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Measurement of piperaquine in plasma by liquid chromatography with ultraviolet absorbance detection

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Abstract

Piperaquine (PQ) is an antimalarial drug enjoying a resurgence of use in combination with an artemisinin derivative because of parasite resistance to standard treatments. Its pharmacokinetic properties have not been characterised. An assay for PQ in plasma was developed using solvent extraction and liquid chromatographic separation on a Waters XTerra™ RP_{18} column, with a mobile phase of 7% acetonitrile in water (containing 0.025% trifluoroacetic acid, 0.1% NaCl and 0.008% triethylamine) and UV detection at 340 nm. The assay was linear up to 1000 μg/l. Intra- and inter-day relative standard deviations were <10% (5–500 μg/l) and <21% (5–500 μg/l), respectively. Inter-day limits of quantitation and detection were 5 μg/l and 3 μg/l, respectively. A preliminary pharmacokinetic study in a patient who received 2.56 g of PQ phosphate orally with dihydroartemisinin as four doses over 32 h found an apparent steady-state volume of distribution of 447 l/kg, an apparent oral clearance 0.93 l/h/kg and a terminal half-life of 17.3 days.
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Keywords: Piperaquine; Antimalarial drugs

1. Introduction

Piperaquine {1,3-bis[1-(7-chloro-4′-quinolyl)-4′-piperazinyl]phosphate; PQ; Fig. 1} is a bisquinoline antimalarial drug that was synthesised in the 1960s by both the Shanghai Pharmaceutical Industry Research Institute in China and Rhone Poulenc in France. The molecule is based on the 7-chloro-4-aminoquinoline structure found in chloroquine (CQ). In the 1970s, PQ was used to prevent and treat CQ-resistant falciparum malaria in the southern

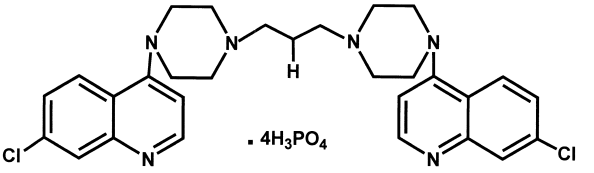

Fig. 1. PQ, 1,3-bis[1-(7-chloro-4′-quinolyl)-4′-piperazinyl]phosphate.

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provinces of China [1–3]. Over the last decade, numerous related bisquinolines have also been synthesized in the search for potent and well tolerated antimalarial drugs that are effective against CQ-resistant *Plasmodium falciparum* [4–6].

Despite the clinical use of PQ in China, its pharmacokinetic properties in humans have not been studied. In recent years, combination antimalarial therapy with a short half-life artemisinin derivative and a long-acting drug such as mefloquine has been recommended as a strategy that maximises cure rates and limits the development of parasite drug resistance [7,8]. Since 1997, three such combination antimalarial products containing PQ have been marketed in China and several other south-east Asian countries. These include CV-8® (dihydroartemisinin (DHA), PQ, trimethoprim and primaquine), Artecom® (DHA, PQ and trimethoprim) and Artekin-2® (DHA and PQ) [9]. This renewed interest in PQ further strengthens the case for the characterisation of its disposition in humans. We have, therefore, developed a robust high-performance liquid chromatographic (HPLC) assay for PQ in plasma, so that its pharmacokinetic properties can be derived and used as a basis for the development of rational dose regimens.

2. Experimental

2.1. Specimens

Serum samples were collected from Cambodian patients with slide-positive falciparum malaria participating in a larger efficacy study using Artekin-2® [10]. The study protocol was approved by the National Ethics Committee for Health Research, Ministry of Health, Cambodia and by the Human Rights Committee of the University of Western Australia. Written informed consent was obtained from the patients. Drug-free plasma samples were collected from healthy volunteer subjects and used for assay development and standard curves. In each case, venous blood samples were centrifuged within 45 min, and the serum or plasma separated promptly and stored below −20 °C. Samples taken in Cambodia were transported to Australia on dry ice before assay.

