Arch Pharm Res Vol 27, No 5, 576-580, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Pharmacokinetics of Primaquine and Carboxyprimaquine in Korean Patients with Vivax Malaria

Yang-Ree Kim*, Hyo-Jeong Kuh[1]*, Mi-Young Kim, Yo-Sook Kim[2], Woo-Chul Chung, Sang-Il Kim, and Moon-Won Kang

Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, 137-040, Korea, [1]Research Institute of New Drug Development, and [2]Infectious Disease Section, Catholic Research Institutes of Medical Science, The Catholic University of Korea, Seoul, 137-040, Korea

(Received February 24, 2004)

Primaquine is used for relapses caused by vivax malaria hypnozoites. No studies on the pharmacokinetics of primaquine (PMQ) has been reported in Korean patients. In our study, thirty vivax malaria patients were given 15 mg primaquine daily for 14 days after 3 days of chloroquine treatment. Plasma samples were taken at intervals after each daily dose of PMQ for 3 days. Plasma concentrations of PMQ and carboxyprimaquine (CPMQ), the major metabolite of primaquine, were measured by HPLC. The PMQ concentrations reached a maximum of 0.28±0.18 μg/mL at 1.5 h after the first dose. The maximum concentration of CPMQ was 0.32±0.13 μg/mL at 4 h. Higher drug concentrations with repeated dosing were observed for CPMQ, but not for the parent drug, PMQ. The elimination half-life was 3.76±1.8 h and 15.7±12.2 h, for PMQ and CPMQ, respectively. Large variation in the plasma concentrations of both drugs was observed. Overall, PMQ is absorbed and metabolized rapidly after oral administration. It was noted that the mean peak plasma concentration of PMQ was significantly higher and that of CPMQ was lower in our patients compared to other studies. This suggests a potential difference of inter-ethnic groups, which warrants further investigations.

Key words: HPLC, Pharmacokinetics, Primaquine, Carboxyprimaquine, Malaria, Ethnic difference

INTRODUCTION

It was considered that the malaria was eradicated in Korea between the later half of 1970s and the earlier half of 1980s. But July 1993, tertian malaria caused by *Plasmodium vivax* was identified in a soldier from Paju (Chai *et al.*, 1994). This marked the beginning of an increase in the number of malaria cases in Korea and lasted until the year 2000 (Anonymous, 2001), after then the number of cases have been declining steadily.

The standard treatment regimen for *Plasmodium vivax* consists of 3 days of chloroquine for blood schizonts followed by 14 days of primaquine (PMQ) administration for hypnozoites. Generally there have been no problems with the standard treatment, but several studies have reported problems (Kimura *et al.*, 1996; Pukrittayakamee *et al.*, 1994; Rieckmann *et al.*, 1993), suggesting that the dosage should be increased in some cases such as Chesson strain infection or relapse (Bunnag *et al.*, 1994; Kimura *et al.*, 1996). There were also reports that treatment dosages of PMQ should be modified according to the strain and region of origin of malaria protozoan (Clyde and McCarthy, 1977). It was reported in Korea that a malaria patient of returning from South America had multiple relapses and was cured by extended administration of PMQ at a higher dosage (Kim *et al.*, 1997).

Several studies evaluated the pharmacokinetics of PMQ (Bhatia *et al.*, 1986; Dua *et al.*, 1996; Ward *et al.*, 1985, Fletcher *et al.* 1981). A wide range of peak plasama concentrations were reported among those studies and the different levels were observed between different ethnic groups (Fletcher *et al.* 1981).

In the present study, we evaluated the pharmacokinetics of PMQ and its main metabolite, carboxyprimaquine

*Equal contribution made by these authors.
Correspondence to: Moon-Won Kang, M.D., Department of Internal Medicine, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Seocho-ku, Seoul 137-040, Korea
Tel: 82-2-590-1402, Fax: 82-2-590-1409
E-mail: infect@catholic.ac.kr

(CPMQ), in Korean patients infected with *Plasmodium vivax*. No other similar studies have been conducted in Korean patients.

