ABSTRACT:

The purpose of this study was to determine the characteristics of intestinal absorption and metabolism of cimetidine. The initial finding of the appearance of cimetidine sulfoxide in rat and human jejunum from cimetidine perfusions had prompted an isolation of mucosal membrane transport and enterocyte metabolism contributions in earlier membrane vesicle and microsomal studies, respectively. In this report, perfusion studies in rat small intestine detail regional differences in intestinal elimination. Cimetidine S-oxide appears to a significantly greater extent in the jejunum compared with the ileum. Jejunal metabolite appearance is shown to be a function of the pH-dependent intracellular uptake of cimetidine. Cimetidine permeability decreases with increasing perfusion concentration in both jejunum and ileum. Similar permeability magnitudes and concentration dependence are observed in both regions. Perfusion studies with inhibitors of cimetidine mucosal transport and inhibitors of microsomal S-oxidation provide an inhibition profile suggesting that jejunal cimetidine permeability decreases with increasing intracellular cimetidine concentration. The data support a reduction in paracellular cimetidine absorption as controlled by intracellular cimetidine. This inference is drawn on the basis of mass balance. Because significant appearance of cimetidine S-oxide was previously found in human jejunal perfusions, this region-dependent intestinal elimination process detailed in rats may be relevant to drug plasma-level double peaks observed in clinical studies. Saturation of jejunal metabolism at typical oral doses may limit paracellular absorption of cimetidine in the jejunum and contribute to the double peak phenomenon and to absorption variability.

Cimetidine was the first H₂-receptor antagonist marketed to control gastric acid secretion (Somogyi and Gugler, 1983). It is currently sold as an over-the-counter product but continues to be remarkable in the number of literature reports documenting its role in drug interactions (Shinn, 1992; Guengerich, 1997). Cimetidine is well absorbed after oral administration but its absorption is highly variable and the appearance of a second plasma concentration maximum provides an unusual pharmacokinetic profile characteristic (Bodemar et al., 1979). Enterohepatic recirculation had been initially proposed to be responsible for the double peak (Veng Pedersen, 1981), but biliary elimination has been shown to not significantly impact drug plasma levels (Kaneniwa et al., 1986a). Gastric pH and emptying variability have been reported to influence the absorption pattern of cimetidine as a function of dose and time of administration (Oberle and Amidon, 1987). However, plasma level double peaks are also observed in human intestinal perfusion of cimetidine (Voinchet et al., 1981). Cimetidine absorption had been shown to be lower in rat jejunum than in duodenum and ileum (Barber et al., 1979) and this absorption site dependence has been projected to account for plasma level double peaks in humans (Witcher and Boudinot, 1996). However, membrane transport contributions to this regional dependence and the mechanistic elements of cimetidine plasma level double peaking and absorption variability have not been resolved.

Most recently, cimetidine absorption in rat proximal jejunum has been shown to be exclusively via the paracellular pathway. This was based on data that indicated that intracellular cimetidine did not appreciably exit the basolateral membrane. Furthermore, intracellular cimetidine levels, as controlled by cellular uptake and elimination, appear to regulate paracellular absorption (Zhou et al., 1999). In this report, the role of jejunal S-oxidation in influencing cimetidine absorption was further explored by varying concentration and using inhibitors of cimetidine metabolism and transport in single-pass small intestinal perfusions in rats.

The current data suggest that cimetidine S-oxidation influences paracellular cimetidine absorption in rat jejunum by determining the magnitude of intracellular drug concentration. As intracellular uptake increases with luminal cimetidine concentrations beyond the capacity of jejunal cimetidine metabolism, intracellular cimetidine levels rise, leading to the restriction of paracellular transport and a reduction of drug absorption into the systemic circulation. The reduction in paracellular transport provides a greater driving force for intracellular uptake. This, in turn, amplifies the contribution of intracellular cimetidine metabolism to jejunal elimination, resulting in elevated cimetidine S-oxide levels in the jejunal lumen. Cimetidine S-oxide is not absorbed and does not enter the rat jejunal lumen from the blood (Hui et al., 1994). Thus, intestinal elimination of cimetidine is enhanced by

1 Abbreviations used are: MES, 2(N-morpholino)ethanesulfonic acid; PEG 4000, polyethylene glycol 4000; BBMV, brush-border membrane vesicles.
inhibition of paracellular transport. At high luminal cimetidine concentrations, this form of self-inhibition depresses rat jejunal absorption. Based on cimetidine metabolite levels in human jejunum, it is proposed that this mechanism may account for double plasma level maxima. The concentration dependence and inhibition profile of this process may also contribute to the broad variability observed in cimetidine oral bioavailability.