2.2. Chemicals and reagents

PQ (CAS registry number 4085-31-8; molecular mass 535.51) was extracted from PQ phosphate (molecular mass 927.48) tablets. Briefly, six tablets (2.7 g total; Shanghai Zhongxi Pharmaceutical, China) were dissolved in 3 ml water, adjusted to pH 10 using 10 *M* NaOH and extracted with 2×20 ml dichloromethane by shaking vigorously. After evaporation of the organic phase, white needle-shaped crystals of PQ base were obtained. In agreement with previous literature, PQ had a melting point of 212–213 °C and UV absorption peaks at λ_{max} 225, 239 and 340 nm [11]. It also chromatographed as a single peak on HPLC (see below). A reference sample of 7-hydroxy PQ was obtained from Lt.-Col. Dennis Kyle at the Walter Reed Army Institute of Research (Rockville, MD, USA). CQ diphosphate (internal standard, I.S.) and trifluoroacetic acid were purchased from Sigma–Aldrich (Castle Hill, Australia). Atovaquone was obtained from the Wellcome (London, UK). HPLC grade dichloromethane and acetonitrile were used, and all other chemicals were of analytical reagent grade.

2.3. Sample preparation

Plasma/serum samples (1 ml) were aliquoted into 10 ml polypropylene tubes, with I.S. (200 ng) and 0.1 ml of 1 *M* NaOH. Dichloromethane (8 ml) was added, and PQ was extracted by shaking vigorously for 10 min. After centrifugation at 1300 *g* for 10 min, the supernatant was aspirated to waste, and the remaining dichloromethane transferred to a clean polypropylene tube. PQ was then back-extracted into 0.3 ml of 0.01 *M* KCl (buffered to pH 2.4 with 0.01 *M* HCl) by shaking vigorously for 5 min. After centrifugation as above, the dichloromethane layer was aspirated to waste and the remaining acidic aqueous extract was transferred to a clean round-bottomed borosilicate glass tubes and centrifuged at 1300 *g* for 20 min to evaporate any traces of dichloromethane that remained. Aliquots (100 μl) of

this final extract were injected onto the HPLC column.

2.4. HPLC instrumentation and chromatographic conditions

The HPLC system consisted of a Waters Millipore Solvent Delivery System coupled to an HP 1100 isocratic pump, autosampler and variable wavelength UV detector (Agilent Technology, Waldbronn, Germany). Separations were achieved on a Waters XTerra™ RP_{18} 3.5 μm, 4.6×100 mm HPLC column with a Waters Symmetry® C_{18} 5 μm, 3.9×20 mm guard column. The mobile phase of 7% (v/v) acetonitrile in water (containing 0.025% trifluoroacetic acid, v/v, 0.1% NaCl, w/v, and 0.008% triethylamine, v/v), was pumped at 1.2 ml/min, and analytes were detected by their UV absorbance at 340 nm. Analysis and quantification of chromatograms (peak areas) were undertaken using CHEMSTATION software (Agilent Technology, Waldbronn, Germany).

2.5. Validation tests

2.5.1. Standard curve

Stock solutions of PQ base (1 mg/ml in 0.05 *M* H_3PO_4) and CQ phosphate (1 mg/ml in water) were stable for up to 50 days when stored at 4 °C. Working standards were prepared in the same diluents at weekly intervals and also stored at 4 °C. For each analytical batch, a 5-point calibration curve (usual range 5–500 μg/l) was prepared by spiking blank plasma with appropriate volumes of working standards. A standard curve for spiked sera was also prepared and compared to that based on PQ added to plasma on the same day.

2.5.2. Precision and accuracy

Five replicates of blank plasma spiked at 5, 20, 200, 500 μg/l PQ (n=20 in total) were used to construct a standard curve. Intra-day accuracy was calculated from the average of the five replicates at each concentration using the formula: Accuracy (%)=(calculated [PQ]/known [PQ])·100. Precision was determined by calculating the relative standard deviation (RSD) of the samples where: RSD (%)=(SD/calculated[PQ])·100. Inter-day precision and accuracy were determined by the assaying of spiked samples at concentrations of 5, 50 and 500 μg/l on separate days, and calibrated against a daily standard curve. Average accuracy and precision were calculated as detailed above for the intra-day data.

2.5.3. Limits of quantification and detection

The limit of quantification (LOQ) was defined as the lowest concentration measured with a RSD of ≤20%, while the limit of detection (LOD) was defined as the concentration with a signal-to-baseline noise ratio of 2.5.

2.5.4. Specificity

Interference by endogenous constituents in plasma was assessed by analysis of blank plasma samples. A range of likely antimalarial (including 7-hydroxy-PQ) and antipyretic co-medications, were also tested for interference in the assay by direct injection of standards onto the HPLC column.

