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Research Paper

Effects of polysorbate 80 on the in-vitro precipitation and oral bioavailability of halofantrine from polyethylene glycol 400 formulations in rats

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Abstract

Objectives The aim of this study was to examine the effects of formulations of polysorbate 80 (PS 80) and polyethylene glycol 400 (PEG 400) on the precipitation and oral bioavailability of the hydrophobic drug halofantrine.

Methods The in-vitro dilution profile of the formulations was evaluated in phosphate buffer and in simulated intestinal fluids using a standard dissolution apparatus. The pharmacokinetic profile of the formulations was investigated in fasted rats at two dose levels, 5 and 17.5 mg/kg, with blood sampling by vein puncture in the tail.

Key findings The solubility of halofantrine was found to be highest in PS 80, and in co-mixtures there was a correlation with the content of PS 80. The in-vitro dilution profile revealed precipitation of halofantrine when dissolved in pure PEG 400, although the precipitation was smaller in the simulated intestinal fluid. Addition of 25% PS 80 to the PEG 400 significantly decreased precipitation. The animals dosed with the PEG 400 formulation had significant lower bioavailability than the PS 80–PEG 400 co-mixtures, possibly due to halofantrine precipitation in the gastrointestinal tract.

Conclusions Addition of PS80 to the formulation increased the bioavailability of halofantrine and the more compound, the more PS80 was needed to prevent precipitation.

Keywords oral bioavailability; polyethylene glycol 400; polysorbate 80; poorly water-soluble drugs

Introduction

Oral intake of drugs is the preferred administration route as it offers a high level of compliance in patients who are able to swallow. Between 40 and 70% of new chemical entities discovered by pharmaceutical companies are poorly water soluble,[1,2] i.e. compounds belonging to Biopharmaceutical Classification System (BCS) class 2 or 4. When formulated in conventional dosage forms, such as tablets and capsules, these physicochemical and biopharmaceutical properties lead to incomplete absorption following oral administration and hence low and variable bioavailability,[3,4] making the definition of a clinical dosage regime very difficult.

Two overall strategies exist for the oral delivery of poorly water-soluble compounds: (i) modification of the dissolution rate or (ii) circumvention of the disintegration and dissolution steps by administering the compound in a solubilised form. In the latter category, lipid-based formulation systems, such as oil solutions, emulsions and self-emulsifying drug delivery systems (SEDDS), have received considerable interest in the pharmaceutical literature.[1,2,5] However, although the compounds have low solubility in water they do not necessarily have high solubility in lipids. Consequently, other vehicles may be needed as co-solvents to solubilise the compounds.

Polyethylene glycols (PEG) are widely used in the pharmaceutical industry as a co-solvent.[6] PEGs solubilise water-insoluble drugs efficiently and have low toxicity.[6–8] They exist as liquids, semi-solids or solids at room temperature, depending on their molecular weight.[9] Liquid PEG formulations are convenient for oral dosing by gavage of animals in pharmacological and toxicological testing,[10] and soft gelatine capsules can be used for humans. However, a potential problem with co-solvents, both *in vitro* and *in vivo*,

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is their miscibility with the aqueous phase, which means that hydrophobic drug solutions may become supersaturated and potentially precipitate on dilution in aqueous media.[11,12] This is often manifested by variable absorption of the administered compounds.[2,3,13]

Surfactants can also be used to solubilise low water-soluble compounds and can therefore also be used as vehicles.[14] However, most surfactants have adverse effects in high concentrations. High surfactant levels in, for instance, SEDDS, can lead to gastrointestinal irritation,[1,15] and the surfactant micelles may even impede absorption by decreasing the free solute concentration.[4,16] Furthermore, most surfactants are highly viscous and in practice more difficult to dose by gavage than low molecular weight PEGs.

Liquid mixtures of polysorbate 80 (PS 80) and PEG 400 combine the high dissolving capacity of the polar PEG and the solubilising capacity of the non-ionic surfactant.[8,17] Despite the capabilities of PEG 400 and PS 80, mixtures of these two frequently used excipients have not, to our knowledge, previously been systematically evaluated with respect to their capacity to improve the oral bioavailability of low aqueous-soluble compounds, in spite of their extended use in pharmacological and toxicological studies. Consequently, the aim of the present study was to examine the effect of the surfactant PS 80 and/or the co-solvent PEG 400 on in-vitro precipitation and in-vivo bioavailability using a BCS class 2 compound, halofantrine, as a model.

