

## Effect of Food on the Pharmacokinetics of Piperaquine and Dihydroartemisinin

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### Abstract

**Background and Objective** Piperaquine–dihydroartemisinin combination therapy has established efficacy for the treatment of malaria; however, a more comprehensive understanding of the pharmacokinetic properties and factors contributing to inter- and intra-individual variability is critical to optimize clinical use. This study assessed the effects of food on the pharmacokinetics of combination piperaquine–dihydroartemisinin administration in healthy volunteers.

**Methods** This was an open-label, single-dose, parallel-group study. Participants were randomly allocated to receive oral piperaquine–dihydroartemisinin either after an overnight fast or immediately after a standardized, high-fat, high-calorie meal. Blood samples were collected for analysis of plasma piperaquine and dihydroartemisinin concentrations, which were utilized for calculation of pharmacokinetic parameters, using a standard model-independent approach.

**Results** Consumption of a high-fat, high-calorie meal resulted in substantial increases in the extent of exposure to piperaquine (ratio between area under the plasma concentration–time curve [AUC] values from 0 to 168 h in the fed and fasted states  $[AUC_{0-168\text{ h FED}}/AUC_{0-168\text{ h FASTED}}] = 299\%$ , 90 % confidence interval [CI] 239–374 %). This likely reflects an increase in the oral bioavailability of the drug, directly related to the fat content of the meal. Co-administration of food was also found to result in both delayed and enhanced absorption of dihydroartemisinin (ratio between AUC values from time zero to infinity in the fed and states  $[AUC_{\infty\text{ FED}}/AUC_{\infty\text{ FASTED}}] = 142\%$ , 90 % CI 113–178 %; ratio between mean transit time [MTT] values in the fed and fasted states  $[MTT_{\text{FED}}/MTT_{\text{FASTED}}] = 135\%$ , 90 % CI 114–160 %).

**Conclusion** Although food was found to significantly impact on the pharmacokinetics of piperaquine and dihydroartemisinin, given the low fat content of standard meals within endemic regions and the anorexic effects of malaria infection, these results are unlikely to impact on the clinical utility of these drugs. However, co-administration of food with these anti-malarials by populations consuming a typical Western diet should be avoided to reduce the risk of toxic side effects. It is therefore a general recommendation that piperaquine–dihydroartemisinin not be administered within  $\pm 3$  h of food consumption.

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## Key Points

The influence of food on piperazine pharmacokinetics has been examined with equivocal findings, whereas the food–drug interaction of dihydroartemisinin has not been investigated; a more comprehensive understanding of the factors contributing to pharmacokinetic variability is critical in order to optimize the use of these drugs in clinical practice.

Consumption of a high-fat, high-calorie meal with administration of piperazine–dihydroartemisinin combination therapy resulted in a substantial increase in the extent of exposure to piperazine and delayed and enhanced the absorption of dihydroartemisinin. For piperazine, the food effect appeared to be directly related to the fat content of the meal.

On the basis of these findings, the general recommendation is that piperazine–dihydroartemisinin combination therapy not be administered within  $\pm 3$  h of food consumption.

## 1 Introduction

The current World Health Organization (WHO) recommendation for the treatment of uncomplicated *Plasmodium falciparum* malaria is artemisinin-based combination therapy comprising two anti-malarial drugs with independent modes of action, specifically a rapid-acting artemisinin-derived compound administered with a longer-acting anti-malarial [1]. The complementary modes of action of these two anti-malarial drugs are thought to increase treatment effectiveness and prevent or delay the emergence of resistance.

The combination of piperazine and dihydroartemisinin is a well-tolerated treatment for uncomplicated falciparum malaria, with established efficacy in practice [2–6]. Despite previous long-term clinical use of these drugs, the pharmacokinetics of piperazine and dihydroartemisinin have been described only within the last decade [7–10]. The pharmacokinetics of piperazine are characterized by slow oral absorption (with a time to reach the maximum plasma concentration [ $T_{\max}$ ] of 5 h in the fasted state), a large apparent volume of distribution ( $\sim 700$  L/kg), extensive protein binding ( $>99\%$ ) and a long elimination half-life ( $\sim 20$  days) [11]. On the other hand, dihydroartemisinin is rapidly absorbed ( $T_{\max}$  1–2 h) and displays a small apparent volume of distribution (0.8 L/kg) and a rapid

elimination half-life ( $\sim 1$  h) [11]. A more comprehensive understanding of the pharmacokinetics of these drugs and the factors contributing to inter- and intra-individual variability is critical in order to optimize their use in clinical practice.

Food–drug interactions are often associated with alterations in drug pharmacokinetics due to changes in gastric emptying, gastric pH or other physiological changes, resulting in a reduction, delay, increase and/or acceleration in drug absorption [12]. Although a number of studies have investigated the effects of food on the pharmacokinetics of various drugs, given the varied contribution of factors such as the physicochemical properties of the drug and the composition and timing of the meal, there is still no scientific basis to predict food–drug interactions.

Previous studies have examined the influence of food on the pharmacokinetic properties of piperazine, with some studies demonstrating substantial alterations in piperazine concentrations after a meal [13, 14], whereas others have reported no significant effects [15–18]. On the other hand, the impact of food on the pharmacokinetics of dihydroartemisinin has not been reported, and while this aspect has been examined for other artemisinin-derived compounds, the results have been equivocal [19–21]. This study was conducted to assess the effects of food on the extent and rate of absorption of piperazine and dihydroartemisinin, administered as a fixed-dose combination, in healthy adult male volunteers.

