JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 1049–1053

Received October 22, 2001
Accepted April 9, 2002
ISSN 0022-3573

Enhanced oral absorption of halofantrine enantiomers after encapsulation in a proliposomal formulation

Dion R. Brocks and Guru V. Betageri

Abstract

In this study, we evaluated the ability of a coated, encapsulated formulation to increase the oral bioavailability of (±)-halofantrine (HF) enantiomers, a drug with low and erratic oral bioavailability. After encapsulation of HF in distearoylphosphatidylcholine, the dried particles were coated with cellulose acetate phthalate. A suspension of the product was made using methylcellulose as a dispersion agent, and the product was administered to Sprague–Dawley rats to provide a HF dose of 7 mg kg^{-1} as the HCl salt. HF HCl powder in 1% methylcellulose with or without liposomal product excipients was also administered to separate groups of rats, which served as control groups. Serial blood samples were obtained from the rats and plasma was assayed by stereospecific high-performance liquid chromatography. There were no significant differences in the area under the concentration–time curve (AUC) or maximum concentration (C_{max}) between the two control groups. Plasma concentrations of both HF enantiomers were significantly higher in the rats given HF as an encapsulated proliposomal formulation compared with the control groups. Compared with methylcellulose control, the encapsulation product resulted in increases of 41 to 47% in the AUC of HF enantiomers, and 90 to 100% in C_{max}. The ability of an encapsulated proliposomal product to significantly increase the oral absorption of HF was clearly demonstrated.

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada

Dion R. Brocks

College of Pharmacy, Western University of Health Sciences, Pomona, CA, USA

Guru V. Betageri

Correspondence: Dion R. Brocks, Associate Professor of Pharmaceutical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada, T6G 2N8. E-mail: dbrocks@pharmacy.ualberta.ca

Note and Funding: This work was presented in part at the 2000 American Association of Pharmaceutical Scientists Annual Meeting, 29 October–2 November, Indianapolis, IN, USA. This work was supported by the Burroughs Wellcome Foundation and AFPE (AACP-NIP grant to D.R.B.).

Introduction

In the generation of new drug formulations, encapsulation of active ingredients into liposomes has been attempted as a means of increasing the solubilization of active ingredients in parenteral products, and as a means of potentially enhancing the ability of drugs to be absorbed across biological membranes. This latter application may have utility in improving oral bioavailability of otherwise poorly bioavailable drugs. In general, however, this methodology has not been optimal due to lack of integrity of the liposomes caused by low pH in the stomach and high concentrations of bile salts in the proximal duodenum. Formulation failure would then result due to introduction of unencapsulated drug at the major anatomical site of gastrointestinal drug absorption, the small intestine. One approach to overcoming the problem is to coat the preformed liposomes with a protective coating, although this may be of limited utility owing to difficulties in applying coating to the suspended particles. Application of coating to dried proliposomal formulations of insulin, however, has been used successfully by several investigators, and sustained reductions in blood glucose levels in rats suggested an improvement in insulin delivery with the resultant formulations (Takeuchi et al 1996; Ramadas et al 2000).

One agent that has been used as a model compound for study of lymphatic absorption of drugs is the phenanthrene methanol agent, halofantrine (HF) (Porter et al 1996a, b; Porter & Charman 2001). HF, which is chiral and administered as the racemate for treatment of chloroquine-resistant strains of *Plasmodium falciparum*, is known to possess incomplete and erratic oral bioavailability in humans and animal species, presumably due to its high lipophilicity (Milton et al 1989; Humberstone et al 1996; Brocks et al 2000). The ingestion of oral lipids is known to cause increases in the oral bioavailability of the drug (Milton et al 1989; Humberstone et al 1996). Although the

enantiomers of HF share similar potency with respect to antimalarial activity (Karle et al 1993), it is nevertheless important to consider the enantioselective pharmacokinetics of the drug because there appears to be stereoselectivity in its cardiotoxic effects (Karle 1997; Wesche et al 2000). Further, stereoselectivity has been established in the pharmacokinetics of the drug when given as the racemate (Gimenez et al 1994; Brocks & Toni 1999; Brocks 2000).

