British Journal of Pharmaceutical Research
15(4): 1-10, 2017; Article no.BJPR.32723
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Pharmacokinetic Characterization of Piperaquine in Nigerian Healthy Volunteers after Co-administration with a Commercial Brand of *Moringa* Tea

Alero Lydia Iseh[1], Adebanjo Jonathan Adegbola[1] and Babatunde Ayodeji Adeagbo[1*]

[1]Translational Pharmacokinetics and Pharmacogenomics Research Group, Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors BAA and AJA designed the study, performed the statistical analysis, wrote the protocol and jointly wrote the manuscript. Authors ALI and AJA collected the samples and managed the bio-analyses of the study. All the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2017/32723
Editor(s):
(1) Nawal Kishore Dubey, Centre for Advanced Studies in Botany, Banaras Hindu University, India.
Reviewers:
(1) Norma-Aurea Rangel-Vazquez, Instituto Tecnologico De Aguascalientes, Mexico.
(2) Mostafa Abbas Shalaby, Cairo University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/18487

Original Research Article

Received 11th March 2017
Accepted 31st March 2017
Published 3rd April 2017

ABSTRACT

This 2-phase cross-over drug–herb interaction study investigated the effect of co-administration of tea prepared from the commercial product of *Moringa oleifera* on the pharmacokinetic parameters of piperaquine. Fourteen healthy subjects were recruited for this study, but ten subjects (age range, 22–30 years) completed the study. Blood samples were collected from the subjects at the Obafemi Awolowo University (Ile Ife, Nigeria) and the bio-analysis took place in the Therapeutic Drug Monitoring Laboratory (TDM) of Obafemi Awolowo University Teaching Hospital Complex, Ile Ife, Nigeria, between January 2016 and October 2016. In the first phase of the study, each of the subjects received three tablets of Dihydroartemisin- Piperaquine (DHA/PQ) combination (artelad® containing DHA/PQP, 40/320 mg). After a washout period of three months, each of the subjects received 25g freshly prepared *Moringa* tea twice daily for 5 days and on the sixth day, received *Moringa* tea concurrently with three tablets of DHA/PQ combination. The plasma concentrations of

**Corresponding author: E-mail: tcubeadeagbo@gmail.com;*

PQ were determined using a validated HPLC method. The results showed a 23.8% significant decrease (P=0. 01) in the Cmax of PQ and a 5.8% decrease in the AUC after co-administration with *Moringa* Tea. The Tmax also increased by 61%, while the half-life increased by 12.2%. There was no difference in the clearance and $C_{28.}$ The geometric mean ratio showed that there is no significant difference in the disposition of PQ with *Moringa* Tea. The study showed wide variability in PQ pharmacokinetics following a single oral dose. The effects of concurrent administration of *Moringa oleifera* tea did not significantly influence the PQ plasma exposure but delayed its absorption.

Keywords: Malaria; drug-herb interaction; pharmacokinetics; bioavailability.

ABBREVIATIONS

ACT : *Artemisinin-based Combination Therapy*
AUC : *Area under curve*
BMI : *Body Mass Index*
CL : *Clearance*
C_{max} : *Maximum plasma concentration*
DHA : *Dihydroartemisinin*
DHA-PQ : *Dihydroartemisinin-piperaquine*
FDA : *Food and Drug Agency*
GMR : *Geometric Mean Ratio*
HPLC : *High Performance Liquid Chromatography*
HREC : *Health Research and Ethics Committee*
IPT_P*-SP* : *Intermittent preventive treatment in pregnancy with sulphadoxine-pyrimethamine*
LOD : *Limit of Detection*
LOQ : *Limit of Quantification*
PQ : *Piperaquine*
RDT : *Rapid Diagnostic Test*
TDM : *Therapeutic Drug Monitoring*
T_{max} : *Time to reach maximum concentration*
V_d : *Volume of distribution*
WHO : *World Health Organization*

1. INTRODUCTION

Malaria is a major public health issue in the world, particularly in sub Saharan African countries like Nigeria. Consequently, the World Health Organization (WHO) now recommends Artemisinin-based combination therapy (ACT) as first line treatment for *Plasmodium falciparum* uncomplicated malaria [1]. Among the recently approved ACTs by WHO, dihydroartemisin-piperaquine (DHA-PQ) regimen is proven to have good efficacy and ease of administration. A recent study even indicated that DHA-PQ regimen was more efficacious when considered for chemoprevention in high risk groups such as pregnant women and infants compared with the standard IPTp-SP [2,3]. Dihydroartemisinin (DHA) is short-acting with a half-life ranging between1-2 hours, whereas piperaquine (PQ) has rather very long half-life ranging from 11-33 days [4,5]. A long-acting PQ serves to eradicate residual *P. falciparum*, prevent renaissance and reduce the likelihood that a resistant strain will emerge [3].

