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Formulation, antimalarial activity and biodistribution of oral lipid nanoemulsion of primaquine

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Abstract

Primaquine (PQ) is one of the most widely used antimalarial and is the only available drug till date to combat relapsing form of malaria especially in case of *Plasmodium vivax* and *Plasmodium ovale*. Primaquine acts specifically on the pre-erythrocytic schizonts which are concentrated predominantly in the liver and causes relapse after multiplication. However application of PQ in higher doses is limited by severe tissue toxicity including hematological and GI related side effects which are needed to be minimized. Lipid nanoemulsion has been widely explored for parenteral delivery of drugs. Primaquine when incorporated into oral lipid nanoemulsion having particle size in the range of 10–200 nm showed effective antimalarial activity against *Plasmodium bergheii* infection in swiss albino mice at a 25% lower dose level as compared to conventional oral dose. Lipid nanoemulsion of primaquine exhibited improved oral bioavailability and was taken up preferentially by the liver with drug concentration higher at least by 45% as compared with the plain drug.

Keywords: Lipid nanoemulsion; Antimalarial activity; Liver uptake; Oral bioavailability

1. Introduction

Malaria, the 'king of diseases' is re-emerging as world's number one killer infection. Every year, malaria is reported to claim about three million lives worldwide. Thus it is a huge challenge to humankind and puts great burden on the economies of especially third world countries (Breman et al., 2004). Malarial parasite is transmitted into mamalian host through bite of female Anopheles mosquito. Asexual reproductive stage of *Plasmodia* takes place in host mammal. In humans, malaria is caused by four species of *Plasmodia* viz. *Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale* and *Plasmodium malaria*. *P. vivax* infection accounts for 70–80 million cases annually. In the case of *P. vivax* and *P. ovale* some of the tissue form persists in the liver in the form of hypnozoites which on reactivation are capable of causing relapse of erythrocytic infection even after years. Primaquine (PQ), a liver schizonticide, is the only drug available against the relapsing form of malaria and it specifically acts on the latent hepatic forms of the parasite (Baird and Hoffman, 2004). The new drug Malarone® (atovaquone and progaunil hydrochloride tablet) acts against early liver stage, but it is not clear whether it can fight against latent tissue form malaria in the liver as 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 (Povinelli et al., 2003). 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 of chloroquine for falciparum malaria as a measure to block transmission (Povinelli et al., 2003).

However primaquine is characterized by dose-limiting side effects like acute hemolytic anemia in patients with G6PD deficiency, methanoglobenemia, leukocytopenia, leukocytosis, GI disturbances and abdominal cramps (Baird and Rieckmann, 2003). Also limited oral bioavailability due to pre-systemic

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metabolism and excretion is reported. Thus it is important to reduce the adverse effects of primaquine considering its crucial role in antimalarial chemotherapy. Targeting primaquine to its site of action, liver would possibly help to reduce therapeutic dose and subsequent reduction in toxicity may be achieved. In one of previous reports, attempts have been made for PQ targeting, comparing plasma clearance, urinary excretion and tissue distribution of radiolabeled free (FPQ) and liposome-entrapped primaquine (LPQ) in mice after i.v. administration. FPQ was eliminated very rapidly from the plasma and excreted predominantly in the urine, probably largely in a metabolized form. However cholesterol-rich multilamellar negatively charged liposomes retained higher drug levels in liver followed by kidneys, lungs and skeletal muscles. Doubled liver accumulation (50% of total dose), prolonged plasmatic half-life and reduced excretion alongwith reduced toxicity was reported (Pirson et al., 1980); Conjugation of PQ to protein carrier to modify the toxicity profiles have also been reported (Pirson et al., 1982; Smith et al., 1983; Arica et al., 1995; Green et al., 2004). Counsell et al. (1983) disclosed a novel method in the prophylaxis and chemotherapeutic treatment of malaria using glycoconjuate of PQ incorporated in the liposome. PQ-loaded polyisohexylcyanoacrylate nanoparticles showed a 21-fold increase in ED_{50} compared with free PQ when tested for *in vitro* antileishmanial activity (Gaspar et al., 1992). Rodrigues et al. (1995) prepared PQ loaded-poly (D,L-lactide) nanoparticles with a unimodal size distribution, ranging from 150 to 200 nm with a narrow distribution. Intravenously injected nanoparticles were well tolerated by healthy and Leishmania donovani-infected mice with 50% reduction in lethal dose when compared to that of free PQ. Albumin and gelatin nanoparticles of PQ have reported to show reduced toxicity in mice (Labhasetwar and Dorle, 1990). Mbelaa et al. (1992) reported higher increased life span index related with the PQ-loaded nanoparticles after administration of the free PQ diphosphate, free poly(DEMM) nanoparticles and PQ-loaded poly(DEMM) nanoparticles as a single intraperitoneal injection to *Plasmodium bergheii* infected female NMRI mice. Bhadra et al. (2005) reported dendrimeric nanoparticles of PQ diphosphate for liver targeting using polypropyleneimine (PPI) dendrite coated peripherally with galactose. Galactose coating prolonged release up to 5–6 days as compared to 1–2 days for uncoated PPI systems. Stensrud et al. (2000) prepared unilammelar PQ-loaded liposome which would pass through fenestrations of endothelial lining of hepatic sinusoid (~100 nm), but no *in vivo* data has been reported. Reconstituted artificial chylomicron emulsion of primaquine has been reported which could preferentially be taken up by hepatocytes following i.v. administration (Dierling and Cui, 2005). Primaquine biphosphate has been complexed with sodium lauryl sulphate prior to incorporation in chylomicron emulsion. Chylomicron has been used to solubilize the oil and drug in the initial steps and later sequential extrusion through membrane had been carried out to get desired particle size. All the above reports include formulation suitable for intravenous administration. However, primaquine is advised not to be administered parenterally because of the risk of marked hypotension (www.pharmacology.tulane.edu/medpharm/DrugProfiles). Thus oral route is preferable for administration of primaquine. Targeting via oral route would be possible by using lipid nanoparticulate systems that would mimic natural lipoproteins with regards to the intestinal absorption and distribution after oral administration.

