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Excipient-mediated alteration in drug bioavailability in the rat depends on the sex of the animal

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ABSTRACT

The pharmaceutical excipient, polyethylene glycol 400 (PEG 400), unexpectedly alters the bioavailability of the BCS class III drug ranitidine in a sex-dependent manner. As ranitidine is a substrate for the efflux transporter P-glycoprotein (P-gp), we hypothesized that the sex-related influence could be due to interactions between PEG 400 and P-gp. In this study, we tested this hypothesis by: i) measuring the influence of PEG 400 on the oral bioavailability of another P-gp substrate (ampicillin) and of a non-P-gp substrate (metformin); and ii) measuring the effect of PEG 400 on drug bioavailability in the presence of a P-gp inhibitor (cyclosporine A) in male and female rats. We found that PEG 400 significantly increased ($p < 0.05$) the bioavailability of ampicillin (the P-gp substrate) in male rats, but not in female ones. In contrast, PEG 400 had no influence on the bioavailability of the non-P-gp substrate, metformin in male or female rats. Inhibition of P-gp by oral pre-treatment with cyclosporine A increased the bioavailability of the P-gp substrates (ampicillin and ranitidine) in males and females ($p < 0.05$), and to a greater extent in males, but had no influence on the bioavailability of metformin in either male or female rats. These results prove the hypothesis that the sex-specific effect of PEG 400 on the bioavailability of certain drugs is due to the interaction of PEG 400 with the efflux transporter P-gp.

1. Introduction

Pharmaceutical excipients are usually considered as “inactive” ingredients according to pharmaceutical regulations and standards (Bhattacharyya et al., 2006). However, a number of excipients have been shown to adversely affect physiological function and drug behaviour. For example, the emulsifying agent cholesterol increases the fluidity of cell membranes (Baggetto and Testa-Parussini, 1990), the suspending agent carrageenan induces inflammatory reactions in rats and mice (Farges et al., 2006; Halici et al., 2007), while the filler mannitol decreases small intestine transit time in a dose-dependent manner leading to a reduction in drug bioavailability (Adkin et al., 1995a; Adkin et al., 1995b). In addition to dose-dependency, the influence of excipients on drug bioavailability can also be sex-dependent. For instance, the absolute bioavailability of the drug celastrol was two-fold higher in the presence of carboxymethylcellulose sodium in female rats, while there was no change in male rats (Zhang et al., 2012). In another study, the area under the curve (AUC) of the drug γ-schizandrin following oral administration of pure γ-schizandrin solution (dissolved in water) was 20 times higher in male rats compared to female ones. Surprisingly, an opposite trend was observed when γ-schizandrin was administered as a solid dispersion with PVP K30 or in a capsule prepared in-house, where AUC in female rats was 6-fold higher than in male ones from both γ-schizandrin formulations (Xu et al., 2008; Zhao, 2010). Though the mechanism of this sex-based difference has not been identified, the surprising influence on γ-schizandrin bioavailability could have been caused by the excipients in the formulations. Clearly, excipients and sex of the organism have a major influence on drug bioavailability and this warrants further investigation.

In our laboratories, we are investigating the sex-based influences of polyethylene glycol 400 (PEG 400) on oral drug bioavailability. PEG 400 is a widely used excipient which is typically employed as a solubility enhancer to improve the dissolution and subsequent bioavailability of poorly-soluble drugs. We have previously shown its sex-related influence on the pharmacokinetics of ranitidine in humans (Ashiru et al., 2008) as well as in rats (Afonso-Pereira et al., 2016), a commonly used animal model. In both humans and rats, PEG 400 had a dose-dependent effect on ranitidine bioavailability in males but not in females.

It is possible that this sex-specific influence of PEG 400 on ranitidine bioavailability could be related to the latter's mechanisms of absorption (Freire et al., 2011). Ranitidine is predominantly absorbed via the paracellular pathway and membrane transporters (both influx and efflux transporters are involved) (Bourdet and Thakker, 2006). Therefore,

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the bioavailability-enhancing effect of PEG 400 in males could be due to the opening of tight junctions by PEG 400 and/or its interactions with membrane transporters. It is known that ranitidine is a substrate for the organic cation uptake transporters (OCTs) (Ming et al., 2009; Han et al., 2013), as well as the efflux transporter P-glycoprotein (P-gp) (Cook and Hirst, 1994; Collett et al., 1999). Meanwhile, PEGs are known to inhibit P-gp in a concentration-dependent manner from 0.1 to 20% (w/v) (Hugger et al., 2002; Shen et al., 2006). Additionally, the activity and expression of P-gp has been reported to be different in males and females (Mariana et al., 2011).

Consequently, we hypothesized that the observed sex-related influence of PEG 400 on the bioavailability of ranitidine could be due to its interaction with the efflux membrane transporter P-gp. The aim of the work discussed in this paper was to test this hypothesis by:

i) determining the influence of PEG 400 on the bioavailability of another P-gp substrate (ampicillin) and a non-P-gp substrate (metformin) in male and female rats,
ii) determining the influence of PEG 400 on drug bioavailability in the presence of a P-gp inhibitor (the immunosuppressive agent cyclosporine A).