Materials and Methods

Materials

Halofantrine crystalline base and internal standard 2,4-dichloro-6-trifluoromethyl-9[1-[2-(dibutylamino)ethyl]]-phenathrenemethanol hydrochloride were kindly donated by GlaxoSmithKline (West Sussex, UK). PS 80, PEG 400, soybean oil and porcine bile salt extract were obtained from Sigma-Aldrich (St Louis, MO, USA). A single lot of porcine bile salt (purity 60%) was used for all the dissolution media to reduce variability. KH_2PO_4 (Merck, Darmstadt, Germany) was used for the phosphate buffer. Glycerol was purchased from Unikem (Copenhagen, Denmark) and lecithin (Lipoid E80, purity 98%) from Lipoid GmbH (Ludwigshafen, Germany). Acetonitrile, glacial acetic acid and *tert*-butyl-methylether were of HPLC grade and were purchased from (Sigma-Aldrich, St Louis, MO, USA). Sodium lauryl sulfate (Sigma-Aldrich, USA) was electrophoresis grade. Water was obtained from a water purification system (Veolia Water Solutions & Technologies, Saint Maurice, France). All other chemicals were of analytical grade.

Methods

Saturated solubility of halofantrine

The saturated solubility of halofantrine was studied in the formulations used in the in-vivo study at 21 ± 1°C and at 37 ± 1°C. Additionally, the saturated solubility of halofantrine was quantified in phosphate buffer and co-mixtures of bile salt/lecithin (molar ratio of 1 : 4) media at 37 ± 1°C, containing 0 : 0 and 15 : 3.75 mM of bile salt/lecithin, respectively. Excess halofantrine base was added into vials with the formulation or in the bile salt/lecithin medium and equilibrated by magnetic stirring for about 48 h. After the equilibrium time, organic/formulation samples were withdrawn and filtered (Millex-FH filter, pore size of 0.2 μm, Millipore Corporation, Bedford, MA, USA) where solids were still present, and the first drop was discarded. Samples were assayed by high pressure liquid chromatography (HPLC) as described in the analytical section.

Halofantrine formulations

Formulations for the in-vitro and in-vivo studies were prepared by weighing halofantrine into a glass vial and adding the relevant amount of PEG 400 and/or PS 80. Subsequently, the content was mixed by magnetic stirring at room temperature until all halofantrine was dissolved. The formulations for the animals were prepared in two concentrations: 1 and 3.5 mg/ml, corresponding to the two dose levels of 5 and 17.5 mg/kg (fixed formulation volume of 5 ml/kg). The formulation was stirred while being drawn into the syringe, prior to dosing, since formulations containing less than 45% PS 80 in PEG are a biphasic system.[8] Formulation $A_{1\ mg/ml}$ refers to formulation A at 1 mg/ml, formulation $A_{3.5\ mg/ml}$ refers to formulation A at 3.5 mg/ml and so forth.

In addition to the oral formulations, an intravenous oil-in-water (o/w) emulsion was prepared, containing 0.1% halofantrine, 20% soybean oil, 2% lecithin, 2.4% glycerol and 75.5% water (w/w). The emulsion was prepared by dissolving halofantrine in lecithin and soybean oil by magnetic stirring under gentle heating (50°C). Glycerol was dissolved in water by magnetic stirring at 50°C. The aqueous phase was added to the lipid phase and the mixture was homogenised for 10 min using an ultrasonic device equipped with a standard microtip, at a power output of 5 (Sonifier Cell Disruptor, Model 450, Branson, Pusan, Korea). The intravenous formulation was aseptically filtered through a sterile 0.45 μm filter (Millipore Corporation, Bedford, MA, USA) into a sterilised glass bottle.

Solubilisation-upon-dilution study

The precipitation of halofantrine on dispersion of formulations A, E and G (Table 1), in either the phosphate buffer (0.028 M, pH 6.5) or phosphate buffer with 15 mM bile salt/3.5 mM lecithin, was determined in a USP dissolution apparatus II

Table 1 Saturation solubilities of halofantrine in formulations at 21 and 37°C

Formulation	Content (%, w/w)		Solubility (mg/ml)	
	PEG 400	PS 80	21°C	37°C
A[a]	100	0	3.3 ± 0.0	5.9 ± 0.1
B	95	5	3.3 ± 0.2	8.9 ± 0.8
C	90	10	4.5 ± 0.2	10.6 ± 0.1
D	85	15	4.9 ± 0.0	11.1 ± 0.4
E[b]	75	25	5.9 ± 1.0	10.5 ± 0.8
F	50	50	8.8 ± 0.4	15.2 ± 0.3
G[c]	0	100	14.8 ± 0.4	30.7 ± 1.3

Values in mg/ml are mean ± SD (n = 3). Amount of halofantrine in vehicles in the *in vitro* precipitation study: [a]3.5 mg/ml; [b]6 mg/ml; [c]14 mg/ml.

