Concentration-Dependent Effects of Polyethylene Glycol 400 on Gastrointestinal Transit and Drug Absorption

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Purpose. The aim of the study was to investigate the effect of different concentrations of polyethylene glycol 400 (PEG 400) on liquid transit through, and ranitidine absorption from, the gastrointestinal tract.

Methods. Six healthy male volunteers received, on four separate occasions, 150 mL water containing 150 mg ranitidine and either 0 (control), 1, 2.5, or 5 g PEG 400. The solutions were radiolabeled with technetium-99m to allow their gastrointestinal transit to be followed using a gamma camera. Urine samples were collected over a 24-h period to assess the amount of ranitidine excreted and hence absorbed.

Results. No significant differences in gastric emptying were noted between the four solutions. In contrast, the presence of 1, 2.5, and 5 g PEG 400 reduced the mean small intestinal transit times of the solutions by 9, 20, and 23%, respectively, against the control. In terms of drug absorption, the mean cumulative amount of ranitidine excreted was reduced by 38% in the presence of both 2.5 and 5 g PEG 400, although it was significantly increased by 41% in the presence of 1 g PEG 400.

Conclusions. The results show that low concentrations of PEG 400 enhance the absorption of ranitidine possibly via modulation of intestinal permeability, while high concentrations have a detrimental effect on ranitidine absorption presumably via a reduction in the small intestinal transit time.

KEY WORDS: polyethylene glycol 400; cosolvent; gamma scintigraphy; ranitidine; paracellular pathway; tight junctions; permeability enhancement, bioavailability.

INTRODUCTION

Solubility in the gastrointestinal fluids is a major factor limiting the oral bioavailability of therapeutic agents. Many drugs are poorly water-soluble and hence comprise a challenge in the formulation of bioavailable dosage forms. An increase in drug solubility can be achieved by the addition of solubilizing agents such as cosolvents into the formulation. Polyethylene glycol 400 (PEG 400), a mixture of oxyethylene glycol polymers with an average molecular weight of 400, is one such commonly used cosolvent. Although PEG 400 has proven solubility-enhancing benefits, recent work has shown that it has a stimulatory effect on gastrointestinal motility and transit, which calls into question the inert nature of this pharmaceutical excipient (1,2). Specifically, 10 g of PEG 400 reduced the mean small intestinal transit time of a liquid formulation in 10 healthy volunteers by 37% (1). Since the small intestine is the primary site of drug absorption, a reduction in contact time with this region of the gastrointestinal tract is therefore likely to impact on the rate and extent of drug absorption. This was confirmed in a follow-up study where it was found that the presence of 10 g PEG 400 caused not only a 35% reduction in the small intestinal transit time, but also a 31% decrease in the oral bioavailability of the H_2-receptor antagonist ranitidine (2). As a small, hydrophilic molecule, ranitidine is believed to be absorbed across the gastrointestinal mucosa via the paracellular route (3) and has been classified as a class III compound (high solubility, low permeability) of the Biopharmaceutics Classification System (4). The absorption of low permeability drugs, such as ranitidine, is likely to be most susceptible to changes in intestinal residence time (5), because their transport across the gastrointestinal mucosa is by definition very slow. Moreover, previous studies in humans have shown marked reductions in oral bioavailability of the H_2-receptor antagonists as a result of accelerated small intestinal transit induced by concurrent administration of the pharmaceutical excipients sodium acid pyrophosphate (6) and mannitol (7).

Although the use of cosolvents such as PEG 400 at amounts as high as 10 g per single dose may be common in early drug development, commercial formulations such as solutions, and soft gelatin capsules usually contain lower doses of these solubilizing agents. Therefore, this study was conducted to investigate the effect of lower quantities of PEG 400 (1, 2.5, and 5 g) on small intestinal transit and their effects on the absorption of the model drug ranitidine.

MATERIALS AND METHODS

Dosage Forms

The solution formulations consisted of 150 mL of water containing 168 mg ranitidine hydrochloride (Glaxo SmithKline, Ware, UK), equivalent to 150 mg ranitidine base, and either 0, 1, 2.5 or 5 g of PEG 400 (Sigma-Aldrich Company, Poole, UK). Each solution was also radiolabeled with technetium-99m (^{99m}Tc) to an activity of 7 MBq using ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) solution. Osmolality measurements indicated that the solutions were all hypotonic in nature: 0 g PEG 400 (4 mOsm kg^{-1}), 1 g PEG 400 (25 mOsm kg^{-1}), 2.5 g PEG 400 (50 mOsm kg^{-1}), 5 g PEG 400 (102 mOsm kg^{-1}).

