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Research Article

Chloroquine Blood Levels After Administration of the Liposome-Encapsulated Drug in Relation to Therapy of Murine Malaria

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In a previous report (P. A. M. Peeters, C. W. E. M. Huiskamp, W. M. C. Eling, and D. J. A. Crommelin. *Parasitology*, 1989, in press) an increase in therapeutic and prophylactic potential was found when chloroquine (CQ) was encapsulated in fluid-state liposomes (lipCQ) and tested in *Plasmodium berghei*-infected mice in comparison to intraperitoneal (i.p.) administration of the free drug. In this study, the same model was used to demonstrate that encapsulation of CQ into gel-state liposomes further increased the preventive and therapeutic effect considerably. CQ determinations in whole blood, plasma, and red blood cells (RBC) after i.p. administration of fluid- or gel-state lipCQ revealed a prolonged availability of the drug in comparison to administration of free CQ. The CQ concentrations were related to the CQ levels needed for prevention or therapy of *Plasmodium berghei* infections in mice.

KEY WORDS: liposomal chloroquine (CQ); *Plasmodium berghei* infections; slow release; CQ blood level; fluid-state and gel-state liposome.

INTRODUCTION

The liposomal delivery of antimicrobial drugs has been subject of numerous studies, reviewed in Refs. 1–3. Several of these studies demonstrated the potential application of liposomes as drug carriers in malaria chemotherapy (4–11).

In a previous study (10) it was shown that encapsulation of chloroquine (CQ)[4] into fluid-state liposomes (phosphatidylcholine:phosphatidylglycerol:cholesterol, 10:1:5) improved the therapeutic activity, reduced the toxicity, and prolonged the availability of the drug in *Plasmodium berghei*-infected mice.

In this study the effect of the liposomal bilayer rigidity on the therapeutic effect of liposome-encapsulated CQ was studied in the same model. In order to elucidate the possible mechanism for this beneficial effect, CQ concentrations were measured in blood, plasma, and red blood cells (RBC) after administration of free or liposomal CQ (lipCQ) and compared to levels found in mice given a minimum effective dose of CQ for the treatment of a *P. berghei* infection. Finally, attempts were made to determine the free CQ plasma concentration after lipCQ administration.

MATERIALS AND METHODS

Materials

Cholesterol (chol), L-α-distearoylphosphatidylcholine (DSPC), and L-α-dipalmitoylphosphatidylglycerol (DPPG) were obtained from Sigma Chemicals (St. Louis, Mo.). Soybean-α-phosphatidylcholine (PC) and egg-α-phosphatidylglycerol (PG) were a gift from Nattermann GmbH (Cologne, F.R.G.). Chloroquine-polyacrylamide (CQ-PA) and horse radish peroxidase (HRP)-labeled anti-CQ monoclonal antibody were kindly donated by Dr. T. Eggelte (Royal Dutch Institute of Tropical Diseases, Amsterdam, The Netherlands). Desethylchloroquine (des-CQ) and hydroxychloroquine (OH-CQ) were gifts from Dr. F. Schobben (Academic Hospital, Utrecht, The Netherlands). Chloroquine diphosphate met the requirements of the British Pharmacopoeia. All other reagents were of analytical grade.

Liposome Preparation

Fluid-state CQ containing reverse-phase evaporation vesicles (REV)—lipid composition-PC:PG:chol (10:1:5)—were prepared according to the method of Szoka and Papahadjopoulos (12) with minor modifications as described ear-

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[4] Abbreviations used: chol, cholesterol; CQ, chloroquine; des-CQ, desethylchloroquine; DPPG, L-α-dipalmitoylphosphatidylglycerol; DSPC, L-α-distearoylphosphatidylcholine; lipCQ, CQ-containing liposomes; OH-CQ, hydroxychloroquine; PC, phosphatidylcholine; PG, egg-α-phosphatidylglycerol; p-mRBC, parasitized mouse red blood cell(s); RBC, red blood cell(s); REV, reverse-phase evaporation vesicle; tCQ, CQ and its metabolites determined with ELISA; BSA, bovine serum album; PBS, phosphate-buffered saline; HPLC, high-performance liquid chromatography; ELISA, enzyme-linked immunosorbent assay.

