ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
28 (2002) 13–22

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Preparation of β-artemether liposomes, their HPLC–UV evaluation and relevance for clearing recrudescent parasitaemia in *Plasmodium chabaudi* malaria-infected mice

B. Chimanuka [a,c], M. Gabriëls [a], M.-R. Detaevernier [b], J.A. Plaizier-Vercammen [a,*]

[a] *Departement Farmaceutische Wetenschappen, Farmaceutische Technologie & Fysische Farmacie, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels, Belgium*

[b] *Farmaceutische Scheikunde & Analyse van Geneesmiddelen, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels, Belgium*

[c] *Laboratory of Medical Parasitology (Malaria), C.R.S.N-Lwiro, D.S. Bukavu, Congo*

Received 18 April 2001; received in revised form 16 August 2001; accepted 3 September 2001

Abstract

Egg phosphatidylcholine-cholesterol liposome formulations containing the antimalarial drug β-artemether have been prepared and analyzed for their encapsulating capacity, chemical stability, leakage, in vitro release and their therapeutic efficiency against *Plasmodium chabaudi* infection. A HPLC–UV analysis of β-artemether liposomes without derivatisation was achieved. A good linearity of $y = 4437.7x + 469.01$ ($R^2 = 0.9999$) with a detection limit of 2 μg ml^{-1} was reached. Prior to this, liposomal formulations composed of different molar ratios of EPC-CHOL were prepared to select β-artemether crystal-free liposome preparations. The formulation corresponding to 4:3 and a total concentration of 300 mg lipids ml^{-1} buffer (pH 7.2), which could incorporate as much as 1.5 mg β-artemether was selected for therapy. A trapping efficiency of nearly 100% was reached, the drug being located in the lipid bilayers. A dialysis test demonstrated that the drug could be reversibly released from the liposomes, reaching equilibrium within 24 h. After 3 months storage at 4 °C, no leakage of β-artemether had occurred indicating a high stability of the liposomes. These liposomes were used to treat mice infected with the virulent rodent malaria parasite *Plasmodium chabaudi chabaudi*, with a 100% cure by clearing the recrudescent parasitaemia.

Keywords: β-Artemether liposomes; HPLC–UV analysis; Recrudescent parasitaemia; Malaria

1. Introduction

β-Artemether (ARM) is one of the artemisinine (QHS) derivatives which has proved to be efficient against acute uncomplicated and severe falci-

* Corresponding author. Tel.: +32-2-477-4598; fax: +32-2-477-4735.

E-mail address: jplaizie@vub.vub.ac.be (J.A. Plaizier-Vercammen).

0731-7085/02/$ - see front matter
PII: S0731-7085(01)00611-2

parum malaria [1,2] and can clear the parasite faster even in multiple drug-resistant falciparum malaria [3]. The chemical structure of this drug and other artemisinine derivatives such as artemether (ART), artesunate (ARS), and dihydroartemisinine (DQHS) (Fig. 1), displays an endoperoxide bridge and lacks UV chromophore appropriate for their routine measurement using UV detection.

This problem of UV detection of ARM has been tackled by using HPLC with pre-column base catalyzed derivatisation [4] or acidic decomposition, inducing the production of a UV detectable degradation product (Fig. 1) [5]. A highly sensitive method was also developed to determine dihydroartemisinin by pre-column derivatisation with diacetyldihydrofluorescein prepared from fluorescein [6]. The use of pre-column derivatisation-based methods allowed to evaluate the bioavailability of ARM and DQHS from human plasma using hydrochloric acid decomposition [7] and ART in rabbit plasma was determined after perchloric acid decomposition [8].

As the detection of a degradation compound is inadequate for the detection of very low concentrations in biological fluids, the reductive electrochemical detection that allows a direct detection of artemisinine and derivatives was developed. This method has been used with good sensitivity and specificity to determine the artemisinine content of crude plant extracts [9], and to study the pharmacokinetics of ART and DQHS in dogs after intravenous and intramuscular injection of ART [10]. The reductive electrochemical method is now used extensively to detect very small concentrations of artemisinine and derivatives ranging from 1.25–2.5 ng ml^{-1} [11] to 3–5 ng ml^{-1} [12,13]. However, this method requires rigorous helium deoxygenation of all solvents and samples and also a strict maintenance of the Ag/AgCl glassy carbon electrode detector making its use in intensive routine work rather expensive and cumbersome.

Capillary electrophoresis has been used for the detection of ARS and DHQS after alkaline derivatisation with KOH [14] and recently, the same method proved to be suitable for quantitative analysis of larger samples of ARS and artelinic acid without derivatisation [15].

