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Omeprazole increases permeability across isolated rat gastric mucosa pre-treated with an acid secretagogue

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Abstract

Triple therapy using proton-pump inhibitors (PPIs) in combination with oral antibiotics for the treatment of *Helicobacter pylori*-associated gastritis has shown increased efficacy for reasons that are still poorly understood. Possible explanations include a direct antibacterial effect of the PPIs or a PPI-mediated increase in bacterial susceptibility to antibiotics. Using an in-vitro model of rat gastric mucosa, we examined fluxes of a radiolabelled marker molecule through the interepithelial tight junctions under normal conditions and under the influence of an acid secretagogue (50 μM histamine) and a PPI (100 μM omeprazole). Paracellular fluxes of the radiolabel (represented by calculation of apparent permeability coefficients) were linear over 2 h. Fluxes of the marker increased significantly after treatment with histamine followed by omeprazole, but were unaltered in paired preparations exposed to the same drugs given in reverse order. Enhancements in paracellular permeability were mirrored in separate experiments using a detergent (Triton X-100), a bile salt (deoxycholate) and an agent that disrupts the cytoskeleton (cytochalasin D) to interfere with tight junctional integrity. The results suggest that exposure of acid-secreting gastric mucosa to omeprazole widens the interepithelial spacing in a manner that may facilitate enhanced macromolecular transport. Increases in antibiotic delivery from the blood to the gastric lumen via such a mechanism may account for the greater eradication rates observed with PPI-based triple therapy in *H. pylori*-associated gastritis.

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Introduction

Therapeutic regimens for the treatment of *Helicobacter*-associated gastritis have for some years relied on triple therapy using oral antibiotics and proton-pump inhibitors (PPIs) in combination with bismuth compounds (Lambert 1996). Such strategies have been highly successful, resulting in bacterial eradication rates of 90–95% for triple therapy compared with 20–40% for antibiotic monotherapy (Peterson 1997). The reasons why antibiotic therapeutic efficacy is enhanced by co-administration of acid inhibitors are, however, still poorly understood (Goddard 1998). Inhibition of gastric acid secretion alone is of benefit in patients recovering from peptic ulcer disease. For example, the PPI omeprazole has been shown to promote healing of duodenal ulcers (Prichard et al 1985a). However, pharmacological inhibition of acid secretion, on its own, fails to eliminate *Helicobacter pylori*, the microorganism firmly associated with peptic ulcer disease pathogenesis.

Thus, since *H. pylori* is still present, the simple inhibition of acid secretion is of limited therapeutic benefit in the treatment of *H. pylori*-associated gastritis.

However, the combination of omeprazole with macrolide antibiotics (such as clarithromycin) has resulted in decreased acid secretion, increased rates of bacterial eradication and increased healing (Cederbrant et al 1994). Potential mechanisms of PPI/antibiotic synergism have been reviewed by Tytgat (1996) and Peterson (1997). These highlight the capacity of omeprazole to directly inhibit bacterial survival at low pH (McGowan et al 1994), and to enhance the activity and stability of antibiotics (Cederbrant et al 1994). In addition, bacterial survival is significantly impaired at the higher pH environments created by treatment with compounds such as omeprazole (Clyne et al 1995).

An alternative perspective underlying PPI/antibiotic synergism can also be considered in terms of altered capacity for drug delivery. It is interesting that antibiotics target gastric lumenal-dwelling *H. pylori* when antibiotic administration is systemic (Goddard et al 1996), and considering that orally administered antibiotics are absorbed in the small intestine and recirculated in the plasma, providing a reservoir from which the drug is secreted into the gastric lumen (Cederbrant et al 1994). The hypothesis that PPIs may alter permeability or transport characteristics across gastric mucosa has, for the most part, been overlooked, but may play a role in delivering systemic antibiotics to their site of action within the lumenal compartment of the *H. pylori*-infected stomach. Furthermore, there is also a possibility that antibiotic pharmacokinetics may be altered by concomitant PPI therapy. For example, omeprazole has been shown to increase intragastric concentrations of intravenously administered amoxycillin, partly by reducing gastric juice volume (Goddard et al 1996). It has also been proposed that omeprazole can reduce gastric juice viscosity and impair mucus barrier function, thus facilitating antibiotic delivery to the site of bacterial colonization (Goddard and Spiller 1996).

