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Research Paper

Polyethylene Glycol 400 Enhances the Bioavailability of a BCS Class III Drug (Ranitidine) in Male Subjects but Not Females

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Purpose. The aim of this study was to investigate the effects of different doses of polyethylene glycol 400 (PEG 400) on the bioavailability of ranitidine in male and female subjects.
Method. Ranitidine (150 mg) was dissolved in 150 ml water with 0 (control), 0.5, 0.75, 1, 1.25 or 1.5 g PEG 400 and administered to 12 healthy human volunteers (six males and six females) in a randomized order. The cumulative amount of ranitidine and its metabolites excreted in urine over 24 h was determined for each treatment using a validated HPLC method.
Results. In the male volunteers, the mean cumulative amount of ranitidine excreted in the presence of 0, 0.5, 0.75, 1, 1.25 and 1.5 g PEG 400 were 35, 47, 57, 52, 50 and 37 mg respectively. These correspond to increases in bioavailability of 34%, 63%, 49%, 43% and 6% over the control treatment. In the female subjects, the mean cumulative quantity of ranitidine excretion in the absence and presence of increasing amounts of PEG 400 were 38, 29, 35, 33, 33 and 33 mg, corresponding to decreases in bioavailability of 24%, 8%, 13%, 13% and 13% compared to the control. The metabolite excretion profiles followed a similar trend to the parent drug at all concentrations of PEG 400.
Conclusions. All doses of PEG 400 enhanced the bioavailability of ranitidine in male subjects but not females, with the most pronounced effect in males noted with the 0.75 g dose of PEG 400 (63% increase in bioavailability compared to control, $p<0.05$). These findings have significant implications for the use of PEG 400 in drug development and also highlight the importance of gender studies in pharmacokinetics.

KEY WORDS: excipients; gastrointestinal transit; gender; H_2 receptor antagonists; oral absorption; metabolism; permeability; solubility; efflux transporters.

INTRODUCTION

Polyethylene glycol 400 (PEG 400) is a well established solubility enhancing excipient which is listed as an inactive ingredient in drug monographs. However, recent work has called into question the inert nature of this excipient (1–3). At doses relevant to pharmaceutical formulations PEG 400 stimulates gastrointestinal motility and accelerates small intestinal transit (1–3). This effect is attributed to the fact that PEG 400 is poorly absorbed from the gut (4). PEG 400 is osmotically active and will "hold" fluid in the lumen of the intestine, leading to an increase in bulk fluid volume, which in turn stimulates peristalsis and hence transit. No such effects on transit have been noted with other commonly used solubility enhancing excipients such as propylene glycol, vitamin E-TPGS, Labrasol and Capmul MCM in man or dog (5,6).

A consequence of rapid transit through the small intestine is a reduction in contact time with the primary site of absorption in the gut and the potential impact on oral bioavailability. A case in point was observed with the drug ranitidine. Ranitidine is a class III compound (high solubility, poor permeability) according to the Biopharmaceutics Classification System (BCS) and is mainly absorbed in the small intestine (7). The excipients PEG 400, sodium acid pyrophosphate and sorbitol have been shown to reduce the bioavailability of ranitidine (2,3,8,9). In the case of PEG 400, studies in male volunteers showed that a 10 g dose of PEG 400 reduced small intestinal transit time by 37% and the absolute bioavailability of ranitidine by more than 30% (2). In addition to transit differences, the reduction in bioavailability may be related to the reduction in the concentration gradient of ranitidine across the mucosa because of the diluting effect of the increased fluid load in the gut lumen in the presence of PEG 400. A subsequent clinical study by Schulze *et al.* (3) (also in male volunteers), looking at the effects of low doses of PEG 400 (1, 2.5 and 5 g) revealed that PEG 400 had concentration dependent effects on transit and drug absorption (3). Small intestinal transit times were decreased by 9%, 20% and 23% respectively. The oral absorption of ranitidine was reduced by 38% in the presence of 2.5 and 5 g PEG 400. However, in the presence of 1 g PEG 400 the absorption of ranitidine was increased by 41%, despite the

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reduction in small intestinal transit time (3). It was proposed that the effect noted with 1 g PEG 400 was due to the ability of PEG 400 to modulate intestinal permeability, an absorption enhancing effect which had been overshadowed at higher concentrations due to rapid passage through the small intestine.