The use of liposomes as artemether carrier has been approached in vitro [16] and an improved liposome formulation of the same drug adminis-

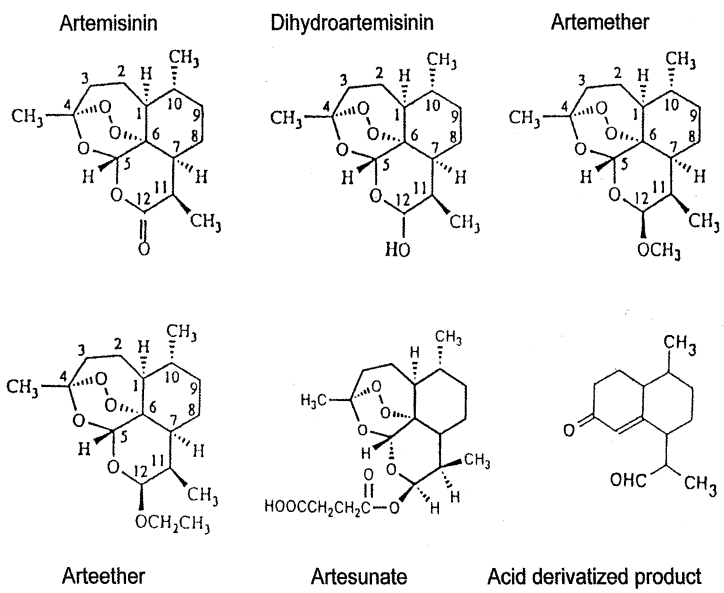

Fig. 1. Chemical structure of the main artemisinine derivatives and their main degradation product [2,4,5].

tered orally and intravenously to rabbits showed a longer elimination half-life [17]. Indeed, although QHS and derivatives are very effective in rapidly reducing the parasitaemia and the fever, their short half-lives lead to recrudescence commonly encountered after treatment with these drugs. A formulation that increases their biodisposition is actually needed to fight this recrudescence post DHQS and derivative treatment. Increasing their dosage in terms of administration frequency could be a solution but not much is known about their toxicity yet, except for some neurotoxilogical effects reported for certain animal species [18]. Liposome formulations combine the advantages of larger dose loads and a slower release of the drug compared to other formulations. In this respect, some other antimalarials have been experimented with. The dosage of chloroquine encapsulated in liposomes has been increased without causing any signs of toxicity, resulting in a 100% efficacy and long-term survival rates of mice infected with *Plasmodium berghei* [19]. Likewise, an improvement of efficacy and a reduction of toxic effects have also been obtained in the case of primaquine by entrapping the drug into liposomes [20,21]. Liposomes have also been used as drug carriers targeted to red blood cells, to the liver, and to the spleen with improved efficacy of chloroquine and primaquine [22,23].

Previous studies on artemisinine derivative encapsulation into liposomes have focused on analytical methods for their detection in plasma, in vitro and in vivo [7,13,24], but not much is known about the efficacy of such formulations for β-artemether up to now. A HPLC analysis of liposome formulation of β-artemether without derivatisation has not been reported. Here we present some physico-chemical characteristics of liposome formulations of β-artemether and the feasibility of analysing β-artemether in liposomes using HPLC–UV detection at 215 nm without prior derivatisation. This β-artemether liposomal formulation has also been evaluated for its therapeutic efficacy against the recrudescence of the virulent rodent malaria parasite strain *Plasmodium chabaudi chabaudi* (*P. c. chabaudi*) IP-PC1 in mice.

2. Experimental

2.1. Chemicals, reagents and instrumentation

β-Artemether (ARM) and a small amount of α-artemether were kindly donated by Arenco (Geel, Belgium); dichloromethane (DCM), cholesterol (CHOL, M_r: 386.7), sodium chloride, potassium chloride, potassium dihydrogen phosphate, disodium phosphate.$12H_2O$, all analytical grade and dodecanol (reagent grade) were obtained from Merck (Darmstadt, Germany); egg phosphatidylcholine (EPC, M_r: 775) was from Lipoid (Ludwigshafen, Germany); acetonitrile from Biosolve (Valkenswaard, The Nederlands); chloroform and methanol were from Labscan (Brussels, Belgium), Mygliol®, a medium-chain triglyceride oil extracted from *Cocos nucifera* L., was provided by Federa (Brussels, Belgium). All solvents used were HPLC-grade. Phosphate buffered saline (PBS) was prepared by dissolving NaCl (136 mM), KCl (2 mM), KH_2PO_4 (1 mM), and Na_2HPO_4 (3 mM) in 100 ml of deionised water, and adjusting the pH to 7.2 with NaOH (1 M). The buffer was kept overnight at 4 °C. All glassware used to prepare sterile liposomes was thoroughly cleaned with ethanol 70% or autoclaved.