Piperaquine (PQ) (Fig. 1), a bisquinoline antimalarial drug is a highly lipophilic base with Log P value of 6.1 [6]. This property is partly attributed to the enhancement of its bioavailability in the presence of high-fat meal [7]. Sim et al. reported that the oral bioavailability of PQ relative to the fasting state was 121% greater after the high-fat meal as the solubility and absorption in the fatty environment are greatly upgraded, whereas a standard Vietnamese meal [8,9] had no such effect on the oral absorption of piperaquine. Unpredictable upsurge and decrease in the systemic exposure of PQ are not usually desirable as high peak PQ level had been associated with the prolongation of QT [10] and the lower peak level was strongly connected with poor therapeutic outcome [11].

As described by Sim et al., food is one of the covariates for variability in pharmacokinetic characteristics of PQ and this observation may be extrapolated to other food supplements. *Moringa oleifera* is one of the herbal plants used as a food supplement and every part of the tree is either useful for nutritional or medicinal purposes [12]. The tea from its leaf powder is used as potential antioxidant, anticancer, anti-inflammatory, anti-diabetic and antimicrobial agent [13]. As a result of the acclaimed versatility in treating certain ailments, its co-administration with myriads of pharmaceutical products such as ACTs may be a common practice. We hypothesized that herb-PQ pharmacological interactions may exist when *Moringa tea is* consumed simultaneously with DHA-PQ.

In order to describe the extent of such interaction, a sensitive bio-analytical procedure is necessary for accurate determination of piperaquine levels in human plasma, which in turn are used to characterize the pharmacokinetics of the drug. A number of sensitive HPLC-UV and LC-MS/MS methods have been reported for quantification of the PQ in plasma [14-19]. However, there are constraints in the applicability of these methods, especially in a resource-limited environment. Such constraints include cumbersome sample pre-treatment [14,16,20], sophisticated analytical method [17,18] and expensive deuterated internal standard [19]. Here, we reported a simple HPLC-UV method using one step protein precipitation and quinine as the internal standard. The plasma levels of PQ were quantified with the aim of describing the effect of co-administration with tea prepared from a commercial product of *Moringa*.

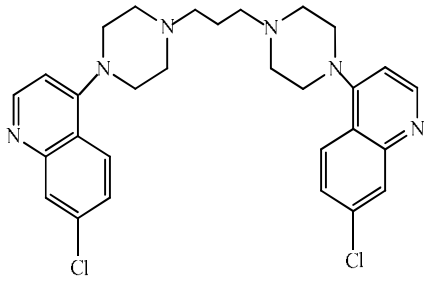

Fig. 1. Piperaquine

2. EXPERIMENTAL

2.1 Chemicals and Reagents

Piperaquine phosphate, PQP, ($C_{31}H_{42}Cl_2N_6O_4P$, MW 664.6, 569.6, purity 98%) was obtained from AK Scientific, Inc. (CA, USA). Quinine ($C_{20}H_{24}N_2O_2$, MW 324.4, purity ≥99%) was purchased from BDH, hydrochloric acid (HCl) and ammonium acetate (NH_4AcO), HPLC grade acetonitrile (MeCN) and methanol (MeOH) were purchased from Fisher Scientific, Inc. (NJ, USA). Artelad® containing 40/320 mg of DHA/PQ was purchased from a retail pharmacy outfit in Ilesa, Osun State, Nigeria and *Moringa* powder for making tea listed by National Agency for Food and Drug Administration (NAFDAC) was purchased from Julie® Pharmacy, Nigeria.

2.2 Study Population

Ethical clearance was obtained from the Health Research and Ethics Committee (HREC) of the Institute of Public Health, Obafemi Awolowo University (Ile Ife, Nigeria). All the subjects were certified healthy by the physician and all of them signed written informed consents after a thorough understanding of the implications of the study. The study was conducted in compliance with the principles of the Declaration of Helsinki and the FDA draft Guideline for the Conduct of Bioavailability and Bioequivalence Studies [21].

Thirty-five healthy subjects who were certified to be healthy and between the ages of 18 and 55 years, non-smoking, non-pregnant with body mass index (BMI) between 18 and 32 and were willing to provide written consent were screened for malaria infection using rapid diagnostic test (RDT). Subjects who had uncomplicated or severe malaria or evidence of any organ damage or had participated in similar studies were out rightly excluded from the study. Only fourteen healthy subjects met the criteria and were enrolled in the study, but ten subjects, with a mean age of 25.2 ± 2.5 years (range, 22–30 years), mean body weight of 65.3 + 4.5 kg (range, 56–73 kg), completed the study.

2.3 Study Design

The study is a 2-phase drug–herb interaction study with a 3-month washout period. All the subjects received either PQ alone or PQ after receiving a twice daily intake of tea drink made from the commercially available brand of *Moringa* powder (Julie® *Moringa* powder).

In the first phase of the study, all the subjects received three tablets of DHA/PQ. The subjects were allowed to eat a standardized Nigerian meal consisted of boiled rice and fish 4 hours after PQ administration. After a washout period of three months, subjects were instructed to report at the study center by 8 am and 6 pm daily for 5 days prior to PQ administration. During each visit, the subjects received a portion of 250 ml *Moringa* tea, freshly prepared by pouring hot water on 25 g of *Moringa* powder and the subjects drank the tea 30 minutes after each preparation. On the dosing day following an overnight fasting, the subjects received *Moringa* tea concurrently with three tablets of artelad® each (containing DHA/PQP, 40/320 mg).

