Sex differences in excipient effects: Enhancement in ranitidine bioavailability in the presence of polyethylene glycol in male, but not female, rats

Francisco Afonso-Pereira[1,2], Sudaxshina Murdan[1], Joao Sousa[2], Francisco Veiga[2], Abdul W. Basit[1*]

1
Department of Pharmaceutics, UCL School of Pharmacy, University College London
29-39 Brunswick Square, London WC1N 1AX, UK

2
Faculty of Pharmacy, University of Coimbra, Portugal

* Corresponding author
Tel.: +44 20 7753 5865
Fax: +44 20 7753 5865
E-mail address:
a.basit@ucl.ac.uk

Abstract

Males and females respond differently to drugs: indeed, sex plays a crucial role in determining drug pharmacokinetics and pharmacodynamics. Excipients have also been shown to enhance the biovailability of drugs differently in men and women. The aim of this work was to investigate whether rodents are a good model in which to study sex-specific effects of polyethylene glycol 400 (PEG 400) on the bioavailability of ranitidine. Ranitidine (50 mg/Kg) was dissolved in water with different amounts of PEG 400 - 0 (control), 13, 26, 51, 77, 103, and 154 mg/Kg; these solutions were dosed orally by gavage to male and female Wistar rats. Blood samples were withdrawn over 480 minutes and assayed via HPLC-UV. Individual ranitidine plasma profiles were constructed for each rat, and standard pharmacokinetic parameters were determined. In the male rats, the change in the area under the plasma ranitidine curve ($AUC_{0\text{-}480}$) compared to the control group, was +18%; +49% ($p<0.05$); +37% ($p<0.05$); +31% ($p<0.05$); +8% and -22% ($p<0.05$) for PEG 400 doses of 13; 26; 51; 77; 103; and 154 mg/Kg respectively. On the other hand, females showed no statistically significant difference between the groups. In conclusion, low doses of PEG 400 enhanced the bioavailability of ranitidine in male, but not female, rats. These findings are in agreement with previously published human data, therefore confirming he validity of the rodent model, and highlight the unusual and clinically significant phenomenon that an excipient can influence drug bioavailability in one gender and not the other.

Key words: polyethylene glycol 400; polyoxyethylene polymers; gender; bioavailability; ranitidine; p-glycoprotein.

1. INTRODUCTION

Variability in drug effects is governed by a multitude of factors. Age, race, genetics, hormones, diet and disease can potentially alter the activity of a drug through either or both its pharmacokinetic and pharmacodynamic parameters (Jamei et al., 2009, Nicolas et al., 2009). Yet sex also acts as an important marker of inter-individual variability: sex-related differences have been evidenced in areas ranging from selective serotonin reuptake inhibitors' efficacy (Berlanga and Flores-Ramos, 2006) to cardiovascular pharmacology (Oertelt-Prigione and Regitz-Zagrosek, 2009, Ueno and Sato, 2012), pain management (Fillingim et al., 2009, Pleym et al., 2003), incidence of adverse drug effect, and drug toxicity (Bigos et al., 2009, Nicolas et al., 2009, Makkar et al., 1993, Aichhorn et al., 2005).

Though there remain wide gaps in our knowledge insofar as gender-specific drug effects are concerned, this should not undermine their wider importance - evidently, sex does matter. Indeed, such sex-related differences in gastrointestinal physiology have been increasingly well documented, including luminal pH (Feldman and Barnett, 1991), acid secretion (Prewett et al., 1991), fluid volume and composition (Lindahl et al., 1997, Bouras et al., 2002), transit times (Sadik et al., 2003, Metcalf et al., 1987), and even the extent to which they have been seen to influence oral drug bioavailability (Woodhead et al., 1991). The implications of such differences relate not only to the achievement of therapeutic benefit in sex groups and circumvention of adverse effects but, likewise, the potential for the personalization of medicines (Freire et al., 2011, Florence and Lee, 2011).

This also has special importance for the understanding of drug and dosage form behaviour in the context of sex specificity; though we might anticipate some differences in drug behaviour owing to such inter-individual variability between the sexes, this would not

necessarily be the case for excipients included in a given formulation. Excipients are small or large molecules, such as mixtures of polymers (Raymond, 2009), incorporated into the final drug product to fulfil a particular role in drug delivery such as lubrication, dilution of the active ingredient or bulking, and are formally defined by the US Food and Drug Administration (FDA) as "inactive" substances. Strikingly, however, we have previously demonstrated the influence of the widely available and utilised solubility enhancer, polyethylene glycol (PEG) 400, on the enhancement of ranitidine bioavailability in men when given at low doses (Ashiru et al., 2008). The same effect was, surprisingly, absent in women – highlighting the complexity of sex influences in the context of oral drug delivery, as well as bringing into question the inert status of PEG 400 and excipients.

