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The formulation of Halofantrine as either non-solubilising PEG 6000 or solubilising lipid based solid dispersions: Physical stability and absolute bioavailability assessment

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Abstract

A non-solubilising solid dispersion formulation (polyethylene glycol 6000) and two solubilising solid dispersions (Vitamin E TPGS and a Gelucire 44/14/Vitamin E TPGS blend) containing the antimalarial, Halofantrine (Hf), were formulated for bioavailability assessment in fasted beagles to determine if the oral absorption of Hf can be enhanced by these delivery systems. Solid dispersions comprising varying proportions of drug to carrier were prepared by the fusion method. Whilst the non-solubilising formulation was assessed according to its dispersion characteristics, the solubilising solid dispersions were assessed by their ability to form microemulsions upon dispersion. Studies in fasted beagles showed that the solid dispersions afforded a five- to seven-fold improvement in absolute oral bioavailability when compared with the commercially available tablet formulation. The delivery of Hf in either a solubilising or non-solubilising solid dispersion did not result in significant differences in oral bioavailability. The physical stability of the solid dispersions was studied using differential scanning calorimetry and X-ray powder diffraction. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Solid dispersions; Amorphous drug; Microemulsions; Halofantrine

1. Introduction

Halofantrine (Hf) is an important antimalarial agent effective against multidrug-resistant strains of *Plasmodium falciparum* (Bryson and Goa, 1992; ter Kuile et al., 1993). However, the incomplete and highly variable absorption following oral administration of the commercially available tablet (Halfan®, 250 mg Hf hydrochloride) has limited the broader clinical utility of this lipophilic drug (Karbwang and Na Bangchang, 1994). Sub-therapeutic Hf plasma concentrations are a concern as this can lead to treatment failures and more importantly predispose the emergence of new multidrug-resistant strains of parasites. Lipidic self-emulsifiying formulations have previously

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been shown to successfully improve the oral bioavailability of Hf (Khoo et al., 1998), however, solubility considerations limit the maximum unit dose. Consequently, solid dispersions were selected for evaluation as an alternative drug delivery system to enable a higher drug loading per unit dose.

Solid dispersions have been explored as potential delivery systems for many poorly water soluble drugs such as griseofulvin, indomethacin and oxazepam (Chiou and Riegelman, 1970; Elliott et al., 1984; Jackowicz et al., 1993). The ability of solid dispersions to afford drug release as fine, dispersed particles has resulted in improvements in dissolution rates of poorly water soluble drugs which have also been reflected in increases in oral bioavailability (Chiou and Riegelman, 1971).

An extensive review of a selection of suitable carriers has been presented by Ford (1986). In addition to water soluble carriers with no intrinsic solubilising properties such as high molecular weight polyethylene glycols (PEG) and polyvinylpyrrolidones (PVP), the use of lipid based carriers with solubilising properties has also attracted much interest recently (Serajuddin, 1999). Two examples of such carriers are the Gelucire range of pharmaceutical excipients (which are hydrogenated food grade oils containing mixtures of glycerides and polyethylene glycol esters) and Vitamin E TPGS (D-α-tocopheryl polyethylene glycol 1000 succinate). These carriers offer the advantage of spontaneously solubilising lipophilic drugs upon contact with an aqueous medium to form either a fine emulsion or microemulsion and hence, further facilitate drug absorption.

In this study, solid dispersions containing amorphous Hf free base were prepared using the fusion method. The amorphous presentation of Hf was preferred as the highly disordered form has a lower energy barrier to overcome for dissolution than the structured crystalline form (Hancock et al., 1995). The free base of Hf rather than the hydrochloride salt was chosen as the former has a much lower melting point and better miscibility with the carriers in molten state. Accordingly, excessively high temperatures and prolonged heating were not required to produce the fusion of drug and carrier. To circumvent additional processing such as pulverization and compression, which may induce drug crystallisation, the molten mixture was filled directly into hard gelatin capsules and cooled rapidly. A rapid cooling process was important for preventing the amorphous drug from attaining a suitable orientation for crystal nucleation and growth. X-ray powder diffraction and differential scanning calorimetry were used to determine and monitor the presence of crystalline Hf.

A non-solubilising PEG 6000 dispersion and two optimised lipid based solubilising dispersions comprising either Vitamin E TPGS alone or in combination with Gelucire 44/14 were selected for an absolute bioavailability study in fasted beagles. The terms 'non-solubilising' and 'solubilising' refer to the ability of the carriers to solubilise the drug upon dispersion in the dissolution media. In the case of solubilising solid dispersions, a micellar solution is formed and complete solubilisation of the drug is usually achieved, whereas the non-solubilising solid dispersions liberate the drug as fine, dispersed particles upon dissolution of the water-soluble matrix. This study was designed to enable a direct comparison of the bioavailability of Hf delivered from non-solubilising and solubilising solid dispersions and to determine the importance of drug solubilisation in the absorption of lipophilic compounds. Physical stability issues pertaining to solid dispersions were also investigated and these are discussed.

2. Materials and methods

2.1. Materials

Halofantrine hydrochloride (Hf.HCl, SmithKline Beecham Pharmaceuticals, King of Prussia, PA), Gelucire 44/14 (Gattefossé s.a., Saint-Priest, France), Vitamin E TPGS (Eastman Chemical Company, Kingsport, TN), and PEG 6000 (Ajax Chemicals, NSW, Australia) were used as received. Intralipid® 10% (Kabi Pharmacia, Sweden), and AR grades of *N*,*N*-dimethylformamide (Ajax Chemicals, NSW, Australia) and triacetin (Henkel, Düsseldorf, Germany) were used to prepare the IV formulation. For the plasma assay, desbutylhalofantrine HCl (Hf metabolite, Hfm) and the internal standard (2,4-dichloro-6-trifluoromethyl-9-{1-{2(dibutylamino)-ethyl]}-phenanthrenemetha-nol HCl) were obtained from SmithKline Beecham Pharmaceuticals (King of Prussia, PA). HPLC grades of acetonitrile (Mallinckrodt, KY) and *tert*-butyl methyl ether (Fluka, Switzerland), and AR grades of absolute ethanol (CSR Limited, Australia), sodium hydroxide, hydrochloric acid, and glacial acetic acid (Ajax Chemicals, NSW, Australia) were used. Sodium dodecyl sulphate was purchased from BDH Laboratory Supplies (Poole, UK) and water was obtained from a Milli-Q (Millipore, Milford, MA) water purification system.