Blood samples were obtained into EDTA tubes by venipuncture - pre-dose (10min before dosing), 1, 2, 4, 6, 8, 10, 24, 48, 72, 168, 336 and 672 hr and centrifuged at 2000×g for 10 min to obtain plasma aliquots which were stored at −-

℃ until analysis. Safety and tolerability of the treatment were assessed by the physician on day 1, 2, 3, 7 and 14 by checking the subjects B.P, pulse rate and filling of safety report form.

2.4 Sample Analysis

The piperaquine plasma concentration was determined by using a modified HPLC-UV method earlier described by Hung et al. [15]. The HPLC-UV condition consisted of quinine as an internal standard and mobile phase was methanol-NH_4OAc buffer $(0.02\,M, pH\ 6.5)$, 80:20 at a flow rate of 1 ml/min and monitored with UV detection at 225 nm. The limit of detection (LOD) and limit of quantification was generated through regression analysis of the calibration curve and the method was validated based on FDA / CDER guidelines.

100 µl of plasma of each the sample was micro-pipetted into an Eppendorf centrifuge tube. To each tube was added 50 µl of 1 µg/ml of the internal standard and 350 µl of CH_3CN. The mixture was vortex-mixed for 30 s and centrifuged at 10,800 × g for 5 min. The clean supernatant (20 µl) was injected into the HPLC column and PQ and IS peak response were followed during routine analysis. Concentrations of PQ were measured in plasma through the response generated from HPLC data.

2.5 Pharmacokinetic and Statistical Analysis

A plot of plasma PQ concentration versus time data was done in Microsoft® Excel 2010, All derived pharmacokinetic parameters were determined using non-compartmental analysis using Kinetica™ Version 4.1, (Inna Phase Corporation, Philadelphia, USA) pharmacokinetics software where the concentration at time 0 (C_0) was assumed to be zero. The AUC_{0-672} or AUC_{last} was computed using the linear method, trapezoidal rule when $C_n > C_{n-1}$ and t_0 was defined as C_0. The AUC_T was estimated as the sum of AUC_{0-672} and$AUC_{672-\infty}$. The$AUC_{672-\infty}$ was estimated from C_{672} and k_e that is by extrapolating the last measurable plasma concentration (C_{last}) to the time axis using the following relationship:

$$AUC_{672-\infty} = \frac{C_{672}}{k_e}$$

k_e is the elimination rate constant that was obtained as the slope of linear regression of the ln transformed plasma concentration–time curve in the elimination phase. Whereas half-life ($t_{1/2}$) was computed from $t_{1/2} = \frac{\ln(2)}{k_e}$, clearance ($CL$) from $\frac{Dose}{AUC_T}$ and volume of distribution (V_d) from $V_d = \frac{CL}{k_e} = \frac{t_{1/2} * CL}{0.693}$.

Power calculation was conducted using a p*ost-hoc* analysis based on the difference between two independent means of C_{max} generated in this study. The data were summarized as mean ± standard deviation. The bioavailability (BA) parameters, C_{max}, $C_{D7,}C_{D28}, AUC_{0-D28}, AUC_{0-\infty}$, for piperaquine with and without *Moringa* tea were log transformed and were subjected to hypothesis test (mean piperaquine alone ≠ mean of piperaquine-*Moringa* co-treatment) using a paired two sample t-test and a two-tailed significance level of P < 0.05 were considered for all parameters. The test for establishing similar disposition or bioavailability between *Moringa*-piperaquine co-administration and piperaquine alone was carried out by transforming BA parameters (C_{max}, $C_{D7,}C_{D28}, AUC_{0-D28}, AUC_{0-\infty}$) to logarithm scale and the data from both arms were compared with the 90% confidence interval (CI) using the ratio of geometric means. *Moringa*-piperaquine co-treatment was considered to have similar bioavailability compared with piperaquine administration alone if the 90% CIs for AUC and Cmax were within the predetermined bioequivalence range of 80% to 125% [21].

3. RESULTS AND DISCUSSION

Ten out of 14 subjects enrolled completed the study. Two subjects were lost to follow-up during phase two while the other two had uncomplicated plasmodium malaria and were treated with the standard dose of a-ACT regimen and acetaminophen. The data collected in phase one from the dropped-out subjects were not included in the overall analysis as they were considered as incomplete. While the subjects who had an episode of acute malaria and promptly treated with ACT were disallowed from completing the study because of possible interference or interaction between the study drug and drugs used for treatment. The high drop-out experienced in this study was reasonably connected with the long wash-out period as a result of the characteristic long half-life of piperaquine. Moreover, the sample size of (10 subjects) had a statistical power of 89% from a

post doc power analysis using t-test of difference means of C_{max} between two matched pairs with probalilty error level of 5% which is sufficient to detect a significant difference.