MATERIALS AND METHODS

Pharmacokinetic study

The patients were all males aged 21-24 years from the ROK Army in northwestern Kyounggi-do, Korea. *Plasmodium vivax* malaria infection was confirmed by examination of peripheral blood smear. They were treated with total 1,500 mg (base) chloroquine for 3 days, and then with 15 mg PMQ daily for 14 days under the standard treatment regimen. A series of 10 mL blood samples were collected at 1, 1.5, 2, 2.5, 3, 4, 12, and 24 h after the first dose of PMQ. On the second and third days, blood was collected at 3 h after administration of the drug. The samples were centrifuged at 1,000 g for 10 min, and the plasma was stored at -70 °C until analysis. This study was conducted after obtaining protocol approval from the institutional review board of Kangnam St. Mary's hospital.

Chemicals

PMQ was purchased from Sigma Co. (St. Louis, MO, USA), and the internal standard 2-methoxy-5-methylalanine (MMA) from Fluka Co. (St. Louis, MO, USA). CPMQ was a generous gift from Dr. McChesney (University Mississippi, MS, USA). PMQ and MMA were dissolved in acetonitrile (HPLC grade, Fisher Scientific, Pittsburgh, PA, USA), and CPMQ was dissolved in acetonitrile/distilled water (50:50 v/v). The solutions were kept in the dark at 4 °C.

Sample preparation

The extraction process was done according to the method previously reported (Endoh *et al.*, 1992). Five hundred μL of plasma was put in a polypropylene centrifuge tube to which was added 0.5 mL of acetonitrile containing 0.2 μg/mL of MMA. The tube was vortexed for 2 min and stored at room temperature for 15 min.

After centrifugation at 1,200 g for 15 min, supernatant was filtered through a 0.45 μm Acrodisc 13-CR membrane (Gelman Science Japan, Tokyo, Japan), and concentrated by using Speed Vac (Savant Instruments, Inc. Farmingdale, NY, USA) at 43 °C for 1.5 h. Twenty μL of the concentrated supernatant was injected into HPLC.

HPLC instrumentation and conditions

The HPLC analysis system was composed of a Gilson 712 HPLC system, a 306 piston pump, an 811C dynamic mixer, a 402 syringe pump, an Asted XL sample processor (Gilson S.A., Villiers-le-Bel, France), and an amperometric detector ICA-5252 (TOA Electronics Ltd., Tokyo, Japan). The Shodex ODSpak F-511 C_{18} reversed-phase column (5 μm, 4.6×250 mm I.D. Shoko Co., Ltd., Tokyo, Japan) was maintained at 40 °C using a temperature regulator (Gilson S.A., Villiers-le-Bel, France). The mobile phase was a mixture of 70 mM phosphate buffer (pH 5.8), 10 mg/mL Na_2EDTA, and acetonitrile (74.5:25.5, v/v). Chromatographic separation was monitored at a flow rate of 1 mL/min.

Calibration and validation

Standard samples were prepared at final concentrations of 0.005, 0.01, 0.02, 0.05, 0.1, 0.5 μg/mL PMQ and 0.01, 0.05, 0.1, 0.5, 1, 2 μg/mL CPMQ. Unipoint (Gilson) was used to construct standard curves using the peak height ratio mode and a weighed regression of 1/y. Calibration curves were constructed for each run.

Recovery rates and variability

Recovery rate was evaluated at the concentrations of 0.1, 0.5, and 1 μg/mL of PMQ and CPMQ (n=3). Intra-run variability were determined at the concentrations of 0.05, 0.1 μg/mL of PMQ and 0.1, 0.5, 1 μg/mL of CPMQ (n=5). The coefficient of variation (CV) was determined and expressed as a percentage.

Pharmacokinetic analysis

Using one-compartment model analysis of the PKCALC program, we determined elimination rate constant (Ke), half life ($T_{1/2}$), area under the curve ($AUC_{0\rightarrow\infty}$), plasma clearance, and the volume of distribution. The plasma clearance was calculated by assuming bioavailability as 0.96 as reported by Mihaly *et al.* (1985). Paired *t*-test was used for statistical comparison and $p < 0.05$ was considered significant.

