—Regular Articles—

Pharmacokinetics of Piperaquine after Single and Multiple Oral Administrations in Healthy Volunteers

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The aim of this work was to study the pharmacokinetics of piperaquine in healthy volunteers. Healthy volunteers received piperaquine and tablets of Artekin by oral administration. The plasma samples were analyzed for piperaquine by liquid-liquid extraction and determined by HPLC-UV. The results demonstrated that the plasma drug concentration-time curves of single and multiple dose of piperaquine were fitted to a two-compartment open model. The pharmacokinetics parameters of piperaquine alone in a single dose were: $t_{1/2(\beta)}=(317.2-/+126.6)$ h, $AUC_{0\to\infty}=(44293-/+12636)$ h×ng/ml, $V_d=(9490.9-/+2161.9)$ ml/kg, and $Cl=(22.83-/+9.83)$ ml/h/kg. In Artekin in a single dose these parameters were: $t_{1/2(\beta)}=(302.8-/+180.7)$ h, $AUC_{0\to\infty}=(46419-/+13670)$ h×ng/ml, $V_d=(10188.6-/+3520.3)$ ml/kg, and $Cl=(25.48-/+10.89)$ ml/h/kg, while in Artekin in multiple doses they were: $t_{1/2(\beta)}=(298.9-/+101.9)$ h, $AUC_{0\to\infty}=(227692-/+56294)$ h×ng/ml, $V_d=(5031.5-/+1097.8)$ ml/kg, $Cl=(11.91-/+3.046)$ ml/h/kg, respectively. The absorption and distribution of piperaquine were quick while the elimination was quite slow. There were significant differences in the pharmacokinetics parameters of piperaquine in Artekin between a single dose and multiple doses ($p<0.001$), suggesting that piperaquine might accumulate in vivo and that attention should be given to its possible adverse drug reactions in clinical treatment.

Key words——piperaquine; pharmacokinetics; HPLC-UV

INTRODUCTION

Malaria is a common and a life-threatening disease in many tropical and subtropical areas. At present it is the developing world's most dreaded killer, accouting for the deaths of over two million people per year according to estimates by the World Health Organization.[1)]

Piperaquine {1,3-bis [1-(7-chloro-4′-quinolyl)-4′-piperazinyl]; PQ} is an anti-malaria drug which was synthesized in the 1960s. In recent years, PQ and many combination anti-malarial products containing PQ such as Artekin® (dihydroartemisinin and PQ phosphate) and CV8® (dihydroartemisinin, PQ phosphate, trimethoprim and primaquine), have also been used effectively to prevent and treat drug-resistant Plasmodium falciparum.[5–7)]

Although PQ has been in clinical use for several decades, the information about its pharmacokinetics is scarce. Chen *et al.*[8)] demonstrated that the half-life of PQ was about 9 days in rats. Roshammar *et al.*[9)] reported that the pharmacokinetics of PQ after repeated oral administration of CV8 in healthy subjects were described by a two-compartment disposition model with a terminal half-life of 11.7 days, and Hung *et al.*[10)] pointed out that the population pharmacokinetics of Artekin in adults and children with malaria were described by a two-compartment disposition model, and the half-life was 23 days in adults and 14 days in children. However, those previous studies did not research the pharmacokinetics' differences between PQ and PQ in the combination anti-malarial drugs nor the differences between male and female. We therefore set out to investigate these differences and develop a safe and rational dose regimen.

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EXPERIMENTALS

1. Chemicals and Reagents PQ and the internal standard (chliroquine, CQ) were obtained from Guangzhou University of Traditional Chinese Medicine (Guangzhou, China). Acetonitrile (HPLC grade) was purchased from Merck (Darmstadt, Germany) and all other chemicals and solvents were of

analytical grade.

2. Instrumentation and Chromatographic Conditions The HPLC system used in the assay consisted of a Waters 515 pump and Waters 486 UV detector (Milford, USA). Data acquisition was obtained from Mellinium[32] Chromatography Station (USA). Separations were achieved on an Alltima C_{18} column (5-μm, 4.6×100 mm) with a 5-μm SecurityGuard C_{18} short guard column (Grace, USA). The mobile phase was acetonitrile–0.1% trichloroacetic acid solution (w/v)–phosphate acid (25:75:0.035, v/v/v) with a flow rate of 0.4 ml/min and UV detected at 345 nm.