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lier (10). Gel-state lipCQ—lipid composition, DSPC:DPPG:chol (10:1:10)—were prepared in a similar way, except that all preparational steps were performed at 75°C (above the phase transition temperature of DSPC and DPPG) instead of 30°C. It was shown earlier (13) by fluorescence polarization measurements that liposomes with a similar composition as mentioned here ("fluid" and "gel" state) indeed differ in their fluorescence polarization behavior (fluid state, $P \approx 0.25$; gel state, $P \approx 0.42$) and therefore in the rigidity of the bilayer.

CQ Determination

The total amount of CQ (expressed as CQ base) in lipCQ suspensions was determined spectrophotometrically at 341 nm, pH 1, as described earlier (10). Briefly, lipCQ were lysed by dilution in 0.1 *M* hydrochloric acid solution containing 0.1% Triton X-100 (v/v). A standard calibration curve (5 to 25 μg CQ/ml) was prepared under the same conditions.

Free CQ in lipCQ dispersions was determined spectrofluorimetrically at pH 9.3 with excitation and emission wavelengths of 330 and 383 nm, respectively. Free CQ was expressed as a percentage of the total CQ content (CQ fluorescence inside the liposomes is completely quenched at the concentration used). LipCQ dispersions were diluted in 100 m*M* glycine buffer (pH 9.3) with (*A*) or without (*B*) 0.1% Triton X-100 (v/v). A standard calibration curve in the same buffer (with or without Triton X-100) was used as a reference. The percentage of free CQ (expressed as CQ base) was calculated by $B/A \times 100\%$.

Total CQ concentrations in whole blood, plasma, and RBC were determined by an enzyme-linked immunosorbent assay (ELISA) (14). In this study the total CQ concentration (tCQ) is defined as the concentration of all compounds with a 7-chloro-4-aminoquinoline group in their molecular structure; CQ was used for the calibration curve (14). Briefly, a 50-μl sample was diluted 10-fold with phosphate-buffered saline (PBS) containing 1% Triton X-100. A serial threefold dilution was prepared. This solution was incubated at 37°C for 1 hr with 50 μl HRP-labeled antibody [diluted in 2% bovine serum albumin (BSA) containing PBS] in a flat-bottom 96-well plate (Costar, Type 3590) which was coated with CQ-polyacrylamide (5 μg/ml 0.05 *M* carbonate coating buffer, pH 9.6). The plates were washed four times with PBS containing 0.05% Tween-20, 100 μl substrate solution (1 mg 5-aminosalicylic acid/ml 0.1 *M* phosphate buffer containing 0.1 m*M* EDTA) was added per well, and the E_{492} was measured. Inhibition of color formation indicates the presence of tCQ in the sample. For every plate a calibration curve was determined.

A similar assay with slight modifications was used for the determination of free tCQ levels in plasma after lipCQ administration. The samples were diluted in PBS without Triton X-100 and were immediately assayed. The washing buffer did not contain Tween-20 and incubation with the conjugate was performed at room temperature (after preincubating the plates with empty liposomes to avoid nonspecific interactions).

