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Research Paper

Effect of bile on the oral absorption of halofantrine in polyethylene glycol 400 and polysorbate 80 formulations dosed to bile duct cannulated rats

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Abstract

Objectives The aim of this study was to examine the effects of bile on the oral absorption of the poorly water-soluble compound, halofantrine, when administered to rats in vehicles consisting of the co-solvent polyethylene glycol 400 (PEG 400) alone or in mixtures with the surfactant polysorbate 80 (PS 80) (95 : 5; 85 : 15; 75 : 25 PEG 400 : PS 80).

Methods Halofantrine (17.5 mg/kg) was administered to bile duct cannulated (BDC) and sham-operated rats in a fixed vehicle volume of 5 ml/kg.

Key findings The bioavailability of halofantrine was significantly lower in BDC rats when dosed with 0–5% PS 80 in PEG 400 compared with BDC rats dosed with >15% PS 80. Increasing the concentration of PS 80 to 15–100% eliminated this difference. A possible explanation for the lower bioavailability of halofantrine in BDC rats when dosed in pure PEG 400 could be the dilution of the vehicle by intestinal fluids, decreased transit time and precipitation in the gastrointestinal tract upon dilution of PEG 400.

Conclusions The addition of PS 80 to the formulation increased its solubilising power upon dilution and may have inhibited precipitation and substituted the absence of bile above a certain level. Adjusting the level of surfactant in drug formulations could therefore be used to minimise variability in the bioavailability from co-solvent systems based upon differences in bile concentration between individuals.

Keywords bile; intestinal absorption; polyethylene glycol 400; polysorbate 80; poorly water-soluble drugs

Introduction

Oral administration is generally the preferred route of drug administration. At present the majority of new chemical entities brought into development by innovative pharmaceutical companies belong to the Biopharmaceutic Classification System (BCS) class 2 or 4, making poor aqueous solubility a general problem. The poor physicochemical and biopharmaceutical properties related to these two classes of the BCS often result in poor and variable bioavailability from conventional oral formulations.

Two formulation strategies exist for the oral delivery of poorly water-soluble compounds: modification of the dissolution rate in solid dosage forms; or circumvention of the disintegration and dissolution steps by administering the compound in solution, i.e. in lipid or surfactant-based formulations. Although the compound is presented to the intestine in solution the variation in bioavailability may be pronounced.[1] This may be caused by inter-individual differences in e.g. gastric emptying rate, intestinal transit time, lipase activity or bile salt/phospholipid levels. It is therefore desirable to study how these different physiological factors can be overcome by the formulation and thereby enable a reduction of the impact of interindividual variation on dosage form performance. A particularly important parameter is the luminal bile concentration, where a considerable variation has been observed between different individuals, and as a function of food intake and disease type or stage.[2–6] Very few in-vivo studies have, however, investigated the effect of bile on the

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absorption of BCS class 2 and 4 compounds from different formulations. Limited structured information is therefore available on the topic.

Frequently used vehicles for administration of poorly soluble drugs are the polyethylene glycol (PEG).[7] PEG often efficiently dissolve water-insoluble drugs and generally pose low toxicity.[7] PEG exist as liquid, semi-solids and solids at room temperature, depending on their molecular weight.[8] The liquid PEG are very suitable as oral vehicles for rodents in pharmacological and toxicological testing or in soft gelatin capsules for humans.[7–9] However, in-vitro studies have shown that poorly water-soluble drugs can precipitate from pure PEG 400 upon dilution.[10–13] A potential disadvantage of the use of PEG 400 as an oral vehicle is therefore the potential loss of solubilisation capacity when diluted in the gastrointestinal tract.[10,11] The aqueous phase may become supersaturated with the poorly water-soluble drug and the active pharmaceutical ingredient may potentially precipitate, leading to lower bioavailability.[12–16]

Tønsberg *et al*.[17] reported that the oral absorption of halofantrine dissolved in PEG 400 increased in rats upon addition of polysorbate 80 (PS 80). Furthermore, in-vitro studies showed a tendency of decreased halofantrine precipitation when the level of bile salts or PS 80 was increased. No previous in-vivo studies have to our knowledge investigated the influence of bile in co-solvent systems. The aim of this study was therefore to examine the effect of bile on the in-vivo absorption of halofantrine when dosed in formulations with increasing levels of the surfactant PS 80 in relation to the co-solvent PEG 400 using bile duct cannulated (BDC) and sham-operated rats.