(Erweka DT70, Heusenstamm, Germany). A total volume of 300 ml was used at 37°C and the paddle speed was set to 100 rpm. One millilitre of the formulation A, E or G was pipetted quickly into the phosphate buffer or bile salt/lecithin medium, $n = 3$. Samples of 1 ml were removed with subsequent replacement of buffer/medium after 2, 5, 10, 15, 30, 45, 60 and 120 min.

The total amount of halofantrine (Q) dissolved at the different sample times was calculated using the following equation:[18]

$$Q = V_s\left(\sum_{n=1}^{n} C_{n-1}\right) + C{\cdot}V_t \qquad (1)$$

where C_n is the concentration in sample n, V_t is the volume of the bile salt/lecithin media and V_s is the sample volume.

HPLC analysis of halofantrine

The halofantrine concentrations in the solubility study, the dilution study and the bioavailability study were quantified by means of reverse-phase HPLC-UV, as previously described by Humberstone *et al.*[19] The HPLC system comprised an L-7100 pump, an L-7300 column oven, an L-7400 UV detector, an L-7200 autosampler and a D-7000 interface, all equipment from Merck (Darmstadt, Germany). The column used was a Phenomenex Luna 5 μm C8 (2), 250 × 4.60 mm (Torrance, CA, USA). The mobile phase consisted of 75% acetronitrile, 25% purified water, 0.2% sodiumdodecylsulfate and 0.2% glacial acetic acid. The flow rate was set to 1.5 ml/min and the absorbance measured at 257 nm. Standard curves were linear in the spiked plasma concentration range 40–3000 ng/ml, and the recovery from the extraction was above 90% in the investigated range.

In the dissolution study, 1 ml samples were immediately transferred to plastic tubes and centrifuged at 15 000 rpm for 20 min. Supernatant (200 μl) was diluted with acetonitrile to appropriate concentrations and assayed on the HPLC. The concentration of halofantrine in the solubility samples and the formulations was determined by dilution with acetonitrile and quantification using an external standard of halofantrine in acetonitrile.

The plasma samples were extracted and analysed using a validated method previously described by Humberstone *et al.*,[19] with a few modifications. Plasma samples of 100 μl were spiked with 100 μl internal standard (2 mg/ml in acetonitrile), 1 ml acetonitrile and 4 ml *tert*-butylmethylether, vortexed twice consecutively for 30 s. The samples were centrifuged for 15 min at 4°C, 2765*g* (Centrifuge Multifuge 1 S-R, Heraeus, Hanau, Germany). Four millilitres of the supernatant had 100 μl 0.005 mM HCl in acetonitrile added to it, and the mixture was evaporated to dryness under a stream of nitrogen at 40°C (TurboVap LV, Caliper Life Sciences, Mountain View, CA, USA). The residue was reconstituted with 100 μl acetonitrile, and 25 μl was injected onto the HPLC.

Animal study

The protocol used was approved by the Animal Welfare Committee appointed by the Danish Ministry of Justice, and all animal procedures were carried out in compliance with EC Directive 86/609/EEC and with Danish law regulating experiments with animals and the NIH guidelines on animal welfare. Male Spraque–Dawley rats (225–250 g) were purchased from Charles River Deutchland (Sulzfeld, Germany). The animals were acclimatised and maintained on standard feed with free access to water for a minimum of 5 days prior to the experiment. Before entry into the experiment the animals were fasted for approximately 16–20 h and randomly assigned to receive one of the treatments.

The animals were dosed by oral gavage with 5 or 17.5 mg/kg of halofantrine solubilised in the oral formulations A–G (Table 1) with a fixed formulation volume of 5 ml/kg. Blood samples of 200 μl were obtained from the tail vein and collected into 0.5 ml EDTA tubes at 1, 2, 3, 4, 6, 8, 10, 24 and 28 h after administration of the oral formulations. Plasma was harvested immediately by 15 min of centrifugation at 4°C, 2765*g* (Centrifuge Multifuge 1 S-R), and stored at –80°C until analysis. The animals were allowed access to drinking water 4 h after oral dosing, and standard feed 6 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. At the end of the experiment, the animals were euthanised by gas.

Pharmacokinetics

Pharmacokinetic parameters from the in-vivo study were calculated using WinNonlin Professional software, version 5.2 (Pharsight Corporation, Mountain View, CA, USA). The plasma concentration profiles of halofantrine after intravenous dosing were fitted to a two-compartment model, while a non-compartmental 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 $A_{1\ mg/ml}$–$G_{1\ mg/ml}$ or $A_{3.5\ mg/ml}$–$G_{3.5\ mg/ml}$ was calculated for the individual animal using the following equation:[20]

$$F_a = \left(\frac{AUC_{PO}}{AUC_{IV}}\right){\cdot}\left(\frac{Dose_{IV}}{Dose_{PO}}\right) \qquad (2)$$

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

Statistical analysis

Sigma Stat for Windows software, 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 subsequent differences between the test formulations were assessed post-hoc using Tukey's pairwise test comparison. P values below 5% ($P < 0.05$) were considered statistically significant.