Ampicillin, metformin and cyclosporine A have been reported to be a P-gp substrate, a non-P-gp substrate and a P-gp inhibitor, respectively (Siarheyeva and Glaubitz, 2006; Song et al., 2006; Liow et al., 2007). Also, considering the PEG 400 inhibition on some uptake transporters such as OATPs (Engel et al., 2012), metformin was chosen because it is also transported via the same uptake transporter, organic cation transporters (OCTs), as ranitidine (Chen et al., 2010). Furthermore, all drugs tested in this study are transported by the paracellular pathway. Thus, any possible influence of PEG 400 on the paracellular pathway would be observed, if applicable. (Lafforgue et al., 2008; Alvi and Chatterjee, 2014) (Details of absorption mechanisms are shown in Table 1).

2. Materials and methods

2.1. Materials and animals

Metformin hydrochloride and ampicillin sodium were obtained from USV Ltd. (Mumbai, India) and VWR International (Lutterworth, UK), respectively. Cyclosporine A was purchased from Cambridge Bioscience (Cambridge, UK). Ranitidine hydrochloride, polyethylene glycol 400, sodium dodecyl sulfonate and HPLC-grade water were supplied by Sigma-Aldrich (Dorset, UK). HPLC-grade reagents such as acetonitrile, methanol and glacial acetic acid were obtained from Fisher Scientific (Loughborough, UK). Analytical grade reagents such as ammomium acetate and sodium dihydrogen phosphate were procured from VWR International (Lutterworth, UK). Male and female Wistar rats (10 weeks old, approx. 250 g and 200 g respectively), were purchased from Harlan UK Ltd. (Oxfordshire, UK).

2.2. Drug solution preparation

Ampicillin and metformin were used at a concentration of 50 mg/kg, which was the same dose as ranitidine used in the previous human and rat studies. PEG 400 was used at a dose of 26 mg/kg as this caused the greatest enhancement in ranitidine bioavailability in rats (Afonso-Pereira et al., 2016).

Ampicillin, metformin and ranitidine solutions containing 25 mg/mL of drugs in the absence or presence of 13 mg/mL of PEG 400 were prepared with distilled water. CsA was suspended in water at 25 mg/mL.

2.3. Influence of PEG 400 on drug bioavailability in the absence or presence of P-gp inhibitor

All the animal work was conducted in accordance with the Home Office standards under the Animals (Scientific Procedures) Act, 1986. The rats were housed at room temperature (25 °C) and in a light-dark cycle of 12 h. They were caged in groups of six, allowed to move freely and provided with food and water before the experiment. The day before the experiment, they were fasted overnight and individually housed in metabolic cages.

On the day of the experiment, each rat was weighed and administered an aqueous solution (see details in following sections) by oral gavage. Subsequently, approximately 250 µL–300 µL of blood was collected from the rats' tail vein into anticoagulant centrifuge tubes (BD Microtainer® K2E Becton, Dickinson and Company, USA) at 0.5, 1.25, 2, 3, 4 and 6 h. At 8 h post-administration, the rats were killed in a CO_2 euthanasia chamber and about 1 mL of blood was taken by cardiac puncture.

2.3.1. Effect of PEG 400 on the bioavailability of ampicillin and metformin in the absence of P-gp inhibitor

Each rat was administered an appropriate volume of aqueous solution, corresponding to a dose of 50 mg/kg ampicillin or metformin with or without 26 mg/kg PEG 400.

2.3.2. Effect of PEG 400 on the bioavailability of ampicillin, metformin and ranitidine in the presence of P-gp inhibitor

The rats were orally administered an appropriate volume of cyclosporine A suspension for a dose of 50 mg/kg. Fifteen minutes later, a solution of ampicillin, metformin or ranitidine at a dose of 50 mg/kg in the absence or presence of PEG 400 (at 26 mg/kg) was administered via oral gavage. After dosing, the rats were placed individually in a metabolic cage and were allowed to move freely until blood collections.

Fifteen minutes before drug administration was chosen as the appropriate time to give the P-gp inhibitor following a study on the influence of timing using ranitidine as the drug. In this study, P-gp inhibition was conducted by orally administering cyclosporine A to animals immediately or 15 min, 30 min or 60 min before dosing with 50 mg/kg ranitidine in the absence or presence of 26 mg/kg PEG 400.

Table 1
Mechanism of Absorption in the Small Intestine.

BCS class III drugs	Active membrane transporters						Paracellular diffusion
	Influx transporters			Efflux transporters			
	PEPTs	OATPs	OCTs	P-gp	BCRP	MRP2	
Ranitidine	× [1][2][3]	× [1][2][3]	√[4]	√[5][6]	× [6]	× [6]	√[6]
Ampicillin	√[5]		× [1][7][8]	√[9]			√[10]
Metformin	× [1][2][3]	× [1][2][3]	√[11]	× [12]	× [1]		√[13]

√ stands for the substrates. × stands for this drug is neither inhibitor nor substrates for the transporter. [1](Konig et al., 2013) [2](Leibach and Ganapathy, 1996) [3](Liang et al., 1995) [4](Muller et al., 2005) [5](Bourdet et al., 2006) [6](Collett et al., 1999) [7](Muller et al., 2012) [8](Tsuji et al., 1981) [9](Siarheyeva et al., 2006) [10](Lafforgue et al., 2008) [11](Chen et al., 2010) [12](Song et al., 2006) [13](Alvi and Chatterjee, 2014)