2.5.5. Storage stability and freeze–thaw stability

The stability of PQ under storage conditions to which the stock solutions and study samples may be exposed was investigated. Stability of PQ stock stored at 4 °C was determined by comparing UV absorbance at $\lambda = 340$ nm over periods of up to 50 days using UV spectrophotometry (UV–Vis Model 916, GBC Scientific Equipment, Melbourne, Australia).

Stability of PQ in plasma was evaluated at 35 and 120 μg/l. At both concentrations, triplicates were assayed when freshly prepared and again after storage at −20 °C. Samples assayed at day 0, and samples stored at −80 °C and assayed with the test samples after 1, 2, 4 and 6 months, were included to provide reference data. Concentration vs. storage time data were analysed by linear regression, and the difference between the gradient of the fitted line and zero was assessed by *t*-test.

Stability of PQ in the final plasma extracts in the 0.01 *M* KCl/HCl backextraction medium was tested using triplicate plasma samples containing 50 and 500 μg/l PQ. The extracts were kept at room temperature in the autosampler and aliquots were

injected onto the HPLC immediately after preparation, and again after 1, 3, 6 and 8 h.

Stability of PQ in plasma subjected to three freeze–thaw cycles was also tested. Triplicate samples containing 50 and 400 μg/l were assayed and the calculated PQ concentration was compared to control aliquots that were assayed immediately.

2.6. Pilot pharmacokinetic study

A 51-kg adult male patient aged 41 years who had blood slide-positive uncomplicated falciparum malaria received two Artekin-2® tablets (Holleykin Pharmaceuticals, Phnom Penh, Cambodia) orally (each containing 320 mg PQ phosphate and 40 mg DHA) at the start of treatment (0 h), and at 6, 24 and 32 h. This schedule is recommended by the manufacturer and reflects conventional divided dose regimens employed for both the artemisinin derivatives and 4-aminoquinoline drugs including chloroquine. Serial blood samples ($n=17$) were collected at various times up to 28 days after the start of treatment and the sera analysed for their PQ concentrations. Serum samples, rather than the preferred plasma sample matrix were collected in this pilot study.

2.7. Distribution of PQ between red blood cells and plasma or buffer

Venous blood was taken from four healthy volunteer subjects into heparinised sample tubes. Aliquots of the blood (2.5 ml) were spiked with PQ at 50, 100, 200 and 500 μg/l. The remainder was centrifuged at 1300 *g* for 10 min after which the plasma and buffy coat were aspirated. The remaining red blood cells (RBCs) were then washed (×3) by resuspension in an equal volume of isotonic phosphate buffer (K_2HPO_4 1.41 g/l, Na_2HPO_4 0.26 g/l, NaCl 8.1 g/l, pH 7.4), followed by centrifugation as above. Finally, the RBCs were resuspended in buffer to achieve a haematocrit of approximately 50%, and aliquots of the RBC: buffer mixture (2.5 ml) were spiked with PQ at 50, 100, 200 and 500 μg/l. Both the whole blood and RBC–buffer mixtures were then incubated in a waterbath at 37 °C for 30 min to allow PQ to equilibrate between the RBCs and plasma or buffer. At the end of this incubation, the haematocrit of each sample was measured using microhaematocrit tubes centrifuged in a Heraeus Biofuge Haemo (Kendro Laboratory Products, Hanau, Germany). Finally, each sample was centrifuged, and PQ concentration in the plasma or buffer supernatant was measured by HPLC as described above. The partition ratio of PQ between RBCs and plasma or buffer was calculated from the formula:

$$\text{Partition ratio} = \left[\frac{\text{Blood PQ } (\mu\text{g/l})}{\text{Plasma or buffer PQ } (\mu\text{g/l})} - (1 - \text{HCT}) \right] / \text{HCT} \quad (1)$$

Preliminary experiments established that PQ equilibrated between RBCs and plasma or buffer during a 30-min incubation (data not shown).

2.8. Data analysis

One-way repeated measures analysis of variance with Dunnett's test was used to investigate time-related differences in plasma PQ in the frozen storage and freeze–thaw experiments, while the Mann–Whitney rank sum test was used to compare peak area ratios for PQ standard curves constructed with plasma and serum (ANOVA; SIGMASTAT Ver. 2.0, SPSS, Chicago, IL, USA). Linear regression analysis of long-term storage stability results was done using BIOSTAT version 1.01©(Perth, Australia). Compartmental pharmacokinetic analysis of plasma PQ concentration–time data was carried out using Kinetica™ Ver. 4.1 (Innaphase, Philadelphia, PA, USA) to yield estimates of the absorption rate constant, apparent volume of distribution, oral clearance and elimination half-life.