2.2. Preparation of Hf free base

Hf free base was prepared by firstly dissolving Hf.HCl (8.6 g) in absolute ethanol (400 ml) to which 1 M sodium hydroxide (20 ml) was added to alkalinize the solution, and water (20 ml) was added to precipitate the Hf free base. The crystalline drug was then filtered and washed with an absolute ethanol:water (1:1) mix and dried under vacuum in a desiccator containing P_2O_5. The purity of Hf free base was determined by HPLC and differential scanning calorimetry.

2.3. Preparation of Hf solid dispersions

The miscibility of drug and the carrier(s) in the molten state enabled the solid dispersions to be prepared by the fusion method. Crystalline Hf free base was firstly heated in a beaker at 90°C on a hotplate to produce the amorphous form prior to the addition of the carriers. The fusion of drug with carrier was then allowed to occur at 70–80°C. Once mixed, the molten mixture was either filled into hard gelatin capsules or directly into precooled suppository molds and placed in a refrigerator (5–8°C) for rapid solidification. Following preparation of the solid dispersions, the chemical stability of Hf was determined by HPLC to ensure that the drug had not undergone chemical decomposition during the fusion process.

2.4. Assessment of solubilisation characteristics of solid dispersions

The solubilisation characteristics of prototype solid dispersions were determined using a standard USP XXIII dissolution apparatus 2 (Erweka, Germany) (US Pharmacopeia, 1995). The studies were conducted in 500 ml of either 0.1 N HCl or Milli-Q water maintained at 37°C. Gentle agitation was provided by rotating the dissolution paddle at 100 rpm. For the solubilising solid dispersions the time required for complete dissolution of the carrier was recorded, the formulations assessed visually according to the final appearance of the emulsion formed and graded using the following grading system: ME, clear or slightly bluish, transparent microemulsion; +, slightly less clear emulsion with bluish white appearance; + +, bright, white emulsion; and + + +, dull, greyish white emulsion. The presence of precipitated material was indicated by the letter ‘p’. In the case of the non-solubilising Hf:PEG 6000 formulation, dissolution of the water soluble carrier led to the formation of a fine milky white supension of Hf.

The dissolution profiles of Hf from the solubilising formulations were determined by collecting a 1 ml aliquot of the dissolution media at various time intervals. The same volume of the appropriate medium was replaced after the removal of each aliquot. Each aliquot was then diluted to 10 ml with acetonitrile and analysed by HPLC to determine the concentration of Hf. For the non-solubilising formulation, the degree of dispersion of Hf after dissolution of the PEG 6000 carrier was also assessed by taking 1 ml samples of the dissolution media over time, diluting to 10 ml with acetonitrile and analysis by HPLC. However, in this case the assay quantified the total concentration of Hf in the sample (both suspended and in solution) and therefore reflects the degree of dispersion of the Hf suspension and not drug dissolution per se.

2.5. X-ray powder diffraction (XRD)

A Scintag model PAD V X-ray powder diffractometer and a solid state Ge detector were used to detect the presence of crystalline Hf free base. Cross-sections of the solid dispersions were taken and held in place on a quartz plate for exposure to CuKα radiation. The samples were analysed at room temperature over a range of 2–60° 2θ with sampling intervals of 0.01° 2θ and a scanning rate of 1°/min.

2.6. Differential scanning calorimetry (DSC)

The thermal properties of the solid dispersions were determined using a Perkin Elmer DSC 7 differential scanning calorimeter which was controlled by a TAC 7/DX controller. Samples (3–5 mg) were accurately weighed into 50 μl aluminium pans and hermetically sealed. The samples were heated from 30 to 100°C at a rate of 10°C/min under a dry nitrogen gas purge.

2.7. Absolute oral bioavailability study

A randomized, four treatment, four way crossover absolute bioavailability study in four healthy male beagles (13–18 kg, 2.5-year-old) was approved and conducted in accordance with the guidelines of the Institutional Animal Ethics Committee. The dogs were fasted for ~24 h prior to Hf dosing, and fed after collection of the 10 h samples. Water was available ad libitum throughout the study period. The individual treatments of the study included an intravenous formulation (2 mg/kg) administered as a 5-min infusion (prepared as described by Humberstone et al., 1996), and three oral solid dispersions (Table 1) administered with 100 ml of water. All oral formulations contained 100 mg Hf free base and had a fill weight of 700 mg. A 7-day washout period was allowed between treatments.

Blood samples (2.5 ml) were taken from either the cephalic vein via an indwelling catheter or by individual venipunctures of the jugular vein at predose (−10 min), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24, 32, 48 and 72 h following oral dosing, and at predose (−10 min), 0 (end of infusion), 15 and 30 min, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 24, 32, 48 and 72 h following IV administration. The blood samples were collected into individual tubes containing dipotassium EDTA. Plasma was separated from whole blood by centrifugation and stored frozen at −70°C until analysis.

The plasma concentrations of Hf and the major metabolite, desbutylhalofantrine (Hfm), were determined using a validated HPLC assay (Humberstone et al., 1995). From plasma concentration versus time profiles of both Hf and Hfm, the peak plasma concentration ($C_{\max}$) and the time at which this occurred ($t_{\max}$) were noted. The area under the curve ($\mathrm{AUC}^{0-\infty}$) was calculated using the linear trapezoidal rule to determine the area from time 0 to the last measurable concentration and then by adding the extrapolated area calculated by dividing the last measurable plasma concentration by the terminal elimination rate constant. The absolute bioavailability of Hf was obtained by comparing the dose normalised $\mathrm{AUC}^{0-\infty}$ values determined after oral and IV administration. To determine the significance ($P = 0.05$) of the differences observed, the data were analysed by one way analysis of variance and the Student–Newman–Keuls multiple comparison test.