The aim of the current work was to investigate the potential of a rat model to identify sex-specific differences in the effects of excipients on drug bioavailability. As such, we aimed to determine the effects of PEG 400 on orally-delivered ranitidine in male and female rats.

2. MATERIALS AND METHODS

2.1 Materials and animals

Ranitidine, polyethylene Glycol 400, glacial acetic acid and sodium acetate trihydrate were obtained from Sigma Aldrich (Dorset UK). Water and acetonitrile were purchased from Fisher Scientific (Loughborough, UK), and were of HPLC grade.

Male and female Wistar rats (8 weeks old) were purchased from Harlan UK Ltd (Oxfordshire, UK).

2.2 Animal procedures

The rats were fasted for twelve hours in metabolic cages prior to the start of the experiment; food was made available four hours after dosing of the animals. A dose of 50mg/Kg of ranitidine was administered via oral gavage in an aqueous solution to all animals. The dose of ranitidine was chosen based on literature (Eddershaw et al., 1996), and on the quantification capabilities of the method used. The groups differed only according to sex and dose of PEG 400 administered (0mg/Kg; 13 mg/Kg; 26 mg/Kg; 51mg/Kg; 77mg/Kg; 103 mg/Kg; 154mg/Kg). The doses of PEG 400 were extrapolated to rats based on the human doses previously used (Ashiru et al., 2008), which produced an increase in the oral bioavailability of ranitidine in human volunteers. The dose extrapolation from human to rat was based on species body surface area (BSA). In order to extrapolate the animal dose from a human equivalent dose (HED), Reagen-Shaw et al. (Reagan-Shaw et al., 2008) explained that BSA would enable the most appropriate conversion as it provides acceptable correlations across different biological parameters, including “oxygen utilization, caloric expenditure,

basal metabolism, blood volume, circulating plasma proteins, and renal function". The following equation was applied for the conversion:

$$HED(mg/Kg) = AD(mg/Kg)\frac{Animal\ Km}{Human\ Km}$$

Where HED is the human equivalent dose, AD the animal dose, Km is the BSA conversion factor which is 37 for humans and 6 for Wistar rats (Reagan-Shaw et al., 2008).

After dosing, the rats were placed in individual metabolic cages and were allowed to move freely. Approximately 200µL of blood was collected from the tail vein into anticoagulant centrifuge tubes (BD Microtainer® K2E Becton, Dickinson and Company, USA) at the following time points: 30min, 1.25h, 2h, 3h, 4h, and 6h. In between sampling, the rats were placed back in the metabolic cages. Blood volumes were taken in accordance with the project license and were stored on ice until the last collection point. After 8h, the animals were sacrificed in a CO_2 euthanasia chamber (Schedule 1 method), and approximately 2mL of blood were obtained via cardiac puncture.

Blood samples were centrifuged at 10000rpm (930g) for 10 min on a Centrifuge 5804R (Eppendorf AG, 22331 Hamburg, Germany) within 8h of sampling. 50µL of the supernatant (plasma) was collected and placed into a 1.5mL Eppendorf tube, and immediately frozen at -20°C prior to analysis.

2.3 Sample analysis

Plasma samples were thawed and assayed for the amount of ranitidine: for each sample, 50µL of plasma was mixed with the same volume of acetonitrile in order to precipitate the plasma proteins. After 1 min of vortex-mixing, a further 100µL of HPLC grade water was added to the mixture, and after subsequent vortex-mixing, the samples were

centrifuged at 4ºC for 10 min at 10000rpm. The resulting supernatant was subjected to HPLC-UV analysis using a previously validated method (Ashiru et al., 2007). The column used was a 5µm Luna SCX (Phenomenex, UK); the mobile phase was a mixture of 20:80 acetonitrile:0.1M sodium acetate pH=5.0 with a flow rate of 2ml/min and 40µL of injection volume. Calibration standards were prepared with blank rat plasma samples spiked with drug subjected to the above-mentioned treatment.

Plasma ranitidine concentration versus time profiles were produced for each animal. C_{max} and t_{max} were taken from these profiles. The cumulative area under the plasma concentration versus time curve (AUC_{0-480}) was calculated using the integration method with OriginPro 9.0 (OriginLab, Northampton, MA, USA).

2.4 Statistical analysis

The overall data was analysed by two-way ANOVA, followed by a Tukey post-hoc analysis and by individual t-student comparisons between individual groups and the appropriate controls, with IBM SPSS Statistics 19 (SPSS Inc., Illinois, USA).

3. RESULTS

The mean variations in the AUC in the presence and absence of PEG 400 are shown in Figures 1, 2 and Table1.

Two-way ANOVA showed a statistically significant influence of sex ($p<0.05$) and different doses of PEG 400 ($p<0.05$) on variation of ranitidine AUC_{0-480} compared with the control.