**Experimental Procedures**

**Chemicals.** Cimetidine, d-glucose, 3-oxyethylglucose, d-mannitol, imipramine hydrochloride, chlorpromazine hydrochloride, erythromycin, neomycin sulfate, lincomycin, L-cysteine, L-methionine, 2(N-morpholino)ethanesulfonic acid (MES), HEPES, and polyethylene glycol 4000 (PEG 4000) were purchased from Sigma Chemical Co. (St. Louis, MO). [14C]PEG 4000 was obtained from New England Nuclear (Boston, MA). Cimetidine sulfoxide was kindly provided by SmithKline Beecham Pharmaceuticals (King of Prussia, PA). All other chemicals were of reagent grade or HPLC grade.

**Rat In Situ Single-Pass Intestinal Perfusion.** Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 250 to 300 g were fasted overnight for 16 to 22 h with free access to water and anesthetized with an intramuscular injection of 87 mg/kg ketamine and 13 mg/kg xylazine before surgery. After anesthesia, rats were placed on a heating pad under a surgical lamp to maintain body temperature. A midline longitudinal abdominal incision was made and an inlet Teflon tube (0.42 cm diameter) was inserted into the jejunum at about 10 cm distal to the ligament of Treitz. Outlet Teflon tubes were inserted 8 to 10 cm proximal to the inlet cannulae. In ileal perfusions, the outlet tubing was inserted 2 cm proximal to the ileocecal junction and the inlet tube 8 to 10 cm proximal to the outlet. After cannulation, the abdomen was rinsed with isotonic saline, and covered with an isotonic saline-wetted gauze. The entire surgical area was then covered with parafilm to reduce evaporation. All inlet tubing was water-jacketed to maintain inlet temperature at 37°C. Inlet cannulae were connected to syringes that were placed in a perfusion pump (Harvard Apparatus Co., South Natick, MA). Drug solutions were perfused through the intestine at flow rates of 0.12 or 0.25 ml/min. After allowing 40 min to reach steady-state outlet concentrations, outlet perfusate samples were collected every 10 or 15 min for 90 min. When the experiment was completed, the perfused intestinal length was measured by dissecting the mesentery and blood vessels and placing the segment flat over a ruler whose surface was wetted with saline to prevent segment elongation.

**Perfusion Solutions.** Cimetidine solutions were made isotonic (290 ± 20 mOsm/kg) and buffered to the desired pH with MES or HEPES buffer. These perfusion solutions contained 5 mM potassium chloride, in the range of 135 mM sodium chloride for isotonicity adjustment, 10 mM MES and were adjusted to pH 5.5 or 6.5 with 10 N sodium hydroxide. In some studies, 10 mM HEPES was used in place of MES to adjust perfusion solution pH to 7.5. A nonabsorbable marker (0.01% PEG 4000) traced with radioactivity counterpart, specific activity 0.8 mCi/g) was included in the buffer to monitor water transport across the intestinal membrane. Perfusion outlet drug and metabolite concentrations were corrected for water absorption or secretion as outlined in the data analysis section. All inlet drug solutions were analyzed to ensure cimetidine stability before initiating rat perfusion and then used to obtain drug-water-corrected outlet-to-inlet cimetidine concentration ratios. Cimetidine sulfoxide concentrations were quantified using standard curves generated with SmithKline Beecham cimetidine sulfoxide. Jejunal perfusions were also performed with 0.4 mC/mmol cimetidine sulfoxide in four rats at pH 6.5 and a perfusion flow rate of 0.12 ml/min.

**Analytical Methods.** Cimetidine and cimetidine sulfoxide were analyzed by an HPLC method (Larsson et al., 1982). Samples were injected onto an HPLC system composed of a WISP autosampler model 710B, Waters 501 HPLC solvent pump (Waters Corporation, Milford, MA), and a nucleosil SA 10-μm cation exchange column, 250 × 4.6 mm (Alltech Associates, Inc., Deerfield, IL). The mobile phase, containing 20% v/v acetoni treitol and 80% v/v buffer (30 mM monobasic potassium phosphate, 3.7 mM dibasic sodium phosphate, and 10 g/liter potassium chloride, adjusted to pH 4.0 with 85% phosphoric acid) was pumped at a flow rate of 1.2 ml/min. The eluting peaks were monitored at 228 nm using a variable wavelength UV detector (Spectroflow 773; Kratos Analytical Instruments, Ramsey, NJ) and the peak height together with peak area were measured with a Shimadzu integrator (Model CR 501 Chromatopac; Shimadzu Corporation, Kyoto, Japan). Retention times for cimetidine and cimetidine sulfoxide were 7.5 and 12.8 min, respectively. The minimal levels of detection for cimetidine sulfoxide and cimetidine were 50 and 80 nM, respectively. A linear response was obtained for cimetidine and cimetidine sulfoxide standard curves (r = 0.997–1.000). Quantification of metabolite was obtained as the mean value of four to five steady-state outlet perfusate samples as determined from the standard curve. The metabolite HPLC peak from perfusions was confirmed as cimetidine sulfoxide by mass spectrometry analysis in a previous study (Hui et al., 1994). The original solutions and the effluent perfusate samples were also analyzed for [14C]PEG 4000 using liquid scintillation counting. A 500-ml aliquot was mixed with 5 ml of scintillation fluid (Ecolite; ICN Pharmaceuticals, Costa Mesa, CA) and the amount of radioactivity was determined in a Beckman LS 6000 SC scintillation counter (Beckman Instruments, San Jose, CA).