Materials and Methods

Materials

Halofantrine crystalline base and the internal standard 2,4-dichloro-6-trifluromethyl-9[1-[2-8-dibutylamino)ethyl]]-phenanthrenemethanol hydrochloride were kindly donated by GlaxoSmithKline (West Sussex, UK). PS 80 and PEG 400 were obtained from Sigma-Aldrich (St Louis, MO, USA). Glycerol was purchased from Unikem (Copenhagen, Denmark) and lecithin (Lipoid E80, purity 98%) from Lipoid GmbH (Ludwigshafen, Germany). Acetonitrile, methanol, and *tert*-butylmethylether were of HPLC grade and were obtained from Sigma-Aldrich (St Louis, MO, USA). Potassium dihydrogen phosphate was from Merck (Darmstadt, Germany). Deionised water was obtained from a water purification system (Elga Labwaters, UK). All other chemicals were of analytical grade.

Halofantrine formulations

Formulations were prepared by weighing halofantrine free base into a glass vial and adding the appropriate amount of vehicle. Subsequently, the components were mixed by magnetic stirring during gentle heating (approximately 40°C) until all halofantrine was dissolved. The formulations were stirred while drawn into the syringe at ambient temperature before dosing, as the formulations containing less than 45% PS 80 in PEG are reported to be a biphasic system at room temperature.[8] The intravenous oil-in-water (o/w) emulsion contained 20.0% soybean oil, 1.2% lecithin, 2.4% glycerol and 76.4% water (w/w) and was prepared as described previously.[18]

Animal study

The protocol was approved by the Animal Welfare Committee, appointed by the Danish Ministry of Justice. All animal procedures were carried out in compliance with EC Directive 86/609/EEC and with the Danish law regulating experiments with animals and the NIH guidelines on animal welfare. Male Sprague-Dawley rats were purchased from Charles River, Germany (Sulzfeld, Germany). Animals were acclimatised and maintained on standard feed (Altromin 1324, Altromin Spezialfutter, Lage, Germany), apples and carrots with free access to water for a minimum of five days before the experiment. The animals underwent a surgical procedure as described by Tønsberg *et al*.,[18] with a few modifications. Rats were divided into two groups: laparotomy followed by placement of a BPU-T30 catheter (Instech Salomon) with three silicone beads (SIL-C30) inserted in the common bile duct and placement of an aortic catheter (Dilab, Lund, Sweden) in the left carotid; or laparotomy in a control group of rats (sham operated). After surgery both groups were placed in a swivel system. Before entry into the experiment the animals were fasted for approximately 16–20 h and randomly assigned to receive one of the treatments. During post-operative recovery animals were allowed lump sugar as described by Karpf *et al*.[19]

The animals (225–403 g) were dosed by oral gavage with 17.5 mg/kg halofantrine dissolved in 5 ml/kg of the oral formulations with PEG 400 and PS 80 in different ratios (Tables 1 and 2). Blood samples of ~200 µl were obtained by individual vein puncture of the tail vein at 1, 2, 3, 4, 6, 8, 10, 24 and 28 h after oral administration into EDTA-coated tubes. Plasma was harvested immediately by centrifugation for 15 min at 2765*g* at 4°C (Centrifuge Multifuge 1 S-R, Heraeus, Hanau, Germany), and stored at −80°C until analysed. The animals were allowed access to drinking water 4 h after oral dosing, and fed with carrots and apples 10 h after dosing. Nine rats were dosed intravenously (1.7 mg/kg) with the halofantrine o/w emulsion in the tail vein. Blood samples of 100–200 µl were collected at 5, 15, 30 min and 1, 2, 4, 6, 8, 10, 24, 28 h after intravenous injection. The animals were killed by gas after 28 h.