Table 1
Composition of the optimised solid dispersions selected for the absolute bioavailability study in fasted beagles

Components	Hf:Vitamin E TPGS (1:6) (mg)	Hf:Gelucire 44/14:Vitamin E TPGS (1:3:3) (mg)	Hf:PEG 6000 (1:6) (mg)
Halofantrine	100	100	100
Vitamin E TPGS	600	300	–
Gelucire 44/14	–	300	–
PEG 6000	–	–	600

Table 2

Dissolution characteristics of solubilising solid dispersions containing 100 mg of Hf free base[a] encapsulated in hard gelatin capsules

Solid dispersion	Fill weight (mg)	Dissolution time (min)		Visual grading[b]	
		0.1 N HCl	Water	0.1 N HCl	Water
Hf:Gel 1:2	300	20	>30	+ +	+ + +, p
Hf:Gel 1:3	400	30	>30	+	+ + +, p
Hf:Gel 1:6	700	>30	>30	ME	+ +, p
Hf:Vit E 1:2	300	15	30	ME	+ + +
Hf:Vit E 1:3	400	20	30	ME	+ + +
Hf:Vit E 1:6	700	30	30	ME	ME
Hf:Gel:Vit E 1:2:1	400	>30	>30	ME	+ + +
Hf:Gel:Vit E 1:2:2	500	>30	>30	ME	+ +
Hf:Gel:Vit E 1:2:4	700	>30	>30	ME	+
Hf:Gel:Vit E 1:3:1	500	25	25	ME	+ +
Hf:Gel:Vit E 1:3:2	600	>30	30	ME	+
Hf:Gel:Vit E 1:3:3	700	>30	>30	ME	ME

[1] Abbreviations: Hf, Halofantrine free base; Gel, Gelucire 44/14; Vit E, Vitamin E TPGS.

[a] Dissolution of blank (drug-free) carriers resulted in the formation of a microemulsion in all cases and was independent of dissolution media pH.

[b] ME denotes a clear or slightly bluish, microemulsion; + denotes a slightly less clear emulsion with bluish white appearance; + + denotes a bright, white emulsion; + + + denotes a dull, greyish white emulsion and p denotes the presence of precipitated material.

2.8. Physical stability study

Hf:PEG 6000 dispersions of varying drug concentrations were prepared as conical shaped solid masses using suppository molds. The physical stability of the solid dispersions was determined by storing the formulations at 30°C, 40°C, 40°C/75% relative humidity, and 50°C. A relative humidity of 75% was obtained by placing the samples in a desiccator containing a saturated solution of sodium chloride at 40°C. Visual observations of the surface characteristics, dispersion studies, DSC and XRD measurements were conducted periodically for a period of 6 months.

3. Results and discussion

3.1. Dissolution characteristics of solubilising solid dispersions

Prototype solid dispersions containing a unit dose of 100 mg Hf free base in a solubilising matrix comprising either Gelucire 44/14, Vitamin E TPGS or combinations thereof were prepared for in vitro evaluation. The studies were conducted in either an acidic (0.1 N HCl) or neutral (water) medium to encompass the range of likely pH values encountered in the GI tract. Table 2 presents the dissolution times for the various carriers and visual assessment of the phases formed on dissolution of the carrier matrices. Ideally, the solid dispersions should have formed either clear solutions, or transparent microemulsions, within 30 min in both dissolution media.

Initially, solid dispersions of amorphous Hf free base in varying proportions of either Gelucire 44/14 or Vitamin E TPGS were studied. Increasing the proportion of solubilising carrier to drug, and hence the self-emulsifying efficiency of the formulation, resulted in an improvement in the visual grading of the emulsions formed. In these studies, Vitamin E TPGS appeared to have slightly better solubilising properties than Gelucire 44/14. For example, a 1:2 ratio of Hf:Vitamin E TPGS was capable of producing a microemulsion in an acidic medium whereas the Hf:Gelucire 44/14 formulations required a higher proportion of carrier (1:6 ratio) to produce a similar result. Of all the formulations studied only the 1:6 ratio of Hf:Vitamin E TPGS formed a microemulsion within 30 min in both acidic and neutral dissolution media.

As multiple daily dosing of Hf may be required for the treatment of malaria, this raised concerns of exceeding the recommended daily intake of Vitamin E should the Hf:Vitamin E TPGS (1:6) formulation be chosen. In an attempt to reduce the amount of Vitamin E incorporated into the formulation, a number of formulations containing both Gelucire 44/14 and Vitamin E TPGS in varying proportions were studied. Although the formulations formed a microemulsion in the acidic dissolution medium, only the Hf:Gelucire 44/14:Vitamin E TPGS formulation in a 1:3:3 ratio, formed a microemulsion in both acidic and neutral dissolution media.

For all the candidate solubilising formulations, dissolution of blank (drug-free) carrier resulted in the formation of a microemulsion, independent of the pH of the dissolution media. It appears therefore that the increased performance of Hf containing formulations (in terms of the ability to form a microemulsion) in acidic dissolution media is related to the presence of drug, and therefore likely reflects the greater degree of ionisation of Hf in the acidic environment.

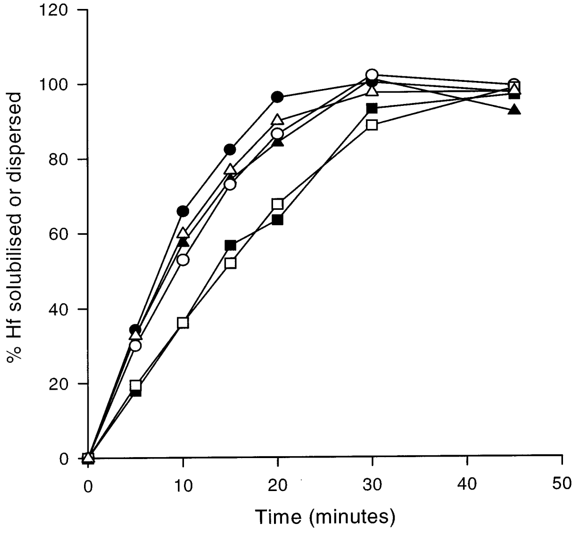

Fig. 1. Representative dissolution profiles of Hf:Vitamin E TPGS 1:6 (●) and Hf:Gelucire 44/14:Vitamin E TPGS 1:3:3 (■), and dispersion profiles of Hf:PEG 6000 1:6 (▲) solid dispersions containing 100 mg Hf free base in water (closed symbols) and 0.1 N HCl (open symbols).