3. Sample Preparation The volume of a 500 μl plasma sample was pipetted into a 10 ml polypropylene tube and 100 ng CQ added as an internal standard. Two-tenths ml of 1 M NaOH was added followed by 30 s of vortex mixing. PQ was then extracted with 5 ml of diethyl-ether by shaking vigorously for 10 min. After centrifugation at 6000×g for 5 min, the organic (upper) phase was transferred into a clean tube and dried under a stream of nitrogen in a 37°C water bath. The residue was reconstituted in 100 μl of mobile phase and 20 μl was injected into the LC system.

4. Subjects Sixteen healthy Chinese volunteers (eight males and eight females) were divided into 2 groups. The characteristics of the male volunteers were: mean age 28.8 years (SD=2.9), mean weight 66.2 kg (SD=3.1); the characteristics of the female volunteers were: mean age 26.6 years (SD=3.1), mean weight 53.2 kg (SD=6.6). None volunteers had smoked or drunk alcohol or coffee or abused other drugs before or during the study, and informed consent was obtained. This project was approved by the local hospital ethics committee for study.

4-1. Single Dose

4-1-1. PQ after Oral Administration All the healthy volunteers received 640 mg of PQ phosphate, the same quantity as in a tablet of Artekin, at 8 a.m with a glass of water (250 ml) and, 2 hours later, ate a standard meal. Venous blood samples (5 ml) were collected pre-dose and at 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 168, 336, 504 and 840 h. Samples were placed in heparinized vacutainer tubes and centrifuged at 5000×g for 15 min and the plasma aliquots were transferred to polypropylene tubes and frozen at −37°C until analysis.

4-1-2. PQ in Artekin after Oral Administration Healthy volunteers completely washed after 90 days and then received two tablets of Artekin (each containing 320 mg of PQ phosphate and 40 mg of DHA) at 8 a.m. The procedures were then the same as "**PQ after Oral Administration**".

4-2. Multiple Doses Eight healthy Chinese volunteers received two Artekin tablets four times orally at the start and 6, 24 and 32 h. This schedule was recommended by the Institute of Tropical Diseases in Guangzhou University of Chinese Medicine. Venous blood samples (5 ml) were collected pre-dose and at 0.25, 0.5, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0, 6.0, 6.5, 7.0, 7.25, 8.0, 8.5, 24, 24.5, 25, 25.25, 25.5, 26, 26.5, 28, 30, 32, 32.5, 33, 33.25, 33.5, 34, 168, 336, 504, 840 and 1008 h. Samples were placed in heparinized vacutainer tubes and centrifuged at 5000×g for 15 min and the plasma aliquots were transferred to the polypropylene tubes and frozen at −37°C until analysis.

5. Data Analysis The results were expressed as $\bar{\chi} \pm s$. All data were statistically analyzed by paired t test. Compartmental pharmacokinetic analysis of plasma PQ concentration vs. time data was carried out using PKsolution2 (USA) to estimate the absorption rate constant, apparent volume of distribution, oral clearance, elimination half-life and other pharmacokinetic parameters.

RESULTS

1. Specificity PQ and the internal standard CQ eluted at 11.0 and 12.9 min, respectively. There were no interfering peaks in the chromatogram (Fig. 1).

2. Linearity, Limits of Quantification (LOQ) and Limits of Detection (LOD) There was a good linearity over the range of 20–1000 ng/ml in the plasma and the calibration curve was $Y=0.0254+5.1376x$ with a correlation coefficient of 0.9993. The LOQ for the assay was 20 ng/ml and the LOD was 10 ng/ml.

3. Precision and Accuracy The data of precision and accuracy are summarized in Table 1. The intra-day relative standard deviation (RSDs) and the inter-day RSDs at the concentration of 20, 200, 1000 ng/ml were less than 12.0% and 11.0%, respectively. The intra-day accuracy ranged from 93.4–106.5% and the inter-day accuracy ranged from 94.8–103.5%, respectively.