The HPLC system consisted of an isocratic pump (Merck Hitachi model L6000A) with an injection loop valve, a UV–Vis detector (Varian model 9050), connected to an integrator (Merck Hitachi D-2000).

2.2. Methods

2.2.1. Determination of β-artemether by HPLC–UV detection

Stock solutions of ARM were prepared in methanol–water (70:30, v/v) at approximately 1.0 mg ml^{-1}, and appropriately diluted with the mobile phase acetonitrile–water (75:25, v/v) to obtain working reference solutions. Aliquots of 100 μl of reference and test solutions were injected on to a Lichrospher–RP-C18 column, 250 mm, 5 μm (Merck Hitachi) at a 1 ml min^{-1} flow rate. The UV detection was set at 215 nm and the peak areas were integrated.

2.2.2. Preparation of β-artemether liposomes

Liposomes were prepared according to the 'film-hydration method' [25,26] slightly adapted. A DCM solution containing specified amounts of EPC, CHOL and ARM was evaporated in a way that a dry film was left on the wall of the container. This pellicle was hydrated with an aqueous buffer solution under gentle shaking, resulting in the formation of a liposomal suspension.

2.2.2.1. Selection of ARM crystal-free liposomes. In a preliminary investigation for the preparation of crystal-free ARM liposomes with the highest entrapment efficiency, a series of liposomes was prepared using the following EPC-CHOL molar ratios of 1:0, 1:1, 2:1, 3:1, 4:1, 3:2 and 4:3, with a total lipid mass of 100, 200 and 300 mg, each hydrated with 1 ml of PBS buffer (pH 7.2). The amount of incorporated ARM varied from 0.5 to 1.5 mg for 100 and 200 mg lipid mass, and up to 6 mg for 300 mg lipids.

The presence of ARM crystals in these liposome formulations was assessed by counting the crystals within 20 squares of a Bürker counting cell of $0.0025 \times 0.0025 \times 0.1$ mm^3 (W. Schreck, Hofheim, Germany) using an optic microscope (Carl Zeiss, Oberkochen, Germany), equipped with a Standard Junior 2 monocular micrometer. A daily control was carried out during the first week followed by a weekly check for 3 months.

2.2.2.2. Preparation of sterile liposomes. As an overall precaution, all glassware, tools and instruments that were brought into contact with liposomes and their components during the preparation were thoroughly cleaned with alcohol 70%, and all manipulations were conducted under a laminar flow hood.

For the preparation of a batch of ARM-liposomes, EPC (3.6 g), CHOL (1.4 g), and ARM (50 mg) were separately dissolved in 20 ml of DCM. Then the three solutions were mixed in the following proportions: 6 ml of EPC, 6 ml of CHOL, and 3 ml of ARM. To make empty liposomes, a similar procedure was followed without the addition of ARM. These solutions were passed through a PTFE 0.22-μm antibacterial filter (Millipore, Molsheim Germany). Then the organic solvent was evaporated under a controlled nitrogen flow while gently rotating the tubes on a Rock'n Roller, leaving a thin lipid film on the wall of the recipients. In order to completely remove all DCM from the lipid film, the tubes were transferred in a lyophiliser under sterile conditions.

For the hydration of the pellicle, 5 ml of the isotonic phosphate buffer (PBS pH 7.2) previously passed through a 0.22-μm antibacterial membrane filter (Millipore filter, Molsheim Germany) together with a few sterile glass beads, were introduced into each of two tubes containing the dry film. The well capped tubes were gently shaken overnight to ensure the formation of the liposomal vesicle suspension. A 1-ml aliquot was immediately checked for sterility at the Department of Microbiology (AZ-Vrije Universiteit Brussels). The two liposomal preparations were transferred into sterile glass serum vials, capped with an aluminum cap crimper (Fermpress H 207) and stored at 4 °C.

2.2.2.3. Trapping efficiency, stability, leakage and dialysis of ARM liposomes. For the determination of the total ARM content in the liposomal suspension, a sample of ± 300 mg was accurately weighed into a 25-ml volumetric flask, dispersed with 20 ml of acetonitrile and the flask was vigorously shaken before it was filled to the mark with the same solvent. An aliquot of the resulting solution was collected and centrifuged at 4000 rpm ($2500 \times g$) for 20 min using a refrigerated centrifuge (Mistral 400, Fisons, UK). Finally, 100 μl of the solution was injected in the HPLC system.

To measure the free (not entrapped) ARM, approximately 1 ml of liposomes suspension was spun at 4000 rpm ($2500 \times g$) for 20 min and an aliquot of the supernatant was analyzed by HPLC.

The leakage of β-artemether from the liposome preparation was evaluated by checking the presence of ARM crystals in the liposomes suspension after storage at 4 °C, and by measuring the free ARM.