Dietary lipids get absorbed through intestinal membrane packed into triglyceride rich emulsion called chylomicron and reach the blood circulation (Windler et al., 1980). In blood circulation lipoprotein lipase hydrolyses the core triglyceride of chylomicron. Many different apolipoproteins like Apo E, Apo B, Apo C, etc. are attracted and anchored on the surface of chylomicron. These modified chylomicron remnants are finally taken up by liver parenchymal cells via apolipoprotein receptors. The chylomicra anchoring specifically Apo E are attracted to the liver (Brown and Goldstein, 1983). Correct particle size is prerequisite for such a specific association. Particle size range between 50 and 200 nm with a narrow size distribution (<300 nm) are best suited for Apo E association which would enhance uptake by liver parenchymal cells (Counsell et al., 1998).

Simple lipid nanoemulsion systems which are easy to scale up have been extensively used for parenteral nutrition over decades. Nanoemulsions are heterogeneous systems comprised of two immiscible liquids in which one liquid is dispersed as droplets in another liquid (Klang and Benita, 1998). The ingredients of nanoemulsions are well tolerated by body owing to their structural and functional similarity with physiological lipids. Nanoemulsion system has been employed in cosmetic and dermatological product to enhance skin penetration (Daniels, 2001; Sonneville-Auburn et al., 2004; Yilmaz and Borchert, 2005). Therapeutic application of nanoemulsion include intravenous nanoemulsion of benzathine penicillin G (Santos-Magalhaes et al., 2000), enhanced oral bioavilability of ubiquinone (Nazzal et al., 2002), increased dissolution and enhanced oral bioavailability of all-trans retinoic acid (Taha et al., 2004), nanoemulsion for ocular delivery to provide reservoir for sustained release drug (Sznitowska et al., 1999) As they are easily taken up by lipoprotein receptors in the liver, they can suitably be exploited to incorporate PQ into lipid emulsion with particle size less than 200 nm for apoprotein E association and thus targeting the drug molecule towards liver. Nanometric size range enables to form kinetically stable systems having long-term stability without any apparent flocculation or coalescence. In present study oral lipid emulsion of primaquine was formulated. Evaluation of antimalarial activity showed that primaquine lipid emulsion could display effective therapeutic activity at a lower dose level than the conventional dose. Also it has been exhibited that improved oral bioavailability and higher levels of primaquine were achieved in the liver by incorporation into lipid nanoemulsion.

2. Materials and methods

2.1. Materials

Primaquine diphosphate of pharmaceutical grade was obtained form IPCA laboratories, Mumbai as a gift sample. Miglylol 812 was obtained as a generous gift sample from Sasol,

Germany. Egg lecithin (Ovathin 160), soyabean lecithin liquid (Topcthin 300) were provided from Degussa bioactives, Deuschland, GmBH. Poloxamer 188 (Lutrol F 68) was a gift sample from BASF, India. Other chemicals like glycerol, sorbitol and solvents of AR grade were purchased from SD Fine Chemicals, Mumbai. Colors and flavors were obtained from Firmenich flavors, USA through Colorcon Asia Pvt. Ltd., Mumbai.

2.2. Preparation and characterization of primaquine free base

Primaquine diphosphate was converted into lipid soluble primaquine free base by alkalanization with ammonium hydroxide at pH 12.0 and extracted twice with chloroform. The organic phase was washed twice with water and twice with a saturated solution of sodium chloride. Before evaporation, the chloroform was dried with anhydrous sodium sulphate. PQ obtained in this way was characterized by infrared spectroscopy using Perkin Elmer 16 PC, ST. Quentin en Yvelines, France.

