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Targeting primaquine into liver using chylomicron emulsions for potential vivax malaria therapy

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Abstract

Primaquine (PQ) exerts a broad spectrum of activities against various stages of parasitic malaria. It remains as the only drug that destroys late hepatic stages and latent tissue forms of *Plasmodium vivax* and *Plasmodium ovale*. However, systems that can target PQ to liver hepatocytes, where malarial sporozoites reside, are needed to minimize the dose-limiting severe toxicities and side-effects caused by PQ. Recently, a reconstituted artificial chylomicron emulsion was generated using commercially available lipids and was shown to be preferentially taken up by liver hepatocytes following intravenous injection. We proposed to target PQ to hepatocytes by incorporating it into this chylomicron emulsion. We have shown that lipophilized PQ can be readily incorporated into the chylomicron emulsion. The PQ remained inside the emulsion without significant release. Moreover, PQ incorporated inside the emulsion was more stable than free PQ when incubated in serum. Finally, when intravenously injected into mice, the PQ-incorporated chylomicron emulsion led to significantly enhanced accumulation of PQ in liver, when compared to the injection of free PQ. This emulsion could be developed into a promising delivery system to target PQ into hepatocytes for vivax malaria therapy.

Keywords: Lipoprotein; Liver uptake; Solubility; Stability; Release

1. Introduction

Malaria is one of the most important parasitic diseases in the world. It is estimated that 270 million people are infected annually, resulting in 1.5–2.7 million deaths (Breman et al., 2004). In human, malaria is caused by 4 distinct species of parasites: *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, and *Plasmodium ovale*. *P. falciparum* is the most threatening species because it is widespread and responsible for almost all malaria-related deaths. However, vivax malaria is also very serious. *P. vivax* accounts for about 70–80 million malarial cases annually (Mendis et al., 2001). It has the widest geographic distribution throughout the world, predominately in Asia, the Western pacific, and the Americas

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(Mendis et al., 2001). *P. vivax* is very different from *P. falciparum*. For example, for *P. falciparum*, once the tissue schizonts rupture and burst into circulation, no form of parasites will remain in the liver. However, for *P. vivax*, some tissue stage parasites persist in the liver to form hypnozoites and are capable of producing relapses of erythrocyte infection months or even years after the primary infection (Mendis et al., 2001). These latent stage vivax malaria in the liver constitute an important reservoir of infection. Thus, in order to completely eradicate *P. vivax*, drugs that kill latent liver stage vivax malaria are required.

Primaquine (PQ), an 8-aminoquinoline, is the only drug available for this purpose and is considered to be a crucial weapon against resurgent vivax malaria (Baird and Hoffman, 2004). The new drug Malarone® acts in the liver, but it is not clear whether it can fight against tissue form malarial reservoir in the liver because there has been a case of vivax malarial infection in spite of Malarone® prophylaxis (Povinelli et al., 2003). PQ exerts a broad spectrum of activities against various stages of the parasite (Baird and Hoffman, 2004). It destroys asexual hepatic stages and latent tissue forms of *P. vivax* and *P. ovale*. It also has a marked gametocidal effect against all four species of plasmodia. The exact mechanism of action of PQ is still unknown, although it is believed that it acts by disrupting mitochondrial membranes. Besides being used to eradicate latent tissue forms of vivax and ovale malaria, PQ is also prescribed for terminal prophylaxis. When taken daily, PQ effectively prevents infection by *P. falciparum* and *P. vivax*. In addition, a single dose of PQ (45 mg) can be administered with standard therapy for falciparum malaria (chloroquine) as a measure to block transmission (Baird and Hoffman, 2004).

However, similar to many other malarial therapeutics, PQ can cause severe dose-limiting side effects (hamolysis and methemoglobinemia), which are particularly prominent in patients who are deficient in glucose-6-phosphate dehydrogenase (G6PD) (Baird and Rieckmann, 2003). The current long period and repeated PQ oral dosing schedule is also associated with some epigastric and abdominal side-effects, such as stomach cramp, nausea, and vomiting (Jong and Nothdurft, 2001), which may cause poor patient compliance and lead to periods of sub-therapeutic concentration of PQ in patient plasma, opening the door for the development of drug resistance.

