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BRIEF COMMUNICATION

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Absence of gender influence on the pharmacokinetics of chloroquine combined with primaquine in malaria vivax patients

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ABSTRACT

Chloroquine is the first-line therapy against the asexual stages of *Plasmodium vivax*. There is a high variation of chloroquine plasma levels after therapeutic doses, which can lead to inadequate exposure to the drug. The gender influence was low regarding the disposition of the drug, which is relevant as there are significant physiological variations between male and female patients. The objective of the study was to investigate whether gender modifies the pharmacokinetics parameters of chloroquine in patients with malaria vivax. A prospective study was performed in male and female adult patients using chloroquine (total dose of 25 mg/kg for three days) combined with primaquine. Serial blood samples were collected at admission and up to 672 h post-administration of the drugs. Chloroquine was measured in plasma samples by high-performance liquid chromatography with fluorescence detection. A non-compartmental analysis was used for modeling the data. A total of 26 male and 25 female patients were enrolled in the study. The pharmacokinetic parameters of chloroquine were similar between male and female patients: a half-life of 9.5 days and 10.2 days, maximum concentration (Cmax) of 1295 ng/ml and 1220 ng/ml, area-under-the-curve (AUC 0–28) of 241 µg/mL h and 237 µg/mL h, observed clearance (CL/f) of 5.8 and 5.5 L/h and the volume of distribution (V/f) of 1869 L and 1936 L. The study results suggest that a similar dose regimen of chloroquine combined with primaquine provides a comparable pattern of exposure in male and female patients.

KEYWORDS: Infectious diseases. Chloroquine. Pharmacokinetic. Malaria.

INTRODUCTION

Chloroquine is a 4-aminoquinoline used for the treatment of malaria caused by *Plasmodium vivax,* as well as for autoimmune and rheumatic diseases[1-4]. The pharmacokinetic parameters of chloroquine were described in a number of population groups and in healthy volunteers, aside from malaria patients. These investigations reported a high variation in the pharmacokinetics of the drug within and between-individuals and also described several predictors of variation of chloroquine blood levels that can affect the exposure and the treatment outcome[5-8]. Only a few studies evaluated the influence of a person's gender on the disposition of chloroquine, which could be relevant, asthe physiological differences between male and female patients can alter the pharmacokinetics parameters of some drugs, which could influence the therapeutic efficacy and the incidence rates of adverse reactions[9].

The present study aimed at investigating whether the gender modifies the pharmacokinetics parameters of chloroquine in patients with malaria vivax, using

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a dose of 25 mg/kg in a three-day course with concurrent administration of primaquine[2].

MATERIALS AND METHODS

Study site and patients

A prospective study was carried out in the municipality of Anajas, Para State, North of Brazil, from August 2018 to July 2019. Patients of bothgenders , aged > 18 years, with mono-infection by *P. vivax* were admitted in the study. The exclusion criteria were the presence of signs or symptoms of severe malaria, positive pregnancy test, regular use of tobacco or ethanol, known hypersensitivity to chloroquine, the use of antimalarial drugs within three months prior to the study, and those who disagreed or did not sign the consent form.

Treatment and follow-up

The treatment followed the recommendations of the World Health Organization, and consisted of chloroquine diphosphate in the total dose of 25 mg/kg for three days with primaquine diphosphate in a total dose of 3.0-3.5 mg/kg for seven days[2]. The drugs were provided by Farmaguinhos - Fundação Oswaldo Cruz/ Rio de Janeiro, Brazil. All treatments were supervised by the research team. Vomiting and other adverse reactions were monitored for 2 hours after the antimalarial drug intake. The patients were invited to return for the follow-up or when there were signs and symptoms suggestive of malaria. A parasite count and clinical interview were performed at each visit.

Blood sample collection

Blood samples (4 mL) were taken for the measurement of chloroquine and for the parasite count on admission to the study (h0) and at 24, 72, 168, 336, 504, and 672 hours after the administration of the drug. On day 1 (24 h), blood samples were collected immediately before the administration of the antimalarial drugs. Whole blood samples were centrifuged at 3,500 g for 10 min at 4 °C for plasma separation, and samples were immediately stored at -80 °C until analysis.