**Pre-treatment with Antibiotics.** In some selected studies, the animals were given a 7-day regimen of a neomycin and lincomycin combination (Illeg, 1981; Ilett et al., 1990). Neomycin sulfate (1.5% v/w) and 0.3% w/w lincomycin were added to their food, and both drugs were also dissolved in their drinking water at concentrations of 2 and 0.5 mg/ml, respectively.

**Data Analysis.** Data were generated from each rat by averaging at least five perfusate collection samples at steady state. The amount of cimetidine sulfoxide detected in the perfusate is calculated as the fraction of drug metabolized (Fmet).

\[
F_{\text{met}} = \frac{\text{luminal cimetidine sulfoxide}}{\text{luminal cimetidine loss}} = \frac{\text{luminal cimetidine sulfoxide (mM)}}{[\text{inlet cimetidine} – \text{outlet cimetidine}](\text{mM})}
\]

Luminal cimetidine sulfoxide and outlet cimetidine concentrations were corrected for water transport across intestinal membrane. Intestinal water flux (J in microliters per minute per centimeter) is calculated at a given flow rate, Q (microliters per minute), per centimeter of perfused intestinal length (L) as 14C disintegration per minute using the following equation (Lu et al., 1998):

\[
J = \frac{14\text{C dpm}_{\text{outlet}} - 14\text{C dpm}_{\text{inlet}}}{14\text{C dpm}_{\text{inlet}}} \times \frac{Q}{L}
\]

Positive values of J imply net water absorption whereas negative values indicate net water secretion. Effective cimetidine permeability (P_{eff}) using a modified boundary model developed by Johnson and Amidon (1988), is computed according to the following equation:

\[
P_{\text{eff}} = \frac{Q}{2\pi RL} \left( 1 - \frac{C_{\text{outlet}}}{C_{\text{inlet}}} \right)
\]

where \(C_{\text{outlet}}\) is the exit cimetidine concentration corrected for water transport in the perfusate and \(C_{\text{inlet}}\) is the input drug concentration. A water transport correction is obtained by multiplying the drug outlet-to-inlet concentration ratio by the marker inlet-to-outlet dpm ratio. R and L are intestinal radius and length of the perfused segments, respectively. The R value used in this study was estimated to be 0.23 cm (Yuasa et al., 1988). The \(P_{\text{eff}}\) is a measure of drug lost across the apical membrane of the intestinal epithelia including both intracellular and paracellular drug transport. Permeability defines the rate of absorption in this rat perfusion system and has been correlated with the fraction of drug absorbed from solution in human clinical studies as assessed by plasma level pharmacokinetic profiles (Amidon et al., 1988). A corrected value for the effective permeability (P_{eff(corrected)}) was calculated using a molar summation of luminal cimetidine and cimetidine sulfoxide as \(C_{\text{inlet}}\) in the above equation. This corrected value is reflective of net systemic drug absorption in the presence of intestinal drug elimination. Data are presented as mean ± S.E. Statistical differences were determined by either Student’s t test or ANOVA, as appropriate.
Results

Previous work had identified cimetidine S-oxide in the lumen after jejunal perfusions of cimetidine in rats and two human subjects (Hui et al., 1994). In the earlier studies in rats, significantly lower levels of the S-oxide had been observed in ileal compared with jejunal cimetidine perfusions (Hui et al., 1994). Furthermore, a maximum ratio of metabolite to initial perfusion drug concentration had been observed in the jejunum above 0.4 mM for perfusion cimetidine concentrations from 0.1 to 4 mM. Therefore, 0.4-mM drug concentrations were used in pH-dependence and metabolism-inhibition studies. Rat jejunal perfusions of 0.4 mM cimetidine S-oxide at pH 6.5 and perfusion flow rate of 0.12 ml/min resulted in water transport-corrected permeabilities of zero, confirming that the metabolite is not appreciably absorbed. Furthermore, reduction of the metabolite to the parent drug was not detectable in the outlet perfusate.