3. Results

3.1. Method development

In the development of our chromatographic assay for PQ, we tried both C_8 and C_{18} reversed-phase columns with a range of mobile phases and pH values. Over the pH range of 3–8, PQ was retained on the column when using ion-pairing with sodium pentanesulfonate with methanol and acetonitrile. An acetonitrile (5–10%, v/v)–buffer system at a pH of

2–3 and a Waters XTerra™ RP_{18} column provided the only conditions under which satisfactory separation was achieved. Peak shape was asymmetrical at a pH of 3 using 100 m*M* KH_2PO_4, but was optimised by using 0.025% trifluoroacetic acid (pH 2.7) together with 0.1% NaCl (w/v) and 0.008% triethylamine (v/v) as modifiers. In developing the extraction method for plasma, dichloromethane gave higher absolute recoveries (~55%) than either chloroform or hexane–isoamyl alcohol (99:1, v/v). The final back extraction into KCl/HCl (pH 2.4) gave cleaner extracts than could be obtained by evaporating the dichloromethane and then reconstituting in mobile phase.

3.2. Validation tests

3.2.1. Specificity

PQ and the internal standard CQ eluted at approximately 5.2 and 11.1 min, respectively. There were no interfering peaks in drug-free plasma or sera at these times or at any other point in the chromatogram (Fig. 2). This was also the case for a range of other drugs likely to be co-administered with PQ (Table 1).

3.2.2. Linearity

For each assay run, a 5-point plasma standard curve was constructed ($r^2 \geq 0.998$) and was linear over the range 5–1000 μg/l. PQ concentrations found in patient samples were infrequently above 500 μg/l and therefore the upper end of the curve was set at this level. Peak area ratios for a standard curve prepared using serum were not significantly different to those for one prepared using plasma (Mann–Whitney rank sum = 37, $P = 0.82$).

3.2.3. Precision and accuracy

These data are summarised in Table 2. The intra-day RSDs were <10% for the concentration range 5–500 μg/l. Inter-day RSDs were <9% at 50–500 μg/l and <21% at 5 μg/l. At the same concentrations, accuracy ranged from 93 to 109% intra-day and from 93 to 106% inter-day.

3.2.4. Limits of quantification and detection

The LOQ for the assay was 5 μg/l (inter-day RSD 20.9%), while the LOD was 3 μg/l.

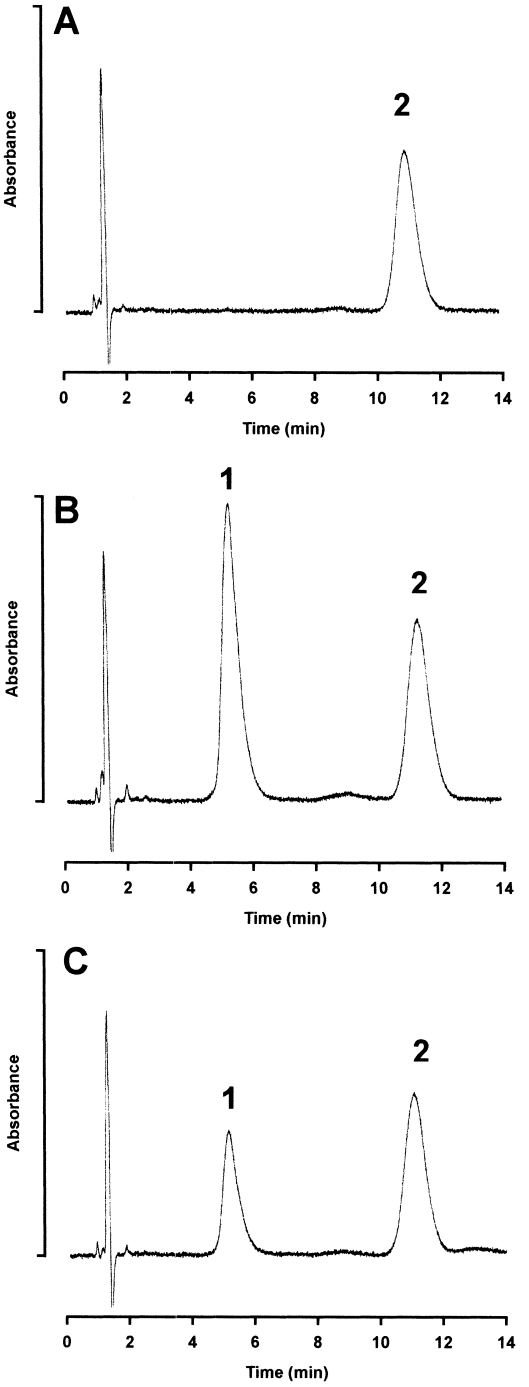

Fig. 2. (A) Chromatogram of an extract of blank plasma spiked with internal standard. (B) Chromatogram of an extract of spiked plasma with PQ concentration 500 μg/l. (C) Chromatogram of a patient serum sample with measured PQ concentration of 206 μg/l; (1) PQ and (2) CQ (internal standard).