4. The Pharmacokinetics of PQ The phar-

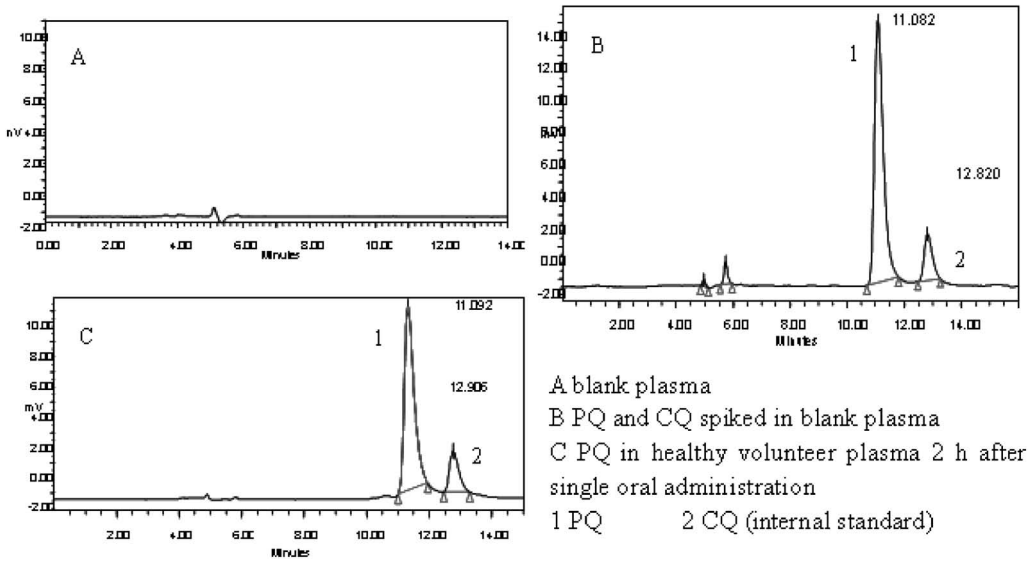

Fig. 1. HPLC Chromatograms of Piperaquine in Human Plasma

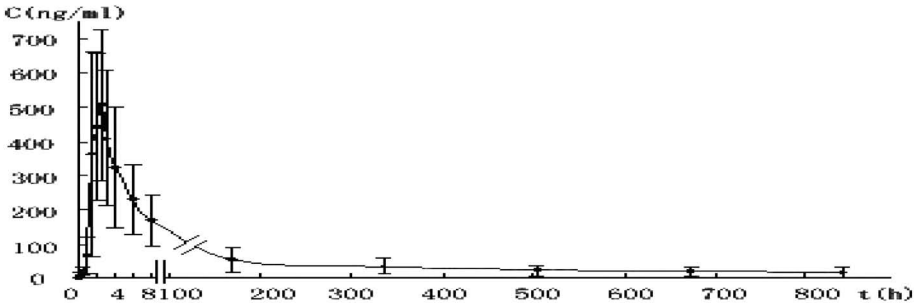

Fig. 2. Mean Plasma Concentration-time Curve of PQ Phosphate in 640 mg after Single Oral Administration in Healthy Volunteers

Table 1. The Precision and Accuracy of the Plasma Concentration for the Assay

Concentration (ng/ml)	Accuracy (%)		RSD (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
20	106.5	103.5	10.8	11.0
200	104.9	102.8	2.4	10.6
1000	93.4	94.8	1.1	2.6

macokinetic property of PQ after single and multiple oral administrations could be described by a two-compartment model. The curves of plasma concentration-time are shown in Figures 2–4 and the main pharmacokinetic parameters of PQ after single and multiple doses of oral administration in healthy volunteers in Tables 2–4.

DISCUSSION

In this study, we found that a two-compartment

Table 2. The Pharmacokinetic Parameters of PQ after Single Dose Administration in Healthy Volunteers

Parameter	Single dose	
	PQ (n=8)	Artekin (n=8)
β (1/h)	0.00240±0.00064	0.003201±0.00166
$t_{1/2(\beta)}$ (h)	317.2±126.6	302.8±180.7
α (1/h)	0.3713±0.30287	0.3562±0.2204
$t_{1/2(\alpha)}$ (h)	3.2402±2.2052	3.0381±2.1387
K_a (1/h)	0.5208±0.3359	0.4744±0.2882
$t_{1/2(abs)}$ (h)	1.9456±1.2509	1.8857±0.8939
$AUC_{0\to\infty}$ (h×ng/ml)	44293±12636	46419±13670
C_{max} (ng/ml)	578.47±242.91	586.93±265.74
T_{max} (h)	2.3±0.5	2.1±0.6
Cl (ml/h/kg)	22.83±9.83	25.48±10.89
V_d (mL/kg)	9490.9±2161.9	10188.6±3520.3
K_{12} (1/h)	0.2993±0.2850	0.2584±0.2165
K_{21} (1/h)	0.0351±0.0309	0.05172±0.0382
K_{10} (1/h)	0.0462±0.0397	0.0523±0.0356