In light of the unexpected result with 1 g PEG 400, coupled with the FDA requirement that the effectiveness and safety data is presented by gender, age and racial subgroups (with gender deemed most important) (10) the objectives of this study were to:

- investigate the effects of low doses of PEG 400 on the bioavailability of ranitidine in male and female volunteers
- determine the optimum PEG 400 dose that would enhance the bioavailability of ranitidine and
- determine if there are any gender differences in the bioavailability of ranitidine in the presence of PEG 400

MATERIALS AND METHODS

Preparation and Characterisation of Dosage Forms

The dosage forms comprised oral solutions consisting of 150 ml of water containing 168 mg ranitidine hydrochloride equivalent to 150 mg ranitidine base (0.1% *w*/*v*) and either 0 (control), 0.5, 0.75, 1.0, 1.25 or 1.5 g PEG 400 corresponding to concentrations of 0.3%, 0.5%, 0.7%, 0.8% and 1% *w*/*v* respectively. The saturated solubility of ranitidine in the different PEG 400 solutions was measured at 37°C. The solubility of ranitidine in the different solutions was: 0 g PEG 400 (558 mg/ml); 0.5 g PEG 400 (547 mg/ml); 0.75 g PEG 400 (559 mg/ml); 1.0 g PEG 400 (569 mg/ml); 1.25 g PEG 400 (544 mg/ml); 1.5 g PEG 400 (524 mg/ml).

Study Protocol

Twelve healthy volunteers (six males and six females)—age range 24–34 years (median 26 years); weight range 50–90 kg (median 68 kg); height range 1.58–1.84 m (median 1.73 m)—participated in a random six-way cross over study after giving informed written consent. All subjects were non-smokers and a health questionnaire was used to screen patients for the study. None of the volunteers had a history of kidney, liver or gastrointestinal disease. Female volunteers were all noted to be at different stages of their menstrual cycle during the study. The experimental protocol was approved by The Joint UCL/UCLH Committees on the Ethics of Human Research. The study was conducted in accordance to the Helsinki guidelines for ethics in research (1965) and its subsequent revisions—Tokyo (1975) and Venice (1983).

The volunteers reported to the study center after an overnight fast and each received, on six separate occasions in a randomized order, 150 ml of drug solution containing 0, 0.5, 0.75, 1, 1.25 or 1.5 g PEG 400. There was at least a 3 day washout period between treatments for all volunteers. A standard lunch consisting of two piece filled sandwich, packet of crisps and a juice drink (Calorie load—750 Kcal) was provided 4 h post dose, and water was available *ad libitum* from this point onwards.

Cumulative urine samples were collected throughout the course of each study day. This involved 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. For each time point, a 20 ml aliquot was retained and stored at −20°C.

Urine Analysis

Urine samples were assayed for the amount of ranitidine and its three metabolites (ranitidine *N*-oxide, desmethylranitidine, ranitidine *S*-oxide).

Frozen aliquots were thawed out at room temperature and 0.65 ml of each sample was mixed with 0.65 ml mixture of 20:80 acetonitrile:water in duplicates. After thorough vortex-mixing, a 10 µl aliquot of each solution was injected onto a Luna SCX (Phenomenex, UK) HPLC column using a validated HPLC-UV method (11). Briefly, the mobile phase was 20:80 acetonitrile/0.1 M sodium acetate with a flow rate of 2 ml/min at 50°C. Calibration standards were prepared with blank human urine, also diluted (50%) with 20:80 acetonitrile/water.

Statistical Analysis

Using SPSS®software, Krusal Wallis was performed on the solubility data to access the effects of the different amounts of PEG 400 on the solubility of ranitidine. The results obtained for the cumulative excretion of ranitidine in urine for males and females were subjected to two-way ANOVA using the General Linear Model in SPSS® to assess differences between gender. This was followed by one-way ANOVA to assess the effects of the difference concentrations of PEG 400 on the bioavailability of ranitidine in males and females separately, and then a post hoc Tukey's test. Statistical significance is considered where $p<0.05$

RESULTS

The bioavailability of ranitidine in each individual was assessed by the cumulative amount of unchanged ranitidine excreted in urine over 24 h. The amount of ranitidine excreted by the six male volunteers is shown in Table I. The mean cumulative amount of ranitidine excreted in the absence of PEG 400 (control) in male volunteers was 35±8 mg, corresponding to 23% of the administered dose (Fig. 1). The mean cumulative quantity of ranitidine excretion in the presence of 0.5, 0.75, 1, 1.25 and 1.5 g PEG 400 in the male volunteers were 47, 57, 52, 50 and 37 mg respectively. These correspond to increases in the bioavailability of ranitidine by 34%, 63%, 49%, 43% and 6% over the control treatment. Pronounced bioavailability enhancement was observed with the 0.75 g PEG 400 dose, a 63% increase over the control ($p<0.05$).