Dissolution of the solid dispersions occurred by erosion, which commenced upon the complete disintegration of the gelatin capsule. Although the gelatin capsule usually disintegrated rapidly at 37°C, on a number of occasions, the gelatin capsule did not completely disintegrate over the time course of the dissolution study. When the gelatin capsule encased the formulation and hindered dissolution, dissolution times in excess of 30 min were recorded. These observations were presumably due to an interaction between the gelatin and the formulation during dissolution. Interestingly, the gelatin capsule also tended to disintegrate more quickly in acidic media resulting in shorter dissolution times for some of the formulations. Subsequently, to avoid variability in the absorption of Hf during the in vivo bioavailability study, the formulations were prepared as a solid mass by filling molten material into standard suppository moulds.

Following the identification of two solubilising formulations, Hf:Vitamin E TPGS (1:6) and Hf:Gelucire 44/14:Vitamin E TPGS (1:3:3), the non-solubilising comparator for the in vivo study was formulated to contain a similar amount of Hf (100 mg) and fill weight (700 mg). PEG 6000 was chosen as the carrier and the resultant formulation was Hf:PEG 6000 in a 1:6 ratio. Unlike the two solubilising carriers, PEG 6000 does not possess any intrinsic solubilising properties, and as a result, a milky white suspension rather than a clear microemulsion was formed upon dissolution of the PEG 6000 carrier. In this case, Hf was likely present in the dissolution medium as fine, dispersed particles. Filtration of the dissolution medium resulted in a clear filtrate and HPLC analysis of the filtrate showed negligible amounts of Hf (in line with the very low aqueous solubility of Hf).

Representative dissolution/dispersion profiles for Hf from all three formulations are shown in Fig. 1. As described in Section 2.4, these data should be viewed with the caveat that whilst the solubilising formulations produced clear microemulsions on dissolution of the carrier, the non solubilising Hf:PEG 6000 formulation resulted in the dispersion of a fine suspension of Hf. To avoid confusion, we have made a distinction in

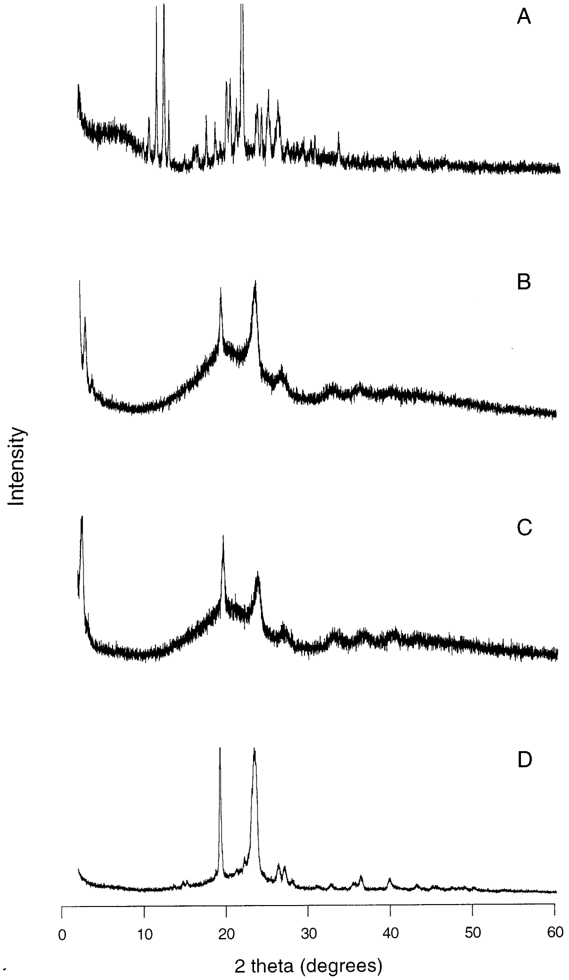

Fig. 2. X-ray powder diffractograms of (A) crystalline Hf free base, (B) Hf:Vitamin E TPGS 1:6, (C) Hf:Gelucire 44/14:Vitamin E TPGS 1:3:3 and (D) Hf:PEG 6000 1:6 solid dispersions.

this study between the dissolution or solubilisation of Hf on release from the solubilising formulations and dispersion of Hf after dissolution of the non-solubilising PEG 6000 carrier.

Notwithstanding these differences, both Hf:PEG 6000 and Hf:Vitamin E TPGS had very similar profiles and were completely dispersed or solubilised respectively, within 30 min. The Hf:Gelucire 44/14:Vitamin E TPGS formulation had a slightly slower dissolution rate, requiring 45 min for complete solubilisation.

3.2. Chemical and physical characterisation of the solid dispersions

The chemical stability of Hf during the fusion process was determined by HPLC. The area of the Hf peak in the HPLC chromatograms of samples taken from all the final formulations accounted for greater than 99.5% of the total peak area. The absence of other peaks indicates that Hf did not undergo chemical decomposition during the fusion process or appear to have interacted with the carriers.

A potential advantage of solid dispersion formulations is their capacity to present drugs to the gastrointestinal tract (GIT) in their high energy amorphous state. Consequently, X-ray powder diffractograms of the three chosen formulations were obtained in an attempt to identify the possible presence of contaminating crystalline Hf free base in the formulations. These diffractograms and the crystalline Hf free base comparator are shown in Fig. 2. The diffractogram of crystalline Hf free base showed a number of characteristic peaks, of which the peak occurring at 22° 2θ had the highest intensity. Diffractograms of the solid dispersions containing Hf free base (Fig. 2) were similar to those of the corresponding blank carriers (data not shown) and showed no evidence of the presence of contaminating crystalline material. XRD studies of physical mixtures of blank carrier and increasing amounts of crystalline Hf showed that XRD could accurately detect the presence of crystalline drug at levels as low as 5% (Fig. 3A). Absence of the characteristic peaks of crystalline Hf free base in the formulations (Fig. 2) suggest that Hf was dispersed in the solid dispersions in the amorphous state.