The ELISA does not discriminate between CQ and its main metabolite des-CQ (14). In order to determine whether des-CQ was formed an HPLC method was used as described by Alvan *et al.* (15) with several modifications. Briefly, the HPLC system consisted of a 6000-A pump, a U6K injector (Waters Associates, Milford, Mass.), and a LS-1 fluorescence detector (Perkin–Elmer, Norwalk, Conn.). The excitation wavelength was 335 nm and a 390-nm filter was used. A normal phase 5-μm spherical silica column (Waters Associates, Milford, Mass.) was used and eluted with acetonitrile–methanol–diethylamine (84.5:15:0.5, v/v) at a flow rate of 0.5 ml/min. Extraction of the CQ and des-CQ was performed as follows: a 200-μl blood sample (if necessary, 10 times diluted) was added to 40 μl internal standard (OH-CQ; 4 μg/ml eluent), 1.2 ml 0.1% (v/v) diethylamine (freshly diluted), and 0.5 ml ml 5 *M* ammonia. After vortexing, 5 ml dichloroethane/diethylether (40/60, v/v; freshly prepared) was added and the mixture was rotated end over end (30 rpm) for 30 min at room temperature. The two phases were separated (15 min, 5000*g*) and the dichloroethane/diethylether phase was removed and evaporated in a new tube by a stream of nitrogen (25°C). The residue was dissolved in 0.5 ml of the eluent and 50 μl was injected. The limit of detection of the method described was 20 ng/ml blood for CQ and 10 ng/ml for des-CQ.

Animals

Outbred male Swiss mice were obtained from colonies of the animal facility of the University of Nijmegen. Five- to six-week-old mice were used in the experiments. They were kept in plastic cages and received standard food (RMH, Hope Farms) and water ad libitum.

Parasite

P. berghei (strain K173) was maintained by weekly subinoculation (i.p.) of 10^5 parasitized mouse RBC (p-mRBC) from infected donor mice into normal mice. Parasitemia was determined from blood smears, made from a drop of tail blood, stained with May Grünwald/Giemsa solutions.

Therapeutic Experiments

Swiss ($N = 6$ to 8 per experimental group) mice were infected i.p. with 10^5 p-mRBC. Therapy (i.p. injection of 0.2 ml per mouse) with (fluid- or gel-state) lipCQ or CQ started at the indicated time before or after infection. Various dosage schedules were used. Parasitemia was determined repeatedly during the whole experiment and the percentage of surviving mice was scored. Experiments were repeated at least three times. Combined data or data of a representative experiment are given. Further experimental details are listed in the figure legends.

Pharmacokinetic Experiments

Male Swiss mice (five or six per group) were injected i.p. with lipCQ (dose and type as indicated in the tables) or 0.8 mg CQ. At regular time points blood samples (50 μl) were taken from the tail vein and collected in a heparinized capillary tube. Plasma and packed RBC (further referred as RBC) were obtained after centrifugation of the glass tubes in a hematocrit centrifuge. The buffy coat (containing all white cells) was removed by cutting out that part of the capillary tube. As information on tCQ levels in buffy coat is lacking,

it is not possible to calculate the total tCQ concentration in whole blood by summation of the data for plasma and RBC. As a routine all samples were diluted 10 times with PBS containing 1% Triton X-100. In cases where free tCQ in plasma was determined, Triton X-100 was left out of the dilution buffer and the diluted samples were immediately used in the ELISA. The mean ± SD of the tCQ concentration is presented. For further details, see the figure legends and tables.

Further Methods Used

Lipid phosphate was determined by the colorimetric method of Fiske and SubbaRow (16). The mean particle size was calculated from data obtained by a dynamic light-scattering technique (17).

RESULTS

Characterization of lipCQ

Analysis of 11 gel-state lipCQ batches and 9 fluid-state lipCQ batches prepared according to the standard procedure yielded a composition of 196 ± 35 μg CQ/μmol phospholipid and a mean particle size of 0.29 ± 0.03 μm. Less than 6% of the total CQ was not associated with liposomes (free CQ). After 9 months of storage at 4°C under nitrogen, no further leakage or change in particle size (due to aggregation or fusion) was observed for either type of lipCQ (results not shown). Nevertheless, in all animal experiments fresh preparations were used.