In this study, we have prepared a dry proliposomal preparation of HF with protective coating applied to the surface of the solid particles, using the same technique used to produce a formulation of glyburide (Kumar et al 2001). In order to assess the viability of this formulation as a means of increasing the oral bioavailability of HF enantiomers, we conducted a study using the rat, the results of which are described here. It was anticipated that on dissolution of the protective coating in the intestines, liposomes containing HF would be formed due to hydration, thus affording increased solubility and absorption, yielding higher plasma concentrations of HF enantiomers.

Materials and Methods

Chemicals

(±)-HF HCl was a gift from SmithKline Beecham Pharmaceuticals (Worthing, UK). (±)-Halothane U.S.P. (Halocarbon laboratories, River Edge, NJ) delivered by anaesthetic machine was used as the inhalation anaesthetic for rats. Heparin was obtained from Elkins-Sinn (Cherry Hill, NJ). All other chemicals were purchased from Fisher Scientific (Fair Lawn, NJ).

Distearoylphosphatidylcholine (DSPC) was purchased from Avanti Polar Lipids (Alabaster, AL). Cellulose acetate phthalate (CAP) was a gift from Eastman (Kingsport, TN). Chloroform (HPLC grade) was purchased from Fisher Scientific Company (Pittsburgh, PA).

Preparation of proliposomal formulation

HF and DSPC (1 : 3) were dissolved in chloroform, and the organic solvent was evaporated under nitrogen. The dry powder was passed through a no. 60 mesh screen. The proliposomal formulation was coated using CAP. The CAP was dissolved in acetone (50 mg mL^{-1}) and sprayed over the dried HF : DSPC mixture, as previously described for a similar formulation of glyburide (Kumar et al 2001).

Animals and pre-experimental procedures

Pharmacokinetic studies were performed using a total of 20 male Sprague–Dawley rats (Harlan Sprague–Dawley; Madison, WI), with a mean bodyweight of 313±16 g at time of use. The study protocol was approved by the Institutional Animal Use and Care Committee at the Western University of Health Sciences. All rats were housed in a temperature-controlled room with a 12-h light–dark cycle for at least 3 weeks. Animals received Harlan Teklad 8604 (Madison, WI) rodent diet containing a minimum 4% crude fat content throughout the housing and experimental part of the study.

The day before the pharmacokinetic experiment, the right jugular veins of all rats were catheterized with Micro-Renathane tubing (Braintree Scientific, Braintree, MA) under halothane anaesthesia. The cannula was flushed with 100 U mL^{-1} heparin in 0.9% saline. After surgery, the rats were transferred to regular holding cages and allowed free access to water, but food was withheld overnight. The next morning, the rats were transferred to metabolism cages and dosing was performed followed by blood sampling.

HF administration and sample collection

Each rat was allocated to one of three groups, and was administered 7 mg kg^{-1} of (±)-HF base as the HCl salt by oral gavage. The control group (n = 6) was administered (±)-HF HCl as suspension dispersed in 1% (w/v) methylcellulose. A second control group (n = 6) was given 0.77% (w/v) methylcellulose and 4.4% acetic acid suspension containing unencapsulated (±)-HF HCl, plus excipients (lipid and CAP) in the same drug/excipient ratio as was used in the encapsulated proliposomal formulation. In the third test group (n = 8), rats were administered proliposomal, encapsulated (±)-HF HCl, dispersed in 0.77% (w/v) methylcellulose and 4.4% acetic acid. Acetic acid was included as part of the suspended proliposomal formulation in order to maintain the integrity of the coating surrounding the components required for formation of liposomes.

Blood samples were obtained from rats administered oral (±)-HF at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 32 and 48 h after dosing. After collection, each blood sample was centrifuged at 2000 *g* for 3 min. The plasma layers were transferred to new polypropylene tubes and stored at −20°C until analysed by high-performance liquid chromatography (HPLC). At 2 h after dosing rats with (±)-HF, animals were allowed food *ad libitum* for the remainder of the study.