Results and Discussion

Drug solutions of co-solvents and surfactants have low toxicity and are widely used in the preformulation and

preclinical development of drugs, as they combine powerful solubilisation and dispersion properties.[8,17] This study provides a detailed investigation of the most commonly used co-solvents and surfactants, PEG 400 and PS 80.

Saturated solubility of halofatrine

The saturated solubilities of halofatrine in the studied formulations are presented in Table 1. The lowest solubility was found in pure PEG 400 (Formulation A) at 21°C, while the highest solubility was found in 100% PS 80 (Formulation G) at 37°C. The data showed a concentration dependency of PS 80 as well as temperature dependence. The solubility of halofantrine in PS 80 found in the present study was in accordance with previously published results.[4]

The addition of 15 mM bile salt/lecithin mixed micelles (4 : 1 ratio of bile salt/lecithin) to the phosphate buffer led to an increase in the saturated solubility of halofantrine. Saturated solubilities at 37°C were determined to be < 0.02 and 712.9 ± 39.7 μg/ml in media containing 0 : 0 and 15 : 3.75 mM of the bile salt/lecithin, respectively. This large increase in solubility of the halofantrine base in bile salt/lecithin while in phosphate buffer is in accordance with previous results described by Humberstone *et al*.[21]

In-vitro dilution of formulations

The formulations in the present study were pipetted into the media so halofantrine precipitation could be studied. The amount of dissolved halofantrine in the formulations investigated is shown in Table 1. The concentration of bile salts in the media was 15 mM, simulating the bile salt concentration in the rat intestine.[22] As previously described by Tejwani *et al.*, not all combinations of PEG 400 and PS 80 are one-phase systems,[8] hence the formulations had to be stirred on pipetting. The use of a disperse system is less optimal than a one-phase formulation but, whatever the combination of PEG 400 and PS 80 used, it will turn into one phase uon dilution[8] and the well-recognised safety profile of PEG 400 and PS 80 makes them relevant to investigate.

The precipitation profiles of the formulations A, E and G in the phosphate buffer and in the bile salt/lecithin media are presented in Figure 1. The equilibrium of halofantrine was reached in less than 5 min and remained almost constant for the entire sampling period. The most pronounced in-vitro dissolution profile was seen for the formulation where halofantrine was dissolved in pure PEG 400, where significantly less was dissolved in the phosphate buffer compared to the bile salt/leclthin media. In the phosphate buffer only around 1% of the halofantrine administered in 100% PEG 400 remained dissolved while 80–90% of the halofantrine administered in 25–100% PS 80 was solubilised (Figure 1). The low solubility of halofantrine in the phosphate buffer resulted in halofantrine precipitation when added in the hydrophilic PEG 400. Furthermore, the difference in precipitation dynamics between the PEG 400 formulation and the PS 80 formulation may be affected by the difference in the formulations' solubilisation capacities when they are solubilised in the phosphate buffer. PEG 400 is soluble in water and thus loses a high part of its solubilising capacity on dilution, whereas PS 80 forms micelles that keep halofantrine solubilised. Gao *et al.* reported a similar tendency for the poorly water-soluble PNU-91325 dissolved in PEG 400.[23] When gelatine capsules with 100% PEG 400/PNU-91325 were dissolved in simulated gastric media, drug precipitation was visually apparent. When PNU-91325 was dissolved in the pure PS 80 formulation, a slower precipitation of the drug compared to the PEG 400 was observed, which is in accordance with the present precipitation study.

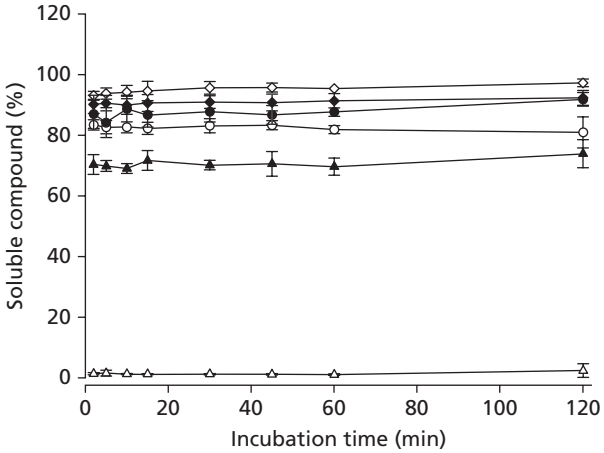

Figure 1 Soluble compound percentage versus incubation time of different formulations. White symbols, formulations in phosphate buffer; black symbols, formulations in bile salt/lecithin (ratio of 15 : 3.75 mM). The graphs illustrate formulations A (Δ and ▲), E (○ and ●) and G (◊ and ♦). Values are mean ± SD (n = 3).