2.3. Preparation of primaquine lipid nanoemulsion

For the preparation of o/w emulsion, Ovathin 160 (1.0%, w/v) and Topcithin 300 (0.6%, w/v) and primaquine (1.0%, w/v) were dissolved in Miglylol 812 at 60–70 °C. Aqueous components like Lutrol F 68 (1.2%, w/v) and glycerol (1.0%, v/v) were added to water and maintained at same temperature. Viscocity modifier like sorbitol (1.0%, v/v), color, flavor and sweetner were added into the aqueous phase. The aqueous phase was added dropwise to the oily phase with constant stirring using a mechanical stirrer and the emulsion was allowed to form. Particle size reduction of the emulsion was done initially using Ultra-Turrax T 25 stirring at high speed of 20,000 rpm and later subjecting to high pressure homogenization using APV2000 at 1000 bars and 5–6 cycles.

2.4. Characterization of primaquine nanoemulsion

2.4.1. Preliminary characterization

Preliminary characterization such as visual observations for creaming, cracking or phase separation were noted over a period of 48 h of undisturbed standing. Freeze–thaw cycling test was carried out at five alternate cycles each of 24 h of storage at ambient and freezing conditions, high speed centrifugation at various speeds starting from 2000 up to 25,000 rpm each for 10 min was carried out.

2.4.2. Particle size determination and TEM

Particle size was determined by photon correlation spectroscopy using N4 plus Beckmann coulter counter after appropriate dilution of the sample. Structure and particle size distribution was confirmed by transmission electron microscopy (TEM). TEM was carried out using ZEISS EM 109, Germany.

2.4.3. Drug content

Encapsulation efficiency was expressed as a percentage of PQ found in the system to the theoretical quantity of the drug added. Drug content was determined by reverse phase HPLC method using C18 Shodex column (4.6 mm × 250 mm, 5 μm) with mobile phase of methanol: phosphate buffer pH 4.0:55:45 at a flow rate of 1 ml/min at 254 nm.

2.4.4. In vitro drug release

In vitro drug release in saline phosphate buffer pH 7.4 as well as in 0.1N HCl was determined using water bath shaker apparatus. Two hundred microliter of the medium was placed in 500 ml stoppered conical flask. Two milliliter of lipid nanoemulsion was packed in a dialysis bag and was placed into the medium. The assembly was stirred at a speed of 50 rpm at 37 ± 2 °C. The aliquots of 5 ml were removed at specific time intervals replacing by fresh medium every time and were extracted and analyzed by using the HPLC method as described above.

2.5. Stability of primaquine lipid nanoemulsion

Stability studies of the developed primaquine nanoemulsion were performed as per ICH guidelines. The samples were kept at four different conditions of temperature and relative humidity (%RH) as 40 °C/75% RH, 30°C/65% RH, 25°C/60% RH and refrigeration condition. The stability was observed over a period of 3 months. The samples were evaluated for particle size, viscosity and drug content.

2.6. Evaluation of antimalarial activity

Antimalarial activity of the formulations was carried out using Peter's 4-day suppressive test (Peters, 1965) using healthy male Swiss albino mice (18–22 g). Parasitic culture of *Plasmodium bergheii yoelii* was maintained in live mice through serial blood transfusion in fresh animals every 4 days. 1 ml of blood was freshly withdrawn through heart puncture of heavily parasitisized (>90%) animal and was diluted to 10 ml in citrated saline. 0.1 ml of the culture was injected intraperitonially (1×10^6 parasites per animal) to the test animals. The animals were divided into 10 groups of five animals per group. After four hours of infection, the animals were treated with the primaquine lipid nanoemulsion (PQ-NE) and plain drug solution (PQ-DS) of Primaquine. The formulations were orally administered daily at four different dose levels as 5.0, 2.0 (equivalent to therapeutic dose in human), 1.5 and 1.0 mg/kg/day over a period of 4 days. One group was administered blank nanoemulsion (placebo) while one group was maintained as untreated control. Blood smears were observed under microscope (100×) to count parasites per 10,000 RBCs/mm^3 by using Neubauer's Chamber. These observations were recorded until all the animals from control group died of malaria. Observations for survival time were made maximally up to 45 days. Mean % parasitemia (M%P) was determined and % suppression of parasitemia was calculated using Eq. (1).

$$\%\text{suppression} = ((A - B)/A) \times 100 \quad (1)$$

where A = % parasitemia in control group, B = % parasitemia in treated group.

Mean survival time (MST) was calculated using Eq. (2) for all the groups.

$$\text{MST} = (n1d1 + n2d2 + n3d3 + n4d4 + n5d5)/5 \quad (2)$$

where $n1, n2, \ldots, n5$ = number of animals that died on a particular day, $d1, d2, \ldots, d5$ = corresponding day on which the animal died.

