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Preparation, characterization and in vivo activity of mefloquine submicron emulsions

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Abstract

Sesame oil-in-water submicron emulsions of mefloquine hydrochloride (compound I) and its potential prodrugs II and III were prepared by ultrasonication. Owing to the low solubility of I in water and oils, two of its lipophilic analogs were synthesized by reacting oleoyl and palmitoyl chlorides with the free base of I. The properties of the submicron o/w emulsions prepared by dispersion of sesame oil solutions of I, II and III in iso-osmotic sorbitol solutions were evaluated before and after autoclaving. Stable submicron emulsions were evaluated for their antimalarial activity in vivo. The physico-chemical properties of the emulsions prepared and their biological activity suggest the usefulness of sesame oil emulsions of mefloquine in malaria chemotherapy.

Key words: Mefloquine; Lipophilic prodrug; Injectable o/w emulsion; Malaria chemotherapy

1. Introduction

The new antimalarials, i.e., artemisin and mefloquine exhibit very low solubility in most solvents. In close relation to this poor solubility, these drugs display considerable variability in their bioavailability, pharmacokinetic and biodistribution. Their poor gastro-intestinal resorption, and consequently, the resulting low blood levels trigger parasite multi-drug resistance (Woerdenbag et al., 1990).

Various drug carriers such as nanoparticles, liposomes and niosomes have been studied with the aim of achieving regular blood levels of drugs, site-specific delivery and lowering of toxicity (Hunter et al. 1988; Krishna and Flanagan, 1989; Titulaer et al., 1990; Couvreur and Vauthier, 1991; Couvreur et al., 1991; Mbela et al., 1992).

A major drawback of liposomes and niosomes is the very low concentration of drugs entrapped. On the other hand, injectable submicron emul-

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sions containing vegetable oils which originally were used for parenteral nutrition or as contrast media could find therapeutic interest in antiparasite treatment (Collins-Gold et al., 1990).

Growing attention is being devoted to the usefulness of injectable lipid emulsions for parenteral drug delivery, particularly owing to their ability to incorporate high drug concentrations, to deliver drugs to particular cells, tissues and organs, to prolong the release of the incorporated drugs, to lower their toxicity and to enhance their bioavailability (Davis and Illum, 1986; Singh and Ravin, 1986; Illum et al., 1989).

The aim of the present study was to develop an injectable mefloquine preparation suitable for the treatment of acute cerebral malaria. Therefore, submicron emulsions of mefloquine were prepared and characterized for their physicochemical properties, droplet size distribution and in vivo antimalarial activity.

To enhance the solubility of mefloquine hydrochloride in oil, its lipophilic pro-drugs were synthesized, purified, dissolved in sesame oil and formulated in 20% sesame oil-in-water submicron emulsions.

2. Materials and methods

2.1. Chemicals

Mefloquine hydrochloride (MFQ) was obtained from Roche (Brussels, Belgium). Acyl chlorides (oleoyl and palmitoyl) and 4-dimethylaminopyridine (4-DAMP) were purchased from Janssen Chimica (Belgium).

α-Tocopherol, sorbitol and sesame oil (ASMA, Antwerp, Belgium) were of pharmacopeial quality for i.v. use. The L-α-lecithin type IV-S from soybean was purchased from Sigma (München, Germany) and used as received, without further purification. The non-ionic surfactant poloxamer 188 (Lutrol® F68) was a gift from BASF (Brussels, Belgium) and used as such without further purification.

All other chemicals were of analytical grade.

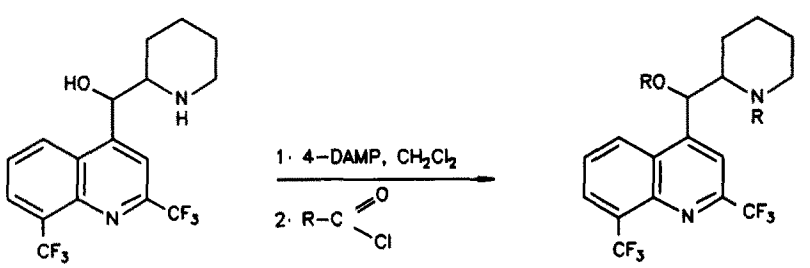
2.2. Synthesis of potential prodrugs

2.2.1. N,O-Dipalmitoyl MFQ

The procedure followed, summarized in Fig. 1, is representative of the general method used for esterification of MFQ base.