The specific aims of this study were to examine whether the PPI omeprazole altered transgastric permeability to an inert marker molecule, mannitol, via a mechanism that could in turn account for enhanced permeability to antibiotics. Permeability was quantified using an arithmetic relationship routinely applied to drug delivery models (Artursson et al 1996). Histamine and omeprazole were used as pharmacological tools with which to stimulate and inhibit acid secretion, respectively. We examined alterations in transport characteristics in response to these alterations in acid secretory status. In separate experiments, we used agents known to disrupt epithelial integrity as positive controls with which to evaluate transport characteristics across pharmacologically compromised epithelia.

Materials and Methods

Rats were bred, housed and maintained under proper veterinary care and management in the Biomedical Centre, University College Dublin. No procedures, other than normal care, were administered before euthanasia. D-[^{14}C]Mannitol was purchased from Amersham International (UK), Caco-2 cells were from the American Type Culture Collection (Rockville, MD) and omeprazole was a kind gift from Astra Laboratories Inc. Ecoscint-A was obtained from National Diagnostics (Atlanta, GA), cell culture materials were from Gibco (UK), and all other drugs were purchased from Sigma Chemical Co. (UK) or British Drug House (BDH, UK). Ussing chambers, DVC 1000 voltage-clamp apparatus and Endohm appliances were purchased from World Precision Instruments (UK). Data acquisition and analysis were performed using a MacLab recording system (AD Instruments, UK).

Rats were killed by stunning followed by decapitation. The stomachs were immediately removed and dissected free of smooth muscle by a blistering technique. Tissues from opposite sides of the corpus, used as matched experimental pairs, were mounted on Ussing chambers under standard voltage-clamp conditions. Tissues were bathed with oxygenated physiological solution with the following composition (mM): NaCl (113), KCl (4.7), KH_2PO_4 (1.2), $MgSO_4.7H_2O$ (1.2), $CaCl_2.2H_2O$ (1.9), $NaHCO_3$ (25) and glucose (12.1). Bathing solutions were maintained at 37°C, gassed and circulated by bubbling with O_2 (95%) and CO_2 (5%). Electrical short circuit current (SCC; reflecting ion transport) and transepithelial electrical resistance (TER; an index of membrane integrity) were continuously monitored. Following stabilization of tissue basal electrical parameters, 0.5 μCi ^{14}C-mannitol was added to the basolateral compartment at time zero. Apical and basolateral samples were removed at 20-min intervals over 120 min. In separate experiments, histamine (50 μM) and omeprazole (100 μM) were each added basolaterally, with the concentrations chosen based on previously published studies (Main & Pearce 1978; Welsh et al 1993). Triton X-100 (0.05%) and deoxycholate (1.2 mM) were applied apically, and cytochalasin D (1 μg mL^{-1}) was applied to both compartments. Equivalent sample volumes were replaced each time with fresh physiological fluid, and the ^{14}C-mannitol content of each sample was determined

by liquid scintillation spectrometry (LKB Wallac 1217 Rackbeta; Finland). Gastric mucosal permeability to mannitol in the secretory direction was then calculated as apparent permeability coefficients (P_{app}) according to the following relationship (Artursson et al 1996):

$$P_{app}\ (cm\ s^{-1}) = (k \times V_R)/(A \times 60)$$

where k is the slope of the graph of time versus cumulative appearance of mannitol in the receiving (luminal) compartment; V_R is the volume (mL) in the receiving compartment; A is the tissue area (cm^2); and 60 is the conversion from minutes to seconds.

To examine whether histamine and omeprazole could influence permeability in model epithelia lacking acid secretory functions, we cultured Caco-2 human colonic enterocytes in Dulbecco's modified Eagle's medium and Glutamax-l, with 10% foetal calf serum, 100 μg mL^{-1} L-glutamine and 100 U mL^{-1} penicillin/streptomycin (Gibco, Long Island, NY). The cells were then seeded at a density of 5×10^5 cells cm^{-2} on optically clear permeable polyester supports with 0.4-μm pore size (Costar Transwell clear; Cambridge, MA). TER and membrane potentials were monitored daily. On reaching confluence, cells were washed and transferred into Hank's balanced salt solution containing 20 mM HEPES. Then, 0.5 μCi ^{14}C-mannitol was added basolaterally at t = 0 min, and samples taken from apical and basolateral compartments (with continuous orbital shaking at 37°C) over 120 min. TER and transmembrane voltages were recorded and the Ohmic relationship was used to estimate SCC.