Assay of HF

A stereospecific HPLC assay was used to measure HF enantiomers in the plasma samples (Brocks et al 1995). The assay was previously validated for use in rat plasma, and had a validated lower limit of quantitation of 25 ng mL^{-1} for each HF enantiomer based on 100 μL of rat plasma (Toni & Brocks 1997). For all analytical runs, quality control samples were incorporated to ensure integrity of the results.

Data analysis

The elimination rate constant (λ_z) was estimated by subjecting the plasma concentrations in the terminal phase to linear regression analysis. Elimination half-life was calculated by dividing 0.693 by λ_z. The area under the plasma

concentration–time curve from time zero to infinity after dosing ($AUC_{0-\infty}$) was calculated using the combined log-linear trapezoidal rule from 0 h after dosing to the time of the last measured concentration, plus the quotient of the last measured concentration divided by λ_z. C_{max} and t_{max} were determined by visual inspection of the plasma concentration–time data.

Statistical analysis

Data are reported as mean ± s.d., unless otherwise indicated. Differences were assessed for significance using one-way analysis of variance or by Student's paired or unpaired *t*-tests (two-tailed) as appropriate. For ranking, Duncan's multiple range test was used. The level of significance was set at $P = 0.05$.

Results

The final encapsulated formulation was solubilized in acetonitrile/methanol/ammonium hydroxide (20:10:1) and assayed for HF content using HPLC. The ratio of HF HCl to excipient components in the final proliposomal product was found to be 1:4.5.

The $t_{\frac{1}{2}}$ value could not be determined in one rat, and in another rat blood sampling could only be carried out for 8 h due to loss of patency of the cannula. From the latter rat, only C_{max} and t_{max} data were obtained. In some other rats, particularly for the (−) enantiomer, plasma concentrations could not be measured further than 24 h due to plasma concentrations falling below the lower limit of detection. Because most of the orally administered dose of HF is absorbed within 24 h in the rat (Brocks & Toni 1999), the AUC truncated to 24 h was evaluated as a measure of extent of drug absorption to allow use of data from as many animals as possible. This approach to bioequivalence has been validated by other investigators (Gaudreault et al 1998; Sathe et al 1999).

Stereoselectivity was present in plasma concentrations of HF enantiomers in each of the three formulations (Figure 1; Table 1). For each of the formulations administered (Table 1), C_{max} and AUC of the (+) enantiomer were significantly greater than the corresponding (−) enantiomer, and no differences were noted in the (+)/(−) ratios between groups. No differences were detected in the t_{max} or in the $t_{\frac{1}{2}}$ between enantiomers or between groups.

With respect to relative ranking using the Duncan's multiple range test, the encapsulated formulation attained higher C_{max} of both enantiomers than did the control and lipid control formulation (Table 1). The AUC_{0-24} of (+)-HF after administration of the proliposomal formulation was higher than in the control formulation. With respect to the ranking between formulations for the (−) enantiomer AUC, no difference could be assigned (Table 1). However, a direct comparison between the (−)-HF AUC_{0-24} of the liposomal formulation and non-lipid control formulation (Student's *t*-test) did detect a significant difference between the mean values ($P = 0.044$). With respect to the sum of the two enantiomers, both the AUC and C_{max} of (±)-HF were significantly higher after administration of the proliposomal formulation than respective values in both control groups, which in turn were not significantly different from one another.