Analysis of halofantrine

The plasma samples were extracted and analysed using a method described previously by Humberstone *et al*.,[20] with some modifications. Plasma samples of 100 µl were mixed with 100 µl internal standard (2,4-dichloro-6-trifluromethyl-9[1-[2-8-dibutylamino)ethyl]]-phenanthrenemethanol, 2 µg/ml in acetonitrile), 1 ml acetonitrile and 4 ml *tert*-butylmethylether, vortexed twice for 30 s. The samples were centrifuged for 15 min at 4°C, 2765*g*. Four millilitres of the supernatant was added to 100 µl 0.005 M HCl in acetonitrile and evaporated to dryness under a stream of nitrogen at 40°C (TurboVap LV, Caliper Life Sciences, Mountain View, CA, USA). The residue was reconstituted in 100 µl methanol; hereof 25 µl was injected into the HPLC system.

The HPLC system comprised a L-7100 pump, a L-7300 column oven, a L-7400 UV detector, a L-7200 autosampler and a D-7000 interface, all from Merck (Darmstadt, Germany). A C-18 column (x-bridge 4.6 × 150 mm, 3.5 µm, MA, USA) was used for the separation. The mobile phase consisted of methanol : 0.025 M potassium dihydrogen phosphate buffer (adjusted to pH 3 with 55 mM perchloric acid) (72 : 28, v/v). The flow rate was set to 1.0 ml/min and the absorbance was measured at 257 nm. Standard curves were linear in the investigated range from 80 to 3000 ng/ml, and the recovery from the extraction was above 90% over this range, accuracy was 99.9% and the relative standard deviation of the procedure less than 5%.

Analysis of bile salts in rat intestinal samples

BDC and sham-operated rats were randomly selected after they had been killed. The abdomen was immediately opened and the small intestine removed. By gentle digital propulsion, samples of the intestinal content were pushed in an aboral direction and collected. The samples were frozen at −20°C until analysed for bile salt concentration and lipase activity.

The bile acids in the luminal content were extracted using a modification of the method described by Scalia[21] and Lee *et al*.[22] In brief, the intestinal samples were weighed and mixed with 0.5 ml methanol, diluted with 1.5 ml 5 mM phosphate buffer (pH 4.5), and centrifuged (4000 rev/min, 10 min). The supernatant was passed through a preconditioned (3 ml methanol and then 6 ml water) Strata C18-E cartridge (500 mg/3 ml, 55 µM, 70A, Phenomenex) and eluted with 1.5 ml 10% (v/v) of methanol in phosphate buffer (5 mM, pH 4.5) and 1.5 ml methanol. The second fraction was centrifuged (15000 rev/min, 2 min) and subsequently analysed.

Bile acids were separated on a ZORBAX Extent-C18 column (4.6 × 150 mm, 3.5 µm particles, Agilent Technologies) with a binary gradient at a flow rate of 1.0 ml/min.[23] Solvent A was 60% of methanol in the buffer (15 mM ammonium acetate, 0.2% triethylamine and 0.5% formic acid), solvent B was 95% methanol in a similar buffer. The gradient started at 100% of solvent A (5 min), and then decreased to 80% of solvent A over 5 min and then further to 100% of solvent B over 10 min and kept constant for 10 min, and finally back to 100% of solvent A and conditioned 5 min. A light-scattering detector (PL-ELS 1000, Polymer Laboratories) was used for detection. The nebulizer and evaporation chamber temperatures were 70 and 50°C, respectively, and the gas flow rate was 1.6 ml/min. This method ensured a detection limit of 0.06 mg/ml with a quantification range from 0.2 to 1.0 mg/ml. This equals a concentration for taurocholic acid of 0.1 mM for the detection limit, which was considered sufficient to provide evidence of significant differences between the sham operated and the BDC rats with respect to intestinal bile acid concentration. Taurocholic acid was used as an external standard for quantification.