Table 1
Retention times of drugs tested for noninterference

Drug	Retention time (min)
Atovaquone	>20
Chloroquine	11.1
Dihydroartemisinin	>20
Doxycycline	>20
Hydroxychloroquine	9.2
7-Hydroxypiperaquine	6.2
Mefloquine	16.6
Paracetamol	2.7
Piperaquine	5.2
Primaquine	4.5, 3.6
Pyrimethamine	>20
Pyronaridine	>20
Quinine	9.2
Sulfadoxine	>20
Tetracycline	15.5

3.2.5. Storage stability

The UV absorbance ($\lambda = 340$ nm) of a working stock solution of PQ (10 ng/μl) was found to be unchanged (0.33 absorbance units) for up to 50 days of dark storage at 4 °C.

There was no significant change in PQ concentration in the back-extraction medium (KCl/HCl buffer). Linear regression of the peak area ratios (PQ/CQ) versus time up to 8 h yielded slopes of 0.001 (95% CI: −0.004 to 0.006) and 0.0044 (95%CI: −0.0041 to 0.013) for extracts from plasma samples containing 50 and 500 μg/l, respectively.

Data for stability of PQ at 50 and 500 μg/l over three freeze–thaw cycles are shown in Fig. 3. At 50 μg/l there was a trend for a decrease in PQ stability but this did not achieve statistical significance ($F = 0.459$, $P = 0.72$). At 500 μg/l, the PQ concentration tended to fall after two cycles ($q' = 1.09$, $P > 0.05$), and there was a significant mean decrease of 21% after 3 cycles ($q' = 3.52$, $P < 0.05$).

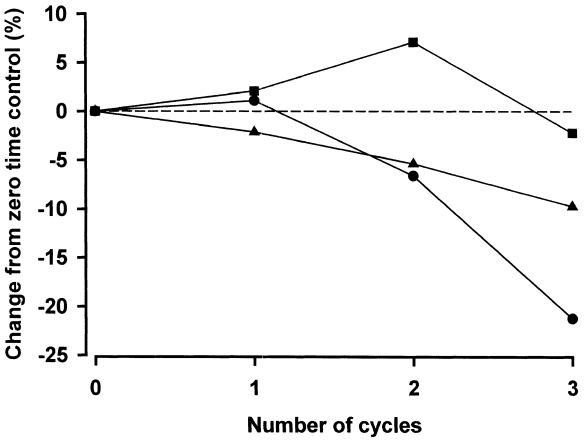

Fig. 3. Freeze–thaw stability of PQ in plasma at 50 μg/l (▲) and 500 μg/l (●). The quality control sample run in parallel with each data set is also shown (■). Mean stability data ($n = 3$) are presented as percent of the zero time control. The dotted line represents no change.

Linear regression analysis of measured concentrations of PQ in plasma samples stored frozen over 6 months showed no significant change in PQ plasma concentrations. At −20 °C, the regression slopes were −1.4 (95% CI = −3.7 to 0.9) at 35 μg/l and 4.6 (95% CI = −4.5 to 13.7) at 120 μg/l. At −80 °C, the regression slopes were −0.5 (95% CI = −3.0 to 1.9) at 35 μg/l and 6.3 (95% CI = −3.1 to 15.6) and at 120 μg/l.