Measurement of chloroquine

A reverse-phase HPLC system with fluorescence detection was used to measure the concentrations of chloroquine (Flexar, Perkin Elmer™, Shelton, MA US). The drug was extracted from plasma using a liquid-liquid procedure as described by Alvan *et al.*[10] with modifications[11]. The column was an RP-18 (X terra 4.6 × 150 mm, i.d. 5-µm) at 25 °C, and the mobile phase was composed of dichloromethane-methanol-(1M) perchloric acid (100:9:1.2; v:v:v) at a flow rate of 1.2 mL/min. The eluate was checked by a fluorescence detector with a wavelength of $E_{xcitation}$ = 340 nm and $E_{mission}$ = 380 nm. The limit of detection was 10 ng/mL, and the limit of quantification was 25 ng/mL. The linear assay range was 25 to 2000 ng/mL. The mean between and within-day coefficients of variation were 8.3% and 10.5%, respectively. The mean recovery was 95%. Desethyl-chloroquine, primaquine, carboxy-primaquine, mefloquine, carboxy-mefloquine, and acetaminophen did not interfere in the detection of chloroquine.

Parasite count

The parasite count was performed on Giemsa-stained thick blood films. An experienced microscopist examined the blood films using 100× (oil immersion) objectives[12].

Samples size

A difference of 30% in the pharmacokinetic parameters of chloroquine between genders could modify the pharmacokinetic parameters of the drug. It was estimated that at least 16 patients completing the follow-up period would provide at least an 80% power of detection, with a 95% confidence interval and 30% difference in the pharmacokinetics parameters assessed between male and female patients[12].

Statistical analysis

Data are presented as means and ranges, frequencies of occurrence, or as medians and ranges. The pharmacokinetic parameters of chloroquine were estimated by a non-compartmental model. The dose of chloroquine was normalized for the patient's weight. The following pharmacokinetic parameters were determined: the maximum concentration (C_{max}) was derived directly from the whole blood concentration-time profile, the area under the curve (AUC_{0-28}) from the time of chloroquine administration to the last measurable concentration was estimated by the linear trapezoidal rule, and the extrapolation to infinity ($AUC_{0-\infty}$) was obtained by dividing the last measurable chloroquine concentration by λZ. The chloroquine concentrations were log-transformed and fitted a linear regression model to the terminal phase of the concentration-time profiles by using the method of least squares, which estimated the terminal elimination rate constant (λZ). The terminal elimination

half-life ($t_{1/2}$) was obtained by dividing ln2 by λZ. The apparent oral clearance per fraction of drug absorbed (CL/f) was derived from the ratio of the dose to $AUC_{0-\infty}$. The apparent volume of distribution (V/f) was obtained from CL/f divided by λZ. The concentrations of chloroquine were compared between the genders in each day of blood sampling by the Student *t*-test. The pharmacokinetic parameters of chloroquine were compared between the genders by the Student *t*-test. The data were analyzed in WinNonlin (version 3.3; Pharsight Corp, Mountain View, CA, USA). The significance level was set at 5%.

Ethical aspects

The research was approved by the Ethical Committee of the Nucleo de Medicina Tropical da Universidade Federal do Para (Brazil), Nº 2.819.240/2018. All patients admitted to the study signed the consent inform.

RESULTS

A total of 26 male and 25 female patients with malaria were admitted in the study. The baseline characteristics are shown in Table 1. All samples collected at admission to the study (h0) had no measurable chloroquine levels. In the clinical interviews, patients of both genders reported comparable frequencies of nausea (75%), pruritus (45%), insomnia (40%), dizziness (40%), and tinnitus (35%). There were no mensurable concentrations of chloroquine in plasma samples collected at admission in the study. Plasma concentrations of chloroquine at 24, 72, 168, 336, 504 and 672 h in male and female patients were 402 ng/mL (145-891 ng/mL) and 451.8 ng/mL (110-745 ng/mL) (p=0.448), 1295 ng/mL (360-2450 ng/mL) and 1220 ng/mL (530-2245 ng/mL) (p=0.629), 527 ng/mL (345-774 ng/mL) and 496.7 ng/mL (295-794 ng/mL) (p=0.387), 201 ng/mL (147-284 ng/mL) and 192 ng/mL (137-236 ng/mL) (p=0.284), 121 ng/mL (48-159 ng/mL) and 124 ng/mL (940-145 ng/mL) (p=0.504), 70 ng/mL (31-104 ng/mL) and 65ng/mL (30-98 ng/mL) (p=0.421). The concentration-time profile is shown in Figure 1. The pharmacokinetic parameters of chloroquine derived from the concentration-time curve are shown in Table 2. Data were similar between male and female patients. All patients showed an adequate therapeutic response up to 28 days of follow-up.