Table II shows the cumulative excretion of unchanged ranitidine in the six female volunteers. The mean cumulative amount of ranitidine excreted on the control day was 38± 12 mg (25% of the administered dose). The mean cumulative ranitidine excretion values with PEG 400 (0.5, 0.75, 1, 1.25 and 1.5 g) were 29, 35, 33, 33, and 33 mg respectively, corresponding to decreases in bioavailability of 24%, 8%,

Table I. Effect of Different Doses of PEG 400 on Ranitidine Bioavailability—Male Subjects

	Cumulative amount of ranitidine excreted in 24 h (mg)					
Volunteer	Control	0.5 g PEG 400	0.75 g PEG 400	1.0 g PEG 400	1.25 g PEG 400	1.5 g PEG 400
1	33	37	49	39	51	39
2	29	53	59	47	39	26
3	47	59	63	67	62	27
4	24	44	70	47	53	26
5	41	61	64	70	63	56
6	34	25	38	42	32	46
Mean	35	47	57	52	50	37
SD	8	14	12	13	12	12
CV (%)	23	30	21	25	24	32
p value		0.530	0.033	0.160	0.266	1.00

13%, 13% and 13% compared with the control; these decreases were not statistically significant ($p>0.05$). However, statistical analysis revealed that the results obtained for female volunteers were different from males ($p<0.05$).

Fig. 2a shows the mean cumulative urinary excretion of the total metabolites whilst the percentage mean of the individual metabolites excreted is shown in Fig. 2b–d. In the absence of PEG 400, the urinary excretion of the three metabolites by the male and female volunteers was 7.7% and 7.3% of the administered dose respectively (Fig. 2a). In the presence of PEG 400, the excretion of the metabolites followed a similar trend to that of the parent drug (Figs. 1 and 2).

DISCUSSION

The mean cumulative amount of ranitidine excreted in the absence of PEG 400 (control leg) in male volunteers was consistent with the value reported by Schulze *et al.* (3) where the average amount of ranitidine excreted was also 23% of the administered dose. The inter-subject variability (coefficient of variation) of ranitidine bioavailability (23%) in the absence of PEG 400 is also comparable to previous studies (12–14). All doses of PEG 400 in this study increased the bioavailability of ranitidine in male subjects. Specifically, the enhancement in drug bioavailability noted with 1 g PEG in the study of male volunteers (49%) is comparable to that obtained by Schulze *et al*, where 1 g PEG 400 enhanced the bioavailability of ranitidine by 41% (3). However, our data clearly show that a lower dose of PEG 400 (0.75 g) has a more pronounced effect on the bioavailability of ranitidine (increase of 63% over the control). Bioavailability enhancement in the presence of 0.75 g and 1 g PEG 400 occurred in each and every male subject in this study (compared to the control); this was also the case in the Schulze study with 1 g PEG 400 (3), thereby confirming the consistency of the effect in males.

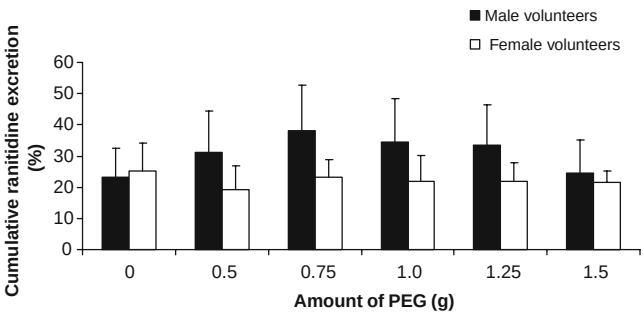

Fig. 1. Mean percentages of ranitidine excreted in urine over 24 h in male and female volunteers.