Table 2
Intra- and inter-day relative standard deviation and accuracy for the assay

Concentration (μg/l)	Accuracy (%)	RSD (%)
Intra-day variation ($n = 5$)		
5	109	6.7
20	93	9.9
200	97	7.4
500	101	5.3
Inter-day variation ($n = 5$)		
5	93	20.9
50	94	6.6
500	106	8.3

3.2.6. Preliminary pharmacokinetic profile for PQ in a Cambodian patient with falciparum malaria

PQ concentration–time data showing the application of the assay to the measurement of PQ in serum from a patient with falciparum malaria who received oral treatment with Artekin-2® tablets are presented in Fig. 4. Pharmacokinetic analysis indicated that a two-compartment model with first order absorption and a lag time gave an optimal fit to the concentration–time data. The model descriptors, and other derived parameters were: absorption rate constant = 0.19/h, absorption lag time = 3.1 h, transfer rate constants between compartments 1 and 2

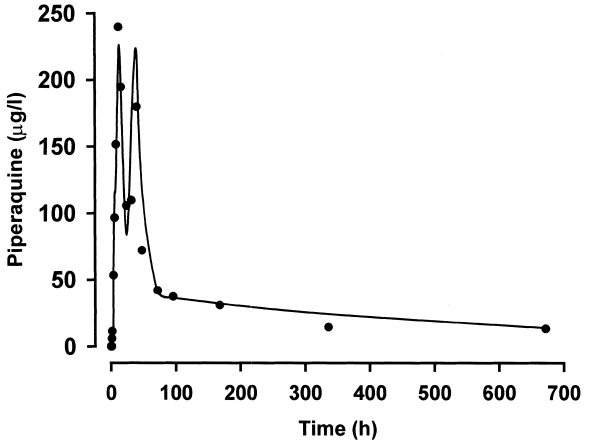

Fig. 4. Plasma PQ concentration–time profile in a Cambodian patient with falciparum malaria who received four doses of 320 mg PQ phosphate and 40 mg DHA orally at 0, 6, 24 and 32 h. The symbols show individual measured concentrations of PQ and the solid line is the computer-generated line of best fit for a 2-compartment open model.

($k_{12}=0.166$/h, $k_{21}=0.008$/h), elimination rate constant (k_{10}) 0.046/h, volume of the central compartment = 20.4 l/kg, steady-state apparent volume of distribution = 447 l/kg, apparent oral clearance = 0.93 l/h/kg and a terminal half-life = 17.3 days.

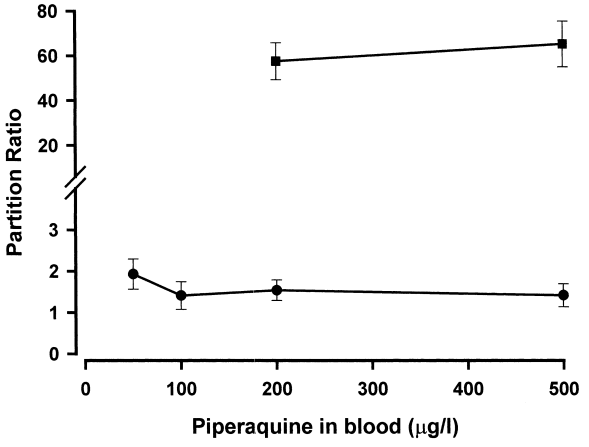

Fig. 5. Mean partition ratios (mean±SD, $n=4$) of PQ between red blood cells and plasma (●) or buffer (■), at total PQ concentrations ranging from 50 to 500 μg/l. PQ in the buffer was close to, or below the assay LOQ in the RBC–buffer samples spiked at 50 and 100 μg/l and hence ratios were not calculated at these two concentrations.

3.3. Distribution of PQ between RBCs and plasma or buffer

The partition ratios over the concentration range 50–500 μg/l in whole blood are shown in Fig. 5. The mean±SD of RBC: plasma partition ratio was 1.5±0.6 over the range 50–500 μg/l at a mean haematocrit of 45.8%. PQ concentration in the buffer was close to, or below the assay LOQ in the RBC–buffer samples spiked at 50 and 100 μg/l and hence distribution ratios were not calculated for these two concentrations. The mean±SD of RBC:buffer partition ratio over the range 200–500 μg/l was 61±18 at a mean haematocrit of 45%.

4. Discussion

Our study is the first to quantify PQ in human plasma during therapeutic use of the drug. The literature contains only two references to the quantification of PQ by HPLC [11,12]. Both were concerned with pharmaceutical quality control of PQ tablets. The authors of the first study could not achieve acceptable chromatography using reversed-phase chromatography on a C_{18} column with either an acetonitrile–water–acetic acid mobile phase or with hexane sulphonic acid ion-pairing in a methanol–water–acetic acid mobile phase [11]. The second study successfully chromatographed PQ using reversed-phase chromatography on a C_{18} column, with an ion-pairing mobile phase of pentane sulfonic acid in methanol–acetonitrile–water–triethylamine (1 ml/min), and UV detection at 240 nm [12].