Effect of Lipid Composition on the Therapeutic Efficacy of lipCQ in the Chemotherapy of *P. berghei* Malaria

In order to study the effect of bilayer rididity of lipCQ on the therapeutic efficacy of lipCQ, fluid- or gel-state lipCQ were administered i.p. in a single injection to Swiss mice on day 5 after infection using several doses of CQ (Table I). The fraction of long-term survivors was significantly higher when 2 or 4 mg CQ was administered in gel-state liposomes than in fluid-state liposomes. This difference disappeared when 6 mg lipCQ was given.

To study the possibility that part of the higher efficacy of gel-state lipCQ (as compared to fluid-state lipCQ) is related to a prolonged availability of CQ or its active metabolite, the two types of lipCQ were injected i.p. into Swiss mice 1, 2, or 3 days before infection with 10^5 p-mRBC. Two dosage levels of lipCQ (4 and 6 mg) were tested (Table II). The data show that gel-state lipCQ are more efficient to prevent an infection than fluid-state lipCQ. The prophylactic effect increased with lipCQ was given shorter before infection and with higher doses of lipCQ. When lipCQ was injected 1 day before infection, both types of lipCQ exhibited the maximum score of negative animals at the 4-mg CQ dose level (Table II).

tCQ Blood Levels After Intraperitoneal Administration of Free CQ or Fluid- or Gel-State lipCQ

In order to analyze the beneficial therapeutic effect of lipCQ in comparison to free CQ, tCQ blood levels were determined after administration of free or liposomal CQ (fluid state), each at a dose of 0.8 mg per mouse [= maximum permissible dose of free CQ (10)]. Figure 1 summarizes the tCQ results obtained with the ELISA. Much higher peak levels and a larger area under the curve (AUC) were obtained after administration of lipCQ in comparison to free CQ. HPLC analysis of the same samples demonstrated an increase in the des-CQ concentration (related to the CQ concentration) from 6% (at T = 10 min) to a maximum of 35% after 24 hr for both regimens, lipCQ and free CQ. Twenty-four hours after lipCQ administration the tCQ blood level was 1.4 ± 0.2 μg/ml blood; after free CQ administration a tCQ blood level of 0.5 ± 0.1 μg/ml blood was found.

In another set of experiments tCQ blood levels were determined after i.p. administration of fluid-state lipCQ at several CQ dose levels (Fig. 2). Increasing the dose resulted in a nonlinear increase in the AUC. In particular, after injection of 6 mg lipCQ per mouse the high tCQ concentration in the blood decreased at a relatively slow rate. Free CQ

Table I. Long-Term Survivors After Intraperitoneal Administration of Fluid- or Gel-State lipCQ, 5 Days After Infection with *P. berghei* in Male Swiss Mice[a]

Dose	Long-term Survivors (N = 16)	
	Fluid	Gel
2 mg lipCQ	1	7
4 mg lipCQ	2	8
6 mg lipCQ	15	16

[a] Long-term survivors after a single i.p. administration of the indicated amount/type of lipCQ 5 days (mean parasitemia was 6 ± 2%) after infection (10^5 *P. berghei*-infected mouse RBC) of male Swiss mice. Total phospholipid dose was 37.5 μmol per animal.

Table II. Prophylactic Effect of Intraperitoneal Administration of lipCQ on the Development of a Patent Infection; Effect of Lipid Composition[a]

Treatment	Negative Animals (N = 10)	
	4 mg CQ	6 mg CQ
Day −3		
Gel	4	8
Fluid	1	1
Day −2		
Gel	10	10
Fluid	4	5
Day −1		
Gel	10	ND
Fluid	10	ND

[a] Male Swiss mice were injected i.p. with the indicated dose of fluid-state (PC:PG:chol, 10:1:5) or gel-state (DSPC:DPPG:chol, 10:1:10) lipCQ (total phospholipid dose, 37.5 μmol per animal) at day 3, 2, or 1 prior to infection (10^5 *P. berghei*-infected mouse RBC). Negative animals means that no parasitemia could be detected during 50 days after infection. Positive animals developed a normal infection and died between day 12 and day 23 after infection. ND, not determined.