Many researchers have attempted to encapsulate PQ into drug delivery systems, such as liposomes, microspheres, and nanoparticles, or to conjugate PQ to protein carriers to modify its toxicity profile (Arica et al., 1995; Gaspar et al., 1992; Green et al., 2004; Hofsteenge et al., 1986; Laakso et al., 1987; Nishi and Jayakrishnan, 2004; Pirson et al., 1982; Pirson et al., 1980; Rodrigues et al., 1994; Smith et al., 1983). For example, encapsulation of PQ into liposomes was shown to reduce the acute toxicity of PQ (Pirson et al., 1980). The LD_{50} and LD_{90} of PQ in mice were estimated to be 21.6 and 25.0 mg base/kg for free PQ, and 79 and 88 mg base/kg for PQ encapsulated into liposomes (Pirson et al., 1980). It was found that this reduced toxicity is due to the change of the distribution pattern of PQ, which led to a high concentration of PQ in liver, and lower concentration of PQ, and thus toxicity, in other organs (Pirson et al., 1982; Trouet et al., 1981). Recently, Stensrud et al. (2000) prepared small unilamellar PQ-encapsulated liposomes (<100 nm), reasoned that smaller liposomes should be able to pass through the ∼100 nm pores of the fenestrated endothelium lining the hepatic sinusoid (Stensrud et al., 2000). However, the authors did not report any in vivo liver or hepatocyte uptake data from their smaller liposomes.

Lipoproteins are natural spherical macromolecular emulsion particles that are involved in intercellular lipid and cholesterol transport in the circulation (Mahley and Innerarity, 1983; Rensen et al., 2001; Schumaker and Adams, 1969). Dietary lipids absorbed through intestine membrane into blood circulation are packed into small triglyceride-rich lipoprotein emulsions named chylomicrons (Hara et al., 1997; Windler et al., 1980). In blood circulation, lipoprotein lipases hydrolyze the core triglycerides of the chylomicron; many different apolipoproteins are attracted and anchored on the surface of the chylomicron. The modified chylomicron remnants are finally taken up by liver parenchymal cells via apolipoprotein receptors. Importantly, Rensen et al. (1995) reported that reconstituted chylomicron (∼100 nm) can be prepared using commercially available natural lipids. When injected intravenously, the reconstituted chylomicron was preferentially taken up by liver hepatocytes (Rensen et al., 1995). We hypothesize that the uptake of PQ by liver can be enhanced by incorporating it into the chylomicron emulsion. In the present study, we have shown

that lipophilized PQ can be readily incorporated into chylomicron emulsion. The PQ incorporated inside the emulsion was chemically more stable than free PQ. Moreover, the PQ remained inside the emulsion without significant release. Finally, intravenous injection of PQ incorporated into the chylomicron emulsion led to a significantly increased accumulation of PQ in mouse liver, when compared to the injection of free PQ.

2. Materials and methods

2.1. Materials

Sodium lauryl sulfate (SLS), egg phosphatidic acid (PA), Sephadex G-75, olive oil (refined), cholesterol (Chol), cholesteryl oleate (CO), and primaquine (PQ) bisphosphate were purchased from Sigma-Aldrich (St. Louis, MO). L-α-Phosphatidylcholine (PC) and lysophosphatidylcholine (lyso-PC) were purchased from Avanti Polar lipids (Alabaster, AL).

2.2. Complexation between primaquine and SLS or PA

In 7-mL glass vials, PQ and SLS in equal moles, dissolved in buffers (100 mM) of different pH, were mixed together with an equal volume of chloroform. The mixture was then vortexed vigorously for 30 s and continuously shaken overnight at room temperature. The buffers were pH 4.0 (citric acid/citrate), pH 6.0 (citric acid/sodium citrate), pH 7.4 (Na_2HPO_4/NaH_2PO_4), and pH 9.0 (glycine/NaOH). After incubation, the mixture was separated into two phases (layers) by centrifugation, and the concentration of PQ in both phases was measured using a DU® 640 UV–vis spectrophotometer (Beckman Coulter, Fullerton, CA) at 254 or 356 nm, depending on the solvent used.

The complexation between PQ and PA was completed similarly in pH 4, 6, and 7.4 buffers, except that the final buffer concentration used was 10 mM.

2.3. Preparation of chylomicron emulsion

The emulsion was prepared as previously described (Hara et al., 1997; Rensen et al., 1995). Briefly, olive oil, PC, lyso-PC, CO, and Chol were dissolved in chloroform in a glass vial at a weight ratio of 70:22.7:2.3:3.0:2.0, respectively. The chloroform was removed by blowing nitrogen gas into the vial, followed by 24 h of desiccation. The dried lipids were hydrated overnight at 4 °C with sterile and de-ionized water to obtain a final lipid concentration of 40 mg/mL. The lipid suspension was vigorously vortexed for 30–60 s, incubated at a 65–67 °C waterbath for 5 min, and then sonicated in a waterbath sonicator (VWR International) for 5 min or until a fluid, clear, and semi-transparent emulsion was formed. The emulsion was then sequentially extruded through 1.0, 0.4, and 0.1 μm filters using an Avanti Mini-Extruder (Avanti Polar Lipids). This emulsion may be sterilized by sterile filtration. Otherwise, a sterile emulsion may also be prepared using aseptic procedure.