RESULTS

Assay validation

The chromatogram showed good separation for PMQ, CPMQ and MMA from patient's plasma. No significant interferences was observed and the peak retention times were 16 min for CPMQ, 21 min for MMA, and 29 min for PMQ.

Calibration curves were obtained for 0.005~0.5 μg/mL of PMQ and 0.01~2 μg/mL of CPMQ with the correlation coefficient 0.99 for both. The recovery rates of PMQ and CPMQ at 0.1, 0.5, 1 μg/mL in plasma were 81.6~92.4% and 86.8~99.0%, respectively. The intra-run variability showed CV of 1.5~3.7% and 1.2~5.5% for PMQ and CPMQ, respectively.

Pharmacokinetics of PMQ and CPMQ

The concentration of PMQ in plasma increased rapidly after drug administration, reaching Cmax of 0.282 ± 0.177 μg/mL at 1.5 h, then decreased to 0.044 ±0 .026 μg/mL at

12 h. On the second and third days, the concentrations were measured at 3 h after the administration of PMQ were 0.156 ± 0.077 μg/mL and 0.162 ±0 .116 μg/mL, respectively (Table I). These concentrations were significantly lower than that of the first day (0.235 ± 0.153 μg/mL, $p<0.05$), hence, no accumulation was noted for PMQ. These data indicate that PMQ was rapidly absorbed and extensively metabolized after oral administration.

The plasma concentration of CPMQ reached a maximum of 0.319 ±0 .126 μg/mL at 4 h after administration of PMQ, and decreased slowly until 24 h. On the second day, the concentration of CPMQ at 3 h post-dosage increased far greater than the peak concentration of the first day ($p < 0.05$), but no further increase was observed in the third day (Fig. 1 & Table I), indicating the accumulation of CPMQ by the second time dosing, but not by the third dosing. Large variation in the plasma concentrations of both drugs was observed in these patients.

Table I. Mean (±SD) plasma concentrations (μg/mL) of primaquine and carboxyprimaquine (n=30)

Time (h)	Primaquine	Carboxyprimaquine
1st day		
1	0.232±0.193	0.198±0.114
1.5	0.282±0.177	0.251±0.110
2	0.272±0.142	0.280±0.113
2.5	0.264±0.139	0.278±0.092
3	0.235±0.153	0.281±0.108
4	0.191±0.110	0.319±0.126
12	0.044±0.026	0.304±0.097
24	0.048±0.033	0.144±0.098
2nd day		
3	0.156±0.077*	0.438±0.189**
3rd day		
3	0.162±0.116*	0.447±0.148**

* $p<0.05$, compared to the concentration of PMQ at 3 h of day 1
** $p<0.05$, compared to the concentration of CPMQ at 3 h of day 1

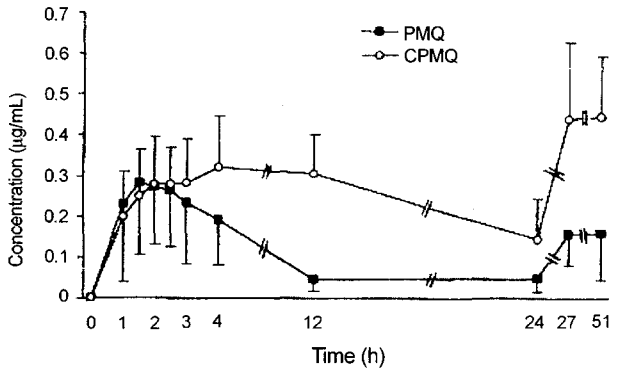

Fig. 1. Plasma concentrations of primaquine (PMQ) and carboxyprimaquine (CPMQ) versus time following oral administration of 15 mg primaquine (n=30)

Table II. Pharmacokinetic parameters of primaquine and carboxyprimaquine in plasma

Pharmacokinetic Parameters	Primaquine	Carboxyprimaquine
Ke (h^{-1})	0.23± 0.11	0.06± 0.03
$T_{1/2}$ (h)	3.76± 1.80	15.7 ±12.2
$AUC_{0\to\infty}$ (μg·h/mL)	1.97± 1.36	9.05± 4.98
Plasma Clearance (L/h)	9.85± 4.60	1.95± 0.93
Vd (L)	46.7 ±19.7	36.6 ±15.7