2.7. Pharmacokinetics and biodistribution studies

Pharmacokinetic and biodistribution studies were carried out using Wistar rats of either sex having weight in the range of 180–220 g. A single dose of 1.5 mg/kg/day of the PQ-NE and PQ-DS were administered to the animals and the animals were sacrificed at specific time intervals of 0.5, 1, 2, 4, 8, 16 and 24 h. Blood samples of the animals were collected through retro-orbital vein into tubes containing heparin before sacrificing and plasma was separated by centrifugation at 2000 rpm for 20 min at 37 °C. All the tissues like liver, spleen, lungs heart, kidneys and brain were isolated and were homogenized in a tissue homogenizer in a small quantity of saline and were maintained in cold condition. The drug from plasma as well as tissue homogenizates was extracted using acetonitrile and the organic layer was evaporated to dryness under nitrogen pressure. The samples were analyzed using a previously established reverse phase HPLC method using C18 Shodex (4.6 mm × 250 mm, 5 μm) column and methanol: phosphate buffer pH 4.0:acetonitrile in the ratio of 5:4:1 at a flow rate of 1 ml/min at 254 nm.

2.8. Statistical analysis

Two-way analysis of variance (ANOVA) and Bonferroni's multiple comparison test was applied to mean survival time in antimalarial activity. For plasma and tissue distribution of the drug, nonparametric t-test was applied. Significance of the differences in mean drug levels in plasma and various tissues was estimated preliminary by one-way ANOVA and further by Turkey's multiple comparison test.

3. Results and discussions

3.1. Preparation and characterization of primaquine base

Primaquine base obtained was highly viscous yellowish brown liquid having boiling point 176 °C (175–179 °C reported) and was free of phosphate residue. The IR spectra of the separated base using 5% (w/v) solution in chloroform were super imposable with the standard spectra as per Indian Pharmacopoeia. N–H bending and stretching vibrations that characterized free amino group were seen at 1519.2 and 3386.1 cm^{-1}, N–H wagging was observed at 752.8 cm^{-1} indicating liquid primary and secondary amine whereas aliphatic C–H linkage was characterized at 1216.0, 1595.5 cm^{-1}.

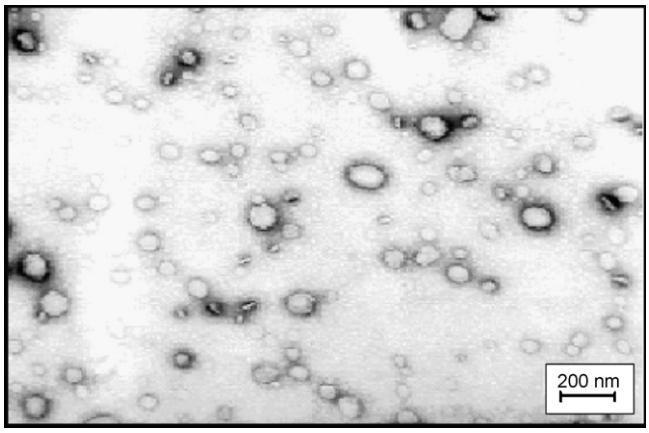

Fig. 1. TEM of primaquine nanoemulsion.

3.2. Preparation and characterization of primaquine lipid nanoemulsion

The coarse primaquine lipid emulsion was formed on addition of aqueous phase to the oily phase which after homogenization resulted into translucent nanoparticulate emulsion. The palatable nanoemulsion had a pH between 7 and 8 and viscosity of 2.42 cps. The emulsion was found to be stable without any incidence of creaming, cracking or phase separation and withstood alternate cyclic storage at ambient and freezing conditions and high speed centrifugation from 2000 to 25,000 rpm.

Particle size distribution of the nanoemulsion was found to be 20–200 nm with a mean of 96.5 nm. TEM micrograph further confirmed the spherical shape and nanometric particle size distribution of the nanoemulsion of primaquine (Fig. 1). Encapsulation efficiency was found to be 95%. *In vitro* drug release in saline phosphate buffer pH 7.4 as well as in 0.1N HCl showed >90.0% of drug release within 8–10 h.

3.3. Stability studies of primaquine lipid nanoemulsion

Stability studies revealed that the samples stored in refrigeration, 25 °C/60%RH and 30 °C/65% RH had stable particle size for 3 months (Fig. 2). No change in the viscosity and drug content was observed within 3 months. The samples stored at 40 °C/75%RH however showed discoloration and phase separation.

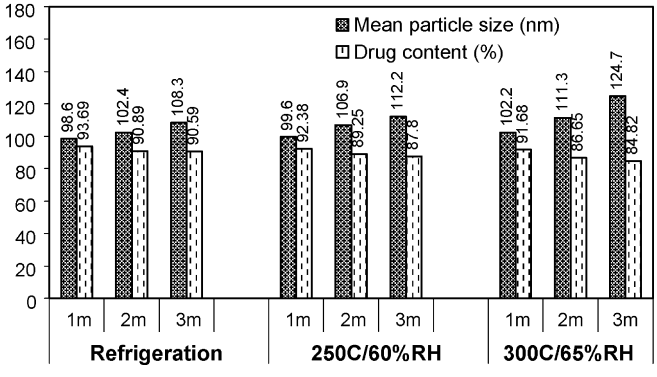

Fig. 2. Three month stability data of primaquine nanoemulsion (PQ-NE).