Determination of lipase activity in rat intestinal samples

Lipase activity was determined in the luminal intestinal samples using the QuantiChrom Lipase Assay (BioAssay Systems, Hayward, CA, USA). In short, the assay is based on lipase hydrolysis of dimercaptopropanol tributyrate (BALB) which forms SH groups that react with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and reacts into a yellow product. The colour intensity, measured at 414 nm, is proportional to the enzyme activity in the sample and was quantified using a calibrator enzyme with known activity. Intestinal samples were weighed and diluted with assay buffer (1 µl/mg), homogenized and centrifuged. Supernatant samples (10 µl) were pipetted into 96-well plates and mixed with 140 µl working reagent containing BALB and DTNB. In to each separate well was added either 150 µl water or 150 µl calibrator. The 96-well plates were then incubated at room temperature for 20 min. After this time the optical density at 414 nm was measured and the lipase activity was calculated compared with the calibrator activity.

Pharmacokinetics

Pharmacokinetic parameters were calculated using WinNonlin Professional version 5.2 (Pharsight Corporation, Mountain View, CA, USA). The plasma concentration–time profiles of halofantrine after intravenous dosing were fitted to a two-compartment model, whereas a noncompartmental model was used to analyse the oral data. The area under the curve (*AUC*) was determined using the linear trapezoidal method and extrapolation of the last measured plasma concentration to infinity for the animals dosed intravenously. The total bioavailability (F_a) of halofantrine from the oral formulations A–E was calculated for the individual animal by using the following equation:

$$F_a = \left(\frac{AUC_{PO}}{AUC_{IV}}\right) \cdot \left(\frac{Dose_{IV}}{Dose_{PO}}\right) \quad (1)$$

AUC_{IV} is the area under the curve following intravenous halofantrine administration and AUC_{PO} following oral administration.

Statistical analysis

The software Sigma Stat for Windows version 3.5 from Systat Software Inc. (Richmond, CA, USA) was used for the statistical calculations. Differences across all formulations were initially assessed using one-way analysis of variance, and subsequently differences between the formulations were assessed post-hoc using Tukey's pairwise test comparison. Two-sided P-values < 0.05 were considered statistically significant.

Results and Discussion

Solutions of drug compound in co-solvents and surfactants are often used for oral dosing of rodents as these vehicles possess low toxicity combined with powerful solubilisation and dispersion properties.[8] Further, the vehicles can be included into a soft gelatin capsule for human administration.[9] This study has provided a systematic approach to elucidate the importance of bile salt on the intestinal absorption of halofantrine from formulations containing co-solvent and surfactant, PEG 400 and PS 80.[17] Bioavailability was determined in BDC rats using sham-operated rats as control.

Table 1 Pharmacokinetic parameters from sham-operated rats following oral administration of 17.5 mg/kg halofantrine in five different formulations

Formulation	Content (weight%)	$AUC_{0\rightarrow 28h}$ (h ng/ml)	T_{max} (h)	C_{max} (ng/ml)	Total bioavailability[a] (%)
A	100% PEG 400	10 996 ± 1090	9.0 ± 0.4 (CDE)	573 ± 54 (D)	12.4 ± 1.4
B	95% PEG 400 : 5% PS 80	9840 ± 297 (E)	6.3 ± 1.3	574 ± 20 (DE)	11.0 ± 0.3 (E)
C	85% PEG 400 : 15% PS 80	11 303 ± 773	3.5 ± 0.2 (A)	927 ± 134	12.1 ± 0.9
D	75% PEG 400 : 25% PS 80	13 800 ± 1997	4.0 ± 0.6 (A)	1092 ± 207 (AB)	15.3 ± 2.2
E	100% PS 80	15 391 ± 1274 (B)	5.5 ± 0.4 (A)	1027 ± 92 (B)	17.1 ± 1.4 (B)

Values are mean ± SEM (n = 4–5). [a]The total bioavailability is calculated as the percentage of halofantrine absorbed to the blood and is estimated from plasma $AUC_{i.v.}^{0\rightarrow 28h}$ relative to the $AUC_{i.v.}^{0\rightarrow\infty}$ obtained after intravenous (i.v.) administration normalised by the dose (AUC after intravenous administration of halofantrine was 8486.91 ± 1251 h ng/ml). The parentheses show from which treatments the denoted formulation is significantly different. PEG, polyethylene glycol; PS 80, polysorbate 80.