To incorporate PQ into the emulsion, PQ/SLS or PQ/PA complexes were prepared at pH 6.0, solubilized in chloroform, and then co-dissolved with the lipid mixture mentioned above in chloroform. The followed steps were similar as mentioned above.

2.4. Gel permeation chromatography

To separate free unincorporated PQ from PQ incorporated inside the emulsion, 0.1 mL of PQ-incorporated emulsion was applied to a Sephadex G-75 column (diameter = 0.7 cm, length = 25 cm) and eluted with water. The concentration of PQ in each elution fraction (1 mL) was measured using a DU® 640 spectrophotometer after it was extracted out using Bligh and Dyer monophase (water/chloroform/methanol, 1:1:2.1, v/v/v) (Bligh and Dyer, 1959). A 5 M NaCl solution, instead of water, was used in the monophase to dissociate the binding between PQ and SLS or PA.

2.5. Particle size determination

The size of the emulsion was determined using a Malvern 2000 MasterSizer (Malvern Instruments Ltd, Malvern, UK).

2.6. Stability of PQ in emulsion

To study the stability of PQ, free PQ or PQ incorporated in emulsion was diluted into sterile de-ionized water, phosphate buffered saline (PBS, 10 mM, pH 7.4, Sigma-Aldrich), or 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA). The dilutions were then

incubated at 37 °C for 4 h. The concentration of PQ remained in each incubating medium was then determined. The experiment was repeated thrice.

2.7. Release of PQ from emulsion

To monitor the release of PQ from the emulsion, GPC-purified, PQ-incorporated emulsion that contains 0.1 mg of PQ in 0.5 mL of PBS (10 mM, pH 7.4) was placed into a cellulose ester dialysis tube (MW cut, 3.5 kDa) (Spectrum Chemicals and Laboratory Products, New Brunswick, NJ). The tube was then placed into 50 mL of PBS (10 mM, pH 7.4) and incubated in a 37 °C shaker incubator at 100 rpm. At predetermined time points, the release medium was sampled for PQ concentration determination. The total volume of the release medium was kept constant by adding fresh PBS medium after sampling.

To verify the feasibility of using the dialysis tube to study the release of PQ from the emulsion, the diffusion of free PQ through the membrane of the dialysis tube was also investigated to make sure the diffusion was not a rate limiting step.

2.8. Uptake of PQ incorporated into the emulsion by mouse liver

Ten- to 12-week-old Balb/C mice were used for liver uptake study. Purified PQ-incorporated emulsion (540 μg PQ in 0.2 mL in 5% dextrose) or free PQ in sterile PBS (pH 7.4, 10 mM) was injected into mice via the tail vein. Mice were lightly anesthetized with pentobarbital prior to the injection of PQ, and were euthanized with CO_2 1 h after the injection. Blood was collected, and liver was harvested. The liver was immediately washed with PBS (pH 7.4, 10 mM) thrice, dried with a paper towel, and weighed. Both blood and liver samples were stored at −20 °C. PQ was extracted from blood and liver samples following a method previously reported with modification (Mayorga et al., 1997). Briefly, known amount of liver tissue was forced through a 70 μm BD Falcon® cell strainer (BD Biosciences, Bedford, MA) and then lysed with 1× Promega Lysis Buffer (Madison, WI). Blood was lysed with the Lysis Buffer only. The lysates were mixed with an equal volume of acetonitrile containing 10 μg/mL of ketoprofen (Sigma-Aldrich) as an internal standard. After 1 h of shake-incubation at room temperature, the mixture was centrifuged. PQ concentration in the supernatant was measured using a HPLC (Waters LC Modules I, Milford, MA) equipped with a Microsorb-TV™ C18 column from Varian Analytical Instruments (Palo Alto, CA). PQ was detected using a UV-detector at 254 nm. The mobile phase was acetonitrile:methanol:perchloric acid (1 M):water (33:6:1:87, v/v/v/v, respectively) (Jeans and Heard, 1999). The flow rate was 1.5 mL/min. The total volume of mouse blood was assumed to be 7.5% (v/w) of the mouse body weight (Cui and Mumper, 2002; Mosqueira et al., 2001).