The mean cumulative amount of ranitidine excreted by the female volunteers in the absence of PEG 400 was similar to the male subjects. The mean and the intersubject variability (coefficient of variation) in bioavailability for the females was also comparable to published studies (13,15). The effects of PEG 400 on the bioavailability of ranitidine in female subjects had not been studied before, and show a clear statistical contrast to that seen in male subjects ($p<0.05$). In the female subjects, the bioavailability of ranitidine was reduced in the presence of PEG 400 and no relationship could be established between the stage of the menstrual cycle and the bioavailability of ranitidine

The bioavailability of a drug is affected by many parameters, including drug solubility. The solubility of ranitide was measured (558 mg/ml) and was in agreement with literature values (10). The different concentrations of PEG 400 had no statistically significant effect ($p>0.05$) on the solubility of ranitidine, thus eliminating it as a reason for the observed bioavailability increases in males.

Consideration is then given to the effect of PEG 400 on the intestinal permeability of ranitidine. It has been reported that ranitidine is primarily transported by the paracellular route and recent studies have suggested paracellular transport *via* tight junctions account for 60% of this movement whilst transcellular processes (*via* absorption transporters such as human organic cation transporter 1) account for the other 40% (16,17). PEG 400 does not have an effect on paracellular transport in Caco 2 cells (18,19), however its effects on OCT transporters is yet to be established. Gender differences between these organic cation transporters have only been examined in animal models to date, but these suggest that there is greater expression of some members of the organic cation transporter family in males compared to females (20).

In addition to drug influx, carrier mediated efflux can be influential in oral absorption. Carrier mediated transporters [P-glycoprotein (P-gp), multidrug resistance-associated protein 1 and 2 (MRP 1, MRP 2) and breast cancer resistance protein (BCRP)] expel absorbed drug back into the lumen of

Table II. Effect of Different Doses of PEG 400 on Ranitidine Bioavailability—Female Subjects

	Cumulative amount of ranitidine excreted in 24 h (mg)					
Volunteer	Control	0.5 g PEG 400	0.75 g PEG 400	1.0 g PEG 400	1.25 g PEG 400	1.5 g PEG 400
1	30	40	42	27	35	37
2	44	26	29	20	25	32
3	32	25	34	51	37	39
4	22	17	26	26	23	25
5	56	44	46	43	45	34
6	43	23	33	30	31	28
Mean	38	29	35	33	33	33
SD	12	11	7	12	8	5
CV (%)	32	38	20	36	24	15
p value		0.624	0.995	0.942	0.934	0.925

the intestine and many drugs are substrates of these transporters; consequently the bioavailability and pharmacokinetics of such drugs are controlled by the expression of these carriers. The efflux carrier P-gp has been implicated in intestinal ranitidine transport (21), whilst cimetidine (of the same pharmacological class as ranitidine) has been identified as both a P-gp and BCRP substrate (21,22) and there has emerged extensive evidence that excipients can inhibit these efflux transporters. The impact of PEG 400 on P-gp efflux has been investigated in excised rat intestine (19) and the authors reported a dose-dependent inhibition of P-gp. PEG 300, PEG 400, vitamin E TPGS and Tween 80 have also been shown to inhibit P-gp in Caco 2 cells without affecting the integrity of the cells tight junctions (19,23) whilst other excipients (Cremophor EL, Tween 20, Span 20, Pluronic P85 and Brij 30) inhibit both P-gp and BCRP and subsequently enhanced the absorption of a BCRP substrate (24). The effects of PEG 400 on BCRP have yet to be studied. The bioavailability