Table 2 Pharmacokinetic parameters obtained from bile duct cannulated rats following oral administration of 17.5 mg/kg halofantrine in five different formulations

Formulation	Content (weight%)	$AUC_{0\rightarrow 28h}$ (h ng/ml)	T_{max} (h)	C_{max} (ng/ml)	Total bioavailability[a] (%)
A	100% PEG 400	5212 ± 269 (CDE)	10 ± 0.0 (DE)	286 ± 14 (CDE)	6.0 ± 0.3 (CDE)
B	95% PEG 400 : 5% PS 80	6426 ± 1070 (CDE)	7.8 ± 1.4	371 ± 44	7.2 ± 1.2 (CDE)
C	85% PEG 400 : 15% PS 80	13 828 ± 895 (AB)	4.5 ± 1.5	755 ± 84 (A)	16.0 ± 1.2 (AB)
D	75% PEG 400 : 25% PS 80	14 741 ± 792 (AB)	3.0 ± 0.0 (A)	1092 ± 207 (AB)	15.3 ± 2.2 (AB)
E	100% PS 80	15 119 ± 2166 (AB)	5 ± 1.2 (A)	888 ± 165 (AB)	16.8 ± 2.5 (AB)

Values are mean ± SEM (n = 4–5). [a]The total bioavailability is calculated as the percentage of halofantrine absorbed to the blood and is estimated from plasma $AUC_{i.v.}^{0\rightarrow 28h}$ relative to the $AUC_{i.v.}^{0\rightarrow\infty}$ obtained after intravenous (i.v.) administration normalised by the dose (AUC after intravenous administration of halofantrine was 8486.91 ± 1251 h ng/ml). The parentheses show from which treatments the denoted formulation is significantly different. PEG, polyethylene glycol; PS 80, polysorbate 80.

Bile salt level and lipase activity

The surgical procedure was intended to minimise the bile level in the intestine of the BDC rats while allowing the presence of pancreatic lipase to facilitate lipolysis. To evaluate the outcome of the bile duct cannulation, intestinal samples were taken from four animals at the end of the experiment and analysed for bile salt concentration and luminal lipase activity. No bile salts could be detected in the intestinal samples of BDC rats i.e. the effect of the surgical procedure was verified and the animals could be considered as bile depleted.

The lipases secreted by the exocrine pancreas have been shown to affect the intestinal absorption of the lipophilic compound penclomedine when dosed in medium chain triglycerides, as the oral bioavailability was twofold lower after addition of the lipase inhibitor tetrahydrolipstatin to the oil.[24] Further, Liu *et al*.[25] reported a reduction of lymphatic transport of vitamin D_3 from 19.2% to 1% after pancreatic duct ligation of rats. Vitamin D was dosed in an emulsion, and when bile salt and pancreatic lipase were co-administered with the emulsion, lymphatic transport of vitamin D returned to the higher level. Though it is not clear if the in-vivo lipase activity will stay at the same level when bile is absent, these studies have demonstrated that to investigate the function of bile salts the lipase activity is important when digestible excipients such as PS 80 are investigated.[26–28] Therefore, the activity of the pancreatic lipase from intestinal content was determined. Although not statistically significant, there was a tendency towards slightly lower lipase activity in the BDC rats compared with the sham-operated animals with an intact bile supply, 8854 ± 4886 vs 15320 ± 4370 U/l (±SEM), respectively. This demonstrated that the surgical technique, with placement of the cannula in the bile duct before the entry of pancreatic juice, was effective in diverting bile salts with a minor effect on pancreatic lipase levels.

The functions of bile salts have classically been related to the intestinal absorption of lipids. However, it has become clear that bile salts have other intestinal and systemic physiological functions recently reviewed by Hofmann[29] and Monte *et al*.[30] When working with an animal model lacking bile, these physiological effects may be absent or taken over by other signalling compounds. This is a general criticism of surgically modified animal models for pharmacokinetic studies; however, it cannot be ruled out that the lack of bile may have other effects on the animals than just differences in the lipid solubilisation capacity of the intestinal fluid. However, considering the short time course of the study, these effects were considered to be limited, which was supported by the similarity between sham-operated and BDC rats with regard to weight recovery and liver parameters, as reported previously.[18]

Bioavailability study

Tønsberg *et al*.[17] showed that increasing the ratio of PS 80 to PEG 400 beyond 15 : 85 increased the absorption of halofantrine, possibly due to PS 80-induced micellar solubilisation of halofantrine and reduced intestinal precipitation.