2.9. Statistical analysis

Student's *t*-test assuming equal variances was used if two groups were compared. When more than two groups were involved, one way analysis of variance (ANOVA) followed by pair-wise comparisons with Fisher's protected least significant difference (PLSD) procedure was used. A p value of ≤ 0.05 (two-tail) was considered to be statistically significant.

3. Results and discussions

As control measures against *P. falciparum* become more effective, residual malarial infections are likely to become that of *P. vivax*. Therefore, we foresee more use of PQ, should effective alternative substances not be available in the future. We proposed to incorporate PQ into an artificial chylomicron emulsion to target it into liver, potentially liver parenchymal cells. This should enhance the therapeutic effect of PQ. Meanwhile, PQ incorporated inside the emulsion should be protected from enzymatic degradation, and thus have an improved stability. In addition, the toxicity of PQ should be masked by the emulsion if it does not release from the emulsion before being taken up in liver.

3.1. Lipophilization of PQ using SLS

The interior of the reconstituted chylomicron emulsion was mainly of lipophilic olive oil (70%) (Rensen et al., 1995). PQ is commercially available in a bisphosphate form and is moderately soluble in water; one part of PQ is soluble in six parts of water. It is an amphiphatic molecule with two pK_a values of 3.2

and 10.4 (Stensrud et al., 2000). To solubilize PQ into olive oil, we proposed to lipophilize it by complexing it with another amphiphatic molecule having an opposite charge. Sodium lauryl sulfate (SLS) was chosen as an example because it is negatively charged in a wide range of pH. Binding of a protonated amine group on PQ molecule by the sulfate group on SLS should lipophilize PQ and bring it into a more lipophilic phase, such as chloroform and olive oil. PQ alone is practically insoluble in chloroform. However, as shown in Fig. 1A, complexation of PQ with SLS significantly increased the solubility of PQ in chloroform (Fig. 1A). From pH 4.0 to 9.0, the partition ratio of PQ (complexed with SLS) in buffer versus chloroform (buffer/chloroform) increased significantly. This is likely due to the reason that, as the pH of the medium increased, a decreasing proportion of PQ was protonated, lost its positive charge(s), and thus unable to bind to SLS. The electrostatic nature of this binding was confirmed by the fact that the PQ/SLS complex can be disrupted using a high concentration of NaCl (5 M, data not shown). As predicted, this partition ratio increment became less dramatic as the pH of the buffer approached the second pK_a value of PQ (Fig. 1A). Also, as the pH increased, the solubility of free-PQ in the chloroform phase should have gradually increased.

Including SLS in an intravenous injectable might raise some toxicity concerns. Thus, we also lipophilized PQ with phosphatidic acid (PA), a more biocompatible natural anionic lipid. As shown in Fig. 1B, PQ was also brought into the chloroform phase by complexing it with PA (Fig. 1B).

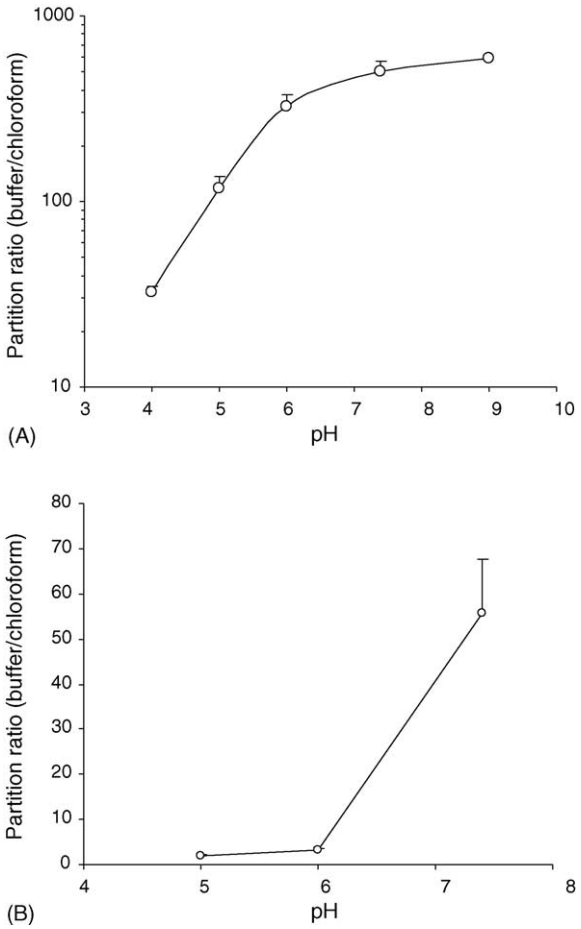

Fig. 1. The effect of pH on the partition of lipophilized PQ into chloroform. (A) PQ and SLS (molar ratio of ∼1:1) dissolved in buffers (100 mM) of various pH were mixed with an equal volume of chloroform and incubated overnight while shaking. After phase separation by centrifugation, the concentration of PQ in the buffer and the chloroform phases was determined. (B) Partition of PQ in buffer/chloroform when PA was used as the complexing agent. The buffer concentration was 10 mM. Data shown are mean ± S.D. ($n = 3$).