The presence of 15 mM bile salt and 3.75 mM lecithin reduced the precipitation of halofantrine from 100% PEG 400 (Figure 1), and approximately 70% more halofantrine was recovered from the bile salt/lecithin media compared to the phosphate buffer. This observation is consistent with the difference in the solubility of halofantrine in the two media, i.e. the solubility of halofantrine in phosphate buffer increased relative to the concentration of bile salts and lecithin. The percentage of halofantrine dissolved in the phosphate buffer medium and the bile salt/lecithin medium was the same when PS 80 was present in the formulation, which suggests that the bile salt/lecithin media was of less importance to the solubilisation of halofantrine when the drug was administered in 25–100% PS 80.

Bioavailability study

The in-vitro dilution study indicated that halofantrine tends to precipitate when administered in the PEG 400 formulation, and that bile salt/lecithin may reduce the precipitation tendency of halofantrine from the PEG 400 vehicle. Formulations with more than 25% PS 80 in PEG 400 showed no precipitation tendency in either the phosphate buffer or in the bile salt/lecithin medium, indicating that PS 80 and/or bile could increase the bioavailability *in vivo*. Based on these interpretations, an in-vivo study was designed to investigate the importance of PS 80 when the concentration of PS 80 was below 25% in PEG 400. Rats were dosed with a fixed formulation volume of 5 ml/kg at two levels of halofantrine (5 and 17.5 mg/kg) in the formulations A–G to

Table 2 Pharmacokinetic parameters obtained following oral administration of 5 mg/kg halofantrine in different formulations

Formulations	$AUC^{0\rightarrow 28\ h}$ (h.ng/ml)	T_{max} (h)	C_{max} (ng/ml)	Total bioavailability (%)[§]
$A_{1\ mg/ml}$	3913 ± 191	7.2 ± 0.3	244 ± 12$^{(F1,D1)}$	15.3 ± 0.7
$B_{1\ mg/ml}$	4059 ± 192	4.6 ± 1.4	255 ± 24$^{(F1,D1)}$	15.8 ± 0.8
$C_{1\ mg/ml}$	4781 ± 362	5.3 ± 0.9	323 ± 18$^{(F1)}$	18.4 ± 1.3
$D_{1\ mg/ml}$	4886 ± 365	3.5 ± 0.9	399 ± 35	18.8 ± 1.3
$E_{1\ mg/ml}$	4702 ± 374	2.7 ± 0.2	343 ± 13	18.4 ± 1.4
$F_{1\ mg/ml}$	5490 ± 342	2.3 ± 0.2	450 ± 46	21.6 ± 1.4
$G_{1\ mg/ml}$	5134 ± 557	3.6 ± 0.9	370 ± 37	20.0 ± 2.1

Values are mean ± SEM (n = 5–10). [§]The total bioavailability is calculated as the percentage dose of halofantrine absorbed to the blood and is estimated from plasma $AUC_{e.v.}^{0\rightarrow 28\ h}$ relative to the $AUC_{i.v.}^{0\rightarrow\infty}$ obtained after intravenous administration normalised by the dose (AUC after intravenous administration of halofantrine was 8486.91 ± 1251 ng.h/ml.). The parentheses in the table show from which treatments the specific treatment is significantly different.

Table 3 Pharmacokinetic parameters obtained following oral administration of 17.5 mg/kg of halofantrine in different formulations

Formulations	$AUC^{0\rightarrow 28\ h}$ (h.ng/ml)	T_{max} (h)	C_{max} (ng/ml)	Total bioavailability (%)[§]
$A_{3.5\ mg/ml}$	8893 ± 629$^{(F3.5,\ G3.5,\ E3.5)}$	8.6 ± 0.8$^{(E3.5,\ D3.5,\ C3.5)}$	561 ± 39$^{(F3.5,\ G3.5,\ E3.5,\ D3.5,\ C3.5)}$	9.8 ± 0.6$^{(F3.5,\ G3.5,\ E3.5)}$
$B_{3.5\ mg/ml}$	9755 ± 776$^{(F3.5,\ G3.5)}$	3.0 ± 0.7	637 ± 80$^{(F3.5,\ G3.5,\ E3.5,\ D3.5)}$	11.0 ± 0.8$^{(F3.5,\ G3.5)}$
$C_{3.5\ mg/ml}$	12480 ± 1208$^{(F3.5)}$	2.2 ± 0.2	876 ± 76$^{(F3.5,\ G3.5)}$	13.9 ± 1.4$^{(F3.5)}$
$D_{3.5\ mg/ml}$	11710 ± 756$^{(F3.5)}$	2.3 ± 0.2	979 ± 47$^{(F3.5)}$	13.3 ± 0.8$^{(F3.5)}$
$E_{3.5\ mg/ml}$	14701 ± 1095	2.3 ± 0.2	1142 ± 67	16.6 ± 1.3
$F_{3.5\ mg/ml}$	17918 ± 1693	2.5 ± 0.2	1396 ± 91	20.1 ± 1.8
$G_{3.5\ mg/ml}$	16386 ± 1424	4.1 ± 0.8	1189 ± 70	18.5 ± 1.8