The plasma concentration time profiles obtained from sham-operated rats dosed orally with formulations A–E are shown in Figure 1a, while the corresponding pharmacokinetic

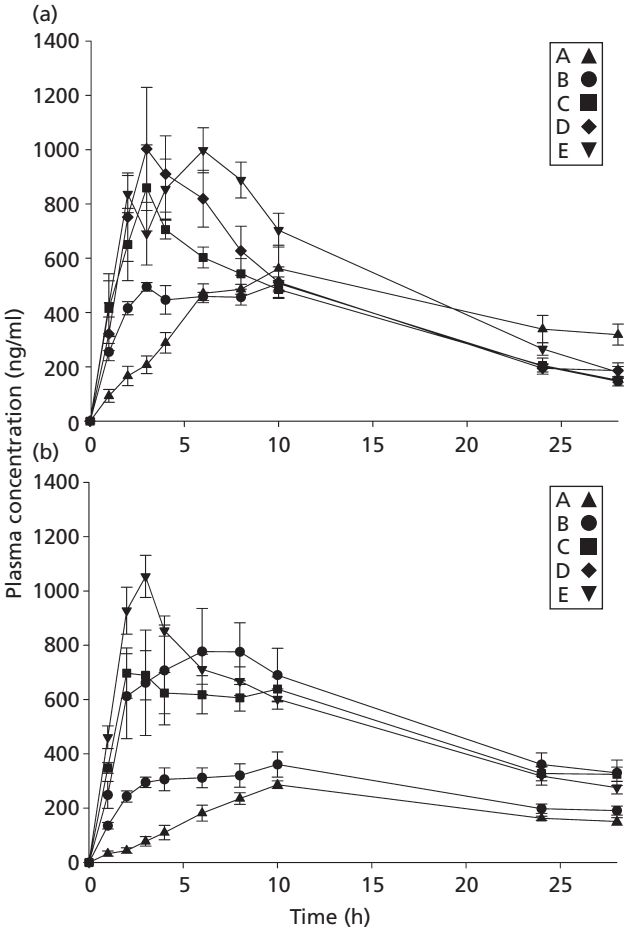

Figure 1 Plasma concentration vs time profiles following oral administration of 17.5 mg/kg halofantrine to rats. (a) Sham-operated rats for formulations: A,100% polyethylene glycol (PEG) 400; B, 95% PEG 400 : 5% PS 80; C, 85% PEG 400 : 15% PS 80; D, 75% PEG 400 : 25% PS 80; E, 100% PS 80. (b) Bile duct cannulated rats for formulations A–E. Values are mean ± SEM; $n = 4–5$.

parameters are presented in Table 1. The difference in the pharmacokinetics between the sham-operated rats in this study and the intact rats of the Tønsberg *et al*.[17] study was not significant when comparing the same formulations. This indicated that the sham operation did not affect the pharmacokinetic profile of halofantrine. The sham-operated animals were treated by a protocol similar to the BDC rats i.e. with antibiotics, presurgical treatment with pain relief and five days of recovery as described by Tønsberg *et al*.[18] This protocol seemed to ensure a low stress level for the animals upon start of the pharmacokinetic study, thereby ensuring consistency between the studies. As the stress levels in sham and BDC rats have been shown to be equal with respect to hepatic parameters (alanine-aminotransferase, aspartate-aminotransferase, alkaline-phosphatase and total bilirubin) and body weight, the difference in the pharmacokinetic profile of BDC animals could be largely ascribed to the absence of bile salts and not the surgical related stress.[19] The mechanism behind the differences in the pharmacokinetic profile of halofantrine when dosed in the PEG 400/PS 80 vehicles to the bile intact animals has been suggested to originate partly from loss of intestinal solubilisation capacity *in vivo*, leading to precipitation in the intestine, a hypothesis consistent with the data from this study.[17] This hypothesis could be investigated further by analysis of the lumen content for halofantrine concentration at defined time points in the PEG 400 group compared with the PS 80 groups, however, this was beyond the scope of this work.