3.2. Preparation of PQ-incorporated chylomicron emulsion

The emulsion, which was mainly comprised of olive oil (70%) and phosphatidylcholine (22.7%), was readily formed by hydration of lipid thin film, followed by vortexing and waterbath sonication (Rensen et al., 1995). We have confirmed that inclusion of 10% (w/w) SLS into the lipid mixture did not change the ability for the lipids to spontaneously assembly into emulsions. However, this spontaneous assembling ability was totally diminished when the SLS concentration was increased to equal to 40% (w/w) of the total lipids. Ultimately, we prepared a PQ-incorporated emulsion by incorporating 4 mg of primaquine bisphosphate, complexed with ∼4 mg of SLS, into 40 mg of total lipids. After being extruded through a 0.1 μm filter, the resulting emulsion had a mean size of 183 ± 45 nm. In a 3-month stability study stored at 4 °C, the size of the emulsion did not change significantly (data not shown).

Fig. 2 showed that unincorporated free PQ in the emulsion preparation can be separated from the emulsion by a Sephadex G-75 column. When compared to the elution profile of free PQ, the unincorporated PQ

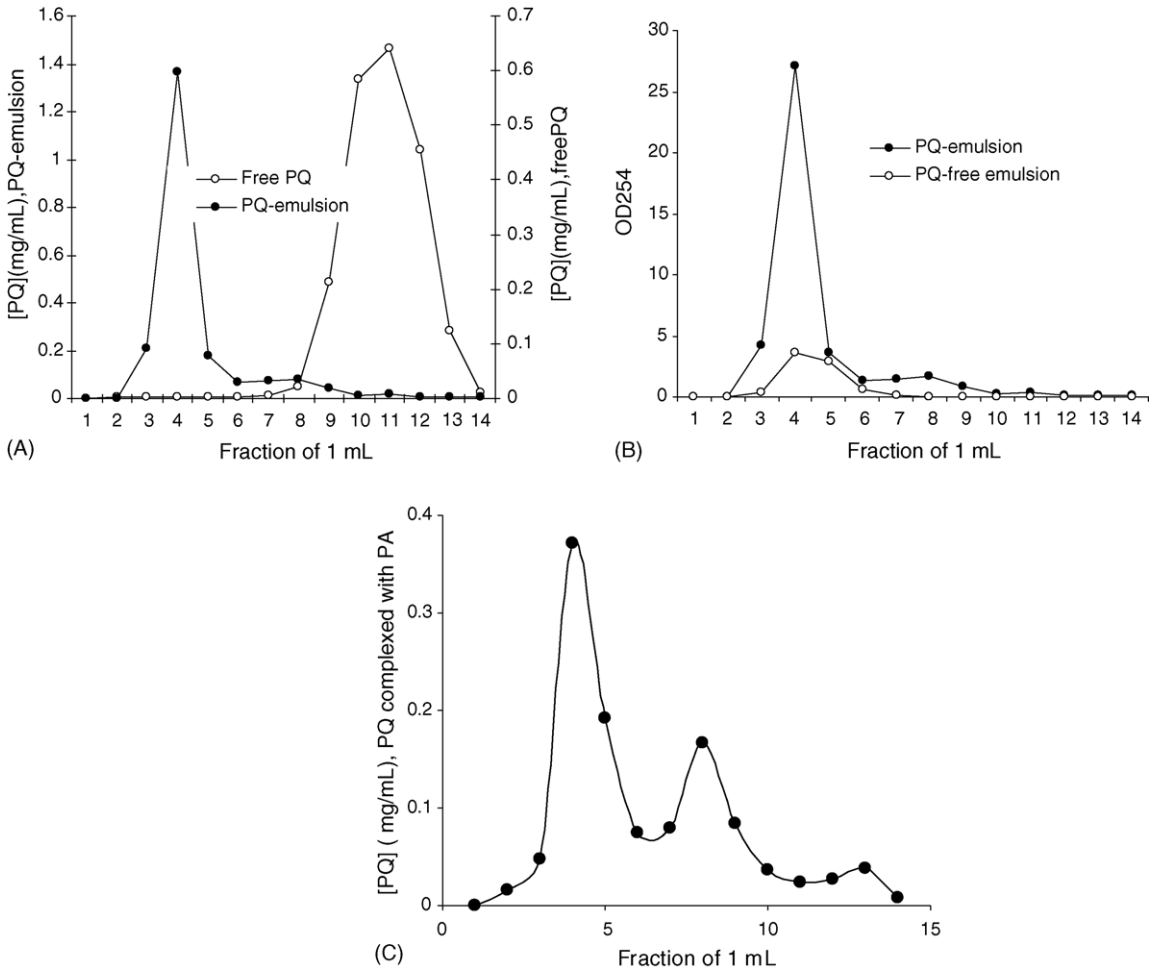

Fig. 2. Unincorporated PQ was removed from the emulsion using GPC. (A) Free PQ or PQ-incorporated emulsion was applied into a Sephadex G-75 column ($d = 0.7$ cm, $l = 25$ cm), and the concentration of PQ in each eluent fraction was determined. (B) As a control and to confirm that PQ incorporated in the emulsion eluted out in the same fraction(s) as the emulsion itself, a blank PQ-free emulsion was also passed through the same column, and the absorbance of each elution fraction at 254 nm was measured. (C) GPC column can also separate unincorporated PQ from PQ/PA complex incorporated in emulsion. The experiments were repeated thrice. Shown is one representative.