To 20 ml of CH_2Cl_2 were added 1.5 g (3.6 mmol) of MFQ-HCl, 1.5 g (15 mmol) of freshly distilled triethylamine and 0.15 g (0.8 mmol) of 4-dimethylaminopyridine (4-DAMP). The mixture was stirred at ambient temperature until the solubilization of MFQ was complete. The mixture was cooled to 0°C in ice and a freshly distilled solution of palmitoyl chloride (2.8 g = 12 mmol in 25 ml of $CHCl_2$) was added dropwise under stirring and cooling. The mixture was stirred for 10 min and heated to 80°C (reflux) overnight.

Some crystalline material precipitated and was discarded by filtration. The filtrate was evapo-



I

II and III

II: R = $CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$

III: R = $CH_3(CH_2)_{14}CO$

Fig. 1. Scheme of synthesis of potential prodrugs (II and III).

rated under vacuum and left a colourless, oily residue which crystallized on storage in methanol. Recrystallization in methanol yielded a white powder, which was checked by IR, GC-MS and ^{1}H-NMR.

2.2.2. N,O-Dioleoyl MFQ

The dioleoyl MFQ derivative was synthesised in a identical way.

2.3. Physicochemical characterization of the compounds

2.3.1. Oil solubility

To estimate the maximum concentration of compounds I–III that could be solubilized in the oil phase of the emulsions at 25 and 70°C, increasing amounts of crystalline I–III were dissolved in 20 g of sesame oil.

The oil suspension was stirred magnetically in a 25 ml glass vial at 25 and 70°C in a water bath for 24 h.

We considered as representative of the maximum solubility of mefloquine hydrochloride and its investigated analogs, the maximum concentration of the drugs giving stable oil solutions and no remaining crystals nor yielding precipitate on cooling at 22°C and on storage of 24 h.

2.3.2. Melting point

The melting points were determined with a Electrothermal apparatus (Labotech, Wiesbaden, Germany) and are not corrected.

2.3.3. Spectroscopic data

The IR spectra were determined on an IR spectrophotometer Acculab 4 (Beckman Scientific Instruments).

The GC-MS spectra were recorded on a gas chromatograph (Hewlett Packard model 5890A) coupled with a mass selective detector (Hewlett Packard model 5970). A 25 m capillary column of 5% phenylmethylsilicone was used.

The ^{1}H-NMR spectra were recorded on a Varian NMR spectrometer EM-360L (Varian Instrument Division).

2.4. Preparation of submicron emulsions

In a preliminary study the effects of the process parameters such as oil phase volume ratio, emulsifier concentration, emulsification temperature and sonication time on the properties of the sesame oil-in-water emulsion were investigated (Higgins and Skauen, 1972; Eberth and Merry, 1983). Mean droplet size and size distribution, which are the most important characteristics of colloidal carrier systems, were determined before and after heat sterilization. From the results obtained and in agreement with Pathak et al. (1990), the following general procedure was chosen. A mixture of 1.2 g of lecithin, 0.02 g of α-tocopherol (as an anti-oxidant) and the appropriate amount of compound I, II or III were dissolved in 20 g of sesame oil under mixing and heating (70°C) in a water bath. The aqueous phase of the emulsion was prepared by dissolving 2.0 g of poloxamer 188 in 80 g of an aqueous iso-osmotic sorbitol solution heated at 70°C. The o/w emulsions were obtained by the addition, under constant mechanical agitation, of the oil phase in a slow stream to the aqueous phase. Agitation with an electric mixer was continued for 5 min after addition of the oil phase. The primary emulsions were then sonicated for 10 min using a Branson B 12 sonifier with an applied ultrasonic intensity of 120 W (Branson Sonic Power, Vésenaz, Switzerland). The sound probe was immersed to constant depth and placed centrally in the emulsion to enhance the reproducibility of droplet size.