The plasma concentration time profiles from BDC rats dosed orally with formulations A–E are shown in Figure 1b, while the corresponding pharmacokinetic parameters are shown in Table 2. Significant differences were found in the *AUC* values between formulation A–B and C–E (Table 2 and Figure 1b). A possible explanation for this observation could be that the solubilised halofantrine had a higher tendency to precipitate upon dilution of the PEG 400 in the intestinal environment when low amounts of PS 80 were present (<15%). When compared with the sham-operated rats, the pharmacokinetic profiles of halofantrine from the BDC rats dosed with low PS 80 levels (0–5%) had a lower *AUC*, C_{max} and a prolonged T_{max} for the same formulation. Since BDC insured the absence of bile salts, pharmacokinetic differences between the two groups of rats given the same formulation (A and B) indicated that bile salts were important to the absorption of halofantrine at low PS 80 levels, which was in line with a rat study by Trevaskis *et al*.[31] The low fraction of halofantrine absorbed in the BDC rats was probably due to the low levels of surfactant needed to prevent the precipitation of halofantrine upon dilution of PEG 400 or due to insufficient solubilisation capacity to solubilise the formed sediment. This interpretation supported conclusions from studies in intact animals and was in accordance with the results published by Kim *et al*.[32] where the absorption of furosemide was higher in bile intact than bile deficient animals.[17] Furosemide is a weak acid and may precipitate in the acidic environment in the stomach where the solubility of the compound is low i.e. the lack of bile may have slowed down the dissolution rate of the precipitate and thereby decreased the bioavailability.[32–34]]

The BDC rats dosed with 15–100% PS 80 had a pharmacokinetic profile very similar to the sham-operated animals dosed with the same formulation. These findings showed that when the level of surfactant became sufficiently high, bile salt became less important for the bioavailability. Plotting *AUC* as a function of PS 80 concentration (see Figure 2) for the sham-operated and BDC rats indicated the range in which bile was important. The effect of bile was most pronounced in formulations with 0–5% PS 80 mixed into PEG 400, while formulations with 15–25% PS 80 showed a smaller difference in the fraction of halofantrine absorbed. This may be used to facilitate a more uniform oral bioavailability in humans, who have a high interindividual variation in bile salt levels in the small intestine.[6] Both PEG 400 and PS 80 have been shown to stimulate bile secretion in rats after duodenal infusion, hence the concentration of PS 80 found to be bile salt independent in rats may be underestimated in a human formulation.[35] Further, it has been demonstrated that PS 80 and its lipolysis product oleic acid increased lipoprotein formation and lymphatic transport.[36–38] This could be relevant for compounds with a high log P, such as halofantrine (clog P 8.5), but also for compounds with a more intermediate log P, that may

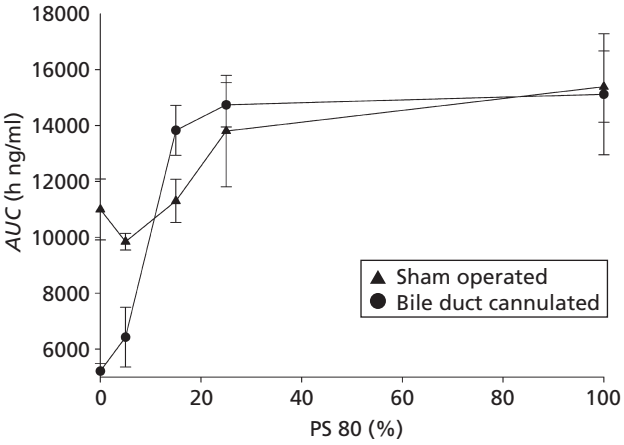

Figure 2 Area under the curve vs concentration of polysorbate 80 formulations dosed to rats. The rats were either sham-operated or bile duct cannulated rats. AUC, area under the curve; PS 80, polysorbate 80.