A longer washout period was adopted in this study on piperaquine bioavailability in human subjects due to its characteristic long half-life from 12-33 days [5,22,23]. We adhered to the average half-live of 19 days before the commencement of the second phase. The second option, parallel design, recommended by the Food and Drug Agency (FDA) for drugs with such a long half-life [21] was not feasible in this study because very few subjects met the inclusion criteria bearing in mind that the region where the study took place is known for endemics of malaria and so to have a large population of healthy subjects without malaria parasite or its symptoms is very difficult. A parallel design without consideration for a wash-out period would be appropriate for BA/BE studies of drugs with a long half-life, however, in our study subjects who were asymptomatic and aparasitic for Plasmodium malaria were limited to form two groups for parallel design so we went for a long wash out period. The chromatogram presented in Fig. 2 showed that the chromatographic resolution between piperaquine and quinine was achieved with a retention time of 6.4 and 3.8 minutes respectively.

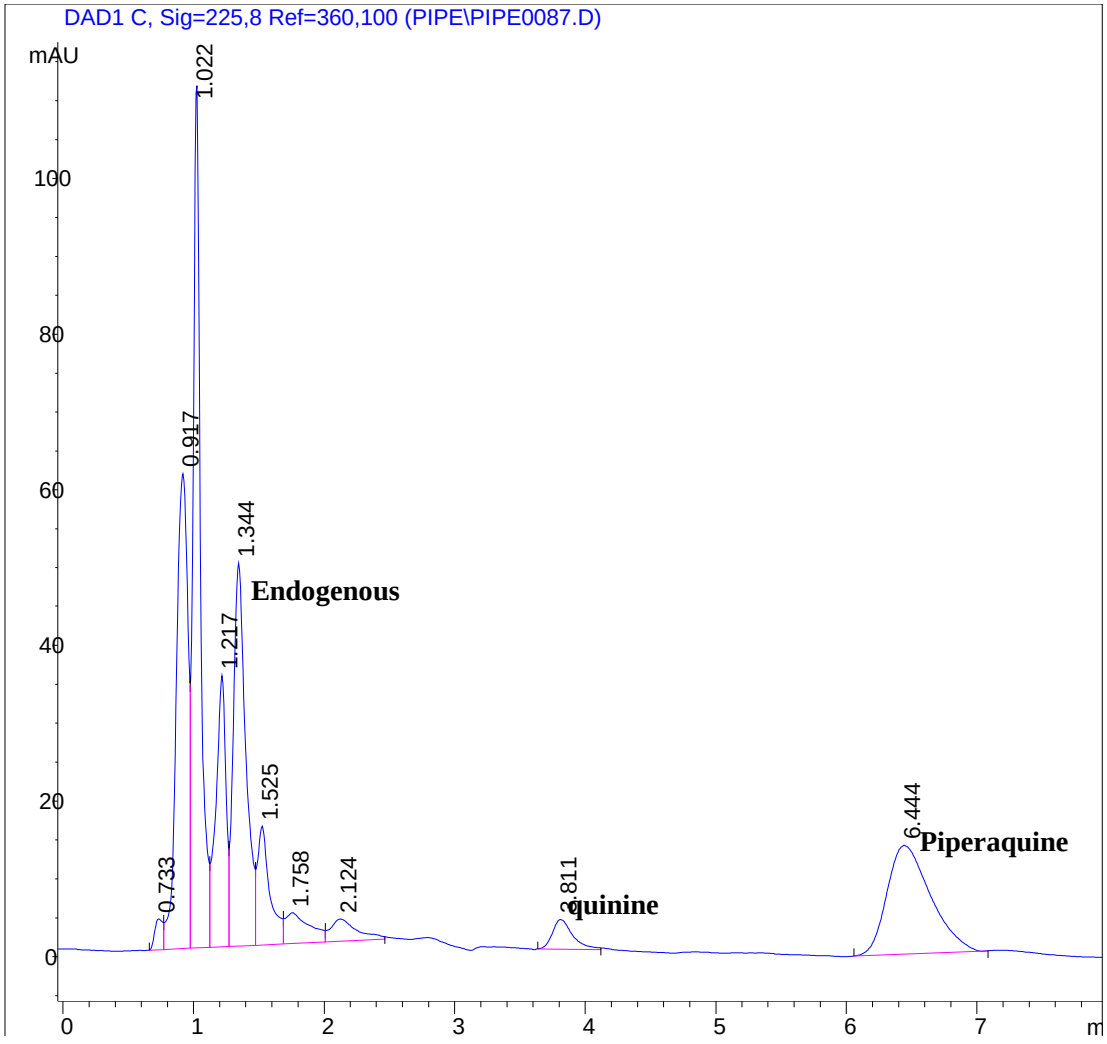

Fig. 2. Chromatogram of HPLC analysis of plasma concentration of piperaquine with quinine as internal standard

Recovery, accuracy, and precision (inter- and intra-day) data for the HPLC method were presented in Table 1 and the LOD and LOQ were 0.0056 µg/ml and 0.016 µg/ml respectively. This method was an improvement on the Hung et al. reported bio-analytical method [15]. The sample preparation was a one-step protein precipitation and we make use of quinine as the internal standard.

