193	0.24	6	0.079
1	Ranitidine	0.338	0.283	0.84	8	0.100
2	Ranitidine	0.208	0.203	0.98	8	0.072
1	Propranolol	4.744	6.240	1.32	8	2.206
2	Propranolol	3.596	4.152	1.15	8	1.468
1	Phenylalanine	3.339	3.994	1.20	8	1.412
2	Phenylalanine	2.511	2.402	0.96	8	0.849
1	PEG 400	0.248	0.131	0.53	8	0.046
2	PEG 400	0.193	0.128	0.66	8	0.045

containing solution, at a rate of 3 ml/min or 2 ml/min for two 90-min periods, was performed according to the protocol described earlier (4).

Drug Analysis

The concentrations of cimetidine, PEG 4000, PEG 400, propranolol, and phenylalanine were determined by high performance liquid chromatography (HPLC) methods. The HPLC system was described earlier (4). For cimetidine, the mobile phase was composed of 0.067 M potassium-sodium phosphate buffer containing potassium chloride 10 g/liter and acetonitrile (60:40 %, v/v) (5). The separation was performed on a Nucleosil 100SA ion exchange column (10 μm, 250 × 4.6 mm, Alltech). The UV absorption was recorded at 228 nm and ranitidine was used as an internal standard. The mixture of the perfusate sample (0.1 ml) and the internal standard solution (250 μl, 200 mg/ml) was filtered (0.45 μm, Acrodisc®, Gelman Sciences), and 20 μl was injected onto the column. The retention times of cimetidine and ranitidine were 7.3 and 14.3 min, respectively. The intraday coefficient of variation (CV) was 1.53 and 0.89 %, and the interday CV was 15.91 and 6.52 %, at 40 mg/ml and 320 mg/ml, respectively. The detection limit was 8 μg/ml. Ranitidine was assayed using the method of Prueksaritanont et al. (6). Analyses of PEG 4000, PEG 400, propranolol, and phenylalanine were carried out using previously described methods (4).

Data Analysis

Estimation of Effective Permeability Coefficients

The estimation of effective permeability, P_{eff}, was carried out using the methodology described previously (4,7–10). The effective permeability coefficients obtained after excluding outliers were then normalized to a fixed segmental radius of 1.75 cm. The mean of the normalized effective permeabilities was then used to calculate the fraction of drug absorbed.

Fraction Dose Absorbed

The fraction dose absorbed, F_a, was estimated using methodology described previously (8).

RESULTS AND DISCUSSION

The estimates of the effective permeabilities, P_{eff}, at steady state for cimetidine and the reference compounds obtained from both perfusion phases at the 2 flow rates, 2 ml/min and 3 ml/min, were found to be statistically insignificant ($p > 0.1$). Thus, the mean effective permeabilities of cimetidine and the reference compounds at the 2 flow rates for both perfusion periods, along with those obtained for ranitidine and the reference compounds for the 2 perfusion periods at 2 ml/min, are shown together in Table I. Both cimetidine and ranitidine exhibited low jejunal permeability relative to the reference compounds propranolol and phenylalanine, consistent with the incomplete absorption observed for both of these drugs (11). The fraction dose absorbed, estimated assuming a mean small intestinal residence time of 3 h and an intestinal radius of 1.75 cm, was about 30% for both cimetidine and ranitidine. This value is at the low end of the highly variable absorption reported for cimetidine (40–90%) and in reasonable proximity to that of 50% observed for ranitidine. The possibility of a significant decrease in the permeability of cimetidine at concentrations greater than 0.4 mM, similar to that reported in both rat jejunum and ileum cannot be ruled out (12). It is also possible that the perfusate pH of 6.5 may have lowered permeability to some extent. More accurate estimates would require consideration of transit, position dependent permeability, motility, and lumenal composition (e.g., pH) variation, as well as the impact of metabolism and P-glycoprotein efflux mechanisms, and is beyond the scope of this report (12,13). The estimated absorption for propranolol and phenylalanine was 100%.