be transported lymphatically.[39] Also physiological considerations may be of importance when translating the results to other species e.g. humans secrete bile in a pulsatile manner in response to chime whereas rats have a continuous secretion into the lumen. Bile composition varies among species; rats only have tauro-conjugated bile salts whereas humans have a combination of tauro- and glycol-conjugations.[40] Last but not least there is a variation in the concentration of bile in the lumen i.e. in humans the reported concentration in the fasted state was typically in the range of 3–10 mM, whereas concentrations in the rat jejunum have been reported to be 9–17 mM.[2–6,41] This means that a number of biological processes may be affected by the use of PS 80 in addition to its solubilisation effect and the inhibition of precipitation, though these are not expected to differ between BDC and sham-operated animals. It is therefore possible that vehicle pharmacology could be important in the interpretation and translation from rats to other species.

The toxicological effects of the vehicles used should be taken into consideration also, though the available public data are sparse for most modern excipients. PEG 400 is generally considered safe in the doses used.[7] Much more controversy, however, exists around the use of PS 80, especially based upon its use in Caco-2 cells. After oral administration to rats the LD50 (lethal dose 50%) was 38 g/kg for PS 80, which was far above the concentrations used in this study.[42] In an older study, Krantz *et al*.[43] dosed 20 ml/kg PS 80 orally to rats and reported no sign of symptoms. The gastrointestinal histology has been investigated following exposure to PS 80 by Swenson *et al*.[44], who described PS 80 to cause less histological changes than bile salts (sodium taurodeoxycholate) or sodium dodecyl sulfate. Further Curatolo and Swenson[45] concluded that 'It should be noted that PS 80 is not an effective intestinal permeability enhancer'. Based on those reported in-vivo data it seemed most likely that PS 80 did not cause serious acute epithelial damage nor affected the morphology of the gastrointestinal tract extensively. The results presented in this work were therefore mainly thought to reflect the different physicochemical behaviour and solubilising capacity of the investigated excipients.

Araya *et al*.[46] investigated the poorly water-soluble compound ER-1258 dosed in a suspension and several lipid based formulations to normal and BDC rats. The highest impact of bile deficiency was found in the suspension and the lowest in a self-emulsifying drug delivery system (SEDDS).[46] Further, the SEDDS reduced the interindividual variation between the animals, which the authors interpreted as an improved emulsification and dispersion due to the presence of surfactants. The data in our work were therefore in accordance with the results published by Araya *et al*.[46] i.e. when halofantrine was dosed in PEG 400, which upon dilution with water seemed to lead to a suspension, a lower bioavailability was observed in BDC rats compared with the sham-operated rats. Araya *et al*.[46] reported differences between the BDC and the control group for all the investigated formulations, which was not seen in this work. This difference may have been formulation dependent, but could also have been differences in the BDC models. Araya *et al*.[46] did not disclose the precise procedure for the bile duct cannulation; however, if the catheter was placed close to the intestinal entry of the bile almost all lipase would have been removed, which may have influenced absorption.[24,25] Though there are some differences between the studies, there seemed to be a general agreement that the addition of exogenous surfactants made the formulation more bile salt independent and should have decreased interindividual variation in bioavailability due to variations in luminal bile salt concentrations. However, further research is needed to understand the mechanisms behind these observations and to enable an application in the development of formulations.

Conclusions

This bioavailability study in sham-operated and BDC rats demonstrated that the intestinal absorption of halofantrine was enhanced by PS 80–PEG 400 co-mixtures compared with pure PEG 400. The total bioavailability and C_{max} of halofantrine increased, while T_{max} decreased significantly when PS 80 was added to PEG 400 in the BDC rats. The limited absorption of halofantrine administered in hyperosmolar PEG 400 was most likely due to precipitation upon the dilution of the vehicle by gastrointestinal fluid and the consequent loss of solubilisation capacity. Adding PS 80 to the formulation could potentially affect this precipitation tendency and from 15% (w/w) of PS 80 no effect from the absence of bile was observed i.e. the bioavailability of the formulation was bile independent.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflict of interest to disclose.

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