The plasma concentration–time curves of piperaquine after oral administration alone and pre-treatment with *moringa* are as shown in Fig. 3. Tables 2 and 3 presented the pharmacokinetic parameters of PQ with or without *moringa* and 90% CI using the ratio of geometric means. The pharmacokinetic parameters generated for 960mg of piperaquine phosphate in this study were concordant with values obtained by other studies [7,8,9,24,25]. Nguyen et al. described the pharmacokinetics of 960 mg piperaquine phosphate using two formulations. Though Nguyen et al. bioequivalence study was conducted among the Vietnamese, the pharmacokinetic parameter such as C_{max}, T_{max}, $t_{1/2}$ and $AUC_{0-\infty}$, Cl and Vd ($0.234\ \mu g ml^{-1}$, $3.0\ hr$, $613\ hr$, $23.31 \mu g * h\ ml^{-1}$, $0.4\ Lh^{-1}kg^{-1}$ and $748\ L\ kg^{-1}$ respectively) were similar following a single oral administration of 960 mg piperaquine phosphate.

Sim et al. and Hai et al. respectively described the effects of a high fat meal and a standardized Vietnamese meal on the pharmacokinetics of piperaquine following an oral single dose of piperaquine. They reported much lower Cmax and plasma exposure of piperaquine compared with the present study, however, T_{max}, $t_{1/2}$ and clearance followed the same pattern previously reported. Lower C_{max} and AUC in their studies were associated with the use of 320 mg of PQP (171.5 mg of PQ base) instead of 960 mg PQP (514.5 mg PQ base) used in this study. The higher dose used in the present study was in line with the recent consideration of the DHA - PQ combination for both uncomplicated Plasmodium malaria treatment and chemoprevention options in special groups such as pediatrics and pregnancy [26,27,28].

The Cmax of PQ was significantly decreased (two-tail P = 0.004) by 23.8% and AUC_T was also decreased by 5.8% after co-administration with *Moringa* Tea. In addition to a reduced C_{max}, half-life of PQ was significantly prolonged when the drug was co-administered with *Moringa* tea (410.4±24.73 hr. versus 460.0±59.46 hr., two-tail P = 0.006) while the Tmax increased by 61%. However, the half-lives of piperaquine with and without *Moringa* were still in within the range of values reported previously [4,29].

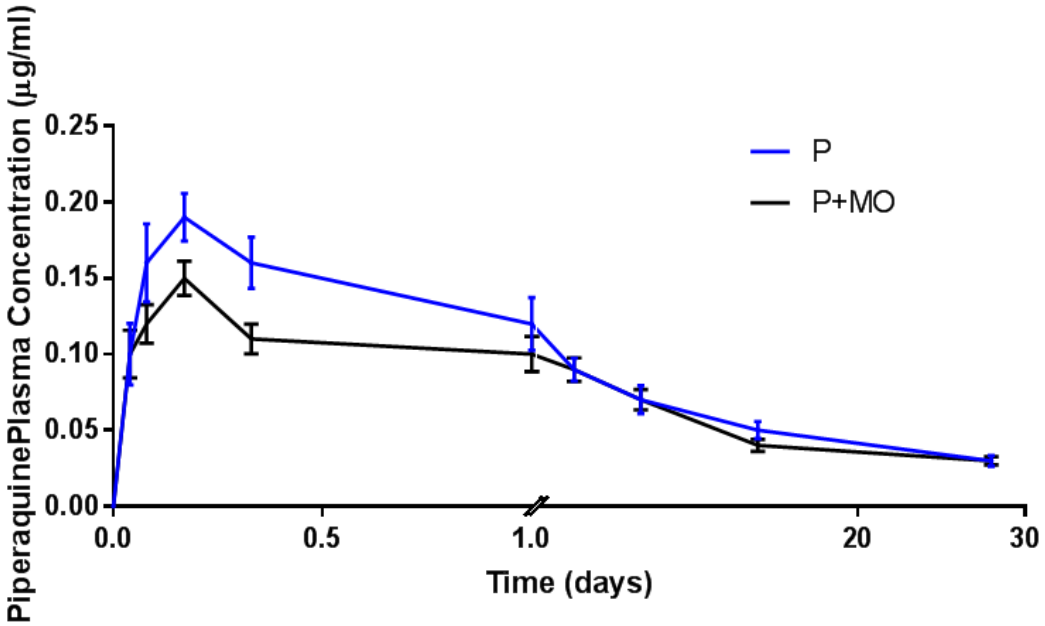

Fig. 3. Plasma piperaquine concentrations versus time after a single oral dose of DHA/PQ with or without *Moringa* tea

Mean ± SD = Mean values ± Standard deviation of ten subjects

Table 1. Recovery, accuracy, and precision of method of analysis

QC concentration	Accuracy (%)	Recovery (%)	Precision	
			Intra-day (%)	Inter-day (%)
0.025 µg/ml	89.4	95.7	7.8	4.0
0.250 µg/ml	98.3	106.2	17.7	14.4
2.5 µg/ml	105	98.3	9.6	6.3

