J. Pharm. Pharmacol. 1998, 50: 1221–1225
Received May 11, 1998
Accepted July 6, 19988

Submicron Oil-in-water Emulsion Formulations for Mefloquine and Halofantrine: Effect of Electric-charge Inducers on Antimalarial Activity in Mice

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Abstract

Stearylamine, oleic acid, phosphatidylserine and dicetylphosphate have been studied to determine their capacity to induce electric charge on non-ionic submicron emulsions containing halofantrine and mefloquine.

The in-vivo antimalarial activity of drug-loaded emulsions, evaluated in mice, was affected by the nature of the additives used. In particular, the electric-charge inducers markedly affected the pharmacological activity of mefloquine, but not of halofantrine. After subcutaneous administration ED50 values (the doses affording 50% protection) were 3 and 15 mg kg^{-1}, respectively, for halofantrine and mefloquine emulsions without charge inducers. The mefloquine-loaded emulsions with charge inducers were active at 10 mg kg^{-1} for dicetylphosphate, 17 mg kg^{-1} for phosphatidylserine, 23 mg kg^{-1} for oleic acid and 27 mg kg^{-1} for stearylamine, again after subcutaneous administration.

This work has enabled the formulation of stable emulsions, incorporating drugs with high antimalarial activity, which are proposed for parenteral delivery of these fairly soluble drugs.

Halofantrine and mefloquine are effective antimalarial drugs against multidrug-resistant strains of *Plasmodium falciparum*. Both drugs have advantageous pharmacological properties such as high activity and long half-lives although their extensive tissue and protein binding, slow clearance and very variable oral absorption should be improved (Karbwang & White 1990; Karbwang & Bangchang 1994). They are also poorly soluble in aqueous media, resulting in difficulties in formulating parenteral injections. A mixture of acetamide and propylene glycol was proposed and used in clinical studies as an injectable vehicle, but it is not devoid of toxic effects (Krishna et al 1993). Formulations of both highly lipophilic drugs as submicron oil-in-water (o/w) emulsions could be an interesting alternative delivery system.

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During the last decade numerous studies of carrier systems such as liposomes (Harashima & Kiwada 1996; Aoki et al 1997), niosomes (Uchegbu et al 1995), nanoparticles (Couvreur & Vauthier 1991; Storm et al 1995) and submicron emulsions (Klang et al 1994) have given strong evidence that their surface charge, hydrophobicity and particle size can affect their biological fate. The current study was performed to evaluate the effect of electric-charge-inducing additives present in the dispersions on the physical properties of non-ionic o/w emulsions and on the in-vivo antimalarial activity of halofantrine and mefloquine.

Materials and Methods

Emulsion ingredients

Pure phosphatidylserine was a gift from Lipoid GmbH (Ludwigshaven, Germany). Poloxamer (Lutrol F68) was obtained from BASF (Brussels, Belgium). Dicetylphosphate and stearylamine were

purchased from Sigma (Munich, Germany). Medium-chain triglycerides (Akomed E) were a gift from Karlshamns (Karlshamns, Sweden). Oleic acid was purchased from Merck (Darmstadt, Germany). Other products and reagents were of analytical or pharmaceutical grade.

Halofantrine hydrochloride (3-dibutylamino-1-[1,3-dichloro-6-(trifluoromethyl)-9-phenantrenyl]-propan-1-ol) was kindly donated by SmithKline Beecham (Brussels, Belgium) and mefloquine hydrochloride (2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinoline methanol) was a gift from Roche (Brussels, Belgium).

Emulsion preparation

Emulsions were prepared as described previously (Mbela & Ludwig 1995). Briefly, glycerol (2·25 g) and poloxamer (2 g) were dissolved in fresh distilled water (80 g) and heated at 50°C. Antioxidant (α-tocopherol, 0·02 g) and the required charge-inducer, i.e. stearylamine (0·3% w/w), dicetylphosphate (0·3% w/w), oleic acid (0·5% w/w) or phosphatidylserine (1·2% w/w) were added to the oil (20 g) at 50°C. The investigated drug (1% w/w) was also dissolved in the oil phase.

The primary emulsion obtained using an Ultra Turrax stirrer (T25, Janke and Kunkel, Stauffen im Breisgau, Germany) was homogenized with an ultrasonic probe at 120 W (Branson B 12 Sonifier, Branson Sonic Power, Vésenaz, Switzerland).

Portions of the samples in sterile vials (glass type I) were thermally stressed by steam sterilization at 121°C for 20 min (Melag Dampf type 23, D autoclave). All samples were stored under refrigeration at 5°C.