in the emulsion preparation eluted out 2–3 fractions earlier when applied to the same column (Fig. 2A). It is possible that PQ that were not incorporated into the emulsion may associate with some micelles formed by SLS and other lipids. Those micelles might be eluted out slightly faster than free PQ. Fig. 2B demonstrated that PQ incorporated into the emulsion was eluted out in the same fractions as the emulsion itself. The incorporation efficiency of PQ in the emulsion was estimated to be $94.7 \pm 0.6\%$ ($n = 3$) using trapezoidal rule. Thus, without any further re-concentrating steps, 3.8–4.0 mg of PQ was incorporated into the emulsion in a volume of 1 mL.

Fig. 2C showed that PQ can also be incorporated into the chylomicron emulsion by complexing it with PA. The incorporation efficient was $\sim 57.0 \pm 0.8\%$.

3.3. Release of PQ from the emulsion

Because we would like to target PQ into the liver, it is important to make sure that PQ will not leak out from the emulsion before the emulsion is taken up by liver

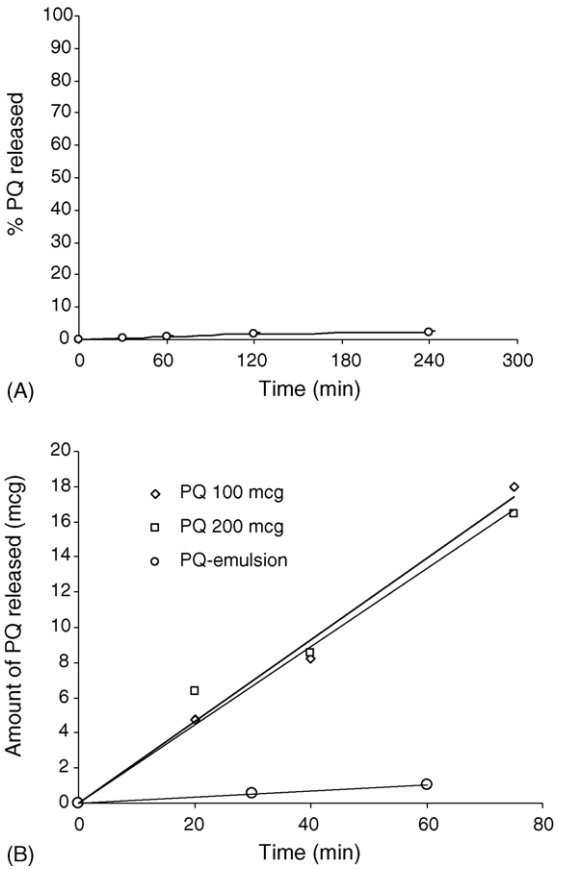

Fig. 3. The release of PQ from the emulsion was slow and limited. (A) To study the release of PQ from the emulsion, emulsion containing 0.1 mg of PQ was placed into a cellulose ester dialysis tube (MW cut, 3.5 kDa). The tube was then placed into 50 mL of PBS (pH 7.4, 10 mM) and incubated at 37 °C. The concentration of PQ remained in the release medium was determined at predetermined time points. The *Y*-axis was scaled from 0 to 100 to clearly show that the release of PQ from the emulsion was very limited. (B) To confirm the feasibility of using the dialysis tube to measure PQ release, the rate for free PQ (100 and 200 μg) to diffuse through the membrane was also determined. Data shown are mean ± S.D. ($n = 3$).