We have developed a sensitive and specific HPLC–UV method for quantifying PQ in plasma. Although for logistic reasons we prefer plasma as a sample matrix, the method also works well for serum. The method has good linearity, precision and accuracy across the concentration range that is seen after administration of usual therapeutic doses of PQ. Although PQ has a very intense UV absorption at 240 nm, we chose to work at its secondary, less intense absorption peak (340 nm), since interference from other analytes in plasma extracts was minimised at this wavelength. Neither endogenous components in plasma or serum, nor drugs likely to be

co-administered with PQ interfered in the chromatogram.

The stability of PQ in the final acid extract was demonstrated over a period of 8 h, as was stability at relevant therapeutic concentrations in plasma stored at both −20 °C and −80 °C for up to 6 months. The plasma PQ concentration tended to decrease with repeated freeze–thaw cycles. Our data suggest that repeated freezing and thawing should be avoided as the loss of analyte may be significant if more than two cycles occur. An understanding of PQ storage stability in plasma is particularly important for pharmacokinetic studies of the drug in the treatment of malaria. On most occasions, sera or plasma are collected in the remote rural tropics, frozen and subsequently transported to a central laboratory for analysis.

Both PQ and its chemically synthesized analog 7-hydroxy PQ have been shown to be effective antimalarial agents [1]. However, there are no studies to indicate whether 7-hydroxy PQ is a metabolite of PQ in vivo. Hydroxylation of the aliphatic chain joining the two quinoline rings of PQ by cytochrome P-450 is theoretically possible, but access to the active site of the enzyme may be sterically hindered by the bulky ring structures. To date, we have analysed multiple serum/plasma samples ($n=4$–14) from each of the 14 adults and 17 children treated with PQ and have not seen any peak at the retention time for 7-hydroxy PQ (data not shown). The LOD for 7-hydroxy PQ in our HPLC system is around 20 μg/l and hence we conclude that either it is an insignificant metabolite of PQ in vivo, or that if formed, it is rapidly cleared via a conjugation pathway.

Since the RBCs are the site of action of antimalarial drugs and are known to concentrate several drugs including CQ, quinine and mefloquine [13,14], we investigated the partitioning of PQ into RBCs. The RBC:buffer ratio of 61 indicates that PQ partitions strongly into RBC. However, the extent of PQ accumulation in RBCs is close to unity in the presence of plasma. Hence we infer that only unbound PQ is able to partition into RBCs and, using our data and Eq. (1) above, that the protein binding of PQ in plasma is 97%. This pattern of distribution in whole blood is also seen with mefloquine, which accumulates extensively in erythrocytes but is almost 98% bound to plasma proteins, resulting in a blood to plasma ratio of 1.1 [15]. Moreover, since the RBC:plasma ratio was similar at all concentrations tested, it can also be deduced that plasma protein binding of PQ is independent of concentration across the 50–500 μg/l range in whole blood.

Further clinical application of our method is shown by the pharmacokinetic analysis of serum PQ concentrations in a patient who received Artekin-2® for uncomplicated falciparum malaria. As might be anticipated for a basic drug ($pK_a=8.5$) with high lipid solubility ($\log P=6.157$) [16], the steady-state apparent volume of distribution of PQ was very large (447 l/kg) and the terminal elimination half-life was long (17.3 days). This latter measure should be interpreted with caution since samples were taken over a period equivalent to only 1.5 times its value. Nevertheless, the derived pharmacokinetic parameters, and efficacy data from other reports [1,2,10,17,18] suggest that, when used together with DHA, PQ should provide highly effective combination therapy for acute malaria infection.

Our preliminary pharmacokinetic data also indicate that usual therapeutic doses [10] resulted in plasma concentrations that exceeded the mean in vitro IC_{50} for sensitive strains of *P. falciparum* (26±16 μg/l) [6,19] for more than 7 of 28 days (approximately three times the average parasite life cycle). The in vitro IC_{50} for resistant strains *P. falciparum* are higher (130±36 μg/l) [20–24] and may justify higher PQ doses, depending on tolerability. Nevertheless, the relationship between in vitro IC_{50} values and the therapeutic response is unclear. In the case of mefloquine, another drug with a long half-life, its combination with artesunate ensured efficacy in an area of emerging mefloquine-resistant strains of *P. falciparum* and also improved parasite sensitivity over time [25,26]. Thus, even in areas where significant PQ resistance has been identified by in vitro testing, treatments such as Artekin-2® may still prove valuable.