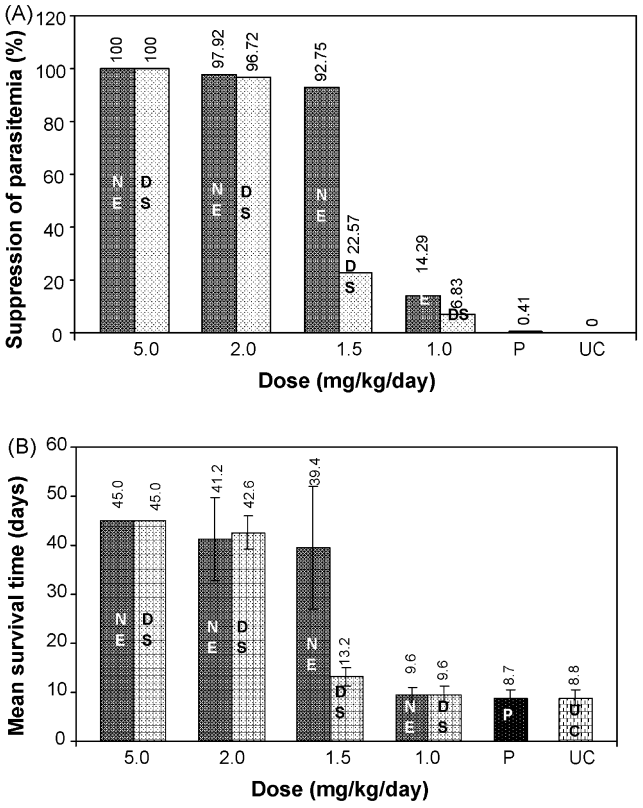

Fig. 3. (A) Suppression of parasitemia (%) in animal groups in antimalarial activity test. NE = PQ-NE, DS = PQ-DS P = placebo, UC = untreated control. (B) Mean survival time of animal groups in antimalarial activity test.

3.4. Antimalarial activity

Placebo and untreated control groups showed upto 40% parasitemia on day 5 as parasites invaded and ruptured RBCs. Parasitemia increased gradually and reached upto 90% and more by day 10. No animal from control group survived after day 11.

Fig. 3 shows the percent parasitemia, percent suppression of parasitemia and mean survival time of the experimental animal groups. Animals treated at the dose level of 5.0 mg/kg/day showed complete suppression of parasitemia and full term survival in case of both the lipid nanoemulsion (PQ-NE) as well as plain drug solution of primaquine (PQ-DS). At the therapeutic dose level of 2.0 mg/kg/day, although some initial appearance of parasites was seen, it was negligible and subsequently disappeared by day 10. Animals were completely cured and almost full term survival of the test animals was observed in the case of both PQ-NE and PQ-DS. At the dose level of 1.5 mg/kg/day, lipid nanoemulsion effectively controlled parasitemia with increased survival time of the test animals than those receiving plain drug solution. Huge difference in the suppression of parasitemia and MST was observed between the two formulations at this dose level. Thus as compared with plain drug solution, desirable therapeutic activity was achieved at 25% reduced dose level of primaquine when administered in the form of lipid nanoemulsion. At the dose level of 1.0 mg/kg/day, however none of the formulations showed therapeutic cure of malaria. However PQ-NE showed higher suppression of parasitemia as compared with the PQ-DS (Fig. 3).

Analysis of variance at 95% limit of confidence ($p = 0.05$) showed high value of F, indicating that change in formulation as well as change in the dose level significantly alters the antimalarial activity. Dose dependence of the suppression of parasitemia and MST was more prominent in the case of PQ-DS as compared to PQ-NE indicating that better suppression of parasitemia was achieved at lower doses with PQ-NE than PQ-DS. In order to determine the formulation and the dose levels that were responsible for significant difference in the activity, ANOVA was followed by Bonferroni's multiple comparison test that compared each of the experimental group individually to all the other groups. Bonferroni's post-test confirmed that at the dose level of 1.5 mg/kg/day significant difference occurred between the MST of the animal groups receiving PQ-NE and PQ-DS.

3.5. Pharmacokinetics and biodistribution studies

Peak drug level of 6.27 μg/ml in the plasma was obtained after administration of PQ-NE which was 1.5 times higher as compared with that after PQ-DS administration. PQ-NE showed 1.3 times higher area under curve (AUC) indicating improved oral bioavailability as compared to PQ-DS (Fig. 4).