The overall mean permeabilities for the various compounds, along with the overall mean values from a previous study with piroxicam normalized to a segmental radius of 1.75 cm, are shown in Table II and Fig. 1. The mean permeabilities

Table II. Comparison of Effective Permeability of Various Compounds with Estimates from Previous Studies

Parameter	Cimetidine this study	Ranitidine this study	Propranolol		Phenylalanine		PEG 400		Piroxicam
			This study	Ref 4	This study	Ref 4	This study	Ref 4	Ref 4
Mean Peff, $\times 10^4$	0.299	0.273	3.878	2.698	3.363	4.313	0.559	0.555	6.738
sd Peff, $\times 10^4$	0.157	0.247	3.940	1.192	2.743	2.112	0.446	0.381	3.933
n	12	16	30	10	30	11	30	11	11
CV	0.53	0.91	1.02	0.44	0.82	0.49	0.80	0.69	0.58
se Peff, $\times 10^4$	0.045	0.062	0.719	0.377	0.501	0.637	0.082	0.115	1.186

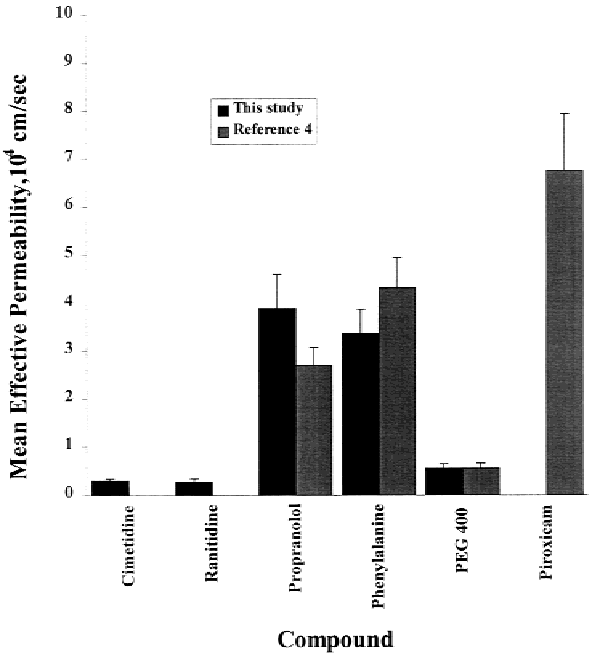

Fig. 1. Comparison of effective permeabilities of various compounds.

of piroxicam and the reference compounds reported in a previous study (4) were determined using a segmental intestinal radius in each subject that was calculated from segmental volume estimates and then normalized to a fixed radius of 1.15 cm. The mean effective permeabilities (± sd) for piroxicam, phenylalanine, propranolol, and PEG 400, obtained in the previous study (4) estimated using an intestinal segment radius of 1.15 cm, was 10.40 ± 5.93, 6.67 ± 3.42, 3.59 ± 1.60, and 0.80 ± 0.46 × 10^{-4} cm/sec, respectively. The mean effective permeabilities (± sd) for phenylalanine, propranolol, and PEG 400, in the present study normalized to an intestinal radius of 1.75 cm, was 3.36 ± 2.74, 3.88 ± 3.94, and 0.56 ± 0.45 × 10^{-4} cm/s, respectively. The value of 1.75 cm was adopted for normalization of permeabilities based on x-ray analysis of barium perfusion studies (2, Lennernas, Personal Communication). The two sets of values can be inter-converted using the ratios of the radii. Thus, the mean effective permeabilities, from the previous study, can be recalculated by multiplication with the factor, 1.15/1.75 or 0.657, to give mean effective permeabilities (± sd) of 6.74 ± 3.93, 4.31 ± 2.11, 2.70 ± 1.19, and 0.56 ± 0.38 × 10^{-4} cm/sec, for piroxicam, phenylalanine, propranolol, and PEG 400, respectively. The two sets of values normalized to a fixed radius of 1.75 cm (Table II and Fig. 1) indicate clearly that a normalized standard procedure is essential for comparison of data obtained from various sets of permeability determinations.

In conclusion, the results of the present study further support the utility of the perfusion methodology for determining human jejunal permeabilities and their correlation with human oral absorption. These determinations constitute an important addition to the database that can be utilized in the construction of several useful correlations. Thus, correlations of human permeability estimates with fraction dose absorbed and with permeabilities from other animal or tissue culture systems would allow both the permeability-classification of drugs (14) as well as the setting of bioequivalence standards for drug product approval.

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