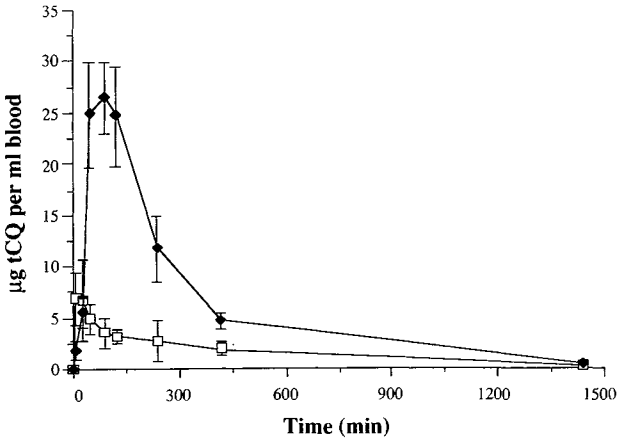

Fig. 1. tCQ blood levels after i.p. administration of free CQ or fluid-state lipCQ. Male Swiss mice (N = 5 per group) were injected i.p. with 0.8 mg (CQ (—□—) or 0.8 mg fluid-state lipCQ (PC:PG:chol, 10:1:5) (—◆—). Total phospholipid dose was 5 µmol per mouse. tCQ was determined with ELISA; data are the mean ± SD. Small SD are not shown.

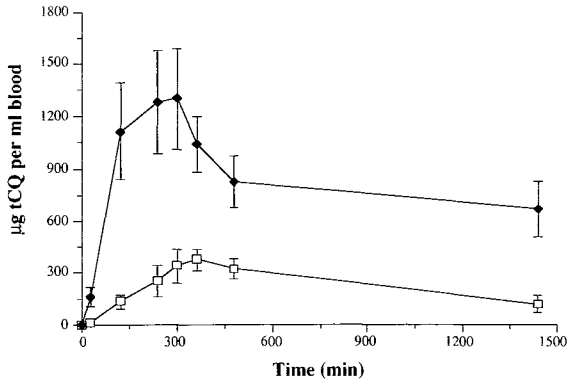

Fig. 3. tCQ blood levels after i.p. administration of gel- or fluid-state lipCQ. Male Swiss mice (N = 5 per group) were injected i.p. with gel-state (DSPC:DPPG:chol, 10:1:10) (—□—) or fluid-state (PC:PG:chol, 10:1:5) (—◆—) lipCQ at a dose of 6 mg CQ and 37.5 µmol phospholipid per mouse. tCQ was determined with ELISA; data are the mean ± SD.

concentrations at this level would cause immediate death of the mice. These findings suggest that the main fraction of CQ circulating in the blood is still liposome encapsulated.

In an additional experiment tCQ blood levels were determined after administration of fluid- or gel-state lipCQ (6 mg CQ per mouse) (Fig. 3). tCQ levels after administration of gel-state lipCQ were considerably higher than after fluid-state lipCQ; 24 hr after administration the tCQ level was about 700 µg tCQ/ml blood for gel-state lipCQ and 120 µg tCQ/ml blood for fluid-state lipCQ. HPLC analysis of the 24-hr samples revealed a des-CQ concentration of 100 µg/ml blood for the gel-state lipCQ and 45 µg/ml for the fluid-state lipCQ.

tCQ Levels in Plasma and RBC After Intraperitoneal Administration of Free CQ or Gel-State lipCQ

In order to determine the distribution of tCQ in blood in relation to the administration of free CQ or lipCQ (gel state), tCQ levels were determined in plasma and RBC. The tCQ concentrations 2 and 6 hr after i.p. administration of 0.8 mg CQ are given in Table III. At both time points tCQ concentrations in RBC were significantly higher than the plasma levels. It should be noted that the tCQ concentrations in whole blood were determined independently, and not by summation of the tCQ concentrations in plasma and RBC (see Materials and Methods).