More specifically, from Figure 1 and Table 1 it can be seen that: PEG 400 influenced ranitidine bioavailability in male but not females rats. The influence of PEG 400 in male rats

was dose-dependent, with three of the doses of PEG 400 leading to significant increases in ranitidine bioavailability, while the highest PEG 400 dose led to a significant decrease in ranitidine bioavailability.

4. DISCUSSION

Our results in the rat model demonstrating the enhancement of ranitidine bioavailability by low doses of PEG 400 in male, but not female, rats is in agreement with results reported for human volunteers (Ashiru et al., 2008). In the latter study, an increase in ranitidine bioavailability of up to 63% ($p<0.05$) was achieved in men, but not in women, when PEG 400 was used at a dose of 0.75 g. High doses of PEG 400 led to reductions in the bioavailability of ranitidine. High doses of PEG 400, by retaining water in the intestinal lumen, reduces the gastrointestinal transit time, thus the time ranitidine has to be absorbed (Basit et al., 2002, Schulze et al., 2003, Basit et al., 2001). Based on the profiles of bioavailability changes in humans (Ashiru et al., 2008) and rats (Figures 1 and 2), this animal model may be considered appropriate for carrying out further studies investigating excipient effects on ranitidine bioavailability.

Ranitidine is a BCS class III drug that possesses a high aqueous solubility but low permeability through gastrointestinal membranes (Kortejarvi et al., 2005). As such, 40% of the overall bioavailability of ranitidine in humans is mediated by membrane transporters, with the paracellular route via tight junctions accounting for the remaining 60% (Bourdet et al., 2006). Ranitidine has also been reported to act as a substrate for efflux and influx membrane transporters and a considerable amount of membrane secretion of ranitidine into the luminal environment, mainly in the small intestine has been observed (Collett et al., 1999). Taking these into consideration, two possible reasons arise for the bioavailability

enhancing effect of ranitidine by PEG 400: intercellular space disorganisation (opening of tight junctions) by PEG 400 with a subsequent increase in the absorption of ranitidine via the paracellular route; or the interactions of PEG 400 with membrane transporters on the intestinal wall. The fact that PEG 400 does not affect the permeation of drugs that permeate exclusively via the paracellular route (Johnson et al., 2002, Rege et al., 2001) without necessitating membrane transporters, indicates that the PEG 400 enhancing effect on ranitidine bioavailability in males occurs by interaction with membrane transporters. Furthermore, studies which have identified regional differences in the absorption of ranitidine from the small intestine (Mummaneni and Dressman, 1994) suggest that the effects of PEG 400 are not necessarily homogeneous throughout the gastrointestinal tract. Regionally differing permeation can be associated with the combination of different transporters (influx and efflux) with regional differences in their expression levels in the small intestine (Kagan et al., 2010, Lindell et al., 2003).

The most extensively described interaction of ranitidine with a membrane transporter is not with uptake transporters, but instead with a transporter responsible for its luminal secretion (efflux transporters). Examples of commonly occurring efflux transporters in biological membranes are P-glycoprotein (P-gp), MDR-associated protein (MRP1 and MRP2), and breast cancer resistance proteins (BRCP) (Collett et al., 1999). It is therefore unsurprising that drugs which function as substrates for these efflux transporters may be subject to variations in their bioavailability when the efflux is somehow affected. While ranitidine is not a substrate for MDR-associated proteins or BCRP (following inhibition of these transporters, no difference was observed in terms of ranitidine secretion) it is a substrate for P-gp (Collett et al., 1999).

Excipients have been reported to elicit effects on efflux transporters (Li et al., 2011, Rege et al., 2001, Johnson et al., 2002, Shen et al., 2006), and specifically in the case of PEG 400, its influence on P-gp transporters has been investigated in excised male rat intestine (Johnson et al., 2002). The latter study revealed an important dose-dependent inhibition of P-gp without affecting the integrity of tight junctions between cells. This data was later corroborated by Ashiru et al (Ashiru-Oredope et al., 2011), who reached similar conclusions, namely that the efflux of ranitidine by Caco-2 cells was effectively inhibited by PEG 400 (Zhang et al., 2008). The reasons as to why Caco-2 permeation do not allow for a full explanation of the PEG 400 effect, including the marked sex difference observed *in vivo*, is that Caco-2 is a male-derived cell line from a colon adenocarcinoma (Sambuy et al., 2005) - there is no corresponding female parental cell line to mimic permeation in females. PEG 400 was also shown to affect the permeation of substances other than ranitidine. Shen (Hugger et al., 2002) found that PEG 400, 2000 and 20000, as well as PEG monooleate and PEG monostearate, inhibited the transport of rhodamine by P-gp across isolated rat intestinal membranes in a dose dependent way.