Paired preparations of stripped rat gastric mucosa were voltage-clamped in Ussing chambers as for the flux studies, and baseline SCC recordings allowed to equilibrate. Following the basolateral addition of histamine and omeprazole at time intervals identical to those used during the flux studies, alterations in SCC were representative of energy-dependent changes in electrogenic ion transport mediated by the drugs.

Statistical analysis

Results are expressed as mean ± s.d. Data were compared using non-parametric two-tailed Mann Whitney tests. For statistical analysis of TER experiments, initial and final TER values are compared over a 2-h period within each tissue/monolayer. In mannitol flux experiments, statistical differences are based on comparison between P_{app} values in control versus treated tissues for each stated time period.

Results

Secretory fluxes of ^{14}C-mannitol were linear over 120 min in isolated preparations of rat gastric mucosa. The P_{app} for ^{14}C-mannitol transfer was 3.8 ± 2.2 cm s^{-1}

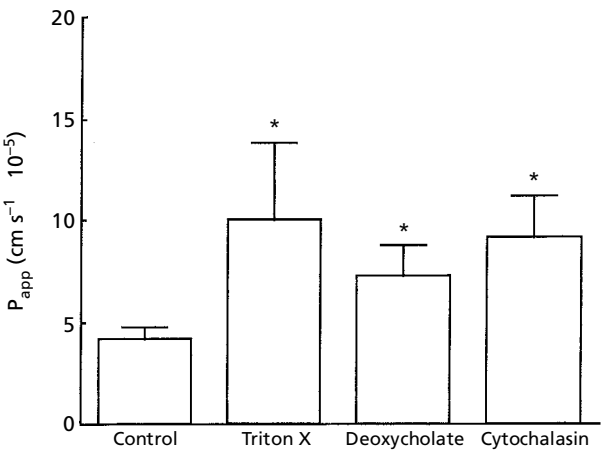

Figure 1 Serosal to mucosal permeability to ^{14}C-mannitol in the presence of agents disrupting membrane integrity. The apparent permeability coefficients (P_{app}) for mannitol flux across rat gastric mucosa were significantly increased over control levels (n = 11) following pharmacological disruption of tissue integrity with either Triton X-100 (0.05%; $*P < 0.05$, n = 5), 1.2 mM deoxycholate (1.2 mM; $*P < 0.05$, n = 7) or cytochalasin D (1 μg mL^{-1}; $*P < 0.05$, n = 6).

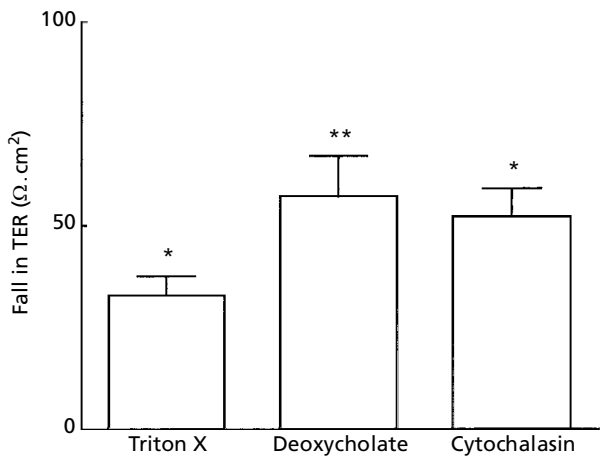

Figure 2 Transepithelial resistances (TER) during membrane disruption. TER fell following exposure to deoxycholate (1.2 mM; n = 7), Triton-X 100 (0.05%; n = 5) and cytochalasin D (1 μg mL^{-1}; n = 6). These reflected overall deficits in epithelial integrity and mirrored mannitol flux results demonstrating an enhancement of macromolecular permeability ($*P < 0.05$; $**P < 0.01$).