Study Protocol

Six healthy male volunteers (age range 21–34 years, median 28 years; weight range 58–98 kg, median 83 kg; height range 1.65–1.87 m, median 1.78 m) participated in an open four-way crossover study after providing written informed consent. All volunteers were non-smokers, were not taking any medication and had no history of gastrointestinal disease.

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The Joint UCL/UCLH Committees on the Ethics of Human Research approved the experimental protocol and the authority to administer radiopharmaceuticals was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC) at the Department of Health. The study was conducted in accordance with the provisions of the Declaration of Helsinki (1964) and its subsequent revisions. The volunteers reported to the study center on the morning of the study, after adherence to an overnight fast. Each volunteer received, on four separate occasions, 150 mL of drug solution containing 0, 1, 2.5 or 5 g PEG 400. To act as anatomic reference markers two small sealed point sources of 0.5 MBq ^{99m}Tc were taped to the abdominal skin at the position of the lower coastal margin at each side. Imaging was performed using a double-headed gamma camera (Maxxus, General Electric Medical Systems, Milwaukee, USA) fitted with two opposed detectors, each having a 508 × 368 mm useful field of view and capable of simultaneous data acquisition. Each detector was fitted with a low energy high-resolution parallel hole collimator suitable for ^{99m}Tc imaging. After administration of the preparations, the volunteer was positioned supine between the two detectors of the gamma camera. Simultaneous anterior and posterior images of 30 s duration were initially acquired every 5 min and then at 10–15 min intervals after the liquid had emptied from the stomach. In between image acquisitions, the volunteer was free to move away from the camera. A standard lunch was provided 4 h post dose, and water was available *ad libitum* from this point onwards. Images were digitally recorded using an integrated computer system (Starcam 3200i, General Electric Medical Systems, Milwaukee, USA) and archived onto optical disc for subsequent analysis. In addition to imaging, cumulative urine samples were collected throughout the course of the study comprising the collection and measurement of bladder output over the following time periods: 0 (pre-dose), 0 to 2, 2 to 4, 4 to 6, 6 to 12, and 12 to 24 h, and the retention and storage of a 20 mL aliquot at –20°C.

Scintigraphic Data Analysis

Processing of image data was performed using a Hermes image processing workstation (Nuclear Diagnostics, Stockholm, Sweden) as previously described in detail (1,2). Briefly, regions of interest were drawn highlighting the stomach, cecum/colon, and anatomic markers. The full sequence of images acquired for each volunteer was corrected for volunteer movement, background radiation, and physical decay of ^{99m}Tc. To account for the differential attenuation of the radiation with varying depth of source the geometric mean of the anterior and posterior counts within the regions of interest was calculated (8). Finally, the geometric mean counts were expressed as percentages of the total counts recorded when all the administered activity was in the stomach or in the cecum/colon for gastric emptying and cecum/colon arrival, respectively, which were plotted as percentage activity in these regions vs. time.

The gastrointestinal transit data were quantitatively assessed using statistical moments to calculate the mean gastric residence time (MGRT) and mean cecum arrival time (MCAT) (9). The difference between the MGRT and MCAT provides a measure of the mean small intestinal transit time (MSITT).

Urine Analysis

After thawing at room temperature and thorough vortex mixing, the urine samples and calibration standards were prepared for ranitidine analysis by diluting 20 μL of sample with 780 μL of a solution consisting of 50% water adjusted to pH 10 with ammonium solution (solvent A) and 50% methanol (solvent B) with sotalol present at 500 μg/mL as an internal standard. Samples requiring dilution to fall into the calibration range were diluted using blank human urine.

The urine samples were assayed for ranitidine content using a validated HPLC-mass spectrometry (MS) method. The HPLC-MS analysis used a Hewlett Packard Series 1100 chromatography system with a CTC PAL autosampler. Aliquots of sample (10 μL) were injected on a 50-mm × 2-mm Luna column (5 μm C18(2); Phenomenex) at 40°C. A binary solvent gradient system was used and the flow rate of the mobile phase was set to 0.8 mL/min. The column was initially equilibrated with 95% solvent A and 5% solvent B. Immediately after sample injection, the concentration of B was linearly increased over 3 min to a concentration of 60% and then reduced to the initial concentration of 5% in the next 0.5 min followed by a 1 min equilibration time before the next sample injection. Subsequent detection of ranitidine was performed via selected reaction monitoring using a Sciex API 3+ mass spectrometer with a turboionspray source at 500°C. Mass detection of ranitidine (*m/z* 315) and the internal standard sotalol (*m/z* 273) was performed in positive single-ion monitoring mode of the corresponding daughter ions (*m/z* 176 and 133, respectively). The retention times for ranitidine and sotalol were 2.40 and 1.25 min, respectively.

Statistical Analysis

A paired Student's *t* test was performed on the scintigraphic and pharmacokinetic data to assess the effect of PEG 400 on gastrointestinal transit and ranitidine absorption.