Table 2. Pharmacokinetic parameters for piperaquine after administration of three tablets of DHA/PQ with or without *Moringa* tea

PK parameters	PQ alone	PQ with *Moringa*	P-value
	mean ± SD	mean ± SD	(Level of sig, P<0.05)
Cmax (µg/ml)	0.21 ± 0.049	0.16 ± 0.0301	0.01
Tmax (h)	3.6 ± 0.84	5.8 ± 6.43	0.3
AUCL (µg/ml*hr)	40.7 ± 13.30	36.3 ± 9.95	0.2
AUCT (µg/ml*hr)	58.6 ± 19.26	55.2 ± 15.04	0.37
T1/2(h)	410.4 ± 24.73	460.0 ± 59.46	0.01
Cl (L/h)	10.0 ± 4.10	10.1 ± 3.68	0.79
Vd (L)	5950.1± 2626.9	6779.8 ± 2941.89	0.26
C28(µg/ml)	0.031 ± 0.0116	0.030 ± 0.0089	0.55

Table 3. Assessment of geometric mean ratio of PK parameters of Piperaquine-*Moringa* co-treatment to piperaquine alone

Parameters	90% CI	Piperaquine + *Moringa*l Piperaquine alone, %
Log10 Cmax	108.0 - 124.3	116.2
Log10 C28	95.0 - 105.7	100.4
Log AUC0-672	92.3 - 102.3	97.3
Log AUC0-∞	94.7- 103.5	99.1

Again, Sim et al. and Hai et al. bioavailability studies described food-drug interaction but the present study revealed the effect of concurrent administration of an herb extract, *Moringa* tea, on the disposition of piperaquine. Food, medicinal products or herbal preparations may affect the pharmacokinetics of drugs at absorption, distribution and elimination phases. There may be delayed, reduced or enhanced absorption, displacement of the drug from plasma protein for drug binding and either induction or inhibition of metabolic enzymes. Drug absorption had been known to be affected in the gastrointestinal tract by the intake of drugs with other agents that have a large surface area upon which the drug can be absorbed, bind or chelate [30]. It is also known facts that substances that could change the pH, alter gastrointestinal motility or modulate transport proteins could also affect absorption of drugs [30]. Numerous phytochemicals such as glucosinolates, flavonoids, phenolic acids, ascorbic acid, β-sitosterol, iron, calcium, phosphorus, copper, α-tocopherol, riboflavin, nicotinic acid, folic acid, pyridoxine, β-carotene, protein, and in particular essential amino acids such as methionine, cysteine, tryptophan and lysine [31] have been isolated from *Moringa*. Of interest to us here is 4-O-(α-L-rhamnopyranosyl-oxy)-benzyl glucosinolate, otherwise known as glucomoringin which has been identified to have antispasmodic property [32,33]. Therefore, there is a possibility that this could have caused the delay in absorption observed with concomitant intake of piperaquine with *Moringa* by altering the gastrointestinal motility. Some of these compounds have also been known to modify gastric pH or form complexes, e.g. ascorbic acid, β-sitosterol, iron, calcium, phosphorus, they could also cause the delayed absorption. Therefore, we assumed that these compounds in *Moringa* extract might have interfered with the transit of piperaquine in the gastro-intestinal tract and delayed its absorption.

Change in C_{max} indicated that the rate, not the extent of absorption of PQ was affected by the concurrent administration of *Moringa* tea extract possibly due to delayed absorption. The geometric mean ratio also showed that there is no significant difference in the disposition of PQ with *Moringa* Tea (Table 3). This showed that the observed significant difference in absorption may not be clinically significant.

Bioequivalence parameters only were considered to measure the effect of *Moringa* tea on PQ disposition as the information on the metabolic behavior of PQ are still limited. Only few studies had recently characterized PQ metabolites [34]

thus its metabolite is not available in order to further characterize the nature of the interactions of between *Moringa* and piperaquine.

The measured day-7 and -28 plasma level, volume of distribution and clearance varied widely among the subjects with a CV of 39, 37, 44 and 41%, respectively within the group taking only PQ while the parameters varied following a similar pattern in 'PQ with *Moringa* group' with a CV of 32, 30, 43 and 36%. In line with this observation, inter-individual variability in PQ pharmacokinetics has been previously reported in African population [35] and in other population in a recent meta-analysis study [36]. Our study reflected day-7 and -28 plasma levels of 0.075 ± 0.03 µg/ml and 0.031±0.01 µg/ml versus 0.066±0.02 µg/ml and 0.030±0.009 µg/ml without or with *Moringa* respectively. The therapeutic target of piperaquine was computed to ≥57ng/ml on day-7 in a population pharmacokinetic study [29] or 20-50 ng/ml plasma level may predict post-treatment prophylactic activity [37]. This showed that Nigerian subjects recruited into both groups achieved plasma levels above the level for a therapeutic target.