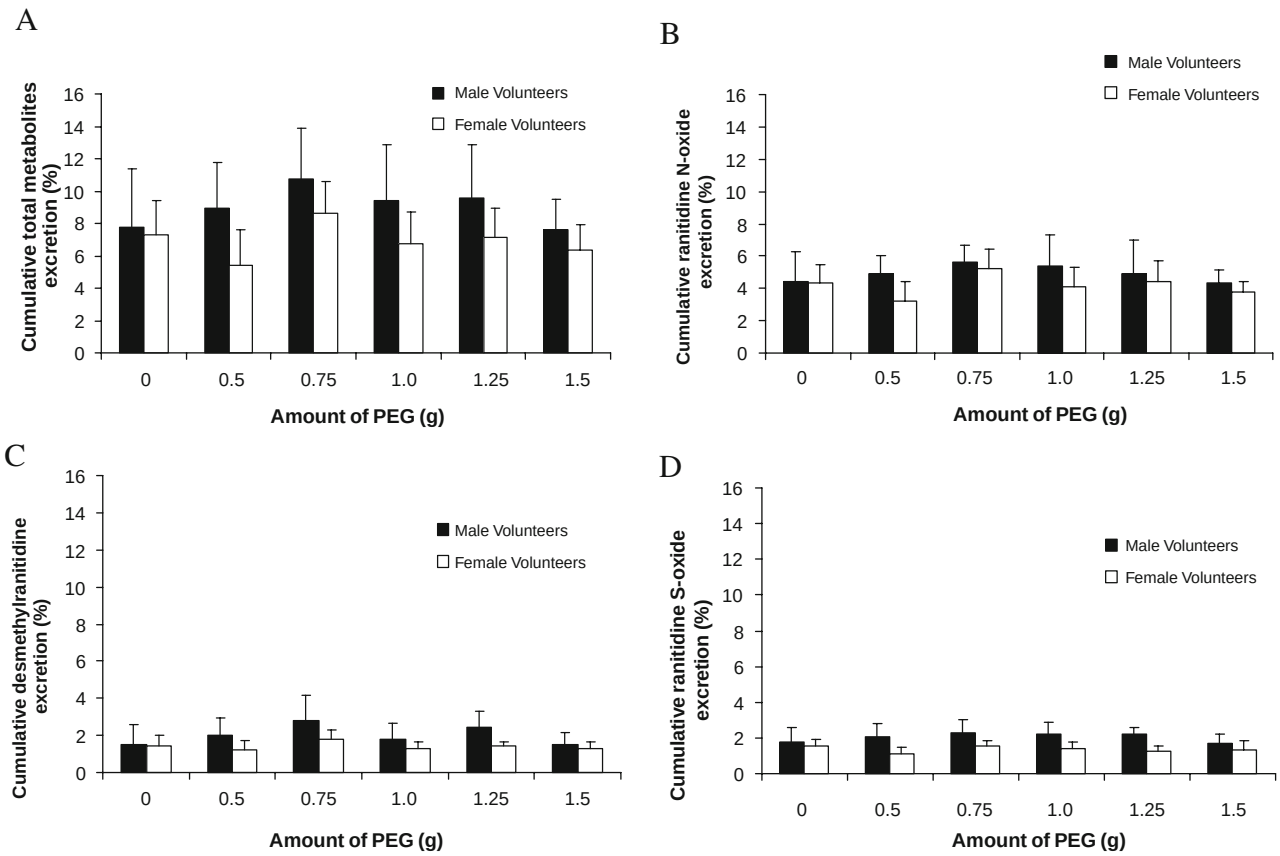

Fig. 2. Mean percentages of **A** all metabolites, **B** ranitidine *N*- oxide, **C** desmethyl ranitidine, **D** ranitidine *S*-oxide excreted in urine over 24 h in male and female volunteers.

enhancement noted with the PEG 400 in males may be due to this inhibition of efflux transporters, an effect which is negated at higher doses due to the previously described transit effects of high dose PEG 400 (1,3).

In light of the gender differences observed here, it is worthy of note that expression of efflux transporters is different in males and females. There is greater expression of intestinal BCRP protein in females compared to males (25). This contrasts to hepatic BCRP and P-gp where expression is higher in males than females (26,27). A higher proportion of gut efflux transporters in females could contribute to the differences in bioavailability noted in this study. Interestingly, Alonso *et al.* (28) showed that healthy women had significantly increased intestinal epithelial permeability compared to men in response to incoming stimuli in the jejunum.

The potential for PEG 400 to affect metabolism should be considered. The major route of elimination of ranitidine is renal (70–80% of total clearance) with the remainder due to hepatic and intestinal secretions (29–31). Ranitidine is metabolized in the liver to *N*-oxide, desmethylranitidine and *S*-oxide metabolites by cytochrome P450, particularly CYP 2C19, CYP 1A2, CYP 2D6 and CYP 3A4/5 (32,33). Cytochrome P450 enzymes are present in the gut wall enterocytes, as well as in the liver (23), and gender differences are noted with cytochrome P450 with women having higher CYP3A metabolism than men. These are attributed to the regulation of their expression and activity, probably due to hormonal influences rather than inherent differences based on allele variations. The effect of PEG 400 on the excretion of ranitidine and its metabolites is shown in Figs. 1 and 2. In the absence of PEG 400 the mean urinary excretion of the three metabolites by the male volunteers was 7.7% of the administered dose (Fig. 2a) and is in agreement with literature values (34). In females the mean metabolite excretion was 7.3%. The excretion of metabolites in both male and female volunteers follows a similar trend to the parent drug excretion (Figs. 1 and 2). PEG 400 has been shown to inhibit CYP 3A metabolism (19), hence it would be expected that if PEG 400 had an effect on the metabolism of ranitidine then the trends seen with the parent drug and its metabolites would be different; for example if PEG 400 inhibits metabolism, then it would be expected that whilst the mean cumulative urinary excretion of ranitidine peaks at the 0.75 g dose in males, the amount of metabolites excreted would be lowest at this PEG 400 dose. The trend noted with the excretion of metabolites show that the observed bioavailability effects of low dose PEG 400 in both male and female volunteers is unlikely to be due to a PEG 400 mediated inhibition of metabolism. The similar trend noted between males and females also suggest that the gender differences observed are not due to metabolism effects.