To check the release of ARM from liposomes, a semi-permeable membrane TE 35 (Schleicher and

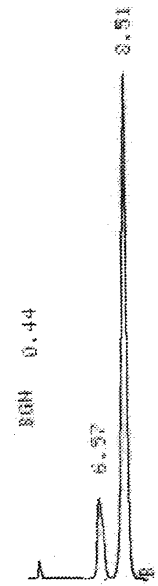

Fig. 2. HPLC chromatogram showing the separation of ∝-artemether ($T_R \pm 6.6$) and β-artemether ($T_R \pm 8.5$).

Schull) was impregnated with dodecanol to selectively allow the passage of ARM. Therefore, 2 ml of ARM liposomes were injected into the donor compartment and 2 ml of empty liposomes into the acceptor compartment of the dialyser. After 24 h of dialysis, a sample was taken from both compartments, and analyzed as described above for the total ARM assay.

2.2.3. Parasite, mice infection and treatment

The infection of mice and the preparation of infected red blood cells were achieved in the same way as previously described [27] with minor modifications. In summary, parasite cryopreserved stabilate of the virulent rodent malaria parasite *P. c. chabaudi* (IP-PC1) provided by the Department of Cellular Immunology (Vrije Universiteit Brussel) and originally from the Pasteur Institute (Paris, France), was thawed and immediately intravenously injected into a mouse that later served as a donor to other mice. OF1 mice (40 g body weight) from Iffa Credo (Brussels, Belgium) were grouped into six batches of six mice each. Four groups (1–4) were inoculated with about 10^6 infected red blood cells from the donor mouse at day 0, while the other two groups (5–6) remained uninfected. The parasitaemia was monitored by examining Giemsa-stained thin blood smears under a light microscope.

The above prepared sterile liposomes composed of 203 mg EPC and 79 mg CHOL ml^{-1}) were used for the treatment of mice. The treatment (day 3 and 4), started when the mean parasitaemia reached a level of 6.3% ($\pm 1.5\%$), by intraperitoneal injection of 150 μl. Each mouse in group 1 received 2×0.172 mg ARM encapsulated in liposomes (ARM-liposomes), mice in group 2 were given empty liposomes (E-liposomes), mice in group 3 received the same dose of ARM as group 1 but dissolved in Mygliol® (ARM-Mygliol®), and mice in group 4 were injected with Mygliol® without ARM (Mygliol®). The remaining non-infected mice received the same amount of empty liposomes (group 5) or Mygliol® (group 6). The number of surviving mice as well as the parasitaemia was recorded daily until 1 month after the treatment.

3. Results and discussion

3.1. HPLC analysis of β-artemether

The UV detection wavelength set at 215 nm was considered as a compromise between the sensitivity of the compound of interest, which does not show any specific UV absorption, and the stability of the baseline. After several trials with different mixtures of acetonitrile, methanol and water, an appropriate mobile phase composed of acetonitrile–water (75:25 v/v) was preferred, since this provided an excellent separation of the α and β isomers of artemether with respective retention times of about 6.6 and 8.5 min (Fig. 2). In these conditions, a calibration curve was constructed within the ARM concentration range of 1–50 μg ml^{-1} showing a linear regression equation of $y = 4437.7x + 469.01$, $R^2 = 0.9999$. The variation between replicate injections of standard ARM solutions ranged between 0.8 and 1.5% R.S.D., except for the two lower concentrations (Table 1). From this observation, a limit of quantification can be estimated at 5 μg ml^{-1}.

3.2. ARM crystal-free liposomes

The highest amount of ARM entrapped in the tested liposome formulations without the presence of crystals was found to be 1.5 mg for 300 mg lipids and 1 ml PBS buffer, with a EPC-CHOL molar ratio of 4:3 (Table 2). This liposomal suspension contained multilamellar vesicles (Fig. 3). The presence of ARM crystals in other preparations depended on the EPC-CHOL ratio, the highest crystals yield occurring when CHOL was half the amount of EPC. The proportion of EPC and CHOL in the composition of liposomes is important for the release and the leakage of the encapsulated drug. We omitted the addition of α-tocopherol, usually added to other liposomes formulations as antioxidant, for the reason that it could diminish the efficacy of ARM due to a possible reduction of the endoperoxide bridge [28] and consequently inhibit the production of free radicals which usually kill the malaria parasite.

3.3. Trapping efficiency, stability and leakage

The determination of total ARM content in the liposomal suspension by HPLC was carried out after isolation of the drug substance either in methanol or acetonitrile. Although comparable peak areas were obtained with both solvents, it appeared that the use of acetonitrile yielded cleaner chromatograms since methanol dissolved a larger part of lipid matrix (Fig. 4).