Recent studies in jejunal epithelia had determined that the cellular origin of metabolite production was the microsomal cell fraction (Lu et al., 1998). Although drug oxidation is rarely mediated by intestinal bacteria (George and Renwick, 1987), this was confirmed by comparing jejunal appearance of metabolite in antibiotic-treated versus untreated rats. Antibiotic pretreatment did increase the variability in S-oxide appearance from 0.4 mM jejunal cimetidine perfusions but metabolite production was not significantly different between treated and untreated animals (F_{met} = 0.66 ± 0.14 antibiotic-pretreated; F_{met} = 0.73 ± 0.04 untreated; n = 4 rats).

It had been shown that jejunal S-oxide appearance was substantial in jejunal jejunal perfusions at pH 6.5 but negligible in ileal perfusions at pH 7.5 (Hui et al., 1994). Jejunal S-oxide levels as a function of perfusion pH showed a maximum at pH 6.5, which coincided with a maximum in jejunal jejunal permeability (Table 1) from drug perfusions at 0.4 mM. Although ileal metabolite levels were higher from perfusions at pH 6.5 versus pH 7.5, they are significantly lower than those from jejunal perfusions at pH 6.5 (Table 1). At a physiologically relevant pH of 7.5 in the ileum, ileal S-oxide appearance is negligible and ileal cimetidine permeability is equivalent to metabolite-corrected jejunal permeability from 0.4 mM cimetidine perfusions at pH 6.5 (Table 1).

Because little metabolite is produced in ileal perfusion, effective permeability calculations are not affected by metabolite production, and the amount of drug loss from the lumen reflects systemic drug absorption. The permeability of cimetidine in the ileum is constant at low concentrations (Fig. 1), indicating that the amount of drug loss is proportional to the amount available for absorption. As had been determined in a previous study, reduced permeability at higher perfusion concentrations is not the result of saturation of carrier-mediated transport at the apical membrane but rather a dose-dependent restriction of paracellular transport (Zhou et al., 1999). If jejunal permeability is calculated from drug loss in perfusate and not corrected for metabolite appearance, an unusual permeability profile is obtained (Fig. 2A). However, when luminal drug concentration is corrected for luminal metabolite appearance, the permeability profile and permeability magnitudes (Fig. 2B) in the jejunum are comparable to those observed in the ileum (Fig. 1). The magnitude of luminal metabolite appearance is also observed to level off at high drug concentrations (Fig. 3). The ratio of jejunal metabolite appearance to drug loss (F_{met}) is proportional to perfusion concentration over the lower perfusion drug-concentration range and levels off at about 0.9 at higher perfusion concentrations (Fig. 4). Given the definition of F_{met}, when metabolism is saturated at higher drug concentrations, the increase in F_{met} indicates that cellular uptake rather than metabolism is limiting metabolite appearance. Otherwise, F_{met} would decrease at higher perfusion concentrations. Permeability was not influenced by perfusion flow rate at any of the concentrations studied (Figs. 1 and 2).

<table>
<thead>
<tr>
<th>Intestinal Segments</th>
<th>pH</th>
<th>F_{met}</th>
<th>P_{eff}</th>
<th>P_{eff,corr}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.5</td>
<td>0.196 ± 0.122</td>
<td>0.289 ± 0.152</td>
<td>0.078 ± 0.042</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>0.726 ± 0.036</td>
<td>0.573 ± 0.055</td>
<td>0.142 ± 0.019</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>0.069 ± 0.037</td>
<td>0.037 ± 0.014</td>
<td>0.035 ± 0.013</td>
</tr>
<tr>
<td>Ileum</td>
<td>6.5</td>
<td>0.120 ± 0.073</td>
<td>0.093 ± 0.025</td>
<td>0.079 ± 0.021</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>0.005 ± 0.003</td>
<td>0.129 ± 0.018</td>
<td>0.128 ± 0.017</td>
</tr>
</tbody>
</table>

Figure 1. Effective ileal permeability (P_{eff}) of cimetidine as a function of steady-state drug perfusion concentration at pH 7.5.

Figure 2. A, effective jejunal permeability (P_{eff}) of cimetidine as a function of steady-state perfusion concentration at pH 6.5 calculated as drug loss from the jejunal lumen; B, effective permeability corrected for drug lost to sulfoxide appearance in the lumen (P_{eff,corr}).
However, at the highest drug perfusion concentration (4 mM), $F_{\text{met}}$ was significantly lower at the slower perfusion rate than at the faster perfusion flow rate (Fig. 4).