Table II showed the pharmacokinetic parameters for PMQ and CPMQ in these patients. The elimination half-life of PMQ in plasma was 3.76 ± 1.80 h and that of CPMQ was 15.7 ± 12.2 h. The volume of distribution of PMQ and CPMQ were 46.7 ± 19.7 L and 36.6 ± 15.7 L, respectively. The area under the curve in plasma was 1.97 ± 1.36 μg·h/mL for PMQ and 9.05±4.98 μg·h/mL for CPMQ.

DISCUSSION

PMQ was introduced for the treatment of malaria in 1950 when it emerged as the drug of choice for the treatment of latent exoerythrocytic stages of *Plasmodium vivax* and *Plasmodium ovale*. The mechanism of action of primaquine is not fully elucidated. CPMQ is the main metabolite of PMQ formed in the liver after oral administration of PMQ (Mihaly *et al.*, 1984). Both primaquine and its oxidized metabolites have antiplasmodial activity, but the antimalarial activity of CPMQ is considerably less than that of primaquine itself (Colin, 1991). Greaves *et al.* (1980), who reported the pharmacokinetics of PMQ in humans for the first time, showed that orally administered PMQ is rapidly absorbed and distributed extensively in the body, and excretion through the kidneys is less than 1% of dosage.

In this study, PMQ was absorbed rapidly with the peak concentration in plasma reaching in 1.5 h post-dosing. The Cmax of CPMQ was achieved later at 4 h, and the concentration was maintained until 12 h before decreasing to a half of that in 24 h. Accumulation was noted for the CPMQ, but not for PMQ, i.e., 1.5 times higher concentration of CPMQ but lower concentrations of PMQ after the second dosing (Fig. 1 & Table I).

Compared to the other pharmacokinetic studies following the administration of the same dose, PMQ concentration was significantly higher and CPMQ concentration significantly lower in our patients. Although the similar elimination half-life (4.4~5.6 h) of PMQ was observed, the volume of distribution was smaller in our studies (46.7 ±

19.7 L vs 292~420 L) (Bhatia *et al.,* 1986; Fletcher *et al.,* 1981). Hence, the peak plasma concentration of PMQ in Korean patients was up to 5 times higher than that in previous studies (0.05 to 0.14 μg/mL) (Bhatia *et al.,* 1986; Dua *et al.,* 1996; Ward *et al.,* 1985), suggesting a potential ethnic difference in pharmacokinetics of PMQ. In fact, Fletcher *et al.* (1981) observed the pharmacokinetic difference between two ethnic groups, i.e., after 45 mg PMQ was administered to Caucasian and Thai male volunteers, the peak plasma concentration of PMQ in Thais (0.233 ± 0.047 μg/mL) was higher than that in Caucasians (0.162 ± 0.020 μg/mL). The difference was attributed to many factors such as age, body weight, diet, gut flora composition, however, the ethnic difference can not be ruled out.

PMQ is metabolized by monoamine oxidase and cytochrome P450 (CYP) (Bangchang *et al,,* 1992; Constantino *et al.,* 1999). It has been reported that PMQ inhibits the metabolism of other drugs by CYP3A4 (Zhao and Ishizaki, 1999) and CYP2D (Law, *et al.,* 2000). Although the exact metabolic pathway of PMQ is not known, the polymorphism of CYP3A4 or CYP2D may be a potential cause of ethnic difference in pharmacokinetics of PMQ (Gashaw, *et al.,* 2003).

The difference was also noticed for the concentration of the metabolite, CPMQ. After administration of 15 mg PMQ, the peak concentration of CPMQ was reported in the range of 0.74 to 2.35 μg/mL, which were significantly higher than those in our study (0.281 to 0.447 μg/mL). Overall, compared to other studies, higher concentrations of the parent drug, PMQ and lower concentrations of the metabolite, CPMQ were observed in our study, which may indicate a slower rate of metabolism of PMQ to CPMQ in Korean patients.