Compound I and to some extent lecithin were not rapidly dissolved in sesame oil. Therefore, organic solvents were used as reported by Hansrani et al. (1983) and by Yu et al. (1993). To reduce the heating time and to avoid excesive degradation, lecithin and compound I, II or III were dissolved in 10 ml of a mixture of diethyl ether and absolute ethanol and maintained at 50°C under reflux. The resulting solution was then incorporated dropwise into the pre-heated sesame oil. After removal of the diethyl ether/ethanol mixture by evaporation under reduced pressure, the oil solution obtained was heated to 70°C and immediately incorporated into the aqueous solution. The primary emulsions were

sonicated and filled in previously depyrogenated type I glass vials. The vials were sealed with butyl-rubber stoppers and sterilized using a steam autoclave at 121°C for 20 min. Following autoclaving the preparations were cooled to room temperature and stored in a refrigerator at 5°C.

2.5. Characterization of the emulsion

The osmolality, pH, rheological behaviour and droplet size distribution of emulsions E0–III, containing no MFQ, compound I, compound II and compound III, respectively, were measured. To allow the emulsions prepared to equilibrate, measurements were carried out 1 day after preparation or sterilization. The influence of storage time was evaluated after 120 days at 5°C.

2.5.1. Osmolality and pH

The osmolality of each emulsion was determined with a vapor pressure osmometer (Model 5500 Wescor, Logan, UT, U.S.A.). The mean of three measurements was calculated.

The pH of the emulsions before and after autoclaving was determined with a Radiometer PH M63 pH meter (Radiometer, Copenhagen, DK).

2.5.2. Rheological measurement

The rheological measurements on emulsions were carried out at 22°C using a Brookfield DV-II$^+$ viscometer (Stoughton, MA, U.S.A.). The viscosity was characterized by means of a single value determined at a shearing rate of $D = 132$ s^{-1}, due to the almost Newtonian behaviour of the emulsions studied.

2.5.3. Droplet size determination

Small droplet size is associated with good stability during storage and low toxicity in the body (Ishii et al., 1990). Therefore, the size of the oil droplets was determined before and after autoclaving by means of a dynamic laser light scattering apparatus (Coulter Counter nanosizer, N_4MD, Luton, U.K.). An aliqout (100 μl) of each emulsion was diluted in 2 ml of filtered distilled water. Measurement was carried out at room temperature. A statistical distribution processor (SDP analysis) provided histograms, mean diameter and SD of the size distribution.

2.6. In vivo activity measurement

The in vivo activity of the emulsions prepared was examined according to the method of Caillard et al. (1992) modified as follows. On day 0, at 9 p.m., outbred male Swiss mice (Iffa-Credo, France) weighing approx. 20 g were inoculated intraperitoneally with freeze-thawed blood infected with *Plasmodium vinckei petteri*. At 9 a.m. the day after infection, different batches of mice received a dose equivalent to 15 mg/kg MFQ-HCl by the intraperitoneal route. Blood smears of all mice were carried out each day until parasitaemia reached 1% according to the Warhust and Folwell (1968) prepatency test. The prepatent period was defined as the number of days before parasitaemia reached 1%. The lengths of the prepatent periods of control mice and mice treated with the drug were compared and the delays were evaluated.

3. Results and discussion

3.1. Synthesis

The yields and physico-chemical properties of the target compounds are summarized in Table 1. The spectra and chromatographic data were consistent with structures II and III (Fig. 1).

Moreover, III crystallized smoothly in methanol. II yielded a semi-amorphous solid in a mixture of methanol-diethyl ether-petroleum ether (1:1:1) after several days of storage at −5°C.

Table 1
Physiochemical characteristics of compounds studied

Compound	Yield (%)	Melting point (°C)	MS (*m/e*)	Oil solubility (mg/mg)
MFQ HCl (I)	–	260–262	378	2.52
MFQ oleoyl (II)	78.1	70– 71	909	16.24
MFQ dipalmitoyl (III)	86.7	66– 67	855	13.98

3.2. Oil solubility

The data in Table 1 demonstrate a significant improvement in the solubility of II and III in sesame oil. Indeed, it is obvious that coupling I with fatty acyl moieties enhanced its solubility in oils about 2.4- and 3.2-fold.

The observed increase in solubility parallels sharply the predicted lipophilicities of the acyl moieties as calculated from the fragmental constants of Rekker (1977).