Fig. 4 presents primaquine levels in various tissues after oral administration of PQ-NE and PQ-DS. Bio-distribution studies revealed that high concentration of the drug was achieved in the target tissue, liver, by PQ-NE as compared with PQ-DS at the peak time of 1.0 h. Statistical t-test revealed that at 95% confidence limit ($p = 0.05$), significant differences of mean occurred for liver drug concentrations after administration of PQ-NE and PQ-DS. The enhancement in the drug levels were 45.5% in the

Table 1
Pharmacokinetic parameters of primaquine after administration of PQ-NE and PQ-DS

Tissue	PQ-NE			PQ-DS		
	T_{max} (h)	AUC (mcg h)	MRT (h)	T_{max} (h)	AUC (mcg h)	MRT (h)
Plasma	2	40.89	5.92	2	31.97	5.63
Liver	1	73.72	7.01	1	52.61	4.86
Kidneys	8	48.44	5.51	8	64.32	6.86
Brain	2	69.23	6.87	2	44.61	6.42
Spleen	2	69.96	7.08	2	46.03	6.49
Lungs	4	61.37	7.23	4	35.08	5.66
Heart	2	33.6	9.15	2	22.89	8.55

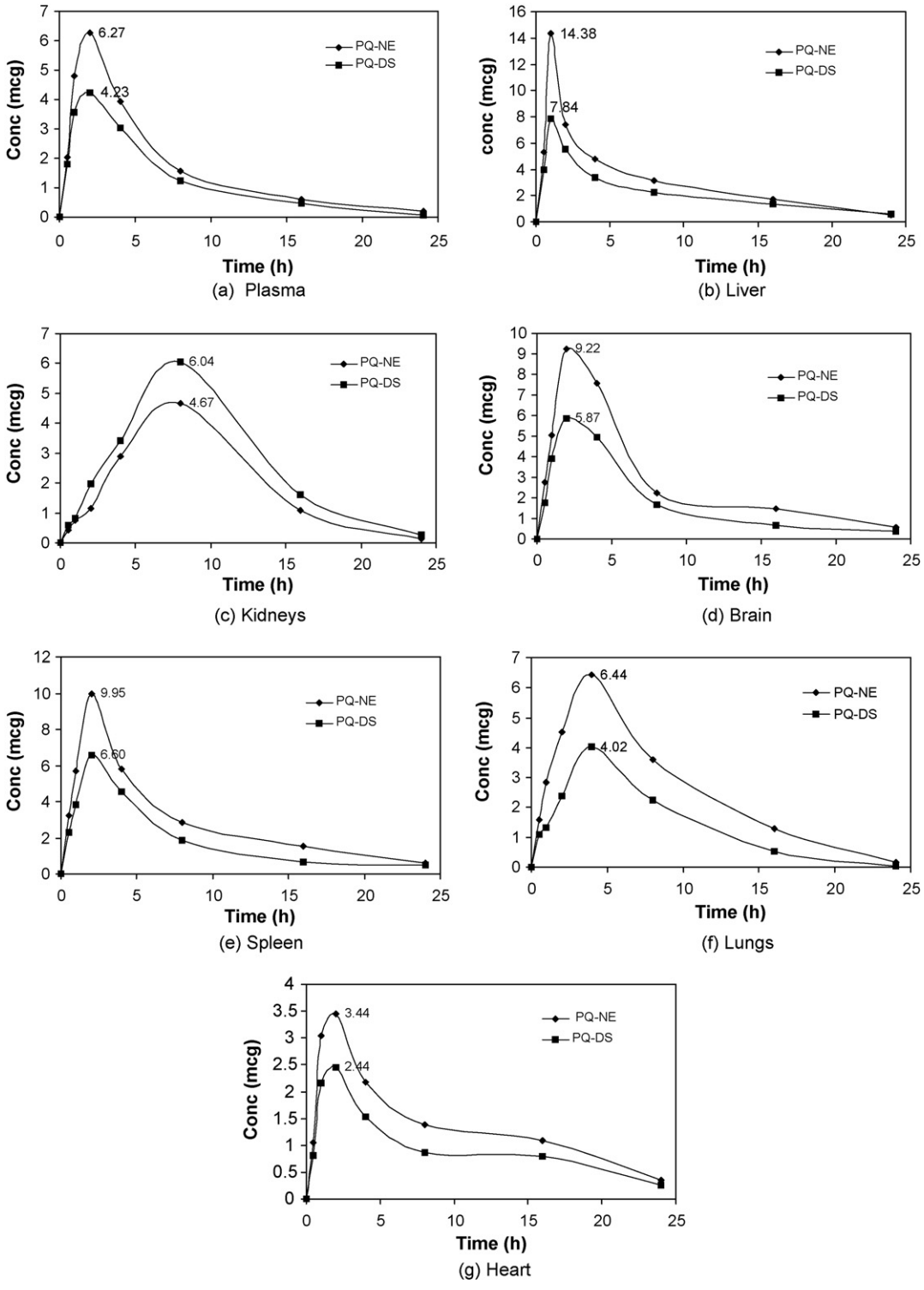

Fig. 4. Drug-concentration time profiles of PQ-NE and PQ-DS after oral administration.