4. CONCLUSION

The study showed that co-administration of PQ with *Moringa* tea could delay its absorption and possibly prolong the effect of the drug, however, the interaction was not found to be clinically significant. Coadministration was also found not to be markedly influence the PQ plasma exposure. The study further established wide inter-individual variability in the PQ pharmacokinetic parameters in Nigerian healthy adult subjects following a single oral dose.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) after a thorough understanding of the implications of the study.

ETHICAL APPROVAL

All authors hereby declare that all the experiments have been examined and approved by the Health Research and Ethics Committee (HREC) of the Institute of Public Health, Obafemi Awolowo University (Ile Ife, Nigeria) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Malaria Report. Geneva: World Health Organization; 2016. Licence: CC BY-NC-SA 3.0 IGO; 2016.
2. Madanitsa M, Kalilani L, Mwapasa V, et al. Scheduled intermittent screening with rapid diagnostic tests and treatment with Dihydroartemisinin-Piperaquine versus intermittent preventive therapy with Sulfadoxine-Pyrimethamine for malaria in pregnancy in Malawi: An open-label randomized controlled trial. Plos Med. 2016;13(9):e1002124.
   DOI: 10.1371/journal.pmed.1002124
3. Sambol NC, Tappero JW, Arinaitwe E, Parikh S. Rethinking dosing regimen selection of piperaquine for malaria chemoprevention: A simulation study. Plos One. 2016;11(5):e0154623.
   DOI: 10.1371/journal.pone.0154623
4. Ahmed T, Sharma P, Gautam A, et al. Safety, tolerability, and single- and multiple-dose pharmacokinetics of piperaquine phosphate in healthy subjects. J. Clin. Pharmacol. 2008;48:166-175.
5. Tarning J, Lindegårdh N, Annerberg A, et al. Pitfalls in estimating piperaquine elimination. Antimicrobial Agents and Chemotherapy. 2005;5127–5128.
6. Warhurst DC, Craig JC, Adagu IS, et al. Activity of piperaquine and other 4-aminoquinoline antiplasmodial drugs against chloroquine-sensitive and resistant blood-stages of *Plasmodium falciparum*. Role of beta-haematin inhibition and drug concentration in vacuolar water- and lipid-phases. Biochem. Pharmacol. 2007;73: 1910–1926.
7. Sim I, Davis TME, Ilett KF. Effects of a high-fat meal on the relative oral bioavailability of piperaquine. Antimicrobial Agents and Chemotherapy. 2005;2407–2411.
8. Hai TC, Hietala FC, Huong NV, Ashton M. The influence of food on the pharmacokinetics of piperaquine in healthy Vietnamese volunteers. Acta Tropica. 2008;107:145–149.
9. Nguyen TC, Nguyen NQ, Nguyen XT, Bui D, Travers T, Edstein MD. Pharmacokinetics of the antimalarial drug

piperaquine in healthy Vietnamese subjects. Am J Trop Med Hyg. 2008; 79(4):620–3.