Table 1
Repeatability of the 100-μl injection of ARM solutions in the HPLC–UV system

	ARM (μg ml^{-1})					
	1 μg	2 μg	5 μg	10 μg	25 μg	50 μg
Mean area ($n = 6$)	4857	8865	23 051	45 609	109 963	221 900
% R.S.D.	4.7	4.8	0.8	1.5	1.1	1.2

Table 2
Presence of ARM crystals in liposomes with different EPC-CHOL molar ratios and lipid mass composition

Lipids (mg)[a]	EPC-CHOL (molar ratio)	ARM (mg)[a]	ARM crystals (20 unit counts)	Crystal size (μm)
100	4:3	0.5	4	2.5–5
100	4:3	1.0	5	2.5–7.5
100	4:3	1.5	9	2.5–15
200	4:3	0.5	2	2.5–12.5
200	4:3	1.0	4	5–25
200	4:3	1.5	6	5–25
300	1:0	0.5	4	2.5–15
300	1:1	0.5	6	2.5–10
300	2:1	0.5	8	2.5–7.5
300	3:1	0.5	4	2.5–5.0
300	3:2	0.5	5	2.5–12.5
300	4:1	0.5	2	5–22.5
300	4:3	0.5	–	–
300	4:3	1.0	–	–
300	4:3	1.5	–	–
300	4:3	2.0	2	5–12.5
300	4:3	4.0	11	2.5–7.5
300	4:3	6.0	2	5–17.5

[a] Amount for 1 ml PBS hydration.

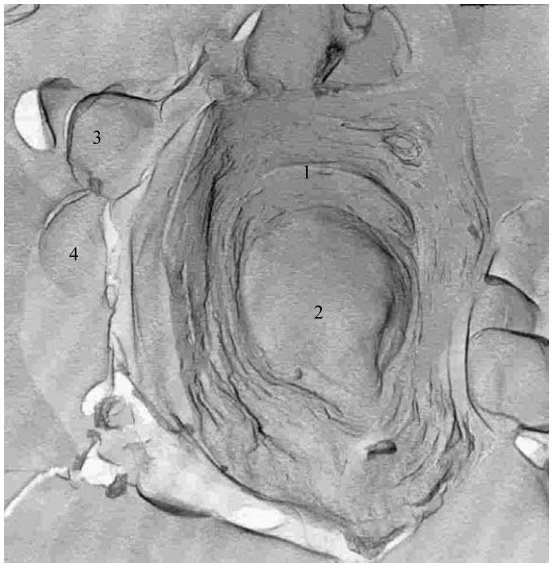

Fig. 3. Electronmicroscopic photograph of multilamelar vesicles liposomes section showing: (1) the lipid bilayers; (2) the vesicle containing the aqueous phase; (3, 4) other entire liposomes.

The trapping efficiency (TE) of crystal-free liposomes, expressed as a percentage, was calculated by subtracting the amount of β-artemether in the aqueous phase (free ARM) from the total amount of β-artemether in the whole liposomes preparation (total ARM), referring to the following equation: $TE\ (\%) = (TA - FA) \times 100/TA$, where TA is the total ARM and FA is the free ARM amount. Three batches of crystal-free ARM-liposomes were prepared separately using the same EPC-CHOL and ARM composition. Each preparation was analysed two times for total and free ARM.

The amount of ARM found in samples of 300 mg liposome suspension averaged 340 μg, corresponding to 1133 μg ml^{-1} liposomes, while free β-artemether had about 8.4 μg ml^{-1}, resulting in a trapping efficiency of 99.3%. This nearly total encapsulation capacity should not be surprising since ARM is very soluble in lipids and not in aqueous solutions.

These liposome suspensions were very stable since after 3 months storage at 4 °C, the analysis of ARM in total liposomes and in the aqueous phase did not reveal the presence of any degradation product. Additionally, no leaking was noticed since no crystals were observed after storage and the trapping efficiency (99%) was comparable to that found for the freshly prepared liposomes. The absence of leakage can be attributed to the presence of an appropriate concentration of cholesterol that increases the stability of the liposome bilayer [29,30]. Moreover, it is known that ARM formulations are stable because of the neutral chemical structure of ARM.

3.4. Release test of β-artemether from liposomes by dialysis

A dialysis experiment was designed to check whether ARM could be released from the liposomes. β-Artemether being practically insoluble in water, a normal dialysis system with an aqueous buffer solution in the acceptor compartment was not considered adequate.

Therefore, empty liposomes were placed in the acceptor compartment. For the choice of the membrane, it had to be taken into account that only the active compound could cross the membrane and not the liposomes. To consider some loss due to the injection of the liposomal suspension, the total concentration of ARM in both compartments was considered as 100%. In this way, we observed that β-artemether passed across the dodecanol-impregnated membrane into the empty liposome compartment, reaching equilibrium within 24 h of dialysis. This is the evidence that the drug could be released from our liposome formulation and that the binding of β-artemether to phospholipids was reversible.