* $p>0.05$

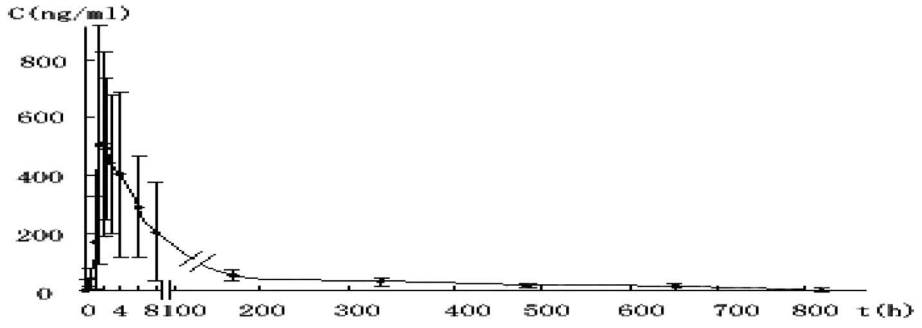

Fig. 3. Mean Plasma Concentration-time Curve of PQ in Two Artekin Tablets after Single Oral Administration in Healthy Volunteers

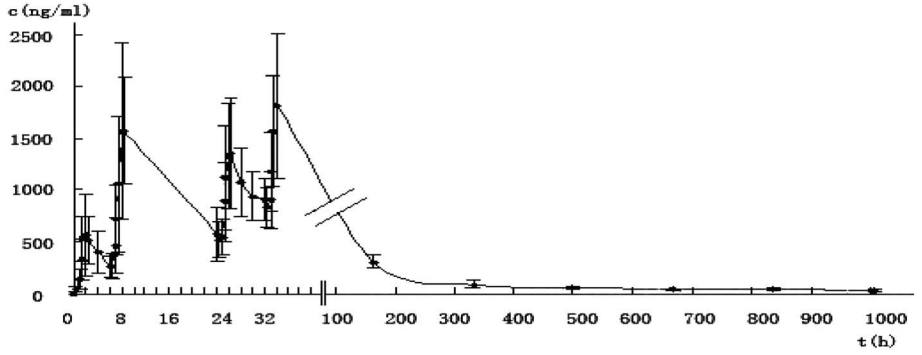

Fig. 4. Mean Plasma Concentration-time Curve of PQ Phosphate in Two Artekin Tablets after Multiple Oral Administrations in Healthy Volunteers

Table 3. Pharmacokinetics of PQ in Artekin in Healthy Volunteers

Parameter	Artekin	
	Single dose (n=8)	Multiple doses (n=8)
β (1/h)	0.003201±0.00166	0.002614±0.00089
$t_{1/2(\beta)}$ (h)	302.8±180.7	298.9±101.9*
α (1/h)	0.3562±0.2204	0.5968±03.619*
$t_{1/2(\alpha)}$ (h)	3.0381±2.1387	1.6035±0.9857*
K_a (1/h)	0.4744±0.2882	0.6837±0.2961*
$t_{1/2(abs)}$ (h)	1.8857±0.8939	1.2284±0.5618*
$AUC_{0\to\infty}$ (h×ng/ml)	46419±13670	227692±56294*
C_{max} (ng/ml)	586.93±265.74	2116.4±732.88*
T_{max} (h)	2.1±0.6	30.3±2.8*
Cl (ml/h/kg)	25.48±10.89	11.91±3.046*
V_d (mL/kg)	10188.6±3520.3	5031.5±1097.8*
K_{12} (1/h)	0.2584±0.2165	0.4512±0.2501*
K_{21} (1/h)	0.05172±0.0382	0.1318±0.1358*
K_{10} (1/h)	0.0523±0.0356	0.0173±0.0108*

* $p<0.001$

Table 4. Pharmacokinetics of PQ after Single Dose Administration of Artekin in Male and Female Healthy Volunteers

Parameter	Female (n=8)	Male (n=8)
β (1/h)	0.00338±0.00101	0.002963±0.002368
$t_{1/2(\beta)}$ (h)	223.9±79.9	356.2±222.2
α (1/h)	0.3276±0.2254	0.3945±0.2283
$t_{1/2(\alpha)}$ (h)	3.4175±2.4902	2.2233±1.1357
K_a (1/h)	0.4576±0.2207	0.4338±0.2115
$t_{1/2(abs)}$ (h)	1.9325±1.1116	1.8233±0.5829
$AUC_{0\to\infty}$ (h×ng/ml)	45721±16675	24017±7730
C_{max} (ng/ml)	726.6±405.3	564.8±268.3
T_{max} (h)	1.9±0.4	2.5±0.6
Cl (ml/h/kg)	19.68±12.74	33.28±17.58
V_d (mL/kg)	6762.5±1765.4	14757±6713
K_{12} (1/h)	0.2093±0.2132	0.3241±0.2217
K_{21} (1/h)	0.0749±0.1419	0.0208±0.0147
K_{10} (1/h)	0.0474±0.0446	0.0535±0.0221

open model could give an optimal fit to PQ drug concentration-time data. The half-life of PQ was 12.5 days, and its absorption and distribution were quick while elimination was quite slow. It was suggested that PQ was absorbed easily *in vivo* due to its strong lipophilic chemicals.[10] We also found multiple peak profiles observed in Fig. 4 in our study. Roshammar

et al.[9] believed this phenomenon was caused by gastric emptying.