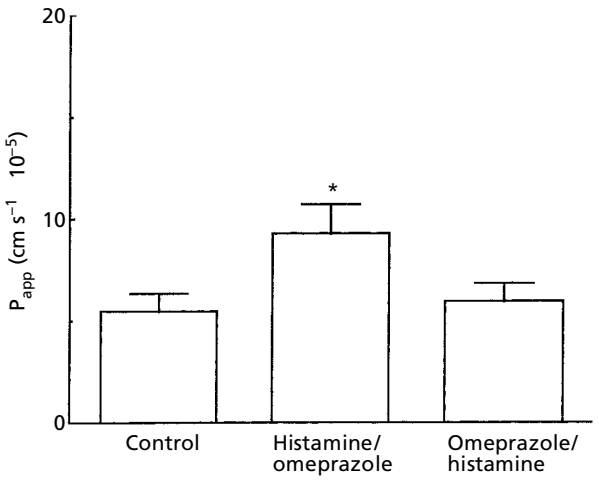

Figure 3 Effects of histamine and omeprazole in combination on ^{14}C-mannitol permeability in rat gastric mucosa. Serosal histamine (50 μM, present for 40 min) followed by serosal omeprazole (100 μM, present for 40 min) caused a significant increase in mannitol flux (*$P < 0.05$, n = 9) with respect to control, untreated tissues (n = 11). In contrast, omeprazole followed by histamine did not alter mannitol permeability (n = 10) over control P_{app} values.

$\times 10^{-5}$ (n = 36). TER (a measure of epithelial integrity) was $38 \pm 18\ \Omega\ cm^2$ (n = 33). Baseline SCC was $83 \pm 29\ \mu A\ cm^{-2}$ (n = 9).

As shown in Figure 1, exposure of rat gastric mucosa to Triton X-100 (0.05%), deoxycholic acid (1.2 mM) or cytochalasin D (1 $\mu g\ mL^{-1}$) significantly increased ^{14}C-mannitol permeability over control levels. TER also decreased significantly compared with control levels following exposure of the gastric mucosa to these agents (Figure 2). After 2 h, TER in control tissues (n = 11) dropped to only 74% of initial TER. In corresponding preparations treated with Triton X-100 (n = 5), deoxycholate (n = 7) or cytochalasin D (n = 6), final TER values were 34, 58 and 53% of initial TER values in each category, respectively. Neither histamine nor omeprazole alone altered TER or permeability to ^{14}C-mannitol. Incubation of rat gastric mucosa with histamine (50 μM) alone for 40 min did not change tissue permeability to ^{14}C-mannitol ($P_{app} = 4.6 \pm 2.6\ cm\ s^{-1} \times 10^{-5}$, n = 11 controls; $6.3 \pm 3.3\ cm\ s^{-1} \times 10^{-5}$, n = 9 with histamine). Similarly, ^{14}C-mannitol permeability in identical preparations was not affected compared with control levels by a 40-min incubation with omeprazole (100 μM) alone ($P_{app} = 4.5 \pm 2.2\ cm\ s^{-1} \times 10^{-5}$; n = 10).

Two test protocols were employed to investigate the combined effects of the acid secretagogue and acid inhibitor on permeability in rat isolated gastric mucosa (Figure 3). Exposure to histamine (50 μM; 40 min) followed by omeprazole (l00 μM; 40 min) produced a significant increase in ^{14}C-mannitol permeability ($P_{app} = 9.3 \pm 4.2\ cm\ s^{-1} \times 10^{-5}$, $P < 0.05$, n = 9). In contrast, exposure to omeprazole followed by histamine did not affect permeability to ^{14}C-mannitol ($P_{app} = 6.0 \pm 1.8\ cm\ s^{-1} \times 10^{-5}$, n = 10).

That enhancement of permeability depended on application of histamine followed by omeprazole was confirmed by experiments in which gastric mucosal tissues were exposed solely to histamine for extended

Table 1 Electrical parameters in rat gastric mucosa.

	Histamine/omeprazole (n = 9)	Omeprazole/histamine (n = 10)
Transepithelial resistance ($\Omega\ cm^2$)	33 ± 15	35 ± 14
Short circuit current ($\mu A\ cm^{-2}$)	86 ± 21	95 ± 31

There was no significant difference in TER of the gastric mucosa following addition of drugs in either order (histamine followed by omeprazole or omeprazole followed by histamine). Baseline short circuit currents were also similar in both types of preparation.

Table 2 Electrical parameters of Caco-2 monolayers treated with histamine followed by omeprazole.

	Control (n = 4)	Histamine/omeprazole (n = 4)
Transepithelial resistance ($\Omega\ cm^2$)	663 ± 63	703 ± 48
Short circuit current ($\mu A\ cm^{-2}$)	1.3 ± 3.4	0.8 ± 1.4

There was no significant change in TER of Caco-2 monolayers following treatment with histamine/omeprazole, with respect to control values. Similarly, SCC values did not vary between treated and control monolayers.

periods of time. In these experiments, there was no difference in permeability when compared with untreated controls. Sequential treatment with either histamine/omeprazole or omeprazole/histamine did not alter electrical characteristics of rat gastric mucosa (Table 1). Thus, alterations in permeability could not be explained simply by drug-induced alterations in electrogenic ion transport rather than by a direct effect on epithelial intercellular junctions.