Recent studies using fluoroscopy and scintigraphy to follow total transit through the gut have shown that gastric emptying, small intestinal transit and colonic transit are all significantly slower in healthy female subjects compared to males (35–37). It is therefore feasible that PEG 400 has greater transit effects in females compared to males even at the lower doses studied, such that the lower doses of PEG 400 in the female volunteers has similar effects to those noted previously with higher doses of PEG 400 in male volunteers (1,3). Similarly, differences in small intestinal fluid volumes were noted in a study by Gotch *et al.* investigating intraluminal fluid volumes in post-mortem humans (38). In the study females were noted to have lower small intestinal fluid volume than males (38). It is therefore possible that the actual intestinal PEG 400 concentration is different between gender; lower fluid volume in females may lead to higher concentrations of PEG 400 in the small intestine of females. This increased osmotic pressure will be normalized by flow of fluid from the systemic circulation to the gut increasing the bulk luminal fluid volume and thereby reducing the concentration gradient for drug absorption across the mucosa and also leading to a stimulatory effect on motility and intestinal transit.

The exact reasons for the lack of bioavailability enhancement in female volunteers compared to male volunteers in the presence of PEG 400 are unclear. It is not uncommon to observe gender differences in the pharmacokinetic handling of drugs. However we show for the first time an unexpected gender difference when an *excipient* is added to a drug which does not normally exhibit gender differences. Prior to 1988, women of child bearing age were rarely included in clinical trials, however with recent guidelines from the regulatory bodies, it is now mandatory for women to be included in clinical trials. Both pharmacokinetic and pharmacodynamic differences have been reported between males and females and these have been reviewed by numerous authors (39–42). These gender related differences, some of which have been discussed, are often associated with molecular factors (efflux drug transporters especially P-glycoprotein and BCRP, drug metabolizing enzymes especially cytochrome P450s-CYP3A, hormonal factors (menstrual cycle and interactions with oral contraceptives and hormone replacement therapy) and/ or physiological factors (body weight, gastrointestinal physiology including gastric acid secretions, gastric emptying and gastrointestinal transit) (40). More recent literature also suggests that extrinsic factors such as dosing regimen and formulation may impart gender related differences (39). Although we did not observe significant gender difference in the bioavailability of our parent drug (ranitidine) when administered on its own (in the absence of PEG 400) (15) many drugs including verapamil, beta-blockers such as labetalol, metoprolol and selective serotonin reuptake inhibitors have been shown to exhibit gender differences either in their metabolism or bioavailability (26,43–47).

The results obtained in this study show that PEG 400, even at low concentrations has a dramatic effect on the bioavailability of ranitidine. The bioavailability enhancing effects observed with PEG 400 in this study are unlikely to be due to its effect on drug metabolism or solubility. It is potentially related to the effect of PEG 400 on ranitidine permeability *via* its effects on the epithelial membrane e.g. transcellular pathways or efflux transporters. Gender differences may also be attributed to differences in the effects of PEG 400 on small intestinal transit in males and females. However definitive transit and mechanistic studies with PEG 400 are needed to elucidate the underlying mechanisms for the intriguing findings. The question remains whether this bioavailability-enhancing effect of PEG 400 in male volunteers is specific to ranitidine alone, other H_2 receptor antagonists or perhaps more generic in nature and applicable to other BCS class III compounds.

CONCLUSION

PEG 400 increases ranitidine bioavailability in males, but not in females. No differences in bioavailability were noted between gender in the absence of PEG 400. This excipient should not be considered an "inactive ingredient". The reasons for the differences between sexes are unclear, but are likely to be a combination of various factors which may include differences in the expression of efflux and influx transporters, transit effects of PEG 400 and fluid volumes in males and females. These findings have significant implications for the use of PEG 400 in drug development and also highlight the importance of gender on drug bioavailability in pharmacokinetic studies.

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