Physical characterization

All measurements were performed at room temperature (22 ± 0·7°C). Measurement of the droplet size of emulsions was performed, after appropriate dilution with the aqueous phase used for the preparation, with a Coulter (Luton, UK) Electronic model N4MD.

The zeta potential was calculated from the mean electronic mobility of the droplets in solutions containing 0·01 M potassium chloride, measured by use of a Coulter Delsa zeta meter 440SX.

The pH of the emulsions was measured with a Radiometer (Copenhagen, Denmark) PH M63 pH meter. The osmolality was measured in triplicate by means of a vapour pressure osmometer (Model 5500; Wescor, Logan, UT).

Determination of drug concentrations

The concentrations of the drugs in the emulsions were quantified by reversed-phase high-performance liquid chromatography (HPLC) with a Merck–Hitachi 655A-11 pump connected to a manual injector, to a Merck–Hitachi L-4000 UV detector set at 252 nm and to a Merck Hitachi D-2500 integrator for data processing (Kapetanovic et al 1983; Hines et al 1985). Appropriate dilutions of the drugs were chromatographed on a C_{18} column (Merck LichroCart Manu-Fix; #15542). The mobile phases were acetonitrile–water–acetic acid, 75 : 25 : 0·1 (v/v) containing 0·001 M lauryl sulphate at pH 3·5 for halofantrine and acetonitrile–water–acetic acid, 48 : 52 : 0·1 (v/v) for mefloquine. Chromatography was performed at room temperature.

The drug concentrations were assessed in the whole emulsions, and separately in the water and oil phases after separation by ultracentrifugation for 7 min at 15 000 *g* and 15°C. The concentrations were estimated from a calibration curve of peak height plotted against the amount of analyte (mL solution)$^{-1}$ using three concentration levels of the drugs (500, 1000 and 2000 ng mL^{-1}).

In-vivo antimalarial activity

The antimalarial activity of the emulsions was evaluated in-vivo by the Peters classic 4-day suppressive test against chloroquine-resistant *Plasmodium yoeli* (Peters 1980). Briefly, erythrozoon-free Swiss male mice (Iffa-Cedo, F), 20 ± 2 g, were infected with 10^7 parasitized cells in 0·9% saline on day 0. Groups of five mice were treated subcutaneously from day 0 to day 3 with increasing doses ranging from 0·0 to 300 mg drug in emulsion. The suppressive effect was estimated on day 4 by comparing, under magnification 1000 ×, Giemsa-stained thin blood smears from the tail vein of treated mice with those from a control group of mice injected with saline. Inhibition (%) was calculated, after counting at least 9×10^3 cells, by use of equation 1:

$$\text{Ratio} = [(1 - P_t)/(1 - P_c)] \times 100 \qquad (1)$$

where P_t is the parasitaemia of the treated animals and P_c is that of the untreated mice. ED50 values (the doses affording 50% protection) were then determined by linear interpolation.

Results and Discussion

Physical characterization

Phospholipids are commonly used as emulsifiers for the preparation of stable parenteral intravenous emulsions. As reported in a previous study, stable emulsions containing stearylamine were obtained only with non-ionic emulsifiers and not with

lecithin-emulsified dispersions. This could be because of the interaction of the cationic group of the stearylamine with the minor lipids associated with phosphatidylcholine in lecithins (Mbela et al 1998).

In the current study the various charge inducers were added to emulsions stabilized by non-ionic poloxamer (Lutrol F68), because their thermal and storage stability was proved satisfactory. Oleic acid, dicetylphosphate and phosphatidylserine were deliberately added as negative-charge inducers and stearylamine to confer a positive electric charge. Oussoren & Storm (1998) have reported the influence of phosphatidylserine on the resorption of liposomes after subcutaneous administration. The emulsions prepared with poloxamer alone were expected to be electrically neutral. The concentration used was selected on the basis of previous studies (Mbela et al 1998).

The physicochemical properties of the various emulsions without and with drug before and after thermal stress are summarized in Table 1. A smaller mean droplet size was measured for dicetylphosphate emulsions without drug but the thermal and storage stability was higher than for emulsions containing stearylamine. The difference between the mean droplet sizes of these two emulsions could be related to the electric charges of the ionizable groups of these substances because both bear similar lipophilic alkyl groups which probably confer identical rigidity to the interfacial film.

Autoclaving had an insignificant effect on the mean droplet size of the stressed emulsions. Interdroplet repulsion seemed sufficient to prevent coalescence. The preparations should be passed through a microfluidizer to reduce droplet size, thus improving the efficiency of absorption of the parenteral emulsions (Mbela & Ludwig 1995; Koster et al 1996).