after intravenous injection. Thus, we monitored the release of PQ from the emulsion. As shown in Fig. 3A, the release of PQ from the emulsion was very slow and limited. After 4 h, only 3.8 ± 0.3% of PQ was released. It has been reported by Rensen et al. (1995) that ∼70% of i.v. injected apo E protein-coated chylomicron was taken up by mouse liver 20 min after injection (Rensen et al., 1995). Even without apo E protein coating, about 30% of the reconstituted chylomicron was accumulated into mouse liver 30 min after injection (Rensen et al., 1995). Thus, with only less than 4% PQ being released in 4 h in vitro (Fig. 3), we believed that, after i.v. injection, the majority of PQ incorporated in the emulsion will remain inside the emulsion before it is taken up by liver.

In this study, we placed the PQ-incorporated emulsion into a semi-permeable dialysis tube to prevent the movement of the emulsion into the release medium. Thus, any PQ released from the emulsion must diffuse through the membrane of the dialysis tube to reach the release medium. To make sure that the release profile shown in Fig. 3A truly represented the release of PQ from the emulsion, we also measured the diffusion of free PQ through the membrane of the dialysis tube. As shown in Fig. 3B, the diffusion rate of PQ through the membrane was 0.228 ± 0.007 μg/min, which was about 13-fold higher than the rate for PQ to release from the emulsion (Fig. 3B). Thus, the 3.8 ± 0.3% truly represented the release of PQ from the emulsion in 4 h.

3.4. Stability of PQ in the emulsion

There had been many reports demonstrating that incorporating chemicals into particles enhanced the stability of the chemicals (Muller, 1998; Muller et al., 2002). Because PQ is resided inside the chylomicron emulsion, we expected that it will be less labile to chemical and enzymatic degradation too. To test this, free PQ or PQ-incorporated emulsion was incubated in de-ionized water, PBS (pH 7.4, 10 mM), or serum at 37 °C, and the concentration of PQ remained in the media after 4 h of incubation was measured. Incorporating PQ into the emulsion apparently protected it from degradation (Table 1). Four hours of

Table 1
Stability of free PQ or PQ incorporated in chylomicron emulsion in different media

	PQ (%)	PQ in emulsion (%)
Water	95.3 ± 1.5	96.1 ± 7.6
PBS, 10 mM, pH 7.4	94.1 ± 1.6	100.2 ± 0.9
Serum (10%)	91.6 ± 0.6[a]	101.1 ± 1.3

Free PQ or PQ-incorporated emulsion was incubated at 37 °C for 4 h. The concentration of PQ remained in the media was determined. Data shown are mean ± S.D. ($n = 3$) of the percent of PQ remained.

[a] ANOVA and *T*-test showed that the value for free PQ in serum is significantly different from that in water ($p = 0.016$).

incubation of free PQ in fetal bovine serum resulted in a significant loss (∼10%) of PQ, while no significant PQ degradation was detected when it was incorporated into the emulsion and incubated in the same condition (Table 1). This enhanced stability, in combination with the slow and very limited PQ release shown above (Fig. 3), was expected to allow an increased proportion of PQ to be taken up by liver when injected.

3.5. Uptake of PQ by liver

To study whether the incorporation of PQ into the emulsion enhanced the uptake of PQ by liver, free PQ or PQ-incorporated emulsion was injected via the tail vein into mice. The amount of PQ accumulated in liver and the amount remained inside blood circulation were determined. As shown in Fig. 4, 1 h after the injection, 47.6 ± 6.0% of the PQ incorporated into the emulsion was recovered in mouse liver, compared to only 20.3 ± 4.3% when free PQ was injected. Apparently, incorporation of PQ into the emulsion significantly enhanced its accumulation in liver. This uptake may be further enhanced if the PQ-incorporated chylomicron emulsion was pre-incubated with apo E proteins as a ligand for apo E receptors located on the surface of liver parenchymal cells. Without pre-incubation with apo E proteins, chylomicron emulsion needs to acquire apo E proteins while circulating in blood. In this process, other apoproteins such as apo C, which might have an opposite effect on receptor binding, may compete with apo E, and thus lead to less accumulation of the chylomicron emulsion in liver (Rensen et al., 1995; Van Berkel et al., 1983; Windler et al., 1980). Rensen et al. (1995) reported that the uptake of a similar chylomicron was predominantly by liver parenchymal cells, with minor uptake by liver endothelial cells and Kupffer cells (Rensen et al., 1995). Therefore, it is likely that that our PQ-incorporated emulsion had delivered the incorporated PQ mainly to liver hepatocytes, where tissue stage malarial schizonts reside (Meis and Verhave, 1988). We will confirm this in future studies.