10. Manning J, Vanachayangkul P, Lon C, Spring M, So M, Sea D, et al. Randomized, double-blind, placebo-controlled clinical trial of a two-day regimen of dihydroartemisinin-piperaquine for malaria prevention halted for concern over prolonged corrected QT interval. Antimicrob Agents Chemother. 2014; 58(10):6056–67.
DOI: 10.1128/AAC.02667-14
11. Sundell K, Jagannathan P, Huang L, Bigira V, Kapisi J, Kakuru MM, et al. Variable piperaquine exposure significantly impacts protective efficacy of monthly dihydroartemisinin-piperaquine for the prevention of malaria in Ugandan children. Malar J. 2015;14(1):368.
12. Gopalakrishnanb L, Doriyaa K, Kumar DS. *Moringa oleifera*: A review onnutritive importance and its medicinal application. Food Science and Human Wellness. 2016;5(2):49–56.
13. Saini RK, Sivanesan I, Keum YS. Phytochemicals of *Moringa oleifera*: A review of their nutritional, therapeutic and industrial significance. Biotech. 2016;6(2): 203.
14. Lindegardh N, Ashton M, Bergqvist Y. Automated solid-phase extraction method for the determination of piperaquine in plasma by peak compression liquid chromatography. J. Chromatog. Sci. 2003; 41(1):4–49.
15. Hung TY, Davis TM, Ilett KF. Measurement of piperaquine in plasma by liquid chromatography with ultraviolet absorbance detection. J. Chromatog. B Analyt. Technol. Biomed. Life Set. 2003; 791(1–2):93–101
16. Lindegardh N, White NJ, Day NP. High throughput assay for the determination of piperaquine in plasma. J. Pharm. Biomed. Anal. 2005;39(3–4):601–605.
17. Singhal P, Gaur A, Gautam A, et al. Sensitive and rapid liquid chromatography/ tandem mass spectrometric assay for the quantification of piperaquine in human plasma. J. Chromatog. B Analyt. Technol. Biomed. Life Sci. 2007;859(1):24–29.
18. Hodel EM, Zanolari B, Mercier T, et al. A single LC-tandem mass spectrometry method for the simultaneous determination of 14 antimalarial drugs and their metabolites in human plasma. J. Chromatog. B Analyt. Technol. Biomed. Life Sci. 2009;877(10):867–886.
19. Kjellin LL, Dorsey G, Rosenthal PJ, Aweeka F, Huang L. Determination of the antimalarial drug piperaquine in small volume pediatric plasma samples by LC-MS/MS. Bioanalysis. 2014;6(23):3081-9.
20. Lindegardh N, Annerberg A, White NJ, Day NP. Development and validation of a liquid chromatographic-tandem mass spectrometric method for determination of piperaquine in plasma stable isotope labeled internal standard does not always compensate for matrix effects. J. Chromatog. B Analyt. Technol. Biomed. Life Sci. 2008;862(1–2):227–236.
21. Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER). Draft Guidance for Industry. Bioavailability and bioequivalence studies for orally administered drug products general considerations; 2014.
Available:http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070124.pdf
(Accessed August 23, 2016)
22. Röshammar D, Hai TN, Hietala SF, Huong NV, Ashton M. Pharmacokinetics of piperaquine after repeated oral administration of the antimalarial combination CV8 in 12 healthy male subjects. Eur J Clin Pharmacol. 2006;62: 335–341.
23. Hung TY, Davis TME, Ilett KF, et al. Population pharmacokinetics of piperaquine in adults and children with uncomplicated falciparum or vivax malaria. Br J Clin. Pharmacol. 2004;57(3):253–262.
24. Nguyen DVH, Nguyen QP, Nguyen ND, Le TTT, Nguyen TD, Dinh DN, et al. Pharmacokinetics and ex vivo pharmacodynamic antimalarial activity of dihydroartemisinin-piperaquine in patients with uncomplicated falciparum malaria in Vietnam. Antimicrob Agents Chemother. 2009;53(8):3534–7.
DOI: 10.1128/AAC.01717-08
25. Liu C, Zhang R, Hong X, Huang T, Mi S, Wang N. Pharmacokinetics of piperaquine after single and multiple oral administrations in healthy volunteers. Yakugaku Zasshi. 2007;127(10):1709-14.
26. Desai M, Gutman J, L'lanziva A, Otieno K, Juma E, Kariuki S, Ouma P, Were V, Laserson K, Katana A, Williamson J, ter Kuile FO. Intermittent screening and

treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomized controlled superiority tria. Lancet. 2015;19;386(10012):2507-19.
27. Kakuru A, Jagannathan P, Muhindo MK, et al. Dihydroartemisin-piperaquine for the prevention of malaria in pregnancy. N. Engl. J. Med. 2016;374:928–939.
28. WHO. Guidelines for the treatment of malaria. Third Edition, Global Malaria Programme, World Health Organization, 20 Avenue Appia- CH1211, Geneva 27; 2015.
29. Tarning J, Zongo I, Somé FA, et al. Population Pharmacokinetics and Pharmacodynamic of Piperaquine in Children with Uncomplicated Falciparum Malaria. Clin Pharmacol Ther. 2012;91(3): 497–505.
DOI: 10.1038/clpt.2011.254
30. Schmidt L, Dalhoff K. Food-drug interaction. Drugs. 2002;62(10):1481-1502.
31. Bennett RN, Mellon FA, Foidl N, et al. Profiling glucosinolate and phenolic in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (Horseradish tree) and *Moringa stenopetala* L. J Agric Food Chem. 2003; 51(12):3546-3553.
32. Cáceres A, Saravia A, Rizzo S, Zabala L, De Leon E, Nave F. Pharmacologic properties of *Moringa oleifera*. 2: Screening for antispasmodic, anti-inflammatory and diuretic activity. J Ethnopharmacol. 1992;36(3):233-237.
33. Vongsak B, Sithisarn P, Gritsanapan W. Simultaneous HPLC quantitative analysis of active compounds in leaves of *Moringa oleifera* Lam. J Chromatogr Sci. 2014; 52(7):641-5.
34. Yang A, Zang M, Liu H, et al. Metabolite identification of the antimalarial piperaquine *in vivo* using liquid chromatography–high-resolution mass spectrometry in combination with multiple data-mining tools in tandem. Biomed. Chromatogr; 2016.
DOI: 10.1002/bmc.3689
35. Staehli Hodel EM, Guidi M, Zanolari B, et al. Population pharmacokinetics of mefloquine, piperaquine and artemether-lumefantrine in Cambodian and Tanzanian malaria patients. Malar J. 2013;12:235.
DOI: 10.1186/1475-2875-12-235
36. Hoglund RM, Workman L, Edstein MD, et al. Population Pharmacokinetic Properties of Piperaquine in Falciparum Malaria: An Individual Participant Data Meta-Analysis. PLoS Med. 2017;14(1): e1002212.
DOI: 10.1371/journal.pmed.1002212
37. Tarning J, Ashley EA, Lindegardh N, et al. Population pharmacokinetics of piperaquine after two different treatment regimens with dihydroartemisinin-piperaquine in patients with *Plasmodium falciparum* malaria in Thailand. Antimicrob. Agents Chemother. 2008;52:1052–61.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18487