Values are mean ± SEM (*n* = 6).[§]The total bioavailability is calculated as the percentage dose of halofantrine absorbed to the blood and is estimated from plasma $AUC_{e.v.}^{0\rightarrow 28\ h}$ relative to the $AUC_{i.v.}^{0\rightarrow\infty}$ obtained after intravenous administration normalised by the dose (AUC after intravenous administration of halofantrine was 8486.91 ± 1251 ng*h/ml.). The parentheses in the table show from which treatments the specific treatment is significantly different.

evaluate the importance of dose on the pharmacokinetics in rats (Tables 2 and 3).

For ease of visualisation, only four of the seven plasma concentration versus time profiles obtained from rats dosed orally with formulations $A_{1\ mg/ml}$, $C_{1\ mg/ml}$, $D_{1\ mg/ml}$ and $F_{1\ mg/ml}$ are shown in Figure 2a, while the corresponding pharmacokinetic parameters for all the seven formulations $A_{1\ mg/ml}$–$G_{1\ mg/ml}$ are set out in Table 2. No significant differences were found in the AUC, T_{max} or the total bioavailability values between any of the seven formulations at the low-dose level of halofantrine, i.e. 5 mg/kg dosed at 5 ml/kg. However, for C_{max} differences were observed. The animals dosed with the pure PEG 400 formulation and the formulations containing 5–10% PS 80 in PEG 400 had a significantly lower C_{max} than the animals dosed with a formulation containing 50% PS 80 and 50% PEG 400 (Table 2). Furthermore, the pharmacokinetic parameters in Table 2 and the graphs in Figure 2a show that the animals dosed with the pure PEG 400 formulation had a slower absorption of the administered halofantrine, when compared to the PS 80–PEG 400 co-mixtures and pure PS 80. This is indicated by a less steep plasma concentration profile for the first 5 h and a lower C_{max} and longer T_{max} for the former formulation.

On dilution, the co-solvent power of PEG 400 with 5 mg/kg of halofantrine was not fully extended, therefore a higher dose of halofantrine was evaluated in order to investigate the biopharmaceutical effect of more saturated PEG 400 formulations and the potential effect of PS 80 in these situations. The plasma concentration versus time profiles of halofantrine dosed orally to rats with the same formulations as previously, containing 17.5 mg/kg ($A_{3.5\ mg/ml}$, $C_{3.5\ mg/ml}$, $D_{3.5\ mg/ml}$ and $F_{3.5\ mg/ml}$), are shown in Figure 2b and the associated pharmacokinetic parameters are reported in Table 3. As for the low-dose treatments, the pure PEG 400 formulation containing 17.5 mg/kg of halofantrine also led to the most divergent plasma profile. The AUC, T_{max}, C_{max} and total bioavailability of halofantrine for the PEG 400 formulation were significantly different to the formulations with 25–100% PS 80 in PEG 400 ($E_{3.5\ mg/ml}$–$G_{3.5\ mg/ml}$, see Table 3). Figure 2b also illustrated another similarity between the pure PEG 400 formulation and the low-dose level group. Halofantrine had a slower absorption rate when administered in the PEG 400 formulation than in the formulations containing PS 80, as can clearly be seen from the long T_{max} and the low AUC obtained (Table 3).