XRD measurements of the solid dispersions performed prior to the start of the in vivo study did not detect the presence of crystalline Hf. However, when repeated on the third week of the in vivo study, the diffractogram of the Hf:Gelucire 44/14:Vitamin E TPGS formulation revealed the presence of crystalline Hf free base. Using the standard curve presented in Fig. 3B which shows a linear relationship ($R^2 = 0.991$) between the integrated peak intensity (area under peak at 22° 2θ) and the concentration of crystalline Hf present, the amount of crystalline Hf present in the Hf:Gelucire 44/14:Vitamin E TPGS formulation after three weeks storage at room temperature (25°C) was estimated to be ~8%.

In addition to XRD, DSC was also used for the physical characterisation of the solid dispersions. DSC thermograms of the three solid dispersions chosen for the oral bioavailability study are shown in Fig. 4. Each thermogram displayed a single melting peak (endotherm) similar to that of the corresponding blank carrier. In agreement with the XRD data, the absence of a second endotherm at ~80–85°C (corresponding to crystalline Hf free base) suggests that the drug was dispersed in the amorphous state. However, the absence of a drug melting endotherm could also have been due to a lack of sensitivity of the method (in our hands XRD appears to be a more sensitive indicator of the presence of low levels of crystalline material) or slow dissolution of drug in the molten carrier.

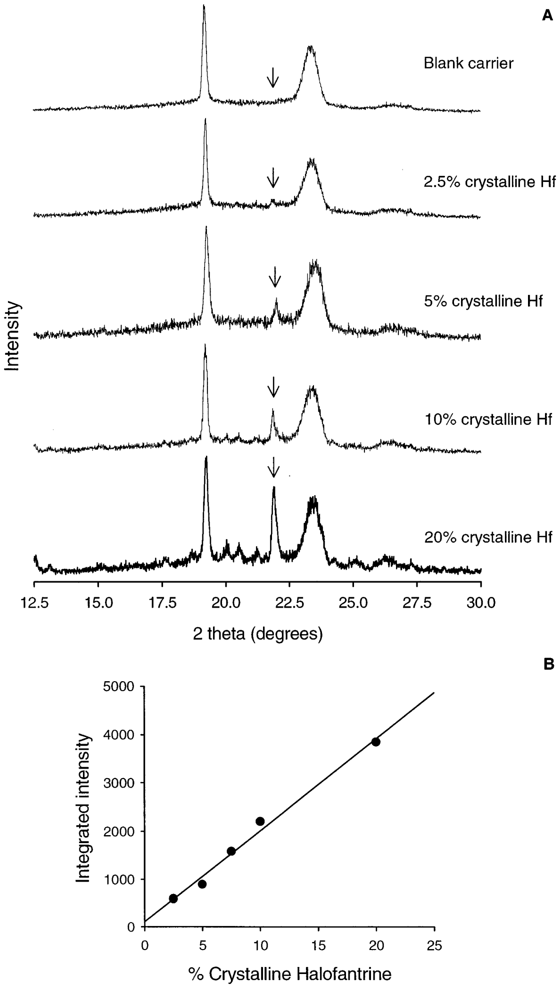

Fig. 3. X-ray powder diffractograms of blank Gelucire 44/14:Vitamin E TPGS carrier and physical mixtures of blank carrier and increasing amounts of crystalline Hf free base (panel A) and the standard curve of integrated intensity of the peak at 22° 2θ (corresponding to crystalline Hf, as indicated by the arrow) as a function of the crystalline content of Hf free base (panel B).

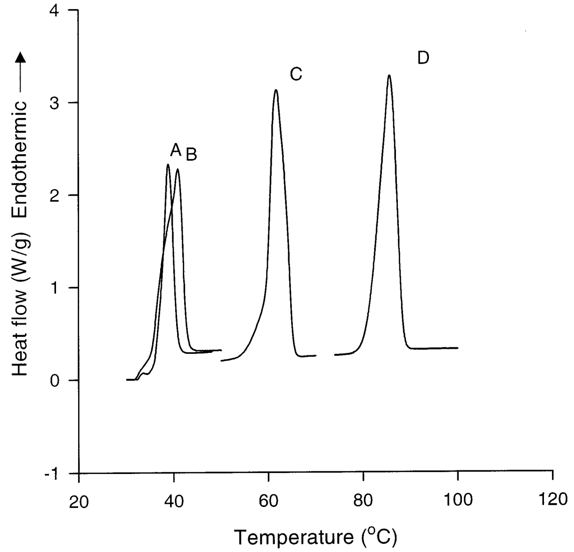

Fig. 4. DSC thermograms of (A) Hf:Vitamin E TPGS 1:6, (B) Hf:Gelucire 44/14:Vitamin E TPGS 1:3:3, (C) Hf:PEG 6000 1:6 solid dispersions and (D) crystalline Hf free base.

The onset and peak melting temperatures and enthalpy changes were measured before the start and again on the fourth week of the in vivo study. Results shown in Table 3 indicate no significant changes in temperature and enthalpy.

3.3. Absolute oral bioavailability of Hf

The formulations of the solid dispersions selected for in vivo evaluation in fasted beagles are shown in Table 1. The Hf:Vitamin E TPGS (1:6) and Hf:Gelucire 44/14:Vitamin E TPGS (1:3:3) formulations had self-emulsifying properties and formed microemulsions upon dissolution whereas the Hf:PEG 6000 (1:6) formulation was non-solubilising, and formed a fine dispersion of drug particles on dissolution of the carrier in vitro. This study was designed to enable a direct comparison of the oral bioavailability of Hf delivered from solubilising and non-solubilising formulations.