5. Conclusion

A sensitive and specific assay for PQ in plasma has been developed using liquid–liquid extraction followed by reversed-phase chromatography on a

C_{18} column and detection at 340 nm. The method is free from interferences arising from plasma or likely drug co-therapy. We plan to use the assay in detailed investigations of the pharmacokinetic properties of PQ in plasma from patients with acute malaria.

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References

[1] L. Chen, Chin. Med. J. (Engl.) 104 (1991) 161.
[2] L. Chen, F.Y. Qu, Y.C. Zhou, Chin. Med. J. (Engl.) 95 (1982) 281.
[3] L. Chen, F.Y. Qu, Y.C. Zhou, Med. J. PLA 4 (1979) 104.
[4] K. Raynes, Int. J. Parasitol. 29 (1999) 367.
[5] K. Raynes, M. Foley, L. Tilley, L.W. Deady, Biochem. Pharmacol. 52 (1996) 551.
[6] J.L. Vennerstrom, W.Y. Ellis, A.L. Ager Jr., S.L. Andersen, L. Gerena, W.K. Milhous, J. Med. Chem. 35 (1992) 2129.
[7] P.B. Bloland, M. Ettling, S. Meek, Bull. World Health Org. 78 (2000) 1378.
[8] F. Nosten, P. Brasseur, Drugs 62 (2002) 1315.
[9] Anonymous. Meeting on Antimalarial Drug Development, World Health Organisation Regional Office for the Western Pacific, Manila, Philippines, 2002.
[10] M.B. Denis, T.M.E. Davis, S. Hewitt, S. Incardona, K. Nimol, T. Fandeur, Y. Poravuth, C. Lim, D. Socheat, Clin. Infect. Dis. 35 (2002) 1469.
[11] B. Sunderland, P. Passmore, M. Boddy, in: Meeting on Antimalarial Drug Development. Shanghai, China 16–17 November 2001, World Health Organisation Regional Office for the Western Pacific, Annex 6, Manila, 2002, p. 41.
[12] W.L. Chen, M.J. Chen, H. Wu, H.Y. Tao, Fenxi Ceshi Yiqi Tongxun 5 (1995) 93.
[13] S. Krishna, N.J. White, Clin. Pharmacokinet. 30 (1996) 263.
[14] N.J. White, Clin. Pharmacokinet. 10 (1985) 187.
[15] H. Tajerzadeh, D.J. Cutler, Biopharm. Drug Dispos. 14 (1993) 87.
[16] Chemical Abstracts Service, Piperaquine. SciFinder Scholar, American Chemical Society, Columbus, OH, 1999.
[17] G. Wang, Chin. J. Inf. Dis. 3 (1985) 78.
[18] X.B. Guo. Zhonghua Yi Xue Za Zhi 73 (1993) 602, 638.
[19] W.B. Guan, W.J. Huang, Y.C. Zhou, W.Q. Pan, Chin. J. Parasitol. Parasit. Dis. 1 (1983) 88.
[20] H. Yang, D. Liu, K. Huang, Y. Yang, P. Yang, M. Liao, C. Zhang, Chin. J. Parasitol. Parasit. Dis. 17 (1999) 43.
[21] B. Fan, W. Zhao, X. Ma, Z. Huang, Y. Wen, J. Yang, Z. Yang, Chin. J. Parasitol. Parasit. Dis. (1998) 460.
[22] H. Yang, D. Liu, Y. Dong, P. Yang, R. Liu, B. Zhang, C. Zhang, Chin. J. Parasitol. Parasit. Dis. 13 (1995) 111.
[23] H.L. Yang, P.F. Yang, D.Q. Liu, R.J. Liu, Y. Dong, C.Y. Zhang, D.Q. Cao, H. He, Chin. J. Parasitol. Parasit. Dis. 10 (1993) 198.
[24] K.Y. Zhang, J.X. Zhou, Z. Wu, Q.L. Huang, Chin. J. Parasitol. Parasit. Dis. 5 (1987) 165.
[25] R.N. Price, F. Nosten, C. Luxemburger, F.O. ter Kuile, L. Paiphun, T. Chongsuphajaisiddhi, N.J. White, Lancet 347 (1996) 1654.
[26] R.N. Price, F. Nosten, C. Luxemburger, M. van Vugt, L. Phaipun, T. Chongsuphajaisiddhi, N.J. White, Trans. R. Soc. Trop. Med Hyg. 91 (1997) 574.