RESULTS AND DISCUSSION

Gastrointestinal Transit

The data for the gastrointestinal transit of the solutions containing 0, 1, 2.5, and 5 g PEG 400 are presented for each individual in Table I, Table II, and Table III. After oral administration the solutions were observed to empty rapidly

Table I. Concentration-Dependent Effects of PEG 400 on Gastric Emptying of the Solutions

	Mean gastric residence time (MGRT) (min)			
Volunteer	Control	1 g PEG 400	2.5 g PEG 400	5 g PEG 400
1	14	13	12	14
2	7	9	7	9
3	9	15	81	10
4	13	9	16	12
5	13	8	12	13
6	19	7	8	10
Mean	13	10	23	11
SD	4	3	29	2
P value		0.400	0.454	0.504

Table II. Concentration-Dependent Effects of PEG 400 on Cecum Arrival of the Solutions

	Mean cecum arrival time (MCAT) (min)			
Volunteer	Control	1 g PEG 400	2.5 g PEG 400	5 g PEG 400
1	305	238	254	263
2	249	279	206	227
3	292	310	313	198
4	212	280	221	156
5	301	210	239	282
6	505	364	339	319
Mean	311	280	262	241
SD	102	54	53	59
P value		0.399	0.133	0.042

from the stomach with no significant difference noted between the treatments (see Table I). On one occasion, however, an unusually long mean gastric residence time (MGRT) of 81 min was obtained for volunteer 3. Although gastric emptying is known to be variable and subject to a variety of factors, the emptying pattern observed for this subject on this particular day appeared to be atypical, since his MGRTs on earlier and later study days proved to be normal and similar to those observed in the other volunteers.

Considerable differences in the mean cecum arrival times (MCATs) were found between the treatments (see Table II). These are the result of marked changes in the mean small intestinal transit times (MSITTs) (see Table III). Transit of the different formulations through the small intestine follows a distinct trend, with the presence of increasing quantities of PEG 400 resulting in decreasing solution transit times. The mean MSITTs for the solutions containing 0, 1, 2.5, and 5 g PEG 400 were 298, 270, 239, and 230 min, respectively. This corresponds to a reduction in the mean MSITT of 9, 20, and 23%, respectively, relative to the control. Although the decrease in the mean MSITT for the 1 g PEG 400 administration did not prove to be statistically significant, the overall trend is indicative of a dose-related effect on intestinal transit. This transit effect is likely to be a consequence of the poor absorption of PEG 400 from the gastrointestinal tract (10). After ingestion, most of the PEG 400 dose remains unabsorbed within the lumen of the gastrointestinal tract and is ultimately excreted unchanged in the feces. PEG 400, being osmotically active, will therefore retain water in the lumen of the gut to attain iso-osmotic conditions. This retention of water will lead to an increase in luminal fluid volume, which in turn stimulates gut motility and, hence, accelerates passage through the small intestine.

The present transit results are in general agreement to the previously obtained data for liquid transit in the presence of 10 g PEG 400, which highlighted a 35–37% reduction in the mean MSITT (1,2). However, the relationship between the amount of PEG 400 present and the observed decrease in the MSITT does not appear to be linear. A 2-fold increase in PEG 400 concentration would perhaps have been expected to result in an approximately 2-fold reduction in the MSITT. Although this may have been due in part to natural inter and intra subject variability in transit, the current results, nevertheless, demonstrate the existence of a concentration-dependent effect of PEG 400 on small intestinal transit.

Drug Absorption

The extent of ranitidine absorption in each individual, as assessed by the cumulative amount of unchanged ranitidine excreted in urine over 24 h, is presented in Table IV. On the control day, the average recovery of ranitidine was 23% of the administered dose, which is in good agreement with literature values (11). In the presence of PEG 400, one would expect the ranitidine absorption results to follow the transit pattern, with increasing concentrations of PEG 400 decreasing not only the small intestinal transit time but also the extent of ranitidine absorption. This was only true, however, for the 2.5 and 5 g PEG 400 treatments, with both showing 38% reductions in the mean cumulative amount of drug excreted, although these were not statistically significant reductions due to the large inter subject variability. The lack of a clear relationship between the reduction in the MSITT and drug excretion in some volunteers is suggestive of additional factors being responsible for changes in ranitidine absorption in the presence of PEG 400. A further possibility may be related to the poorly absorbable and osmotically active nature of PEG 400, which after administration will retain fluid in the lumen of the gut. This increased fluid load will decrease not only the concentration of the drug present in solution but also the concentration gradient across the mucosa, which may further hinder ranitidine absorption. Along similar lines, a previous study in humans has shown that the absorption of a series of com-