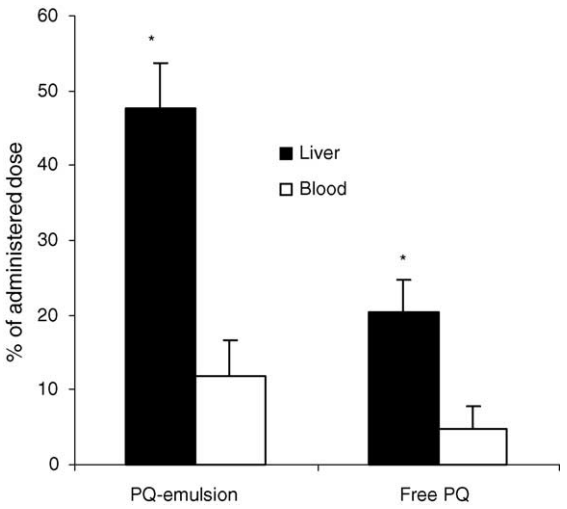

Fig. 4. Intranvenous injection of PQ incorporated into the emulsion resulted in enhanced PQ concentration in liver than injection of free PQ. GPC-purified, PQ-incorporated emulsion or free PQ in PBS (pH 7.4, 10 mM) was injected into Balb/C mice via the tail vein. The concentration of PQ in liver and blood were determined 1 h after injection. Shown are mean ± S.D. (n = 4–5). (*) Indicates that, for PQ recovered in liver, the value from PQ-emulsion was significantly different from the value from free PQ. The accumulation of PQ in blood after the injection of PQ-emulsion was not different from that after the injection of free PQ alone.

The deposition of free PQ into liver agrees well with what had been reported (Pirson et al., 1982). For example, Pirson et al. (1982) reported that when free PQ was injected i.v. into TB_{ESP} male mice, ∼25% of the injected PQ was recovered in the liver 60 min after injection (Pirson et al., 1982). The percentage of PQ that remained in blood was estimated to be about 12% for PQ incorporated into the emulsion and about 5% for free PQ. Again, this 5% agrees with what was reported by Pirson et al. (1982). Interestingly, Mayorga et al. (1997) reported that as high as 16–17% of free PQ were still in the plasma 1 h after it was injected (i.v.) into Swiss mice (Mayorga et al., 1997).

In summary, we have reported a promising approach to target PQ into liver with the potential to treat latent stage malaria and to minimize the toxicity of PQ. The PQ-incorporated chylomicron emulsion has many advantages. For example, (i) the formation of the chylomicron emulsion was a spontaneous assembling process. Thus, high torque and energy consuming mechanical homogenization procedures required for other emulsion preparations are not required. (ii) The final emulsion was stable when stored in buffers and serum. (iii) Incorporation of PQ into the emulsion helped to increase the stability of PQ by preventing it from chemical and enzymatic degradation. (iv) All the components, except the SLS, in the PQ-incorporated

emulsion are, or can readily be metabolized into naturally occurring and biocompatible materials. Even the SLS can be replaced by other more biocompatible and safer materials, such as phosphatidic acid (PA) and cholic acid. (v) Finally, it is necessary to point out that there is another very unique advantage in using this chylomicron emulsion to deliver PQ to the liver. Recent reports suggested the involvement of host lipoprotein clearance pathways in malaria sporozoite invasion of liver. Apo E-enriched β-VLDL was found to inhibit the development of *Plasmodium berghei* sporozoites in HepG2 cells in vitro. Also, LDLR knock-out mice maintained on a high fat diet were found to be less susceptible to infection by *Plasmodium yoelii* sporozoites (Shakibaei and Frevert, 1996; Sinnis et al., 1996). These observations suggested that apolipoprotein E competes for the same binding sites with circumsporozoite (CS) proteins on the surface of malarial sporozoites (Shakibaei and Frevert, 1996). Because malaria invade hepatocytes by their sporozoites, apo E-coated chylomicron emulsion itself may inhibit the entrance of malarial sporozoites into hepatocytes.

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