Formulation $A_{1\ mg/ml}$ had a faster absorption rate than the corresponding $A_{3.5\ mg/ml}$ formulation (Figure 2), and a higher bioavailability of halofantrine, as the AUC only increased by a factor of two when the dose was increased by a factor of 3.5. The occurrence of this pharmacokinetic difference with the same formulation used in the two situations can be explained by the degree of drug saturation on administration to the animals. Formulation $A_{1\ mg/ml}$ only contained halofantrine equal to approximately 17% of saturation at 37°C, whereas in the $A_{3.5\ mg/ml}$ formulation the amount of halofantrine was increased to a level of approximately 60% of the saturation solubility at 37°C (Table 1). Theoretically, this difference makes the $A_{1\ mg/ml}$ formulation more robust

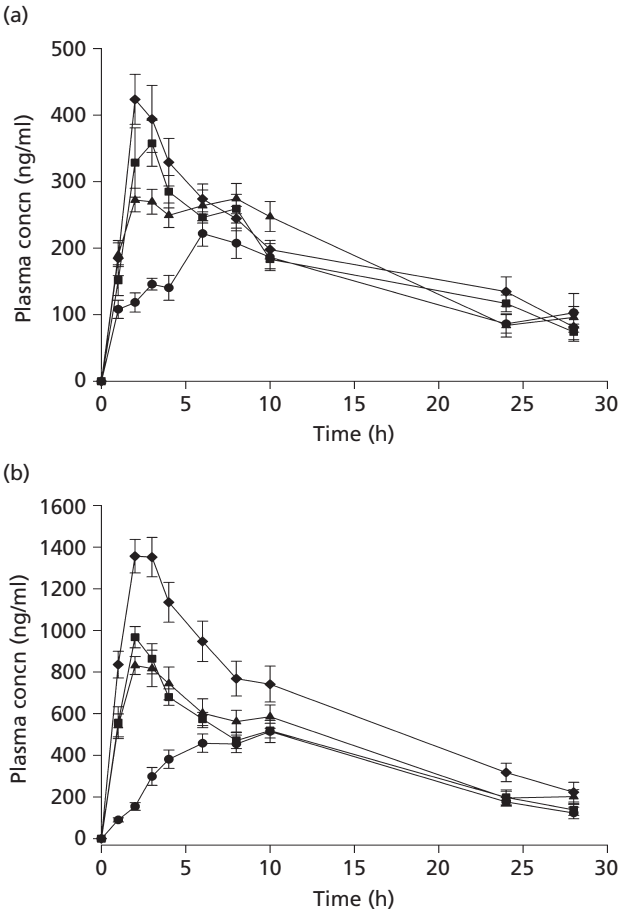

Figure 2 Plasma concentration versus time profiles following oral administration of halofantrine. (a) At dose level of 5 mg/kg in formulations A (●), C (▲), D (■), and F (♦); (b) At dose level of 17.5 mg/kg in formulations A (●), C (▲), D (■), and F (♦). Values are mean ± SEM; n = 5–10).

towards dilution in the gastrointestinal secretions than the $A_{3.5\ mg/ml}$ formulation, as indicated by previously published in-vitro studies.[12] Yalkowsky and Valvani reported that aqueous dilution of a co-solvent containing two different drug concentrations results in two different dilution curves, as the co-solvent loses its capacity to solubilise the compound exponentially.[12] In other words, interpreted in relation to the present study the high dose formulation has a higher risk of supersaturation and consequently precipitation than the low-dose formulation when they are diluted by the same amount of intestinal fluid. Besides these physico-chemical perspectives, the dilution of the PEG 400 formulation was presumably accelerated by a net water influx caused by the hyperosmolar PEG 400 and the increased amount of intestinal fluid.[24,25] This influx of water may lower the bioavailability (i) as more water further increases the risk of oversaturation when compared to the original amount of water in the lumen, due to the exponential relation ship between PEG concentration and solubility, and (ii) as a higher water volume may decrease the intestinal transit time, hence the precipitated compound may have less to time to get dissolved. On precipitation of halofantrine in the intestine, the formulation is converted into a suspension, so that the drug needs to be dissolved before absorption. Halofantrine has a relatively low intrinsic dissolution rate in 15 mM sodium taurocholate,[21] which means that dissolution becomes the rate-limiting step for the absorption of the compound. Together, increased volume of the intestinal fluid, reduced transit time and intestinal precipitation of halofantrine from PEG 400 may lead to the observed in-vivo flattening of the halofantrine concentration gradient between the bowel lumen and the blood, thereby delaying the absorption. This intestinal precipitation of halofantrine from PEG 400 is in accordance with similar in-vitro and in-vivo studies with hydrophobic JNJ-25894934, where orally dosed griseofulvin precipitated from PEG 400 in the gastrointestinal tract on administration to canines and rabbits.[3,26]

For the six other formulations (B–F) consisting of PEG 400 and PS 80 co-mixtures, the differences between the formulations were also most marked for the high-dose group. For animals receiving the formulations containing 5 mg/kg of halofantrine, a significantly lower C_{max} was seen for the formulations containing 5 and 10% (w/w) of PS 80, when compared to the formulation containing 50% PS 80, whereas no differences were observed in the other pharmacokinetic parameters. The bioavailability had a tendency to increase gradually from the PEG 400 formulation up to the formulation containing 10% (w/w) PS 80 after which no changes were seen. For the animals dosed with the formulations containing 17.5 mg/kg of halofantrine statistically significant differences were seen for the formulations containing 0–15% (w/w) PS 80 with respect to C_{max} and bioavailability – again as for the low-dose group – when compared to the formulation containing 50% PS 80. The gradual increase in the bioavailability was therefore clearer for the high-dose group and seemed to plateau at a higher PS 80 concentration. The total oral bioavailability of halofantrine when administered in pure PS 80 was approximately 20% for both the low and high dose groups, which is in accordance with previous findings reported in the literature.[4] As for the PEG 400 formulation, both physico-chemical as well as physiological differences may explain some of the differences observed for the formulations with PS 80.