The bioavailability enhancement of ranitidine in the presence of low doses of PEG 400 in male rats may, therefore, be partially due to the inhibition of P-gp. The mechanism by which P-gp might be inhibited by excipients is still unclear. Nevertheless there have been some studies trying to elucidate this. Surfactants and solvents, for example, by fluidising the lipid bilayer have been shown to modulate P-gp activity (Lo, 2003); Pluronics on the other hand lead to an ATP depletion by reducing the ATPase activity of P-gp (Batrakova and Kabanov, 2008); other interactions between excipients and P-gp have been suggested as down regulation of the MDR1 gene (the gene responsible for P-gp expression) (Sachs-Barrable et al., 2007) and steric impediment or inhibition of the protein kinase C activity (Cornaire et al., 2004). As far as PEG 400 is concerned, no study on the nature of the

inhibition mechanism was found, but the idea of modification of the fluidity of the polar head regions of the cell membranes with PEG 300 has been put forward (Werle, 2008), and most likely will be an analogous situation with PEG 400.

From the above information it may be possible to find an explanation for the bioavailability-enhancing effect in the male rats. If intestinal membrane P-gp is inhibited by PEG 400, ranitidine absorbed into the enterocytes will not be secreted back into the intestinal lumen. This will raise the concentration of ranitidine inside the enterocyte, in turn leading to enhanced absorption into the blood stream, raising the overall exposure of the male rat to ranitidine. The fact that such enhancement of bioavailability does not occur in females indicates sex-based differences in: the level of expression of epithelial transporters and/or on the location(s) of the small intestine where these transporters are expressed in higher amounts. It is hypothesised that females will be less susceptible to P-gp inhibition than males, likely resulting from either an overall lower amount of active P-gp transporters in the gut or in lower expression of P-gp at the preferred absorption sites of ranitidine in the small intestine.

Indeed, sex differences in gut transporter levels have been reported. For example, an important influx transporter is the OCT/OCAT (organic cation uptake transporter) whose substrates include antagonists of the histamine type 2 receptor (Bourdet et al., 2006, Bourdet et al., 2005). Though no reports on the relative amount of the OCT/OCAT in different regions of the intestinal mucosa have been found, their expression in rat kidneys is known to be much higher in males than in females (Urakami et al., 2000). This may provide an alternative explanation for our results. Sex-related differences have also been identified in the renal and hepatic expressions of OAT (organic anion transporters) (OAT1-3 and SLc22a6-8) in mice (Buist and Klaassen, 2004), although whether these differences in the kidney translate to

differences in the intestinal mucosa is unknown. It is also known that, some efflux transporters such as BCRP are generally more prevalent in females than males (Zamber et al., 2003), except for the liver, where the levels of BCRP and P-gp are higher in males (Schuetz et al., 1995, Merino et al., 2005). Cummins et al. (Cummins et al., 2002) and Chen (Chen, 2005) suggested that differences in the clearance of CYP3A4/P-gp substrates could be due to different expression of P-gp in males and females and not so much due to differences in the amount of the related enzymes.

It stands that lower levels of gut P-gp in females could therefore contribute to the differences in bioavailability, as noted in this rat study and in others which have shown healthy women to have significantly increased intestinal epithelial permeability compared to men in response to incoming stimuli in the jejunum (Alonso et al., 2012). Assuming these sex differences are similar to those in rats, the potential inhibitory effect of PEG 400 would not be as pronounced as in males, which could provide an explanation as to why, with the used doses of PEG 400, the bioavailability of ranitidine in female rats only varied slightly from -10% to +7%.

5. CONCLUSION

We have shown that the widely-used so-called “inert” excipient PEG 400 influences the bioavailability of ranitidine in rats in both a gender-specific and dose-dependent manner. Here, high doses of PEG 400 were seen to reduce ranitidine absorption, by decreasing intestinal transit time. Low doses of PEG 400 had the contrary effect of increasing absorption of ranitidine in male rats but not in females. The exact mechanism for these effects remains unknown, but may be related to the role of intestinal P-gp drug efflux transporters present in different regions and at different concentrations in male and female gastrointestinal tracts.

Other factors such as influx transporters, GIT luminal fluid volumes, and GIT transit time may additionally play a role in these gender differences. Furthermore, these PEG 400 effects may be extendable to other BCS III drugs and those drugs which also function as substrates for intestinal membrane transporters. Consequently, the potential for an "active" role of excipients in pharmaceutical formulations should not be underestimated, and particularly in the case of formulations for poorly permeable and/or soluble compounds belonging to BCS classes III and IV. This also applies to the potential for previously unrecognized gender-specific effects, which may prove to be dangerous in the case of some drug compounds should these excipient effects be replicable, predisposing to significant decreases and/or increases in drug bioavailability in one sex but not the other.

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