A 10-fold reduction in jejunal cimetidine permeability (uncorrected for $S$-oxide appearance) is observed when 0.4 mM cimetidine is coperfused with 5 mM methimazole (Fig. 5A). Reductions in permeability (3- to 4-fold) are generated by cimetidine coperfusion with L-cysteine, L-methionine, chlorpromazine, and imipramine. However, when steady-state cimetidine permeability is corrected for the appearance of metabolite, only methimazole and the amino acids actually reduce cimetidine absorption (Fig. 5B). Methimazole eliminates measurable appearance of cimetidine $S$-oxide in the jejunal lumen (Fig. 6) in parallel with the reduction in corrected permeability to near zero (Fig. 5B). Although both chlorpromazine and imipramine significantly reduce metabolite levels (Fig. 6), they do not affect the corrected cimetidine permeability (Fig. 5B). Decreases in the appearance of cimetidine $S$-oxide are statistically significant with coperfusion of L-methionine, whereas metabolite variability in the presence of erythromycin does not result in statistically significant changes in $F_{\text{met}}$ (Fig. 6).

**Discussion**

Cimetidine plasma profiles from oral administration under fasted conditions often show a secondary maximum that is not observed when the drug is administered with a meal (Bodemar et al., 1979). The two plasma level maxima typically occur between 1 and 2 h and between 3 and 4 h after oral cimetidine administration. Given the relatively short elimination half-life of cimetidine and the fact that this double peak time frame coincides with intestinal transit time, variable absorption rate down the length of the intestine is anticipated to contribute to drug plasma level observations. Enterohepatic recirculation (Veng Pederson and Miller, 1980), intestinal bacterial reconversion of biliary metabolite (Gugler et al., 1981), variable gastric emptying (Oberle and Amidon, 1987), and region-dependent absorption (Hui et al., 1994) have all been proposed to account for these observations.

The extent of biliary secretion of cimetidine and metabolites has been shown to be insufficient to contribute to a second plasma level peak (Kaneniwa et al., 1986a). Although variable gastric emptying has been indicated to play a partial role (Oberle and Amidon, 1987), plasma level double peaks are also obtained from human intestinal perfusion of cimetidine (Voinchet et al., 1981). This study indicates that a decreased rate of absorption in the jejunum as compared with...
cimetidine transport by a paracellular rather than transcellular path-
way (Zhou et al., 1999). It is projected that the basolateral membrane
limits transcellular cimetidine absorption and that intracellular cimeti-
dine levels will rise with methimazole inhibition of intracellular
cimetidine sulfoxidation (Fig. 6). The observed depression in cimet-
dine absorption rate (Fig. 5, A and B) could be projected from a
reduction in drug concentration gradient driving force across cellular
mucosal membranes resulting from an elevation of intracellular drug
levels. High intracellular drug concentrations would be projected to
promote passive permeation across the basolateral membrane. At high
cimetidine perfusion concentrations, the fact that almost all drug
returns to the jejunal lumen either as metabolite or unmetabolized
cimetidine indicates that the basolateral membrane is the primary
barrier for transcellular absorption. This is consistent with in vitro data
in Caco-2 cells (Gan et al., 1998) and rat small intestinal tissue
(Collett et al., 1999), showing that H2-antagonists are preferentially
transported in the basolateral to apical direction.

Cimetidine transport into jejunal epithelial cells across the lateral
membrane lining the paracellular pathway may account for the sur-
prising increase in mucosal absorption observed over the concen-
tration range from 0.08 to 0.4 mM (Fig. 2A). To explain this concen-
tration dependence, “systemic” permeability (corresponding to
corrected-Peff) is defined as the rate of absorption corrected for the
appearance of luminal metabolite. This determines the rate at which
drug would be available to the systemic circulation from paracellular
transport, because intracellular drug that is not metabolized is secreted
back into the intestinal lumen. Systemic cimetidine absorption is
unchanged over this concentration range, as indicated by the corrected
permeability data (Fig. 2B). Defining “apical” permeability (corre-
sponding to P eff) as the rate of drug lost from the intestinal lumen
refers to drug traversing the paracellular pathway and drug that enters
epithelial cells beyond the apical membrane. The increase in apical
permeability (uncorrected for metabolism) from 0.08 to 0.4 mM is the
result of cellular cimetidine uptake across both apical and lateral
membranes. Over this lower concentration range, intracellular drug
concentrations may not be sufficiently high to saturate metabolism,
and epithelial elimination increases the inward cimetidine flux across
the apical and lateral membranes by maintaining sink conditions
inside the cell. Essential to this interpretation, it has been shown that
cimetidine sulfoxide does not compete with cimetidine for secretory
transport across rat jejunal brush-border membrane vesicles (BBMV;
Piyapolrungroj et al., 1999) and that the metabolite is not absorbed
in jejunal perfusions. Thus, as cimetidine perfusion concentration in-
creases, metabolism becomes saturated and intracellular cimetidine
levels increase to affect a dose-dependent restriction of the paracel-
lar transport.