The saturated solubility of halofantrine correlates positively with increasing levels of PS 80 in the co-mixtures (Table 1). In other words, the degree of saturation in the formulation becomes lower compared to the PEG 400 formulations and may consequently display a different precipitation pattern. Moreover, PS 80 forms micelles on dilution in aqueous media, thereby forming an additional phase, which together with the bile salts and phospholipids already present within the gastrointestinal tract can solubilise halofantrine. As PS 80 has a very low critical micellar concentration, it must be anticipated that micelles are formed at all PS 80 concentrations. Gao *et al.* demonstrated that the content of surfactant in S-SEDDS (supersaturated-SEDDS) dictates the amount of drug solubilised in micelles and the precipitation kinetics (a function of the degree of supersaturation and/or crystal growth rates).[23,27] A similar effect of PS 80 on halofantrine may be present, as suggested by the

in-vitro study with formulation E. As discussed above, a higher concentration of PS 80 was required in the high-dose group to reach the AUC plateau, which is in agreement with all three possible explanations for the biopharmaceutical effect of PS 80 in the formulations.

Formulations used in preclinical studies are not necessarily biologically and pharmacologically inert. As a general consideration, when comparing different pharmaceutical vehicles, although toxicology reports may suggest that the excipients are acceptable, the impact of local intestinal mucosal damage for the concentrations used is not known. Local mucosal damage, not identified by general toxicological studies, may shorten the signal window or mask bioavailability differences between formulations. More specifically, for the excipients used in the present study the inertness is related to the hydrolysis of PS 80 to oleic acid (C18 : 1) and the corresponding polyoxyethylene sorbitan alcohol by pancreatic lipases in the small intestine.[28,29] Oleic acid has several physiological effects *in vivo,* e.g. induction of immediate and long-lasting increase in water secretion in rats,[30] and prolongation of the small intestinal transit time by activation of the ileal brake.[31] In humans this has been shown to improve the bioavailability of drugs[32] and in relation to the present study it may be speculated that the digested PS 80 may active the ileal brake for some of the formulations, thereby increasing the transit time and potentially also the bioavailability. Furthermore, the undigested part of the dosed PS 80 vehicle can stimulate bile secretion in rats.[33] A precipitation and subsequent solubilisation of halofantrine in the intestine occurs for the formulations with the lowest PS 80 content, therefore these effects are very difficult to observe in the pharmacokinetic profile of halofantrine in the present study. Another important effect of oleic acid in relation to halofantrine is its induction of chylomicron formation and enhancement of intestinal lymphatic transport.[34] For animals dosed with high PS 80 concentrations, a quantitatively greater lymphatic transport of halofantrine may thus be induced, which would circumvent the portal vein and hepatic first-pass metabolism and partly explain the higher oral bioavailabilities of the PS 80–PEG 400 co-mixtures.

For low-aqueous-soluble compounds used in pharmacological or toxicological experiments, the combination of PEG and a surfactant can be used as an approach to maximise the exposure to a given compound. The use of pure co-solvents may improve the solubility, but there is a risk of precipitation, which may lower the bioavailability of the compound. On the other hand, use of high concentrations of surfactants may not be feasible from an animal welfare and toxicological point of view. PEG–PS 80 co-mixtures could be a way to get the best out of both formulation methods without compromising the biological considerations.

Conclusions

In conclusion, the present bioavailability study in rats demonstrates that the intestinal absorption of 17.5 mg/kg of halofantrine is enhanced by PS 80–PEG 400 co-mixtures compared to pure PEG 400, since AUC, total bioavailability and C_{max} of halofantrine increase while T_{max} decreases significantly when PS 80 is added to PEG 400. This limited absorption of halofantrine administered in the hyperosmolar PEG 400 is most likely due to formulation dilution in the gastrointestinal fluid and loss of solubilisation capacity, which entails halofantrine supersaturation and consequently precipitation. PS 80 mixed into PEG 400 increases the intestinal solubilisation and presumably reduces intestinal precipitation of the poorly water-soluble drug halofantrine.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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