Table 1 - Baseline characteristics of patients.

characteristics	male (n=26)	female (n=25)
Age (years)	26 (18-34)	25 (20-32)
weight (kg[a])	64 (54-82)	59 (55-78)
parasitemia on admission[b]	1260 (3.52)	1145 (2.21)
parasite clearance time (hours)	60 (24-96)	60 (24-96)
previous episodes of malaria (%)	100	90
fever at admission, (%)	80	80
Hemoglobin (g/dL[a])	13.2 (12-14)	12.6 (11-14)
haematocrit (%[a])	39 (35-43)	34 (30-38)
platelets(mm^3 X 1000[a])	280 (210-340)	270 (190-320)
total dose of chloroquine administered (mg/kg)	23.9 (22-27)	24.6 (23-26)

[a]Results are expressed as means and ranges; [b]results are expressed as geometrical means and standard deviations

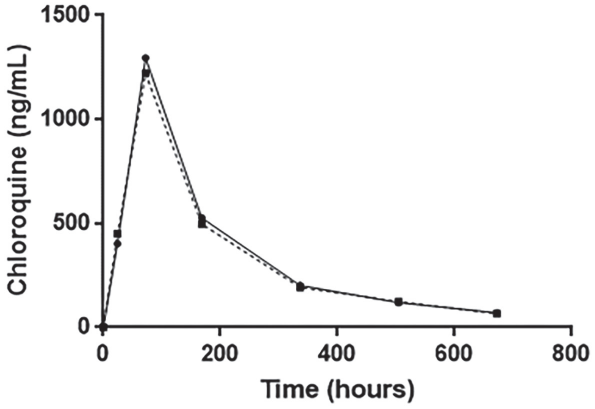

Figure 1 – Concentration time-curve of chloroquine in female (■) and male patientes (●) with *P. vivax* malaria. Data are presented as mean values

DISCUSSION

In Brazil, there are no studies comparing the pharmacokinetic parameters of chloroquine betweengenders, which is relevant, as males and females are at risk for *Anopheles* bites[13]. Chloroquine is the antimalarial drug of choice to eliminate the asexual stages of *P. vivax* due to its safety and low cost[8]. The present study investigated whether the patient's gender would change the pharmacokinetic parameters of the drug, which is relevant as the hormonal cycle of females, the use of oral contraceptives, pregnancy, body weight and fat distribution can influence the pharmacokinetic and the treatment outcomes of some drugs[9]. The pharmacokinetic parameters of chloroquine were estimated by a non-compartmental modeling, which provides a reliable assessment of pharmacokinetics parameters of the drug in sparse samples[12,14]. Despite whole blood samples tend to present with higher concentrations of chloroquine than plasma and serum samples, most studies assessing chloroquine exposure were performed in plasma or serum samples[14-17]. Furthermore, there was no significant pharmacokinetic interaction between chloroquine and primaquine, a partner drug, for the treatment of *P. vivax* malaria[15].

Table 2 - Pharmacokinetic parameters of chloroquine in male and female patients with malaria vivax.