The mean droplet size of the mefloquine-loaded emulsions was in accordance with parenteral intravenous use. That autoclaving had no significant effect on mean droplet size implied that the zeta potential created at the surface of the oil droplets conferred marked stability and thermal resistance. But even for stearylamine, which induces only a small zeta potential, emulsions resisted thermal stress. From the zeta potential data of Table 1 it could be concluded that mefloquine will be at least partly present in the interface as the emulsion without charge inducer had a zeta potential of −14 mV, compared with −2 mV for the blank emulsion. Halofantrine, which should also be present at the interface, influenced the zeta potential of the emulsion to a lesser extent (−4 mV).

The droplet size for the halofantrine-loaded emulsions was smaller (approx. 400 nm) than for mefloquine-loaded droplets (mean droplet size 680 nm), and the distribution was unimodal. According to Lundberg et al (1996) the stability of the emulsions should be excellent because the mean droplet size is indicative of a good physical stability which is dependent on small particle diameter.

The osmolality of all preparations was 294 ± 4 mOsm kg^{-1}, and did not change significantly after autoclaving and storage at 5°C. Except for the drug-free and stearylamine-containing emulsions, the pH of which was near 7, the pH of the drug-loaded dispersions was 3. The pH did not decrease substantially as a result of heating; in general the drop was approximately 0·5 to 0·8 pH

Table 1. Mean droplet size, zeta potential and in-vivo antimalarial activity after subcutaneous administration, of mefloquine- and halofantrine-loaded o/w submicron emulsions containing different electric-charge inducers.

Composition		Concn (%)	Mean droplet size ± s.d. (nm)		Zeta potential (mV) After autoclaving	Antimalarial activity ED50 ± s.d.‡ (mg kg^{-1})
Drug	Charge inducer		Before autoclaving†	After autoclaving		
None	None	–	902 ± 30	915 ± 16	− 2·22	–
None	Dicetylphosphate	0·5	700 ± 54	707 ± 48	–	–
None	Stearylamine	0·3	990 ± 30	996 ± 32	–	–
Mefloquine	None	–	595 ± 38	589 ± 32	− 14·14	15·00 ± 0·05
Mefloquine	Dicetylphosphate	0·5	326 ± 29	341 ± 21	− 11·07	10·00 ± 0·01
Mefloquine	Oleic acid	0·5	421 ± 32	470 ± 39	− 10·68	25·00 ± 0·03
Mefloquine	Phosphatidylserine	1·2	670 ± 30	675 ± 74	− 7·20	17·00 ± 0·01
Mefloquine	Stearylamine	0·3	616 ± 25	600 ± 46	+ 0·72	27·00 ± 0·05
Halofantrine	None	–	293 ± 24	302 ± 22	− 4·26	3·25 ± 0·01
Halofantrine	Dicetylphosphate	0·5	306 ± 29	299 ± 18	− 11·45	3·10 ± 0·01
Halofantrine	Oleic acid	0·5	390 ± 10	396 ± 23	− 12·02	3·20 ± 0·01
Halofantrine	Phosphatidylserine	1·2	329 ± 23	320 ± 25	− 8·76	3·15 ± 0·01
Halofantrine	Stearylamine	0·3	263 ± 17	285 ± 22	+ 5·02	3·25 ± 0·01

The emulsions all contained 2% surfactant F68, 20% medium-chain triglycerides and were isotonized with 2·25% w/w glycerol. †20 min at 121°C. ‡Dose affording 50% protection.

Table 2. Concentrations of mefloquine and halofantrine hydrochlorides in the whole emulsions and in the oil and water phases.

Composition		Concn (%)	Emulsion		Aqueous phase	Oil phase
Drug	Charge inducer		Before autoclaving†	after autoclaving		
–	–		0·0±0·0	0·0±0·0	0·0±0·0	0·0±0·0
Mefloquine	–	–	95·0±0·8	96·3±0·6	1·3±0·1	97·3±0·4
	Dicetylphosphate	0·5	97·5±0·1	96·4±0·3	1·5±0·7	96·6±0·2
	Oleic acid	0·5	95·0±0·4	97·0±0·2	1·3±0·8	96·1±0·4
	Phosphatidylserine	1·2	98·2±0·3	98·6±0·2	1·5±0·1	96·9±0·2
	Stearylamine	0·3	97·2±0·2	98·4±0·1	1·8±0·2	96·2±0·1
Halofantrine	–	–	100·7±0·4	100·2±0·4	< 1·0	99·3±0·3
	Dicetylphosphate	0·5	100·3±0·2	98·4±0·3	< 1·0	98·7±0·6
	Oleic acid	0·5	101·2±0·7	99·5±0·8	< 1·0	98·6±0·3
	Phosphatidylserine	1·2	100·0±0·3	95·0±0·5	< 1·0	99·1±0·5
	Stearylamine	0·3	101·0±0·1	98·5±0·2	< 1·0	98·4±0·3

Results are expressed as percentages of the initial amounts of drugs added to the oil phase. †20 min at 121°C.

unit and pH also remained constant during storage at 5°C for 720 days, indicating that the emulsions were satisfactory—pH is a good indicator of the age and the stability of o/w emulsions (Washington & Davis 1987).