liver. A much higher availability of the drug was obtained in the liver as seen from AUC values (Table 1). Ratio of peak drug concentration from PQ-NE and PQ-DS was highest in liver as 1.84 followed by the ratios in brain, spleen and lung being 1.57, 1.51 and 1.6, respectively. The ratio in plasma, heart and kidney was 1.2, 1.4 and 1.37, respectively. Relative ratio of uptake was calculated as the ratios of tissue: plasma (T:P) drug concentration at 1 h after administration of PQ-NE and were found to be 3.0, 0.16, 1.05, 1.18, 0.6, 0.63 for liver, kidney, brain, spleen, lungs and heart, respectively. The corresponding T:P ratios in case of PQ-DS were 2.2, 0.23, 1.1, 1.08, 0.37 and 0.61. Significantly improved mean residence time (MRT) of 7.01 h with PQ-NE was observed in contrast with 4.86 h after administration of PQ-DS. Higher AUC and MRT ensured higher drug avail-

ability at the site of action over a prolonged period of time. The targeting efficiency of PQ-NE was expressed as the ratio of AUCs of drug concentration-time profile in various tissues and plasma as compared with the corresponding AUCs obtained with PQ-DS. The drug levels in the kidneys were found to be lower with the PQ-NE than those obtained with PQ-DS. AUCs in plasma, liver, spleen, brain, lungs and heart were 1.28, 1.40, 1.57, 1.55, 1.75 and 1.47 times higher respectively, while AUC in the kidneys was 1.33. times lower with PQ-NE as compared to PQ-DS.

ANOVA for comparison of the drug levels in various tissues obtained after administration of PQ-NE revealed statistically significant differences. However these differences were less significant for PQ-DS as compared to PQ-NE. In the case of PQ-NE, this difference was most prominent at 1.0 h while in the case of PQ-DS the maximum difference was observed at 0.5 h. Turkey's multiple comparison revealed that amongst the paired comparisons of drug levels in various tissues, the highest difference was obtained for mean drug levels in the liver against kidneys at 1.0 h of oral administration. Similarly, in the case of PQ-DS liver drug levels were highest as compared to plasma and all the other tissues.

Thus lipid nanoemulsion of primaquine could achieve significantly higher drug levels in the liver after oral administration. Earlier reports of PQ targeting to liver have been obtained after intravenous administration. It is likely that oral emulsion being lipid based would be taken up as intestinal chylomicron and reach blood circulation. While in circulation the nanoemulsion would acquire apoprotein. Association of Apo E would be favored due to narrow size (50–200 nm), which would enhance the drug uptake by liver paranchymal cells. Improved antimalarial activity may be attributed to the enhanced availability of the drug at the site of action when delivered in the form of lipid nanoemulsion as compared with the plain drug solution.

4. Conclusion

Palatable lipid nanoemulsion of primaquine with narrow particle size range of 50–200 nm were developed using medium chain triglycerides and stabilized with lecithins and poloxamer. Effective antimalarial activity at a 25% reduced dose was achieved with novel primaquine nanoemulsion as compared with the plain drug solution after oral administration. Improved therapeutic efficacy of primaquine may be attributed to enhanced oral bioavailability and higher drug levels at the site of action, liver, on incorporation in lipid nanoemulsion. Thus, lipid nanoemulsion holds a great promise for delivery of primaquine to liver with potential to treat latent stage malaria and minimize toxicity. With therapeutic efficacy at reduced dose these systems can also be explored for chemoprophylaxis of malaria in high-risk areas.

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References

Arica, B., Ozer, A.Y., Ercan, M.T., Hincal, A.A., 1995. Characterization, *in vitro* and *in vivo* studies on primaquine diphosphate liposomes. J. Microencapsul. 12, 469–485.

Baird, J.K., Hoffman, S.L., 2004. Primaquine therapy for malaria. Clin. Infect. Dis. 39, 1336–1345.

Baird, J.K., Rieckmann, K.H., 2003. Can primaquine therapy for vivax malaria be improved? Trends Parasitol. 19, 115–120.

Bhadra, D., Yadav, A.K., Bhadra, S., Jain, N.K., 2005. Glycodendrimeric nanoparticulate carriers of primaquine phosphate for liver targeting. Int. J. Pharm. 295, 221–223.

Breman, J.G., Alilio, M.S., Mills, A., 2004. Conquering the intolerable burden of malaria: what's new, what's needed: a summary. Am. J. Trop. Med. Hyg. 71, 1–15.

Brown, M.S., Goldstein, J.L., 1983. Lipoprotein receptors in the liver, control signals for plasma cholesterol traffic. J. Clin. Invest. 72, 743–747.

Counsell, R.E., Longino, M.A., Weichert, J.P., Bakan, D.A., 1998. Hepatocyte-selective oil-in-water emulsion. US patent, US5,851,510.

Daniels, R., 2001. Galenic principles of modern skin care products. Skin Care Forum, 25.