tCQ concentrations were determined in different blood fractions 2, 6, 24, 48, and 96 hr after i.p. administration of gel-state lipCQ. The tCQ levels in whole blood and RBC as well as the free tCQ concentration in plasma are depicted in Table IV. Again, the tCQ concentration in whole blood were considerably higher than the free tCQ concentrations in plasma at all time points. From 6 hr after injection of lipCQ onward, the tCQ concentration in RBC was at least 10 times higher than free tCQ plasma concentrations. The low free tCQ plasma concentrations (in comparison to the tCQ whole-blood concentrations and the low tCQ levels in RBC) indicate that the main part of CQ was still liposome encapsulated (at least until 24 hr after administration). The determination of free tCQ concentration in plasma (with the adapted ELISA) was possible only for gel-state lipCQ, be-

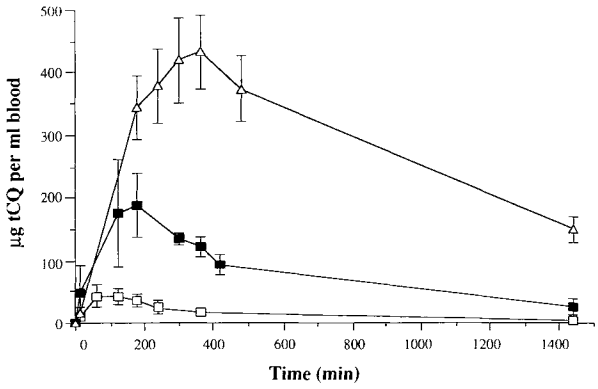

Fig. 2. tCQ levels after i.p. administration of fluid-state lipCQ. Male Swiss mice (N = 4 per group) were injected i.p. with fluid-state lipCQ (PC:PG:chol, 10:1:5): 1 mg (—□—), 3 mg (—■—), and 6 mg (—△—) lipCQ. tCQ was determined with ELISA; data are the mean ± SD. Small SD are not shown.

Table III. tCQ Levels in Whole Blood, Plasma, and RBC After Intraperitoneal Administration of 0.8 mg free CQ[a]

	µg/ml	
	2 hr	6 hr
Plasma	2.05 ± 0.37	0.21 ± 0.03
RBC	3.30 ± 0.48	0.54 ± 0.08
Whole blood	4.95 ± 0.62	1.07 ± 0.18

[a] Male Swiss mice were injected i.p. with 0.8 mg free CQ. The data represent tCQ levels ± SD (N = 4 per group) determined by ELISA.

Table IV. tCQ Levels in Whole Blood and RBC After Intraperitoneal Administration of Gel-State lipCQ; Determination of Free tCQ in Plasma[a]

	μg/ml				
	2 hr	6 hr	24 hr	48 hr	96 hr
Free tCQ in plasma	2.8	0.44	2.3	1.5	0.16
RBC	7.5	4.8	35	16	4.0
Whole blood	850	1678	405	27	5.4

[a] Male Swiss mice were injected i.p. with 6 mg lipCQ (DSPC:DPPG:chol, 10:1:10). tCQ was determined with ELISA; the mean data of four animals are presented (all SD were less than 15%). Free tCQ in plasma was determined in the same ELISA (no Triton X-100 in diluted samples).

cause fluid lipCQ started to leak during the ELISA (results not shown).

tCQ Levels in Plasma and RBC in Relation to the Minimal Effective CQ Dose for Prevention or Therapy of a *P. berghei* Infection in Swiss Mice