3.5. Efficacy of ARM liposomes in P. c. chabaudi-infected mice

The sterile ARM liposomes were used to treat mice infected with *P. c. chabaudi* and cured mice which recovered from recrudescent parasitaemia whereas mice treated with β-artemether diluted in Mygliol® died due to a recurrent infection (Table 3, Fig. 5). The dose of 4.8 mg/kg (2×0.172 mg ARM) which was used in this experiment was intended to follow the action of the drug on the recrudescent infection, demonstrates the superior-

ity of ARM-liposome formulation against ARM-Mygliol® formulation. One could expect that the ARM-Mygliol®-treated mice should better survive the infection than mice treated with ARM-liposomes because of an expected slower disposition of the drug encapsulated in liposomes, but this was not the case. However, the parasitaemia in the ARM-Mygliol® group decreased faster than in the ARM-liposomes group, but finally the former did not survive from the recrudescent parasitaemia. In all the groups, the deviation of the parasitaemia varied from 0.15–1.8 S.D. at lower parasitaemia (0.8– < 6%) to 3.7–4.9 S.D. at higher parasitaemia (32–44%). This clearance of recurring parasites by ARM-liposomes could be associated with the immune response, the route of administration playing a certain role in the initiation of the process.

In fact, such clearance of the recrudescent parasitaemia after treatment of a *P. c. chabaudi* (IP-PC1) infection may be attributed to a protective cellular immune response mediated by $CD4^+$ T

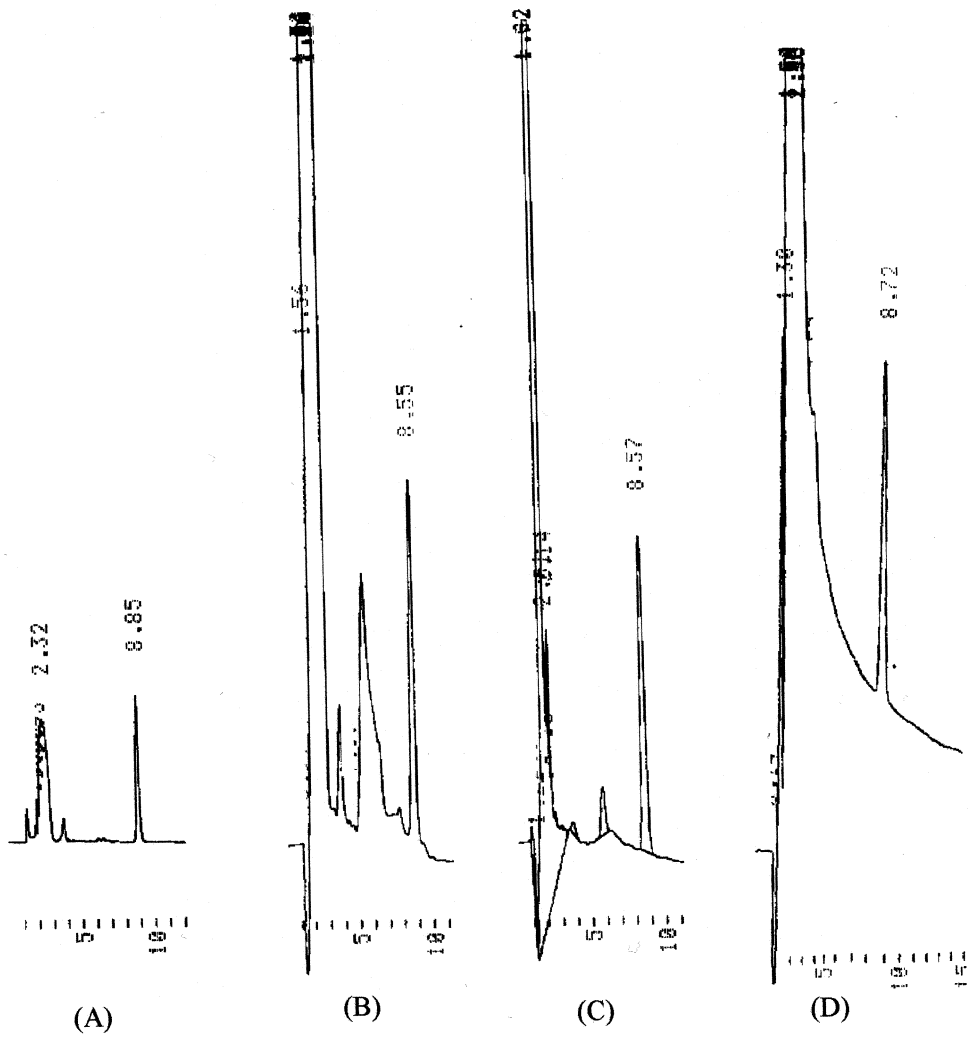

Fig. 4. HPLC chromatograms of β-artemether, detection 215 nm; mobile phase: acetonitrile–water (75:25 v/v), (A) 50 μg ml^{-1} β-artemether in methanol–water (60:40 v/v); (B) ARM liposomes extracted by methanol; (C) ARM liposomes extracted by acetonitrile; (D) β-artemether in liposomal aqueous phase.