The data in Table 2 indicate that the drug-content of the emulsions remained constant (up to 98% w/w) for up to 720 days of storage at 5°C. Occasionally mefloquine (retention time 3·95 min) decomposed slightly, yielding ±2% w/w of a by-product (retention time 2·92 min). Halofantrine did not decompose and was not affected by the additives, by heat sterilization or by storage.

Both antimalarials dissolve mainly in the inner phase of the o/w emulsion—after phase separation by centrifugation approximately 98% of the drugs was located in the oil phase. The small amount of mefloquine present in the aqueous phase could be solubilized by micelles of poloxamer or charge inducer possibly present in the preparation.

In-vivo antimalarial activity

The antimalarial activity of mefloquine- and halofantrine-loaded emulsions containing different charge inducers is summarized in Table 1. All formulations had high in-vivo antimalarial activity after 360 days storage at 5°C. Reference halofantrine- and mefloquine-loaded emulsions containing no charge inducer were active at 3 mg kg^{-1} and 15 mg kg^{-1}, respectively, after subcutaneous administration. The activity of the reference mefloquine emulsion is in accordance with that reported earlier (Mbela et al 1994). The additives significantly affected activity. The greatest activity was observed for formulations containing dicetylphosphate; oleic acid and stearylamine markedly reduced mefloquine activity with the lowest activity being recorded for stearylamine-containing emulsions (ED50 27 mg kg^{-1}; s.c.). Phosphatidylserine at 17 mg kg^{-1} did not significantly influence mefloquine schizonticidal activity after subcutaneous administration; the activity was similar to that of mefloquine alone and to that observed previously in lecithin-stabilized mefloquine-loaded emulsions. This indicates that the nature of the emulsifiers, namely lecithin or non-ionic substance, had no effect on activity. As expected, emulsion containing no drug and no charge inducers (blank) was devoid of antimalarial activity.

This study did not enable reliable correlation between the antimalarial activity (ED50) observed and the droplet size or the zeta potential. The in-vivo activity should be assessed in-vitro or ex-vivo in cell and tissue cultures to gain insight in the influence of the test model used on the pharmacological profile of the drugs. Humberstone et al (1998) pointed out the influence of culture media on the inhibition of the growth of the parasite.

Nevertheless, the activity of mefloquine recorded in this study is consistent with that observed in the prepatent antimalarial test (Mbela et al 1994) and suggests that the drug activity might not be significantly modulated by the antimalarial test employed. The variation in activity was related both to the nature of the additives present in the medium and to the intrinsic activity of the drugs.

All halofantrine-loaded emulsions had similar antimalarial activity (Table 1). The mean ED50 recorded after subcutaneous administration was 3 mg kg^{-1} and so the effect of the additives on its pharmacological activity was not apparent from the Peters antimalarial test in which halofantrine has marked antiparasite activity.

Because the characteristics of the emulsions, e.g. droplet size, pH and zeta potential, and the concentrations of the drugs in the preparations did not change significantly during storage, the variation in

activity observed for the mefloquine series could not be related to these properties. The increased performance of mefloquine in emulsions containing dicetylphosphate might be a result of better absorption—peak level might have been higher, and reached more quickly, compared with values for the control. On the other hand, in positively charged mefloquine emulsions stearylamine reduces the absorption of the drug. The additives had no effect on halofantrine, possibly because its high lipophilicity and marked antimalarial activity render the influence of charge inducers on the effect the drug insignificant.

Conclusions

Non-ionic emulsifier with electric-charge inducers enable the preparation of stable parenteral emulsions loaded with halofantrine or mefloquine. The emulsions are chemically stable and the antimalarial activity of the incorporated drugs is high. In contrast with mefloquine the activity of halofantrine-loaded emulsions is not affected by the charge inducers added.

This work showed that the availability of poorly soluble drugs can be increased by formulating them as o/w submicron emulsions in the presence of additives selected for their positive effect on drug absorption.

Acknowledgements

The authors thank Professor V. Préat (UCL, Brussels) for the use of the Coulter Counter nanosizer and Professor R. Finsey (VUB, Brussels) for performing zeta potential measurements. T. N. wishes to thank the University of Antwerp for financial support.

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