The mean (± S.E., $n = 4$) plasma concentration versus time profiles of Hf and the major metabolite, desbutylhalofantrine (Hfm) following oral administration of the three solid dispersions containing 100 mg Hf free base are shown in Fig. 5A and 5B, respectively, and the corresponding mean (± S.D.) pharmacokinetic parameters are presented in Table 4. Although the mean $C_{\max}$ and AUC values of Hf following oral administration of the two solubilising formulations tended to be higher than the non-solubilising formulation, the values were not significantly different ($P > 0.05$). The rate of absorption of Hf from all three oral formulations was also very similar, resulting in insignificantly different $t_{\max}$ values. Examination of the Hfm data showed no significant differences in the rate and extent of Hfm formation after oral administration of the three formulations.

The mean absolute bioavailability of Hf free base for all three oral formulations ranged from 44 to 62%. In spite of the presence of crystalline drug (estimated to be ~8% by XRD) in the Hf:Gelucire 44/14:Vitamin E TPGS formulation on the third week of the in vivo study, this formulation still provided the highest absolute oral bioavailability. When compared to previous data from this laboratory where the absolute bioavailability of the commercially available tablet (containing 250 mg Hf.HCl) in fasted beagles was 8.6 ± 5.3% (Humberstone et al., 1996), these results represent a five- to seven-fold improvement. Results from this study are also comparable to data obtained after administration of a number of prototype lipidic self-emulsifying formulations which resulted in a six- to eight-fold improvement in absolute bioavailability over the commercial tablet after administration of a 50 mg dose of Hf free base (Khoo et al., 1998).

It is apparent therefore, that the delivery of amorphous Hf in either the solubilising or non-solubilising dispersions results in significant enhancement of Hf absorption. Interestingly, however, the absolute bioavailability value obtained for the non-solubilising Hf:PEG 6000 formulation was similar to that of the corresponding solubilising formulations, suggesting that the extent of solubilisation in vitro did not necessarily reflect enhanced bioavailability in vivo. It is possible, therefore, that the primary advantage afforded by all three formulations was the presentation of Hf to the GIT in the highly disordered, thermodynamically metastable amorphous form and that the energy barrier for the drug to enter solution from the fine suspension formed on dissolution of the PEG 6000 carrier was lowered to the point where the in vivo dissolution and absorption of Hf was comparable to the solubilised form.

3.4. Physical stability issues

The chemical and physical instability of most amorphous pharmaceutically active agents has precluded their widespread use in solid dosage

Table 3

Changes in melting temperatures and enthalpy of crystalline Hf free base and the three oral formulations over the 4-week period of the in vivo study

Formulation	Onset temperature (°C)		Peak temperature (°C)		Enthalpy (J/g)	
	Week 1	Week 4	Week 1	Week 4	Week 1	Week 4
Crystalline Hf base	80.9	81.7	84.9	85.3	64.1	65.9
Hf:Vit E (1:6)	36.8	34.0	38.8	36.8	39.2	39.9
Hf:Gel:Vit E (1:3:3)	36.7	39.3	40.8	41.4	63.9	64.2
Hf:PEG 6000 (1:6)	59.2	56.0	61.6	59.9	148.5	144.2

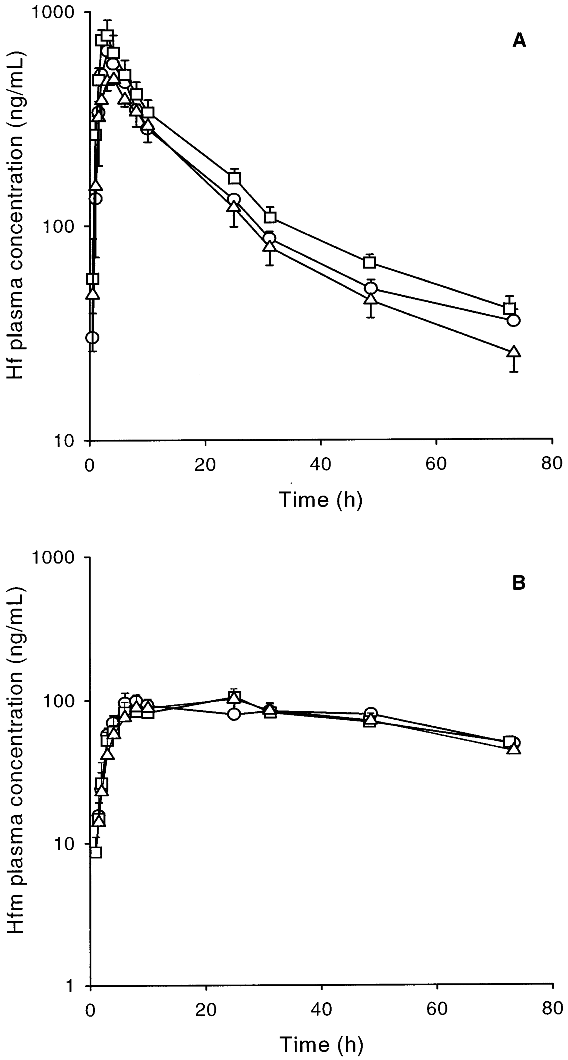

Fig. 5. Mean (± S.E., $n = 4$) plasma concentration versus time profiles of Hf (panel A) and the major metabolite, desbutylhalofantrine (Hfm, panel B) following the oral administration of Hf:Vitamin E TPGS 1:6 (○), Hf:Gelucire 44/14:Vitamin E TPGS 1:3:3 (□) and Hf:PEG 6000 1:6 (△) solid dispersions containing 100 mg Hf free base to fasted beagles.

forms (Hancock et al., 1995). Being thermodynamically metastable, the amorphous drug will tend to spontaneously revert to the more stable crystalline form. This is highly probable if the carrier in which the amorphous drug is dispersed is insufficiently viscous or rigid to stabilise the drug, or if the carrier is susceptible to ageing processes, enabling the dispersed drug to attain the orientation required for crystal nucleation and growth. Such changes are not desirable as they can affect the dissolution rate of the drug and result in diminished absorption. Therefore, following the promising results of the bioavailability study, a physical stability study was established to determine storage conditions that may hasten the rate of drug crystallisation.