Reduced paracellular absorption is coincident with increases in
intracellular drug uptake and controls jejunal elimination at higher
cimetidine concentrations. At low cimetidine perfusion concentrations
(0.08 – 0.4 mM), both apical permeability (Fig. 2A) and metabolized
fraction (Fig. 4) increase proportionally as intracellular cimetidine
is eliminated by microsomal metabolism and metabolite export into the
jejunal lumen. At high concentrations (0.4 – 4 mM), the capacity for
metabolism and/or apical elimination is exceeded. Under these con-
ditions, it is projected that intracellular drug concentrations increase
to levels that can affect paracellular restriction of drug absorption. This
is supported by the fact that both P eff and corrected P eff decrease as
concentrations increase over the higher concentration range (Fig. 2)
whereas Fmet levels off between 0.8 and 0.9 (Fig. 4). The fact that the
ratio of metabolite appearance to drug loss is close to 1 at high
luminal cimetidine concentrations suggests that most of the drug
uptake beyond the apical membrane is not absorbed systemically but
secreted back across the apical membrane as the S-oxide metabolite.
Permeability and $F_{\text{met}}$ were not dependent on perfusion flow rate except at 4 mM cimetidine where a statistically lower $F_{\text{met}}$ was recorded with slower versus faster perfusion flow rate (Fig. 4). Luminal sulfoxide appearance at 2 mM cimetidine was statistically greater at the slower versus the faster flow rate (Fig. 3). Because cimetidine paracellular transport is inhibited more extensively at higher luminal cimetidine concentration, luminal drug loss predominately represents intracellular drug uptake with subsequent metabolite elimination into the lumen. $F_{\text{met}}$ at higher cimetidine concentration is close to 1 and so a statistical difference in flow rate or concentration dependence would not be expected. The higher production of metabolite at the slower flow rate (Fig. 3) indicates that greater drug uptake rather than lower drug metabolism accounts for lower $F_{\text{met}}$ values. A slower perfusion flow rate would be projected to provide a longer jejunal residence time and, therefore, a greater potential for drug uptake and luminal metabolite appearance. A possible explanation is that a decrease in the thickness of an aqueous diffusion boundary layer at the higher flow rate reduces the aqueous resistance to cellular drug uptake. This would provide the strongest dependence of metabolite production on flow rate at a concentration approaching metabolic saturation.

Ileal cimetidine absorption demonstrates a similar self-inhibition profile (Fig. 1) in the absence of metabolite elimination into the lumen. Although the ileum might be expected to be more sensitive to paracellular restriction in the absence of cimetidine $S$-oxidation, the ileum is typically exposed to lower concentrations of drug because it is distal to upper intestinal absorption sites. The similarity in ileal and jejunal permeability concentration dependence (Figs. 1 and 2B) indicates that jejunal elimination and a restricted paracellular pathway promotes less cimetidine absorption in the jejunum as compared with the ileum based on exposure to higher luminal drug concentration. Region-dependent absorption in rats has been reported, showing cimetidine plasma levels were higher from duodenal and ileal administration than from jejunal administration (Kaneniwa et al., 1986b). A similar finding has been documented in human subjects for ranitidine (Gramatte et al., 1994).

Regional differences in permeability and metabolite production are also observed with respect to perfusion pH. Whereas the uncorrected permeability reflects the sum of intracellular drug uptake and paracellular absorption into the systemic circulation, metabolite-corrected permeability defines the rate of systemic drug absorption. The fact that changes in perfusion pH significantly alter $F_{\text{met}}$ and $P_{\text{eff}}$ but do not alter corrected $P_{\text{eff}}$ indicates that changes in pH primarily affect cimetidine intracellular uptake and subsequent luminal secretion. This is consistent with the fact that intracellular pH and metabolism should not be influenced by changes in perfusion pH. Jejunal cimetidine $P_{\text{eff}}$ was maximal at pH 6.5, whereas ileal $P_{\text{eff}}$ was relatively insensitive to the luminal pH change. Initial cimetidine uptake overshoot of equilibrium in rat jejunal BBMV was maximal at an intravesicular pH of 5.7 when extravesicular pH was 7.4 (Piyapolrungroj et al., 1999) indicative of mediated cimetidine secretion across the apical membrane. Cimetidine sulfoxide did not compete with cimetidine uptake in this vesicle preparation. The in vitro and in vivo data indicate that the relative contributions of cimetidine secretion and absorption to jejunal permeability are highly dependent on mucosal microclimate pH, which is lower in the jejunum than in the ileum (Daniel et al., 1989).