It is known that PMQ is well absorbed in the gastrointestinal tract but there is a wide range of individual variation in peak blood concentration even at the same dosage (McEvoy, 2002). The wide variation was also noted in plasma concentrations of PMQ and CPMQ between individuals in our study. Such great inter-individual differences can be attributed to the differences in absorption, metabolism, binding with proteins, excretion, and other factors, such as disease severity. PMQ is known to bind to α_1-glycoprotein (AGP) of the acute phase reactant in plasma but not with other inflammatory proteins such as C-reactive protein or albumin (Kennedy and Frischer, 1990). That is, if the quantity of AGP varies with the severity of disease, the concentration of the drug in plasma may vary.

In summary, this study showed that PMQ is absorbed and metabolized rapidly after oral administration and that the plasma concentration of CPMQ, the main metabolite of PMQ, was higher and the half-life was longer than that of PMQ. The concentrations of PMQ and CPMQ in plasma showed large variations among patients as seen in other studies. It was noted that the mean peak plasma concentration of PMQ in our study was much higher, and that of CPMQ was much lower compared to other studies, indicating a potential difference of inter-ethnic group, which warrants further evaluation.

ACKNOWLEDGMENT

The authors thank to Prof. Sook-Hyang Lee for careful reading of this manuscript.

REFERENCES

Anonymous., Epidemiology of malaria in Korea, 2000. *CDMR.*, 12, 5-10 (2001).

Bangchang, K. N., Karbwang, J., and Back, D. J., Primaquine metabolism by human liver microsomes; effect of other antimalarial drugs. *Biochem. Pharmacol.*, 44, 587-590 (1992).

Bhatia, S. C., Saraph, Y. S., Revankar, S. N., Doshi, K. J., Bharucha, E. D., Desai, N. D., Vaidya, A. B., Subramanyam, D., Gupta, K. C., and Satoskar, R. S., Pharmacokinetics of primaquine in patients with P. vivax malaria. *Eur. J. Clin. Pharm.*, 31, 205-210 (1986).

Bunnag, D., Karbwang, J., Thanvibul, A., Chittamas, S., Ratanapongse, Y., Chalermrut, K., Bangchang, K. N., and Harinasuta, T., High dose of primaquine in primaquine resistant vivax malaria. *Trans. R. Soc. Trop. Med. Hyg.,* 88, 218-219 (1994).

Chai, I. H., Lim, G. I., Yoon, S. N., Oh, W. I., Kim, S. J., and Chai, J. Y., Occurrence of tertian malaria in a male patient who has never been abroad. *Kor. J. Parasitol.,* 32, 195-200 (1994).

Clyde, D. F. and McCarthy, V. C., Radical cure of Chesson strain vivax malaria in man by 7, not 14, days of treatment with primaquine. *Am. J. Trop. Med. Hyg.,* 26, 562-563 (1977).

Colin, D., Primaquine. In Colin., D., Boobis, A. R., Burley. D., Davies, D. M., Davies., D. S., Harrison. P. I., Orme. M. L., Park. B. K., and Goldberg. L. I. (Eds.). Therapeutic Drug. Edinbergh:Churchill Livingston, New York, pp.209-212, (1991).

Constantino, L., Paixao, P., Moreira, R., Portela, M. J., Do Rosario, V. E., and Iley, J., Metabolism of primaquine by liver homogenate fractions. Evidence for monoamine oxidase and cytochrome P450 involvement in the oxidative deamination of primaquine to carboxyprimaquine. *Exp. Toxicol. Pathol,.* 51, 299-303 (1999).

Dua, V. K., Kar, P. K., Sarin. R., and Sharma, V. P., High-performance liquid chromatographic determination of primaquine and carboxyprimaquine concentrations in plasma

and blood cells in Plasmodium vivax malaria cases following chronic dosage with primaquine. *J. Chromatogr. B.*, 675, 93-98 (1996).

Endoh, Y. S., Yoshimura, H., Sasaki, N., Ishihara, Y., Sasaki, H., Nakamura, S., Inoue, Y., and Nishikawa, M., High-performance liquid chromatographic determination of pamaquine, primaquine and carboxyprimaquine in calf plasma using electrochemical detection. *J. Chromatogr.,* 592, 123-129 (1992).