Given that the Hf:Gelucire 44/14:Vitamin E TPGS and Hf:Vitamin E TPGS formulations had relatively low melting temperatures, it was anticipated that these two formulations would more likely produce long term stability problems. Whilst the use of polymers such as PVP could be included to reduce the rate of crystallisation in carriers with low melting temperatures (Yoshioka et al., 1995), this was not explored. In view of the similar bioavailability but likely improved stability of the higher melting Hf:PEG 6000 formulation, the stability of this system was investigated in detail.

An accelerated stability study was conducted to investigate the stability of the Hf:PEG 6000 dispersions at different storage temperatures and conditions (i.e. 30°C, 40°C, 40°C/75% relative humidity and 50°C) for a period of 6 months. The effect of drug loading (8, 10, 12 and 14% w/w Hf) on the rate and extent of crystallisation was also investigated.

Visual observations of the surfaces of the solid dispersions over a period of 24 weeks have been tabulated in Table 5. Visual examination of the surface of the solid dispersions revealed that the smooth, glossy white appearance slowly changed to a dull and sometimes yellowish appearance over a period of time. This was often followed by the formation of fine talc-like powdery material and in some cases, the surfaces became rough and brittle. Generally, the formulations stored at higher temperatures (40 and 50°C) and under humidified conditions lost their glossy appearance more rapidly (after two to four weeks), while those stored at 30°C remained glossy after 6 months. The rate at which the powdery material formed on the surfaces of the dispersions stored at 50°C ranged from 4 to 6 weeks, and was more rapid in formulations having the highest drug

Table 4
Pharmacokinetic parameters (mean ± S.D., $n = 4$) of halofantrine (Hf) and the major metabolite, desbutylhalofantrine (Hfm), following oral administration of solubilising Hf:Vitamin E TPGS (1:6) and Hf:Gelucire 44/14:Vitamin E TPGS (1:3:3), and non-solubilising Hf:PEG 6000 (1:6) solid dispersions containing 100 mg Hf free base, and after intravenous administration of Hf (2 mg/kg) to fasted beagles

Parameters	Hf:Vitamin E TPGS (1:6)	Hf:Gelucire 44/14:Vitamin E TPGS (1:3:3)	Hf:PEG 6000 (1:6)	Intravenous Hf
Hf				
C_{max} (ng/ml)	651 ± 66	831 ± 250	540 ± 107	
t_{max} (h)	3.0 ± 0.0	2.1 ± 0.6	3.1 ± 1.2	
$AUC^{0-\infty}$ (ng h/ml)	11435 ± 1461	13902 ± 3224	10089 ± 2213	7046 ± 1488
Absolute BA (%)[a]	51.2 ± 7.8	62.3 ± 14.7	44.4 ± 5.0	
Hfm				
C_{max} (ng/ml)	107 ± 28	106 ± 16	102 ± 36	
t_{max} (h)	16.8 ± 10.1	20.8 ± 8.6	24.9 ± 1.1	
$AUC^{0-\infty}$ (ng h/ml)	8797 ± 779	8471 ± 841	8145 ± 1855	1305 ± 456
Hfm/Hf AUC ratio	0.78 ± 0.10	0.64 ± 0.18	0.84 ± 0.27	0.19 ± 0.07

[a] Absolute bioavailability of Hf = $(AUC_{oral}/AUC_{IV}) \times (dose_{IV}/dose_{oral}) \times 100$.

Table 5
Visual observations[a] of the changes in surface characteristics of Hf:PEG 6000 dispersions of varying drug concentrations stored at different storage conditions for a period of 24 weeks

Formulation	Storage condition (°C)	Time (weeks)				
		0	4	8	12	24
14% Hf w/w	30	Glossy	Glossy	Glossy	Glossy	Glossy
	40	Glossy	Dull	Dull	Dull	Dull
	50	Glossy	Powdery	Powdery	Powdery	Brittle
	40/75%RH	Glossy	Powdery	Powdery	Powdery	Brittle
12% Hf w/w	30	Glossy	Glossy	Glossy	Glossy	Glossy
	40	Glossy	Glossy	Dull	Dull	Dull
	50	Glossy	Dull	Powdery	Powdery	Powdery
	40/75%RH	Glossy	Powdery	Powdery	Powdery	Powdery
10% Hf w/w	30	Glossy	Glossy	Glossy	Glossy	Glossy
	40	Glossy	Glossy	Sl. Dull	Dull	Dull
	50	Glossy	Dull	Powdery	Powdery	Powdery
	40/75%RH	Glossy	Powdery	Powdery	Powdery	Powdery
8% Hf w/w	30	Glossy	Glossy	Glossy	Glossy	Glossy
	40	Glossy	Glossy	Glossy	Glossy	Glossy
	50	Glossy	Dull	Powdery	Powdery	Powdery
	40/75%RH	Glossy	Dull	Powdery	Powdery	Powdery

[a] Glossy — smooth, shiny surface; dull — smooth but not shiny surface; powdery — fine talc-like material on surface; brittle — rough and flaky surface.

loading. The amount of powdery material formed also appeared to increase with increasing drug loading.

Using DSC and HPLC, the powdery and flaky material on the surface was identified as crystalline Hf free base. It was usually present in small amounts (~ 3–5 mg) and was difficult to quantitate accurately, as the powdery material was easily lost during handling.

The mechanism by which surface crystalline Hf free base formed was not explored, although it may reflect crystallisation of Hf present on the outer surface of the solid dispersion since amorphous drug present on the surface may more readily revert to the stable crystalline form than Hf dispersed within the rigid matrix. Alternatively, sublimation of Hf at the higher temperatures employed in the stability study could have led to the formation of powdery material, however, this seems unlikely since solids usually have a low vapor pressure and sublimation is not common.