Comparing the pharmacokinetic parameters of PQ with that of Artekin, we found that there were no significant differences in single dose ($p>0.05$). This indicated that DHA in Artekin did not affect or even change PQ's absorption, distribution and elimination inside a human when used clinically. However, there were quite significant differences in the pharmacokinetic parameters of PQ in Artekin between single dose and multiple doses ($p<0.001$). As is clearly shown by Fig. 3, the value of C_{max} of multiple doses was four times larger than that of a single dose, but the value of V_d and Cl of multiple doses were less than these of a single dose. This phenomenon suggested that PQ might accumulate in the human body and that attention should be given to its potential adverse drug reactions in clinical treatment.

Some previous studies reported[11] that the pharmacokinetic parameters of PQ not only differenced in healthy volunteers and patients but also in rats.[8] So the values found in this study were not similar to those obtained from some previous studies.[9-10]

Also some studies pointed out[11] that the pharmacokinetic parameters of many kinds of the drug were influenced by human gender.[11] To develop the rational dose regimen of PQ, this study investigated its pharmacokinetic property in male and female. Because there was a difference in the mean body weight in the male and female volunteers in this project ($p<0.05$), in order to evaluate the difference of pharmacokinetic parameters related to gender, the factor of body weight had to be calculated. Therefore, all the pharmacokinetic parameters were corrected by the body weight (W) and dose (D). The correction coefficient (f) was calculated by D/W and the values of correction were obtained as follows:

$$\beta^* = f \times \beta,$$
$$t^*_{1/2}(\beta) = f \times t_{1/2(\beta)}$$
$$\alpha^* = f \times \alpha,$$
$$t^*_{1/2(\alpha)} = f \times t_{1/2\alpha)},$$
$$AUC^*_{0\to\infty} = f \times AUC_{0\to\infty},$$
$$C^*_{max} = f \times C_{max},$$
$$T^*_{max} = f \times T_{max},$$
$$Cl^* = f \times Cl,$$
$$V^*_d = f \times V_d.$$

All the values of correction in Table 4 were obtained from the D/W of female and male and analyzed by single-factor analysis of variance (ANOVA). The results are shown in Table 5.

Table 5. Analysis of Variance (ANOVA) of Pharmacokinetic Parameters

Parameters	p value
β^*	<0.05
$t^*_{1/2(\beta)}$	<0.05
α^*	<0.05
$t^*_{1/2(\alpha)}$	<0.05
$AUC^*_{0\to\infty}$	<0.05
C^*_{max}	<0.05
T^*_{max}	<0.05
Cl^*	<0.05
V^*_d	<0.05

The results demonstrated that there were distinct discriminations in the values of correction of pharmacokinetic parameters depending on gender. Namely, there was obvious difference in the parameters of PQ after administration of a single dose of Artekin in male and female healthy volunteers.

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REFERENCES

1) Zucker J. R., Campbell C. C., *Infect Dis Clin North Am.*, **7**, 547–566 (1993).
2) Chen L., Qu F. Y., Zhou Y. C., *Chin. Med.*, **95**, 281–286 (1982).
3) White N. J., *Parassitologia.*, **41**, 310–308 (1999).
4) White N. J., Olliaro P. L., *Parasitol. Today*, **12**, 399–401 (1996).
5) Raynes K., Forley M., L Tilley., *Biochem. Pharmacol.*, **52**, 551–559 (1996).
6) Vennerstrom J. L., Ellis W. Y., Ager A. L., *Med Chem.*, **35**, 2129–34 (1992).
7) Sunderland B., Passmore P., Boddy M., 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.
8) Chen Q., Deng J., Wu D., *Pharm. Ind.*, **8**, 19–23 (1979).
9) Roshammar D., Hai T. N., Ashton M., *Eur.*

J. Chin. Pharmacol., **62**, 335–341 (2006).
10) Hung T. Y., Davis T. M., Ilett K. F., *Br. J. Clin. Pharmacol.*, **57**, 253–262 (2004).
11) Deng M., Liu H. C., *The Chinese Journal of Clinical Pharmacology*, **20**, 67–71 (2004).