We used a cultured epithelial cell model (Caco-2 monolayers) to examine whether transepithelial permeability of this non-acid-secreting model was sensitive to histamine/omeprazole exposure in the same manner as acid-secreting gastric mucosa. Results obtained for Caco-2 monolayers exposed to an experimental design identical to that used for rat gastric mucosa revealed no enhancement in permeability (Table 2). These data exclude a direct action of the drug combination on epithelial permeability in non-acid-secreting epithelia.

Discussion

Under voltage-clamp conditions and in the absence of electrical, chemical, osmotic or hydraulic gradients, serosal to mucosal permeability to the marker molecule, mannitol, was determined (Artursson et al 1996) and was linear over time. TER and basal SCC values were stable over the time course of the experiments and were in keeping with magnitudes previously reported (Curtis & Gall 1992). That the permeability of our in-vitro model could be pharmacologically enhanced was demonstrated in a number of ways. The detergent, Triton X-100, as well as the unconjugated bile salt, deoxycholic acid, increased P_{app} by direct detergent actions on the membrane (Baird & Cuthbert 1985).

Cytochalasin D also increased macromolecular permeability compared with levels in control, untreated tissues, probably through a rearrangement of cytoskeletal elements (Madara et al 1986). These increases in macromolecular permeability were accompanied in each case by a concomitant decrease in TER, although changes in electrical resistance are not always mirrored by altered permeability to macromolecules (Balda et al 1996).

Histamine, which stimulates acid secretion in rat isolated gastric mucosa (Main & Pearce 1978) had no effect on mannitol flux or TER. Similarly, the PPI omeprazole, which reduces basal as well as stimulated gastric acid secretion in rats, dogs (Larsson et al 1983) and humans (Prichard et al 1985b), did not alter mannitol flux or TER. Baseline electrical measurements were in keeping with magnitudes reported by others (Curtis & Gall 1992). In contrast, histamine followed by omeprazole caused a significant increase in mannitol flux, whereas omeprazole followed by histamine caused no measurable difference. This indicates that proton pump inhibition can positively influence epithelial permeability in this model, but only following pre-exposure to an acid secretagogue. Should the same principles apply to gastritis in-vivo, suppression of an overactive proton pump by PPIs might facilitate the secretion or diffusion of antibiotics from the blood to the gastric lumen. This would facilitate access to the site of bacterial colonization in *H. pylori*-associated gastritis. Thus, an important factor in the synergism between PPIs and oral antibiotics could be a transient alteration in gastric permeability to macromolecules.

The effects of omeprazole on the kinetics of antibiotic delivery into human gastric juice, plasma and saliva have been examined in a related approach (Jessa et al 1997). Although omeprazole had minimal impact on the plasma and salivary concentrations of antibiotics administered systemically or orally to healthy volunteers, concentrations in gastric juice were significantly reduced, suggesting increased transepithelial delivery of antibiotics (Jessa et al 1997). Since the patient group had no reported alterations in acid secretory status, it could therefore be speculated that the normal acid environment of the stomach is sufficient to activate the pro-drug omeprazole to its active form. However, if increases in antibiotic delivery can be accounted for by omeprazole-induced enhancement in permeability, our results suggest that stimulation of acid secretion is important in creating a microenvironment conducive to such enhancement. There is evidence in several studies that *H. pylori* eradication rates are greater in patients with duodenal ulcer compared with non-ulcer dyspepsia (Huang & Hunt 1998a, b). This is perhaps explained by the fact that patients with duodenal ulcer exhibit higher acid secretion than patients with non-ulcer dyspepsia. Acid inhibition, particularly with PPIs, has been proven as the most effective way to heal ulcer symptoms in combination with antibiotics (Huang & Hunt 2001). Therefore, under such conditions, a contribution of omeprazole to the enhancement of transepithelial antibiotic transport, as well as acid inhibition, cannot be discounted. *H. pylori*-induced antral gastritis is associated with a 6-fold increase in acid secretion and, under such conditions, antibiotics might be more effective in eradicating *H. pylori* in combination with a PPI. *H. pylori*, by secreting ammonia locally, changes the pH below the mucous layer resulting in increased gastrin secretion, which in turn results in more acid being

secreted (McColl et al 2000). In addition, *H. pylori* might be selectively toxic to cells that secrete somatostatin. A decrease in somatostatin will result in increased gastrin secretion being unopposed and more acid being secreted (Calam 1995).