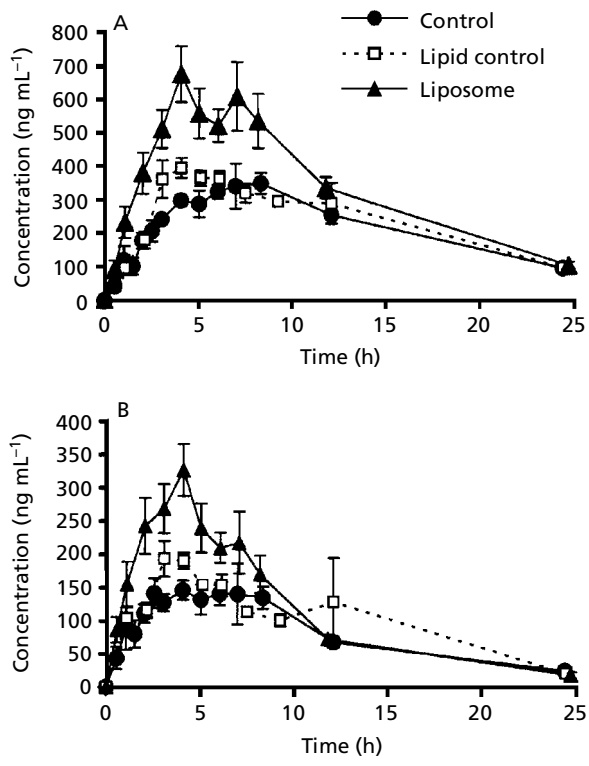

Figure 1 Mean ± s.e. plasma concentration–time data of (+)-halofantrine (A) and (−)-halofantrine (B) after oral administration of three different formulations to rats. Formulations were halofantrine HCl powder as methylcellulose suspension (control), halofantrine HCl powder as methylcellulose suspension plus DSPC:CAP (lipid control), and encapsulated halofantrine in DSPC:CAP proliposomes suspended in methylcellulose (liposome).

Discussion

The most stable liposomal formulations, containing DSPC and cholesterol, may deteriorate with subsequent leakage of encapsulated drug in the small intestine owing to the presence of bile salts (Rowland & Woodley 1980), although recent evidence suggests that modification of the cholesterol content may be helpful in increasing their stability (Kokkona et al 2000). Liposomes composed of stable and indigestible components are reportedly also ineffective (Deshmukh et al 1981). The use of polymerized liposomes administered by the oral route has attracted some attention (Regen 1987). For example, Okada et al (1995) showed that over 6 days in simulated gastric fluids, the release of ^{14}C-BSA or ^{14}C-sucrose was about 70% from polymerized liposomes compared with 30% from conventional liposomes. Using polymerized radiolabelled liposomes, it

Table 1 Pharmacokinetic data for halofantrine enantiomers after administration of 7 mg kg^{-1} racemic HCL salt as one of three different formulations.

Parameter	(+) Halofantrine			(−) Halofantrine			(+)/(−) Ratio		
	Control	Lipid control	Liposome	Control	Lipid control	Liposome	Control	Lipid control	Liposome
AUC_{0-24} (ng h mL^{-1})	5229±788	5564±735	7704±1813[b]*	1859±529	2274±732	2618±656[b]	2.98±0.78	2.55±0.46	2.97±0.42[b]
C_{max} (ng mL^{-1})	391±59.2	478±68.5	784±236[c]*	196±42.3	263±71.4	381±93.0[c]*	2.03±0.28	1.88±0.32	2.07±0.39[c]
t_{max} (h)	7.02±2.81	5.44±3.49	5.21±2.06[c]	4.14±2.33	5.40±3.48	4.64±1.43[c]	–	–	–
$t_{\frac{1}{2}}$ (h)	12.9±2.9	14.3±6.1	18.2±2.9[c]	10.7±3.3[a]	11.7±6.7	21.2±14.6[c]	–	–	–

The formulations were halofantrine HCl powder as methylcellulose suspension (control), halofantrine HCl powder as methylcellulose suspension plus DSPC:CAP (excipient control), and encapsulated halofantrine in DSPC:CAP proliposomal particles suspended in methylcellulose (liposome). Data are mean±s.d., n = 6, unless otherwise indicated ([a]n = 5; [b]n = 7; [c]n = 8). *Liposome group significantly ($P < 0.05$) higher than both control groups; no difference between the two control groups (Duncan's multiple range test).

has been demonstrated that liposomes may retain their integrity in the gastrointestinal tract (Chen et al 1996). Another group developed and administered enteric-coated capsules containing a powdered mixture of freeze-dried preformed liposomes and antigen (Childers et al 1995). On dissolution of the capsule in the intestine, the contents of the capsule were released and hydrated to form antigen-entrapped liposomes 100 nm in size. The antigen from this formulation was taken up by the small intestine, thereby inducing a humoral immunological response. In our experiments, the particles coated with CAP would be expected to dissolve at neutral and basic pH only, preventing disruption of any liposomes that would form in the acidic environment of the stomach.