For the evaluation of the therapeutic relevance of the free tCQ concentrations in plasma and tCQ concentrations in RBC, an estimate was needed of the tCQ concentrations in plasma and RBC when a minimally effective dose of CQ was given in *P. berghei* infections. Therefore, Swiss mice either were given CQ containing drinking water (during 1 week) before they were infected with 10^5 p-mRBC (preventive CQ level; treatment A in Table V) or received CQ-containing drinking water after infection with 10^5 p-mRBC (mean parasitemia was 6 ± 2%) from day 5 onward (treatment B in Table V). As can be derived from the data presented in Table V the minimum effective concentration of tCQ in whole blood to prevent a lethal infection after inoculation with 10^5 p-mRBC was about 0.21 μg/ml whole blood (treatment A, 25 mg CQ/liter drinking water). The corresponding free tCQ plasma concentration was about 0.07 μg/ml plasma and the tCQ concentration in the RBC was about 0.15 μg/ml. As over a day considerable fluctuations in tCQ concentrations in plasma and RBC occur, these minimum effective tCQ concentrations should be considered as approximate values. The data represent samples taken at 8:30 AM. To eradicate an infection with a parasitemia of 6 ± 2% (equivalent to 6 ± 2 × 10^8 p-mRBC), only two times higher tCQ levels in plasma and RBC were needed to cure these infected animals radically.

DISCUSSION

In a previous article the beneficial therapeutic effects of CQ encapsulation in fluid-state liposomes, in comparison with the free drug, was demonstrated in a *P. berghei* mice model (10). In this report it was shown in the same model that encapsulation of CQ in gel-state liposomes resulted in an even larger increase of the therapeutic efficacy after i.p. administration of gel-state lipCQ in comparison to fluid-state lipCQ (Tables I and II). To investigate the mechanism(s) involved, tCQ blood concentrations after free or lipCQ administration were determined using an ELISA (Figs. 1–3). The advantage of using the ELISA over the HPLC technique is that, with the ELISA, both total (liposomal and free) CQ and only free tCQ could be determined without complicated sample preparation procedures, which can introduce artifacts such as leakage of CQ during the isolation of liposomes from the plasma. Apart from the ELISA an HPLC technique was used in a number of experiments. The reason is that, in contrast to the ELISA, the HPLC method is able to discriminate between CQ and its main metabolite (des-CQ) in man (15). No information is available on the biotransformation of CQ in mice (18). In this study no attempts were made to gain insight into the pharmacokinetics of CQ in mice.

The results obtained with the ELISA were in reasonable agreement with the sum of CQ and des-CQ concentrations determined with the HPLC technique (results not shown). This observation indicates that CQ and des-CQ are the main compounds reacting in the ELISA.

The results clearly demonstrate that (i) after administra-

Table V. tCQ Levels in Plasma, RBC, and Whole Blood of Mice Receiving CQ-Containing Drinking Water; Determination of Minimal tCQ Plasma/RBC Level to Eradicate or Prevent *P. berghei* Infections in Swiss Mice[a]

Drinking Water (mg CQ/liter)	μg tCQ/ml			Negative Animals	
	Plasma	RBC	Whole Blood	Treatment A	Treatment B
6.25	ND	ND	ND	0 (6)	ND
12.5	ND	ND	0.09 ± 0.02	2 (6)	0 (8)
25	0.07 ± 0.02	0.15 ± 0.03	0.21 ± 0.04	6 (6)	0 (8)
50	0.12 ± 0.02	0.32 ± 0.04	0.53 ± 0.07	6 (6)	7 (7)
100	0.13 ± 0.02	0.72 ± 0.08	0.84 ± 0.10	6 (6)	8 (8)

[a] Presented are combined data of mean tCQ levels ± SD (determined by the ELISA) of two independently performed experiments (N = 3 or 4 per group) in male Swiss mice used in treatment A. *Treatment A:* The animals received the CQ-containing drinking water during 7 days; blood samples were taken at days 3 and 7. At day 7, mice were infected with 10^5 *P. berghei*-infected mouse RBC and kept on the indicated drinking water. Presented are the number of negative animals (initial number of mice within parentheses). *Treatment B:* At day 5 after infection (10^5 *P. berghei*-infected mouse RBC; mean parasitemia, 6 ± 2%), the indicated CQ-containing drinking water was supplied. Presented are the number of negative animals (initial number of mice within parentheses).