Dierling, A.M., Cui, Z., 2005. Targeting primaquine into liver using chylomicron emulsions for potential vivax malaria therapy. Int. J. Pharm. 33, 148–152.

Gaspar, R., Opperdoes, F.R., Preat, V., Roland, M., 1992. Drug targeting with polyalkylcyanoacrylate nanoparticles: *in vitro* activity of primaquine-loaded nanoparticles against intracellular *Leishmania donovani*. Ann. Trop. Med. Parasitol. 86, 41–49.

Green, M.D., D'Souza, M.J., Holbrook, J.M., Wirtz, R.A., 2004. *In vitro* and *in vivo* evaluation of albumin-encapsulated primaquine diphosphate prepared by nebulization into heated oil. J. Microencapsul. 21, 433–444.

Klang, S., Benita, S., 1998. Design and evaluation of submicron emulsions as colloidal drug carriers for intravenous administration. In: Benita, S. (Ed.), Submicron Emulsions in Drug Targeting and Delivery. Drug Targeting and Delivery, vol. 9. Harwood Academic Publishers, Amsterdam, pp. 119–152.

Labhasetwar, V.D., Dorle, A.K., 1990. Nanoparticles—a colloidal drug delivery system for primaquine and metronidazole. J. Control. Rel. 12, 113–119.

Mbelaa, T.K.M., Poupaertb, J.H., Dumont, P., 1992. Poly(diethylmethylidene malonate) nanoparticles as primaquine delivery system to liver. Int. J. Pharm. 79, 29–38, 1.

Nazzal, S., Smalyukh, I.I., Lavrentovich, O.D., Khan, M.A., 2002. Preparation and invitro characterization of a eutectic based semisolid self-nanoemulsified drug delivery system (SNEDDS) of ubiquinone: mechanism and progress of emulsion formation. Int. J. Pharm. 235, 247–265.

Peters, W., 1965. Drug resistance in *Plasmodium berghei* I. Chloroquine resistance. Exp. Parasitol. 17, 80–89.

Pirson, P., Steiger, R.F., Trouet, A., Gillet, J., Herman, F., 1980. Primaquine liposomes in the chemotherapy of experimental murine malaria. Ann. Trop. Med. Parasitol. 74, 383–391.

Pirson, P., Steiger, R., Trouet, A., 1982. The disposition of free and liposomally encapsulated antimalarial primaquine in mice. Biochem. Pharmacol. 31, 3501–3507.

Povinelli, L., Monson, T.A., Fox, B.C., Parise, M.E., Morrisey, J.M., Vaidya, A.B., 2003. *Plasmodium vivax* malaria in spite of atovaquone/proguanil (malarone) prophylaxis. J. Travel. Med. 10, 353–355.

Rodrigues Jr., J.M., Fessi, H., Bories, C., Puisieux, F., Devissaguet, J.P., 1995. Primaquine-loaded poly(lactide) nanoparticles: physicochemical study and acute tolerance in mice. Int. J. Pharm. 126, 253–260.

Santos-Magalhaes, N.S., Pontes, A., Pereira, V.M.W., Caetano, M.N.P., 2000. Colloidal carriers for benzathine penicillin G: Nanoemulsions and nanocapsules. Int. J. Pharm. 208, 71–80.

Sonneville-Auburn, O., Simonnet, J.T., L'Alloret, F., 2004. Nanoemulsions: a new vehicle for skin care products. Adv. Colloid. Interface Sci. 108–109, 145–149.

Smith, J.E., Pirson, P., Sinden, R.E., 1983. Studies on the kinetics of uptake and distribution of free and liposome-entrapped primaquine, and of sporozoites by isolated perfused rat liver. Ann. Trop. Med. Parasitol. 77, 379–386.

Stensrud, G., Sande, S.A., Kristensen, S., Smistad, G., 2000. Formulation and characterization of primaquine loaded liposomes prepared by a pH gradient using experimental design. Int. J. Pharm. 198, 213–228.

Sznitowska, M., Zurowska-Prychkowska, K., Janicki, S., Jarviuen, T., 1999. Miotic effect and irritation potential of pilocarpine prodrug incorporated into a submicron emulsion vehicle. Int. J. Pharm. 184, 115–120.

Taha, E.I., Al-Saidan, S., Samy, A.M., Khan, M.A., 2004. Preparation and invitro characterization of self nanoemulsified drug delivery systems (SNEDDS) of all-trans retinoic acid. Int. J. Pharm. 285, 109–119.

Windler, E., Chao, Y., Havel, R.J., 1980. Determinants of hepatic uptake of triglyceride-rich lipoproteins and their remnants in the rat. J. Biol. Chem. 255, 5475–5480.

Yilmaz, E., Borchert, H.H., 2005. Design of a phytosphingosine-containing positively charged nanoemulsion as a colloidal carrier system for dermal application of ceramides. Eur. J. Pharm. Biopharm. 60, 91–98.