Table 3
Survival rates of OF1 mice infected with *P. c. chabaudi* (IP-PC1) after treatment with β-artemether entrapped in EPC-CHOL multilamelar liposomes

Group ($n = 6$)	Infection	Treatment	Survival rates
1	+	ARM-liposomes	6/6
2	+	E-liposomes[a]	0/6
3	+	ARM-Mygliol®	0/6
4	+	Mygliol®	0/6
5	−	E-liposomes	6/6
6	−	Mygliol®	6/6

[a] Empty liposomes.

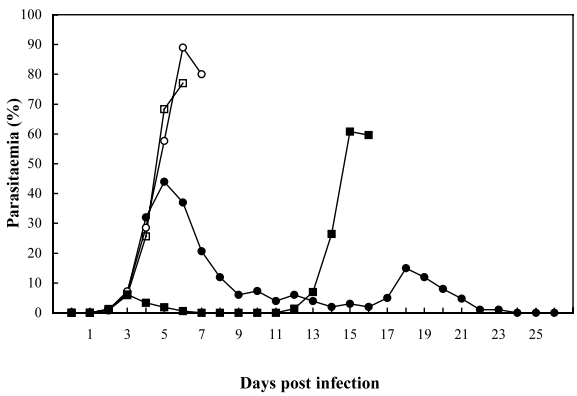

Fig. 5. Evolution of the parasitaemia following the treatment of *P. c. chabaudi* (IP-PC1) infected OF1 mice. Treatment at days 3 and 4, day 0 being the day of infection. Each point represents the mean value obtained from six mice. Group 1: ARM-liposomes (●), group 2: empty liposomes (○), group 3: ARM-Mygliol® (■), group 4: Mygliol® (□).

cells of the TH_2 type as previously reported [31,32]. Moreover, it has been demonstrated that immunization of mice infected with *P. chabaudi* AS strain with phosphatidylcholine could significantly reduce the parasitaemia, and that antibody raised against *P. chabaudi* could comparatively reduce the peak parasitaemia [33].

This hypothesis is also supported by the fact that liposomes are usually taken by macrophages and lymphocytes by phagocytosis or endocytosis and that some antimalarial drugs may act by inducing a protective immune response. Indeed, a study of maloprim prophylaxis on Gambian children demonstrated that lymphocyte proliferation and gamma interferon production were higher in children on chemoprophylaxis than in other children under a curative therapy [34]. Moreover, mice infected with *P. chabaudi* were given subcurative doses of either pyrimethamine or IgG alone or their combination, and it appeared that a much greater effectiveness occurred when the drug was combined with the antibody, implying an additive effect between the drug and the immune response [35]. To elucidate the fate and distribution of the liposome trapped ARM, it would be interesting to make another set of liposomes composed of radioactive labelled ARM, EPC and CHOL. This would allow to localize these components within the organism, and to understand the function of macrophages and the phagocytosis in the parasitaemia clearing process.

4. Conclusions

In this study it is shown that liposomal suspensions containing multilamelar vesicles, and composed of EPC-CHOL in a 4:3 molar ratio could incorporate as much as 1.5 mg of the antimalarial drug β-artemether in 1.3 ml suspension without any crystal formation. A good stability of these liposomes (3 months storage) and a trapping efficiency of nearly 100% were demonstrated. This ARM liposome formulation was successfully used to circumvent the recrudescent parasitaemia in mice infected with *P. c. chabaudi*. Furthermore, evidence is produced that a simple liquid chromatographic method based on a C_{18} stationary phase with an aqueous acetonitrile mobile phase, with UV detection at low range of UV wavelength (± 215 nm) is suitable for the determination of ARM, as long as its concentration is not lower than 3 μg ml^{-1}. This method should be easily applied to other pharmaceutical ARM formulations as well as to its analogues.

References

[1] T.T. Hien, Trans. R. Soc. Trop. Med. Hyg. 88 (1994) 7–8.