Table III. Concentration-Dependent Effects of PEG 400 on Small Intestinal Transit of the Solutions

	Mean small intestinal transit time (MSITT) (min)			
Volunteer	Control	1 g PEG 400	2.5 g PEG 400	5 g PEG 400
1	291	225	242	249
2	242	270	199	218
3	283	295	232	188
4	199	271	205	144
5	288	202	227	269
6	486	357	330	309
Mean	298	270	239	230
SD	99	54	47	59
P value		0.412	0.042	0.037

Table IV. Concentration-Dependent Effects of PEG 400 on Ranitidine Absorption

	Cumulative amount of ranitidine excreted in 24 hours (mg)			
Volunteer	Control	1 g PEG 400	2.5 g PEG 400	5 g PEG 400
1	29	46	29	27
2	31	46	19	25
3	25	40	14	17
4	41	52	23	23
5	15	35	20	16
6	62	68	18	18
Mean	34	48	21	21
SD	16	11	5	5
P value		0.001	0.116	0.117

pounds was adversely influenced by the presence of a non-absorbable osmotic load in the gut (12). Therefore, in addition to accelerated small intestinal transit, trans-mucosal fluid fluxes may also play a role in the reduced absorption of ranitidine in the presence of 2.5 and 5 g quantities of PEG 400.

Surprisingly, the presence of 1 g PEG 400 had an effect in the opposite direction, as the mean extent of ranitidine absorption was significantly increased by 41%. Enhanced absorption was observed in each individual. The presence of low concentrations of PEG 400 may therefore increase the permeability of the gastrointestinal epithelium. The intestinal mucosa normally provides a natural barrier to the absorption of xenobiotics. Such mucosal barriers include the intestinal efflux transporter P-glycoprotein, which expels absorbed drug back into the lumen of the intestine, and the P450 class of drug metabolizing enzymes. PEG 300, a lower molecular weight analogue of PEG 400, was found to inhibit P-glycoprotein in Caco-2 cells, at very high concentrations (13). The impact of PEG 400 on P-glycoprotein efflux and P450 3A metabolism was investigated in excised rat intestine (14), and the authors reported a dose-dependent inhibition of both enzymes. Such an inhibiting effect with PEG 400 is unlikely to majorly impact on ranitidine transport, since ranitidine, although a reported substrate for P-glycoprotein (15,16), has been shown to be mainly absorbed via paracellular pathways and not to enter cells, or be subjected to first-pass metabolism to a significant extent (3,17,18). As an alternative explanation, PEG 400 may be increasing the permeability of the gastrointestinal epithelium via an affect on the paracellular pathway. Such effects have been shown to occur *in vitro* with certain pharmaceutical excipients for purposes of penetration enhancement (19). Moreover, specific chemicals have recently been shown to interact with tight junctional proteins such as occludin in cell lines and human tissues (20–22). This interaction resulted in disruption of the tight junctions and an increase in paracellular permeability. PEG 400, like ranitidine, traverses the epithelial membrane primarily via tight junctions (23) and in doing so could interact with a component of the tight junction structure to open up the paracellular pathway and hence enhance the absorption of ranitidine.

A recent study using Caco-2 cell monolayers reported that H_2-receptor antagonists themselves inhibit their own transport across the epithelial lining (24). It was suggested that ranitidine causes a tightening of the paracellular pathway by modulating interactions among tight junctional proteins, hence contributing to its poor permeability and limited bioavailability in man. Using this information as a basis, it is possible that the absorption-enhancing effect of PEG 400 could also be via an inhibition of the interaction between ranitidine and the tight junctional proteins, thereby increasing both tight junction and paracellular permeability. Contrary to this, a separate Caco-2 study has shown that PEG 400 neither alters the integrity of tight junctions nor the transport of ranitidine (25). It should be appreciated, however, that *in vitro* cell culture systems do not always resemble or accurately predict *in vivo* absorption due to the complex and dynamic nature of the human situation.

Although the precise mechanism behind the absorption enhancing effect of low concentrations of PEG 400 remains somewhat unclear, it is postulated that the polymer may directly influence gastrointestinal permeability. The question remains, however, as to whether the absorption-enhancing effect of PEG 400 is specific only to ranitidine or whether it is more generic in nature and equally applicable to other drugs.

CONCLUSIONS

This study clearly shows that PEG 400 has a concentration-dependent effect on gastrointestinal transit and ranitidine absorption. At low concentrations PEG 400 significantly enhances the absorption of ranitidine possibly via modulation of intestinal permeability. At high concentrations PEG 400 accelerates the rate of passage through the small intestine, which negates the absorption-enhancing effect of the polymer, and causes a decrease in the extent of ranitidine absorption. These findings therefore have ramifications for the use of PEG 400 in pharmaceutical formulations.

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