Fig. 2 shows a plot of the relative oil solubility of the compounds against the theoretical lipophilicity calculated according to Rekker (1977). The calculated log P values are 3.84 for compound I, 21.29 for compound II and 13.42 for compound III, respectively.

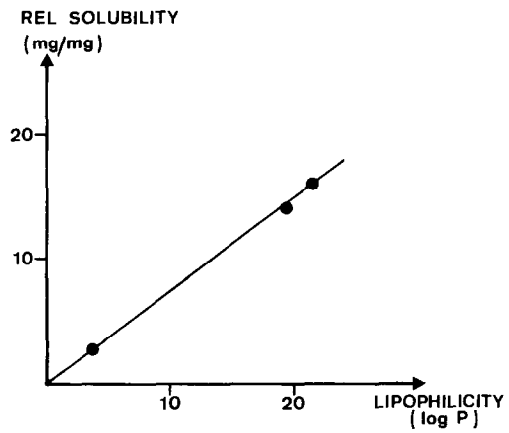


Fig. 2. Relationship between relative oil solubility and theoretical lipophilicity.

3.3. Preparation of emulsions

In the present work, the diethyl ether/ethanol mixture enhanced significantly the dissolution of MFQ or its potential prodrugs and lecithin. In this way, it was possible to dissolve these compounds much more rapidly and at low temperature (30–50°C) in sesame oil.

To avoid drug precipitation during storage, the emulsions were prepared by using sesame oil solutions of I–III containing drug concentrations less than those mentionned in Table 1.

3.4. Emulsion characterization

The results of the osmolality, pH and viscosity measurements before and after autoclaving are presented in Table 2. These characteristics remain in the range of values compatible with parenteral use (Benita and Levy, 1993).

All emulsions display a pH value around 6.3. Autoclaving the emulsion led to a very small change in pH. It has been reported that heat sterilization results in the production of free fatty acids and consequently a drop in pH value (Chaturvedi et al., 1992). On the other hand, phosphatidylcholine, a major constituent of lecithin, exhibits minimal hydrolysis under weakly acidic to neutral pH conditions (Grit et al., 1989). In our study, however, no extended hydrolysis occurred under the preparation conditions applied and during heat sterilization. The pH increase observed with the medicated emulsion EII need further investigation.

Droplet size determination was carried out before and after sterilization. An example of the histograms obtained in the case of emulsion E0 is shown in Fig. 3.

Table 3 summarizes data on the mean diameter ($\pm$SD) of the droplet size distribution of the

Table 2
Physicochemical properties of different emulsions before and after sterilization

Emulsion	[Drug] (mg/mg)	pH		Viscosity (mPa s)		Osmolality (mosm/kg)
		Before	After	Before	After	
E0	0.00	6.34	6.34	4.3	4.3	372 ± 3
EI	0.42	5.58	5.60	4.4	4.4	330 ± 4
EII	2.50	6.32	6.41	4.4	4.4	353 ± 6
EIII	2.37	6.39	6.43	5.2	5.0	345 ± 2

Table 3
Mean dropsize (±SD) of different emulsions before and after sterilization

Emulsion	Mean diameter (±SD) (nm)	
	Before sterilization	After sterilization
(A) 1 day after preparation		
E0	615±51	626±52
EI	562±narrow	562±18
EII	328±19	336±20
EIII	359±23	370±26
(B) After 120 days of storage at 5°C		
E0	626±48	622±51
EI	572±42	562±22
EII	340±22	332±19
EIII	362±22	364±25

different emulsions studied. The primary requirement for i.v. emulsions is the lack of large ($d > 1$ μm) emulsion droplets in order to avoid oil embolism.

The different emulsions show a unimodal dispersion with a size maximum below 800 nm. Autoclaving also did not significantly alter the size distribution and homogeneity of the population. Only a slight increase in the mean diameter was measured.

Chaturvedi et al. (1992), however, observed a significant increase of the droplet size in acidic emulsions upon autoclaving. In contrast, several authors reported that the variations in pH of emulsions altered neither the mean droplet size nor distribution profiles (Pathak et al., 1990; Yu et al., 1993). Our results are consistent with these observations.