The results presented here indicated that when HF HCl was encapsulated in the enteric-coated proliposomal formulation, significant increases occurred in both C_{max} and AUC_{0-24}, but not t_{max}, of HF enantiomers compared with the methylcellulose-only suspension control group (Table 1). In the liposomal group, the mean AUC_{0-24} of the (+) and (−) enantiomers were 47% and 41% higher, respectively, than in the corresponding non-lipid control group. The mean C_{max} was affected even more, with the C_{max} of the (+) and (−) enantiomers being 100% and 94% higher, respectively, than in the corresponding non-lipid control group. This suggested an increase in the extent of absorption of the HF enantiomers, presumably, although not definitely, as a consequence of successful introduction of HF-containing liposomes into the small intestine. In contrast, there were no differences between the AUC and C_{max} of HF enantiomers in the two control formulations. Taken together, the results suggest that it was not merely the presence of the DSPC and CAP that enhanced the bioavailability of the drug, but rather the encapsulation process that led to the enhanced absorption.

The second excipient-containing non-encapsulated formulation of HF was included in the study for two reasons. First, the clearance of HF has recently been demonstrated to be decreased by the ingestion of orally administered lipids (Humberstone et al 1998). This was an unlikely outcome because of the small amount of lipid (~ 10 mg) given as part of the formulation. Nevertheless, this second control formulation was included, in part to rule out the possible effects of increased lipoprotein binding and hence higher plasma HF concentrations as a result of ingestion of the lipid components of the formulation. Second, it might be possible that increases in bioavailability of the encapsulated product were due not to absorption of the HF as part of the encapsulation process, but rather due to a lipid-mediated solubilization of drug in the intestinal tract as a result of the presence of phospholipids in the formulation. Given the similarity in pharmacokinetic data between the two control groups (Figure 1; Table 1), it appears that encapsulation rather than decreased clearance or enhanced solubilization due to the presence of phospholipid was responsible for the increase in bioavailability in the rats given the proliposomal formulation.

HF is a highly lipophilic drug and has been reported to possess erratic absorption and incomplete bioavailability (Milton et al 1989; Humberstone et al 1996). In the rat, absolute bioavailability of the enantiomers lies between 20 and 30% in the fasted state (Brocks & Toni 1999). The enantiomers of HF possess a low hepatic extraction ratio (Brocks & Toni 1999), and therefore the incomplete bioavailability is likely attributable to incomplete absorption from the intestinal tract. Intestinal drug metabolism may also play a role in the incomplete bioavailability of HF, because HF is known to be a substrate for hepatic cytochrome P-4503A enzymes (Baune et al 1999), which are present in the small intestine in significant concentrations. Although the proliposomal product tested here did increase HF oral bioavailability by nearly 50%, this still represents an absolute bioavailability of less than 50%. Therefore, beyond the approach described here, more could potentially be done to increase the absorption of the enantiomers, such as modifying the particle size of the pre-coated proliposomal particles, or by changing the phospholipid/drug ratio. If intestinal drug metabolism does play a significant role in the low bioavailability of the drug, there may be practical limitations on the ability of formulation changes to increase the extent of bioavailability of HF when administered by the oral route.

In conclusion, an enteric-coated proliposomal formulation displayed a significant improvement in the oral bioavailability of HF when administered to the rat. Studies using other model drugs are required to demonstrate the general utility of this approach for increasing the bioavailability of poorly absorbed compounds.

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