Amorphous systems exhibit varying degrees of physical and chemical instability and spontaneously crystallise at different rates below and above the glass transition temperature (T_g) (Hancock and Zografi, 1997). The T_g of pure amorphous Hf, measured during the current studies (using DSC at a heating rate of 10°C/min) was approximately −8°C. This value is in line with the predicted T_g of Hf (− 10°C) determined using the formula proposed by Hancock and Zografi (1997). An accurate estimation of the T_g of Hf when incorporated in the PEG 6000 dispersions was difficult due to the low loading of drug in the formulations and the relatively small sample size afforded by the DSC. However, previous studies with indomethacin suggest that the T_g may decrease in PEG 6000 formulations (Ford and Timmins, 1989). It is likely therefore that the solid dispersion formulations used in this study were stored above the T_g of Hf even at ambient temperatures and certainly at all the storage temperatures of the stability study. The increased rate of crystallisation observed at the higher compared with the lower storage temperatures is therefore probably associated with enhanced molecular mobility of Hf within the dispersion at the higher temperatures and not related to a specific glass transition.

Whilst the formation of crystalline material on the surface of the matrix could be crudely detected by visual observation, both DSC and XRD were employed during the stability study for the determination of the presence of crystalline drug within the formulations. As previously discussed, a limiting factor associated with DSC is the sensitivity of this technique and a well-defined Hf peak could only be observed in the DSC thermogram when a physical mix of ~ 15% of crystalline Hf free base was present in the matrix. The small sample size (3–5 mg) required for each DSC measurement was another limiting factor as the results were therefore only representative of the area from which the sample was taken and not the entire matrix. Consequently, for DSC, sampling from different areas of the matrix was required for a more accurate result.

Using DSC, crystalline Hf free base was first detected in the formulations containing 12 and 14% w/w Hf after 8 weeks of storage at 40°C/75% relative humidity. The thermograms showed a slight raising in the baseline around the region where crystalline Hf free base melts (80–85°C). This then increased to become slightly larger and broader peaks. Under the same storage conditions, crystalline Hf free base was not detected in the 10% w/w Hf formulation until the 17th week and for the 8% w/w Hf formulation, crystalline drug was not detected throughout the duration of the study. Storage at 50°C resulted in the formation of small amounts of crystalline drug in the formulations with 10, 12 and 14% w/w Hf, which was only detected after 6 months. The elevated storage conditions did not appear to significantly affect the melting characteristics of the formulations indicating that the matrix had not undergone significant ageing processes throughout the duration of the study.

XRD was also used to quantitate the amount of crystalline material present. XRD has the advantage of examining a larger section of the matrix and also allows the detection of lower amounts of crystalline drug (5%). Unlike DSC, XRD measurements only detected the presence of crystalline Hf in samples stored at 40°C/75% relative humidity for 6 months. The area of the Hf

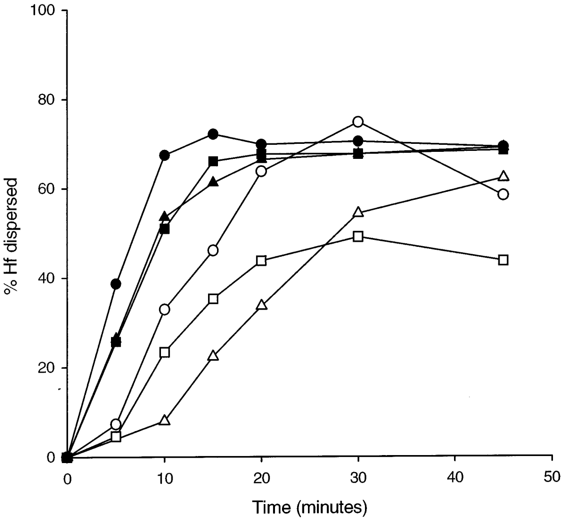

Fig. 6. Representative dispersion profiles of 10% w/w Hf:PEG 6000 formulations stored at either 40°C (closed symbols) or 40°C/75% relative humidity (open symbols) for 4 (●), 12 (▲) and 17 weeks (■).

peak increased with increasing percentages of drug in the formulations and was estimated to range between 8 and 15%. Unlike DSC, XRD studies did not detect any crystalline material in the samples stored at 50°C.

The apparent inconsistencies in the results obtained by DSC and XRD, in terms of the appearance of crystalline material, likely reflect the sampling and sample size differences between the two techniques. DSC samples were obtained by removing small samples from the formulation and were more likely to suffer from contamination from surface crystalline material, whereas XRD was run using a full cross-section of the formulation. The most likely explanation, therefore, is that crystalline Hf free base first appears on the surface of the matrices where it may be observed visually and is preferentially detected by DSC, but the presence of crystalline drug in the bulk of the formulation is limited (as indicated by XRD) except at extreme storage conditions (40°C/75% RH) for 6 months. This is further supported by observations that after the powdery material has been scraped off for DSC, the surface below appeared glossy.

In accordance with the trend in drug crystallinity changes within the formulations (as identified by XRD), the dispersion characteristics of the solid dispersions did not change, except when stored at the most extreme storage conditions (40°C/75% relative humidity) and for long periods (Fig. 6). The dispersion characteristics of the Hf:PEG 6000 formulations stored at the other conditions did not change significantly throughout the study period.

In summary, it appears that Hf:PEG 6000 dispersions containing low percentages of Hf (< 10%) were more stable than those containing a higher percentage of Hf. Similarly, increasing the storage temperature increased the rate at which crystallisation occurred, and this was further hastened by storage in humidified conditions (which could be minimised by appropriate packaging).

4. Conclusion

Solubilising and non-solubilising solid dispersions containing amorphous Hf free base were successfully prepared using a simple fusion method requiring temperatures as low as 70°C. Three formulations were evaluated in vivo in fasted beagles and in comparison to the commercial tablet, these afforded a five- to seven-fold improvement in absolute oral bioavailability. This study also showed that when Hf was delivered in the amorphous form, the solubilising formulations did not offer significant bioavailability advantages over the non-solubilising formulation. A stability study of the non-solubilising Hf:PEG 6000 formulation showed that it was stable at temperatures below 40°C and that a drug loading of 10% or less improved the stability of the formulation over longer periods.

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