Parameter	Male (n=26)	Female (n=25)	*P**
Kel (L/h)	0.0035 (0.0015-0.0058)	0.0033 (0.0015-0.0066)	*0.641*
$T_{1/2}$ (d)	9.5 (4.8-19.1)	10.2 (4.33-19.2)	*0.579*
C_{max} (ng/mL)	1295 (424-2380)	1220 (530-2245)	*0.737*
AUC_{0-28} (µg/mL*h)	241(167-313)	237 (158-284)	*0.765*
AUC_{0-00} (µg/mL*h)	268 (179-333)	263 (171-342)	*0.655*
MRT_{0-00} (h)	280 (194-365)	276 (176-355)	*0.851*
V/f (L)	1869 (990-2100)	1936 (920-2310)	*0.721*
CL/f (L/h)	5.8 (4.4-8.3)	5.5 (4.3-8.7)	*0.851*

Data are expressed as means and ranges; *Student *t* test; Kel = constant of elimination; $T_{1/2}$ = half-life; C_{max} = maximum concentration; AUC_{0-28} = area under the curve at the least observation; AUC_{0-00} = area under the curve extrapolated to infinite; MRT_{0-00} = mean time of residence; Vd = Apparent volume of distribution; CL = Clearance

The baseline characteristics were comparable in male and female patients. There were no significant adverse reactions that could lead to the interruption of treatment, which confirms the safety and tolerability of the drug at a total dose of 25 mg/kg[1,2]. The total doses administered were similar in male and female patients, but the mean values were below the recommendations of the World Health Organization in both genders[2]. However, the low doses found in some patients did not modify the therapeutic response up to 28 days of follow-up, which suggests a downward revision of the total dose of 25 mg/kg proposed by WHO to treat the disease in different endemic scenarios.

Chloroquine tablets present oral bioavailability estimated at 89% in healthy volunteers. The main pharmacokinetic characteristics of the drug are the large volume of distribution and the long elimination half-life. Chloroquine is metabolized in the liver by CYP2C8 and, to a lesser extent, via CYP3A4 and CYP2D6 enzymes. The primary metabolite is desethyl-chloroquine, which retains the pharmacological activity of the parent drug[5-8]. The pharmacokinetic parameters of the study patients agreed with the long half-life and the large volume of distribution of the drug. Additionally, there was a high inter-individual variation in the estimated parameters, which is in line with previous reports on the pharmacokinetics of the drug[5-8].

The comparison of pharmacokinetic parameters of chloroquine between genders showed no significant differences, suggesting that there was no significant influence of the patient's gender on the disposition of the drug. The mean C_{max} found in both genders was within the values of 996-2446 ng/mL reported in male Thai patients with *P. vivax* malaria under similar dosing regimen[16]. The $t_{1/2}$ agreed with values of 8.27-9.33 days found in Thai patients, as well as with values of 6-12.4 days reported in healthy Nigerians after a single dose of 600 mg[16,17]. The clearance in both genders was similar to the median values reported for Thai and Burmese patients with vivax malaria of 6.13 L/h[18]. The median value of the apparent volume of distribution was similar to the apparent volume of distribution in the peripheral compartment of 1600 L and within the range found for the sum of apparent volume of distribution of the central and peripheral compartments of 2068 L found in the above population groups[18].

The schedule of blood sampling of the study, with a small number of time points in the initial phase of distribution of the drug, could lead to an underestimation of the AUC_{0-28} and of the apparent volume of distribution. For instance, most of the values of AUC_{0-28} found in the study were within the values reported in Thai male patients, but the mean values found in both genders were lower than those found in the referred study, although higher than in healthy male Thai. There are several causes of disagreements in pharmacokinetic studies such as the dose regimen, blood sampling schedule, pharmacokinetic model used to estimate parameters, and parasite count on admission[12,19]. Thus, the differences between the data of the present study with those found in the Thai study could be related to the frequency of administration of chloroquine within 24 h of treatment[16].

CONCLUSION

In conclusion, the data of the present study revealed that chloroquine presents a similar pharmacokinetic disposition in bothgenders. . Thus, the same dose regimen of chloroquine provides a comparable pattern of exposure in male and female patients.

AUTHORS' CONTRIBUTIONS

All authors were involved in the conception and design of the study, the analysis and interpretation of data, drafting the article and revising it critically regarding the scientific content and the all approved the version to be submitted for evaluation. MVDF and AGCNM performed the laboratory

analysis, LWPS and JLFV performed the statistical analysis and data interpretation.

CONFLICT OF INTERESTS

None

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