tion of lipCQ much higher tCQ blood levels than after CQ administration were measured (Fig. 1), and that (ii) tCQ blood concentrations after gel-state lipCQ administration were significantly higher than tCQ blood levels after fluid-state lipCQ administration (Fig. 3). Since i.v. injection of 0.5 mg CQ per mouse (20 g) is lethal (18; personal observation), the high whole-blood levels observed after administration of lipCQ (Figs. 2 and 3) indicate that the main part of CQ was still liposome encapsulated. The data in Table IV support this because they show that the free tCQ concentrations in plasma as well as the tCQ concentrations in RBC were low compared to the tCQ levels in whole blood. The mechanism by which liposomes pass through the peritoneal barrier and enter the vascular system has been studied previously (19). Like other high molecular weight and particulate materials, liposomes are removed from the peritoneal cavity via the lymphatics of the diaphragm, probably by leaving the cavity through small pores (stomata) (19).

HPLC analysis of samples taken 24 hr after administration of lipCQ showed, however, that the relative whole-blood concentrations of des-CQ compared to CQ (free and liposomal CQ) are 14 and 37% for gel- and fluid-state lipCQ, respectively. Des-CQ concentrations were much higher than free tCQ concentrations after administration of gel-state lipCQ (Table IV). It was not further investigated in which fraction of blood cells des-CQ accumulated. As no reliable data on the pharmacokinetic profile of des-CQ in mice are available, no estimation of the total amount of CQ converted to des-CQ can be made.

CQ accumulates in granulocytes and thrombocytes (20,21), leading to CQ values for whole blood two or three times higher than the plasma concentrations (20). This might be the reason for the apparent discrepancy between the tCQ concentrations for whole blood and the calculated sum of the tCQ concentrations for plasma and RBC as shown in Table III.

In Table IV a dip in the concentration of free tCQ in plasma and in whole blood was reported 6 hr after administration of 6 mg gel-state lipCQ. This observation might be ascribed to uptake of lipCQ by the macrophages of the mononuclear phagocyte system (MPS) and a subsequent release of CQ and its metabolite into the bloodstream (22).

More information on the biotransformation of CQ and the pharmacokinetics of CQ and its metabolite(s) is needed, because the antiparasitic effect of des-CQ is comparable to that of CQ in CQ-sensitive (18) but less than that in CQ-resistant strains (23–25).

CQ has complicated pharmacokinetic properties (26, 27). The pharmacokinetics profile of CQ in the blood after administration of lipCQ is dictated mainly, however, by the fate of the liposomes. The more than proportional increase in the AUC of tCQ with increasing lipCQ administration in mice (Fig. 2) can be ascribed to saturation of the MPS (28). The occurrence of MPS saturation after lipCQ administration in our model is under investigation.

The route of administration is a further point of discussion. Parenteral administration of lipCQ might be useful in cases of severe malaria (comatose patients). Oral therapy is not possible for these patients; they receive in the clinic a continious infusion of CQ or quinine. At the moment we are comparing therapeutic and toxic effects after i.p., i.m., and s.c. injection in order to select the proper route of administration of lipCQ in the murine malaria model.

In summary, CQ encapsulation in gel-state liposomes provides a considerably improved efficacy to prevent or treat a *P. berghei* infection in mice compared to fluid-state lipCQ. The data presented in Tables III and IV show that 96 hr after i.p. administration of 6 mg lipCQ (gel state), significantly higher whole-blood and RBC tCQ levels were present than 6 hr after administration of the free drug (0.8-mg dose). Free tCQ values 96 hr after administration of 6 mg gel-state lipCQ still exceeded tCQ concentrations in whole blood and RBC found when treatment with CQ prevented or cured an infection (Table V). This suggests that the 3-day period between administration of gel-state lipCQ and infection (Table II) can be further increased.

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