That histamine followed by omeprazole influenced P_{app} could not be accounted for by separate pharmacological actions of each of the drugs. We assessed the possibility that histamine had a delayed effect on the tissues. This was not the case, since prolonged incubation with histamine alone failed to alter permeability. It was thus a necessary condition that omeprazole was added after histamine in order to produce an increase in mannitol flux through rat gastric mucosa.

We turned to a model epithelial monolayer, widely used in drug transport studies, to address whether the influence of co-treatment with histamine and omeprazole produced altered transepithelial permeability in a non-acid-secreting tissue. Fluxes of ^{14}C- mannitol across Caco-2 human intestinal epithelial cells were not enhanced by histamine/omeprazole treatment under the experimental conditions that were established as effective in rat gastric mucosa. Therefore, we speculate that the ability of histamine/omeprazole to alter transepithelial permeability is intrinsically linked to alterations in acid secretory status, and is unlikely to be accounted for by a direct action on the epithelial cells under non-acid-secreting conditions.

Increases in macromolecular permeability caused by histamine/omeprazole in rat gastric mucosa were not mirrored by decreases in TER. Electrogenic ion transport did not differ in response to either combination of secretagogues, suggesting that the capacity of histamine followed by omeprazole to increase paracellular permeability was also independent of rapid alterations in ion transport characteristics. Similarly, in the human epithelial cell model, TER and SCC did not change with respect to control following treatment with histamine/omeprazole.

The observation that omeprazole enhanced macromolecular permeability in histamine-treated stomachs is interesting in terms of drug delivery across a gastric mucosa which harbours *H. pylori*. There is abundant evidence that *H. pylori* infection increases both transcellular (Matysiak-Budnik et al 2001) and also paracellular movement of macromolecules (Terres et al 1998). Thus, bacterial infection by itself could facilitate increased transepithelial delivery of antibiotics. The inflammation status of the patient must also be considered, since inflammation-induced alterations in the mucus layer and epithelium may positively influence antibiotic permeation (Spiller 1999). Antibiotic formulations also play an important role in the struggle to eliminate *H. pylori*. Chitosan microspheres containing either amoxycillin or metronidazole were found to penetrate easily through the gastric mucin layer (Shah et al 1999), facilitating access to the bacterial "sanctuary site" between the mucus and the epithelium. Until more is understood about the pharmacokinetics of antibiotic delivery across the gastric epithelium, it cannot be excluded that PPIs have a direct effect on epithelial permeability that is secondary to their suppression of acid secretion.

Conclusions

In conclusion, these data present novel evidence that the PPI omeprazole in conjunction with an acid secretagogue, can increase gastric epithelial permeability to an inert macromolecule. Altered fluxes through the tight junctions were quantitatively similar to those induced pharmacologically by a bile salt, a detergent and an agent that disrupts the F-actin cytoskeleton. Overall, multiple phenomena may account for the success of PPI-based combination therapy in *H. pylori*-associated gastritis (Peterson 1997). Our model shows that omeprazole-mediated enhancement in permeability of acid-secreting gastric mucosa can alter paracellular permeability to macromolecules. Therefore, we speculate that a physical effect of omeprazole on epithelial permeability (secondary to its ability to suppress acid secretion) may contribute to synergism between oral antibiotics and PPIs by facilitating antibiotic drug delivery to the site of bacterial colonization. Despite obvious molecular weight and structural differences between mannitol and antibiotics such as clarithromycin, the ability of clarithromycin to diffuse readily into numerous mucosal compartments (Rodvold 1999) may not preclude extrapolation from our mannitol flux studies to antibiotic delivery in-vivo. An interesting recent study has also shown that antibiotic penetration into rat gastric mucosa is enhanced when gastric pH is increased by co-administration of the antibiotic with lansoprazole (Endo et al 2001). Additionally, there is abundant evidence that *H. pylori* eradication is more successful in patients with duodenal ulcer compared with non-ulcer dyspepsia (Huang & Hunt 1998a, b), which may relate to higher levels of acid secretion in the former. Nonetheless, our model suggests the potential value and therapeutic significance of looking beyond the traditional role of PPIs as simply acid inhibitors, and further exploring the possibility that they exert a direct influence on transepithelial permeability.

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