Imipramine strongly inhibited initial cimetidine uptake by rat jejunal BBMV (Piyapolrungroj et al., 1999) but proved a weak inhibitor (compared with methimazole) of cimetidine $S$-oxidation in jejunal microsomes (Lu et al., 1998). The perfusion data obtained in this study, indicate that cimetidine transport across the mucosal membrane is inhibited by imipramine because both uncorrected $P_{\text{eff}}$ and $F_{\text{met}}$ are decreased by imipramine but corrected $P_{\text{eff}}$ is not. This contrast in inhibition results between methimazole and imipramine also suggests that intracellular cimetidine is controlling absorption of cimetidine by rat jejunal epithelia. Imipramine’s inhibition of cimetidine uptake and methimazole’s greater inhibition of metabolism favor greater intracellular cimetidine concentrations with methimazole.

Methionine inhibition of cimetidine $S$-oxidation in jejunal microsomes was similar in magnitude to that observed with imipramine (Lu et al., 1998). In contrast to imipramine, methionine did not inhibit cimetidine uptake by jejunal BBMV (Piyapolrungroj et al., 1999). Because methionine does not reduce cellular cimetidine uptake but does inhibit metabolism, a reduction in both $P_{\text{eff}}$ and corrected $P_{\text{eff}}$ is observed. The fact that no significant change is observed in $F_{\text{met}}$ may be accounted for by the fact that cimetidine metabolism parallels luminal drug loss. This is consistent with projecting greater intracellular levels of cimetidine with methionine compared with imipramine coperoxidation. This data further support a role for intracellular cimetidine levels in regulating cimetidine transport across paracellular pathways.

Based on these in situ studies as well as BBMV transport and microsomal metabolism studies, a mechanism for cimetidine transport and metabolism in the jejunum is proposed (Fig. 7). Cimetidine is absorbed paracellularly and intracellular uptake is mediated by pH-dependent and potential-dependent processes in parallel with facilitative uptake across the lateral membrane lining the paracellular pathway. Paracellular transport is regulated by intracellular cimetidine concentrations as a function of intracellular uptake and metabolism. Although cimetidine elimination is substantial in the jejunum and not the ileum, self-inhibition of absorption is significant in both regions. Cimetidine elimination in the jejunum is amplified by self-inhibition of transport at higher drug concentrations. This will not usually occur in the ileum because it is distal to sites of upper intestinal absorption and therefore is exposed to lower drug concentrations. This is projected to result in region-dependent absorption underlying the clinical observation of plasma level double peaks. Based on this absorption mechanism, luminal variables including cimetidine dose, rate of dosage form release, and meal ingestion should affect cimetidine oral bioavailability and absorption variability through their impact on luminal cimetidine concentrations in upper versus lower small intestine.

Although our earlier report indicated that cimetidine $S$-oxide appearance in the jejunal lumen was greater in rats than humans (Hui et
al., 1994), surface area to volume considerations indicate that cimetidine elimination in the human jejunum is substantial (see geometric verification in the appendix). In this regard, this mechanistic data obtained in rats should provide perspective on clinical observations with cimetidine. This data in rats suggest that intestinal elimination will contribute to a lower rate of drug delivery to the systemic circulation from the jejunum than from the ileum. Because inhibition and elimination will be greater at higher luminal concentrations, the appearance of double plasma peaks and reductions in cimetidine bioavailability would be expected to be more prominent under fasted-than fed-state administration conditions. Meal reductions in luminal drug concentrations should result from a slower drug delivery rate from the stomach and dilution effects in the fed-state as compared with fasted-state administration. Whereas double plasma level peaks are primarily a function of reduced jejunal permeability, variation in bioavailability is predominantly a function of jejunal elimination as determined by luminal drug concentration. In this regard, formulation factors that influence the release pattern of a cimetidine will also influence drug bioavailability. As supported by previous clinical data, tablet formulations of cimetidine produce greater drug plasma levels than equivalent doses in solution because luminal concentration is limited by dosage form release rate (Walkenstein et al., 1978). The fact that greater plasma level variability is observed at higher cimetidine doses is also consistent with self-inhibition of jejunal absorption (Grahnen et al., 1979; Somogyi and Gugler, 1983).