Fletcher, K. A., Evans, D. A., Gilles, H. M., Greaves, J., Bunnag, D., and Harinasuta, T., Studies on the pharmacokinetics of primaquine. *Bull. World Health Organ.,* 59, 407-412 (1981).

Gashaw, I., Kirchheiner, J., Goldammer, M., Bauer, S., Seidemann, J., Zoller, K., Mrozikiewicz, P. M., Roots, I., and Brockmoller, J., Cytochrome P450 3A4 messenger ribonucleic acid induction by rifampin in human peripheral blood mononuclear cells: correlation with alprazolam pharmacokinetics. *Clin. Pharmacol. Ther.*, 774, 448-457 (2003).

Greaves, J., Evans, D. A., Gilles, H. M., Fletcher, K. A., Bunnag, D., and Harinasuta, T., Plasma kinetics and urinary excretion of primaquine in man. *Brit. J. Clin. Pharmacol.,* 10, 399-405 (1980).

Kennedy, E. and Frischer, H., Distribution of primaquine in human blood: drug-binding to α_1-glycoprotein. *J. Lab. Clin. Med.,* 116, 871-878 (1990).

Kim, J. H., Park, K. S., Lee, J. M., Choi, J. H., Lee, I. S., Kim, M. Y., Kim, Y. R., and Kang, M. W., A case of primaquine-resistant tertian malaria. *Kor. J. Infect. Dis.,* 29, 503-507 (1997).

Kimura, M., Tomizawa, I., Takizwa, Y., and Ohtomo, H., A study of relapsed cases of vivax malaria after the standard primaquine therapy. *Kansenshogaku Zasshi.,* 70, 1086-1091 (1996).

Law, M. Y., Slawson, M. H., and Moody, D. E., Selective involvemnet of cytochrome P450 2D subfamily in *in vivo* 4-hydroxylation of amphetamine in rat. *Drug Metab. Dispos.*, 28, 348-353 (2000).

McEvoy. G. K., Primaquine phosphate. In McEvoy, G. K., Miller. J. L., Snow. E. K., and Welsh. O. H. (Eds.). AHFS Drug information. ASHP Inc., Bethesda, pp.754-756, (2002).

Mihaly, G. W., Nicholl, D. D., Ward, S. A., Edwards, G., Orme, M. L., and Breckenridge, A. M., Pharmacokinetics of prmaquine in man: Identification of the carboxylic acid derivative as a major plasma metabolite. *Brit. J. Clin. Pharmacol.*, 17, 441-446 (1984).

Mihaly, G. W., Ward, S. A., Edwards, G., Nicholl, D. D., Orme, M. L., and Breckenridge, A. M., Pharmacokinetics of primaquine in man. I. Studies of the absolute bioavailability and effects of dose size. *Brit. J. Clin. Pharmacol.*, 19, 745-750 (1985).

Pukrittayakamee, S., Vanijanonta, S., Chantra, A., Clemens, R., and White, N. J., Blood stage antimalarial efficacy of primaquine in Plasmodium vivax malaria. *J. Infect. Dis.*, 169, 932-935 (1994).

Rieckmann, K. H., Yeo, A. E., Davis, D. R., Hutton, D. C., Wheatley, P. F., and Simpson, R., Recent military experience with malaria chemoprophylaxis. *Med. J. Aust.,* 158, 446-449 (1993).

Ward, S. A., Mihaly, G. W., Edwards, G., Looareesuwin, S., Phillips, R. E., Chanthavanich, P., Warrell, D. A., Orme, M. L., and Breckenridge, A. M., Pharmacokinetics of primaquine in Man. II. Comparison of acute vs chronic dosage in Thai subjects. *Br. J. Clin. Pharmacol.*, 19, 751-755 (1985).

Zhao, X. J. and Ishizaki, T., A further interaction study of quinine with clinically important drugs by human liver microsomes: determinations of inhibition constant (Ki) and type of inhibition. *Eur. J. Drug Metab. Pharmacokinet.*, 24, 272-278 (1999).