All the medicated emulsions (EI–EIII) showed smaller mean droplet diameters compared to the blank emulsion (E0). Thus, the incorporation of drugs results in a decrease in droplet size. The influence of drugs on the properties or stability of the emulsifying interfacial film should be investigated.

Although droplets of 300–800 nm were obtained in this work, smaller droplets of about 200 nm were prepared during preliminary experiments using a microfluidizer (Microfluids, Newton, MA, U.S.A.) (results not shown). Due to the reduced volumes used in the present study, it was not possible to process the emulsion for several cycles through a microfluidizer.

To assess the magnitude of change due to coalescence during storage, the droplet size distribution analysis was repeated after 120 days. The results given in Table 3 indicate that the physical stability of the prepared emulsions was satisfactory. This good stability could be attributed to the probable formation of a complex film at the oil/water interface. Indeed, lecithin stabilizes emulsions either by formation of a mechanical barrier together with poloxamer or by electrostatic charge on the droplet surface (Benita and Levy, 1993). The physicomechanical properties of the mixed interfacial film are likely to be strong enough to prevent significant droplet coalescence upon random collisions or under

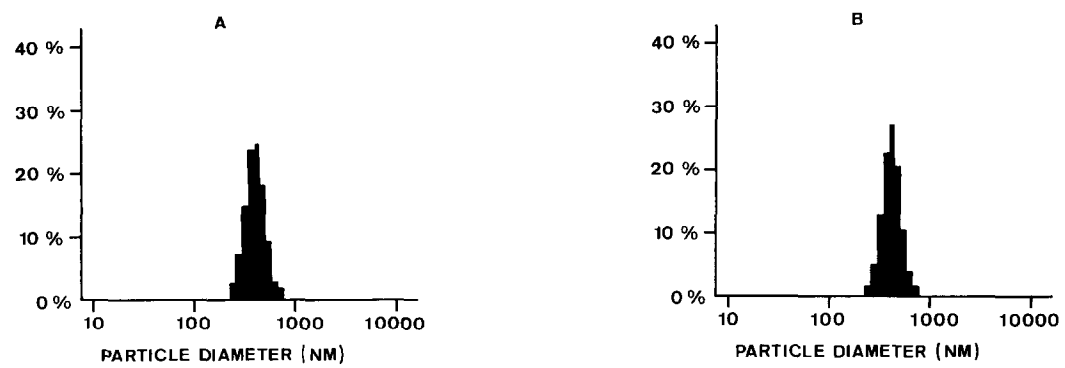


Fig. 3. Histogram of droplet size distribution of emulsion E0. (A) Before sterilization; (B) after sterilization.

Table 4
Mean prepatent period (PPP) in mice treated with different emulsions

Treatment	Mean PPP (days)
E0	2
EI	6
EII	2
EIII	2

thermal stress. This indicates that the preparations under investigation could be stored and handled even in tropical countries without major alterations in droplet size.

3.5. *In vivo activity*

The emulsion containing MFQ HCl (EI) displayed a significant antimalarial activity (Table 4). However, there was no significant difference between the prepatent periods of mice treated with emulsions containing compound II (EII) and III (EIII) and with emulsion without drug (E0).

The lack of activity of the compounds synthesised could be attributed to physicochemical properties or to biological and pharmacological parameters. Compounds II and III would be active after enzymatic hydrolysis mainly by esterases and to a lesser extent by amidases. Probably due to their high lipophilicity, compounds II and III are confined to the oil phase and are not released into the blood stream, since they are rapidly cleared by macrophages. They could also be metabolized in a different way compared to MFQ-HCl, due to the strength of the amide bond. The fate of compounds II and III in mice needs further investigation.

4. Conclusions

The use of o/w emulsions as a vehicle for mefloquine is interesting owing to its poor solubility in most of the current solvents. This work showed that submicron emulsions containing therapeutic concentrations of mefloquine could be prepared.

The formulation proposed allows a physically stable and pharmacological active MFQ emulsion to be obtained. The chemical stability and pharmacokinetic characteristics of the preparation, however, need further investigation.

The in vivo activity exhibited by the mefloquine emulsion may stimulate research into the development of more appropriate prodrugs with antimalarial activity.

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