Appendix

Intestinal metabolism is a sequential process of mass transfer and enzyme reaction. Drug first diffuses from the lumen to the epithelia and is then metabolized in the epithelia with subsequent metabolite transport back into the lumen. Any of these processes or any combination of these steps could be rate limiting. Although the intestine varies in size across different species, a valid comparison of intestinal metabolism among species should consider the influence of geometric differences on the rate-limiting step. An exact mathematical description of the process is complex, however, a meaningful comparison can be projected by considering extreme cases where either cellular metabolism or luminal mass transfer is the rate limiting process. Because intestinal cimetidine metabolism occurs intracellularly, metabolism is positively related to the intestinal surface area. Assuming identical enzyme activity, membrane transport and regional distribution across species, the extent of intestinal drug metabolism can be compared in different species as follows:

1. Enzymatic reaction in the intestinal epithelia is rate limiting.

When the enzymatic reaction is rate limiting, the metabolite is generated in the epithelia at a constant rate $R$. Perfusion through an intestinal segment of length $L$, and radius, $r$, at a different flow rate, $F$, would not directly influence the metabolism but change the final volume of collected perfusate. Therefore,

$$C_{\text{metabolite}} \propto \frac{2\pi rL \cdot R}{F} \times \frac{r \cdot L}{F}$$

and the scaling factor, $j$, between rat and human is

$$j = \frac{C_{\text{rat}}}{C_{\text{human}}} = \frac{L_{\text{rat}}}{L_{\text{human}}}, \frac{r_{\text{rat}}}{r_{\text{human}}}, \frac{F_{\text{human}}}{F_{\text{rat}}}$$

2. Mass transport of drug or metabolite is rate limiting.

When mass transport is rate limiting, the extent of intestinal drug metabolism is proportional to the probability, $Q$, of a drug molecule encountering the surface in different diameter tubes, assuming even distribution of membrane transport and enzymatic metabolism. As shown in the following figure, the transit time for drug solution flow through a tube is:

$$t = \frac{L}{F} = \frac{\pi r^2 L}{F}$$

The radial diffusion time for flow from the luminal center to the wall of the tube is:

$$T \propto \frac{r^2}{F}$$

The ratio of the tube surface area to volume is:

$$f = \frac{\text{Area}}{\text{Volume}} = \frac{2\pi rL}{\pi r^2 L} = \frac{2}{r}$$

The probability, $Q$, of a drug molecule encountering the surface is positively related to $t$, $T$ inverse, and $f$ as follows.

$$Q \propto t \cdot f \cdot \frac{1}{T} \propto \frac{r \cdot r \cdot F}{r \cdot F} \propto \frac{r^2}{r^2}$$

Therefore, the scaling factor, $j$, between rat and human is

$$j = \frac{Q_{\text{human}}}{Q_{\text{rat}}} = \frac{L_{\text{rat}}}{L_{\text{human}}, \frac{r_{\text{rat}}}{r_{\text{human}}}, \frac{F_{\text{human}}}{F_{\text{rat}}}$$

In a previous report by Hui et al. (1994) comparing intestinal cimetidine metabolite production from rat versus human jejunal perfusions, the jejunal, diameter, length, and perfusion rate were 0.23 and 1.25 cm, 10 and 50 cm, 0.12 and 10 ml/min in rat and human, respectively. Using the scaling factor-derived estimates outlined above, cimetidine metabolism is projected to be 88-fold greater in rat than human when luminal mass transfer is rate limiting. When metabolism is rate limiting, cimetidine metabolism is projected to be 2-fold greater in rat than in humans. Experimentally, cimetidine metabolism was measured to be 3 and 6% of initial drug concentration over 50 cm of jejunum in two human subjects. Based on geometric considerations, the corresponding metabolism in rats would be projected to be over 100% and 9 to 18% of initial drug perfusion concentration for luminal mass transport and metabolism rate limits, respectively. Given that metabolism formation occurred at 20% in the rat in this study, metabolism limitations would appear to play a more significant role in controlling the extent of intestinal metabolism production than luminal transport differences. Given the assumptions made, these calculations also indicate that the extent of metabolism production in the human jejunum is significant and similar to that in rat jejunum. Lumenal metabolite formation results from the sequential events of luminal drug transport to the epithelial membrane, intracellular drug uptake, intracellular drug metabolism, and metabolite excretion into the lumen. Thus, the fact that luminal drug transport is not rate limiting to metabolism production is consistent with membrane control of intracellular cimetidine uptake and/or subsequent metabolism and metabolite elimination into the intestinal lumen as detailed in Results and Discussion.

References