[2] T.T. Hien, N.J. White, Lancet 341 (1993) 603–608.
[3] D. Bunnag, J. Karbwang, T. Harinasuta, Southeast Asian J. Trop. Med. Public Health 23 (1992) 762–767.
[4] S. Zhao, M.Y. Zeng, Anal. Chem. 58 (1986) 289–292.
[5] J.K. Baker, J.D. McChesney, H.T. Chi, Pharm. Res. 10 (1993) 662–666.
[6] X.D. Luo, M. Xie, Chromatographia 23 (1987) 112–114.
[7] C.G. Thomas, S.A. Ward, G. Edwards, J. Chromatogr. 583 (1992) 131–136.
[8] A.A. Al-Angary, M.A. Bayomi, S.H. Khidr, M.A. Al-Meshal, K.M. Lutfi, Anal. Lett. 27 (1994) 2689–2702.
[9] N. Acton, D.L. Klayman, I.J. Rollman, Planta Med. (1985) 445–446.
[10] V. Melendez, J.O. Peggins, T.G. Brewer, A.D. Theoharides, J. Pharm. Sci. 80 (1991) 132–138.
[11] V. Navaratnan, S.M. Mansor, L.K. Chin, M.N. Mordi, M. Asokan, N.K. Nair, J. Chromatogr. B 669 (1995) 289–294.
[12] J. Karbwang, K. Na Bangchang, P. Molunto, V. Banmairuroi, K. Congpuong, J. Chromatogr. B 690 (1997) 259–265.
[13] K. Na Bangchang, K. Congpuong, L.N. Hung, P. Molunto, J. Karbwang, J. Chromatogr. B 708 (1998) 201–207.
[14] A. D'Hulst, P. Augustijns, S. Arens, L. Van Parijs, S. Colson, N. Verbeke, R. Kinget, J. Chromatogr. Sci. 34 (1996) 276–281.
[15] M. Gabriëls, M. Jimidar, J. Plaizier-Vercammen, J. Chromatogr. B 21 (1999) 193–198.
[16] A.A. Al-Angary, M.A. Al-Meshal, M.A. Bayomi, S.H. Khidr, Int. J. Pharm. 128 (1996) 163–168.
[17] M.A. Bayomi, A.A. Al-Angary, M.A. Al-Meshal, M.M. Al-Dardinri, Int. J. Pharm. 26 (1998) 1–7.
[18] T.G. Brewer, J.O. Peggins, S.J. Grate, J.M. Petras, B.S. Levine, P.J. Weina, J. Swearengen, M.H. Heiffer, B.G. Schuster, Trans. R. Soc. Trop. Med. Hyg. 88 (1994) 33–36.
[19] P.A.M. Peeters, C.W.E. Huiskamp, W.M.C. Eling, D.J.A. Crommelin, Parasitology 98 (1989) 381–386.
[20] P. Pirson, R.F. Steiger, A. Trouet, J. Gillet, F. Herman, Ann. Trop. Med. Parasitol. 74 (1980) 383–391.
[21] P. Pirson, R.F. Steiger, A. Trouet, Biochem. Pharmacol. 31 (1982) 3501–3507.
[22] P.A.M. Peeters, C. Oussoren, W.M.C. Eling, D.J.A. Crommelin, Biochim. Biophys. Acta 943 (1988) 137–147.
[23] M. Owais, G.C. Varshney, A. Choudhury, S. Chandra, C.M. Gupta, Antimicrob. Agents Chemother. 39 (1995) 180–184.
[24] K. Na Bangchang, J. Karbwang, C.G. Thomas, A. Thanavibul, K. Sukontason, S.A. Ward, G. Edwards, Br. J. Clin. Pharmacol. 37 (1994) 249–253.
[25] D. Lichtenberg, Methods Biochem. Anal. 33 (1988) 339–463.
[26] R.R.C. New, Liposomes A Practical Approach, Oxford University Press, Oxford, 1990.
[27] B. Chimanuka, T. Vanden Driessche, J.N. Lisgarten, J. Plaizier-Vercammen, Biol. Rhythm. Res. 30 (1999) 54–81.
[28] R.K. Sudaratana, Y. Yuthavong, Trans. R. Soc. Trop. Med. Hyg. 81 (1987) 710–714.
[29] D. Chapman, Lipid dynamics in cell membranes, in: G. Weissmann, R. Claiborne (Eds.), Cell Membranes, Biochemistry, Cell Biology and Pathology, H.P. Press, New York, 1975, pp. 13–22.
[30] A.J. Quinn, D. Chapman, CRC Crit. Rev. Biochem. 8 (1980) 1–117.
[31] J.L. Grun, W.P. Weidanz, Infect. Immun. 41 (1983) 1197–1204.
[32] A.W. Taylor-Robinson, R.S. Phillips, Infect. Immun. 62 (1994) 2490–2498.
[33] G. Bordmann, W. Rudin, N. Favre, Immunology 94 (1998) 35–40.
[34] E.N. Otoo, Ph.D. thesis, University of London, 1991.
[35] G.A.T. Targett, Parasitology 105 (1992) S61–S70.