## 2 Methods

### 2.1 Ethical Considerations

The study was reviewed and approved by the Bellberry Human Research Ethics Committee (HREC; reference no. C24/10). Participants were fully informed of the study procedures and provided written informed consent prior to study initiation. The study was conducted in accordance with the Declaration of Helsinki, the National Statement on Ethical Conduct in Human Research issued by the National Health and Medical Research Council (Australia) and the principles of Good Clinical Practice.

### 2.2 Study Design

This was a randomized, open-label, single-dose, parallel-group study conducted to evaluate the effects of a high-fat, high-calorie meal on the pharmacokinetics of piperazine and dihydroartemisinin in 36 healthy, adult male volunteers (18 subjects planned per treatment group). Given the long half-life of piperazine ( $\sim 20$  days), a parallel study design was considered appropriate to address the study objectives.

The clinical component of the study was conducted by CPR Pharma Services (Adelaide, SA, Australia) at clinical sites within Australia.

Prior to treatment administration, volunteers were screened and were required to meet the eligibility criteria for the study: male; Caucasian; aged 18–50 years; body weight >75 kg; body mass index 19.0–27.0 kg/m<sup>2</sup>; free from clinically significant illness or disease as determined by medical and surgical history, physical examination, vital signs, electrocardiogram and clinical laboratory determinations; provision of written informed consent.

Participants were randomly allocated to receive a single oral dose of 1280 mg of piperazine phosphate/160 mg of dihydroartemisinin, either with 240 mL of water after an overnight fast of at least 10 h or with 200 mL of water after a standardized, high-fat, high-calorie meal (consumed within 30 min prior to dose administration).

The study treatment was administered as Eurartesim<sup>TM</sup> tablets containing 320 mg of piperazine phosphate and 40 mg of dihydroartemisinin, manufactured by Sigma-Tau Industrie Farmaceutiche Riunite SpA (Rome, Italy; batch no. PP091166). Subjects randomized to the fed treatment were provided with a high-fat, high-calorie breakfast (50 % fat/800–1000 kcal), comprising two fried eggs, two strips of bacon, two slices of toast with two serves of butter, 4 oz (114 g) of hash brown potatoes and 8 oz (240 mL) of whole milk.

Blood samples were collected for analysis of plasma piperazine concentrations prior to dosing (at 0 h) and 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 30, 36, 42, 48, 60, 72, 96, 120 and 168 h after treatment administration. Blood samples were collected for analysis of plasma dihydroartemisinin concentrations prior to dosing (at 0 h) and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 h after treatment administration.

### 2.3 Analytical Methods

Plasma samples were analysed for piperazine and dihydroartemisinin concentrations by CPR Pharma Services (Adelaide, SA, Australia).

Piperazine and dihydroartemisinin concentrations were quantified by high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) methods validated according to US Food and Drug Administration (FDA) guidelines. Validation studies and study sample analyses were conducted according to the principles of Good Laboratory Practice (GLP).

Piperazine and the internal standard (deuterated piperazine) were purified from plasma by protein precipitation followed by chromatographic separation and MS/MS detection. To 50 µL of human plasma, 250 µL of 0.5 % trifluoroacetic acid in acetonitrile was added; after 1 min of mixing, 50 µL of supernatant was transferred to a

polypropylene tube and a further 100 µL of ultra-pure water was added, after which samples were injected into the HPLC–MS/MS system. A Waters X-Bridge<sup>TM</sup> 3.5 µm C18 column was used for chromatographic separation under gradient conditions at a 0.3 mL/min flow rate. Mobile phase (MP) A was 10:90 acetonitrile/0.1 % trifluoroacetic acid in water (v/v), and MP B was 90:10 acetonitrile/0.1 % trifluoroacetic acid in water (v/v); the gradient program was set from 0 to 3.00 min: 0 to 40 % MP A; from 3.00 to 3.10 min: 40 to 80 % MP A; from 3.10 to 3.40 min: 80 % MP A; from 3.40 to 3.50 min: 80 to 0 % MP A; and from 3.50 to 5.00 min: 0 % MP A. Piperazine and the internal standard were monitored by an MS/MS detector in positive multiple reaction monitoring (MRM) mode. The single charged Q1/Q3 transitions were 535.2/288.3 atomic mass units (amu) for piperazine and 541.4/294.1 amu for the internal standard. The typical retention time was 2.70 min for both piperazine and the internal standard. The lower limit of quantification (LLOQ) was 5 ng/mL, and the calibration curve range was 5–500 ng/mL. Independent quality-control samples had concentrations of 15.0, 75.0 and 375 ng/mL of piperazine. Samples were analysed for piperazine determination in a total of 11 analytical runs. The linearity and reproducibility of the calibration curves were evaluated from repeated analysis of the calibration curve samples. The inter-assay accuracy and precision were within 5.0 % and 8.0 %, respectively. The mean *R*<sup>2</sup> value was 0.9968. The accuracy and precision evaluated from repeated analysis of the quality-control samples were within 3.0 and 11.0 %, respectively.

Dihydroartemisinin and the internal standard (artemisinin) were purified from plasma by liquid/liquid extraction followed by chromatographic separation and MS/MS detection. All sample processing was carried out in ice, i.e. from thawing through addition of the extraction solvent. To 100 µL of human plasma, 3 mL of chlorobutane was added; after 10 min of mixing, samples were centrifuged at 3000 rpm for 5 min and placed in a freezer at  $-80 \pm 15$  °C for 15 min. The upper extraction solvent was then transferred to a polypropylene tube and evaporated to dryness under a gentle stream of nitrogen; samples were reconstituted with 50:50 methanol/10 mM ammonium acetate [pH 4] (v/v) before injection into the HPLC–MS/MS system. A Waters X-Bridge<sup>TM</sup> 3.5 µm C18 (50 × 2.1 mm) column equipped with a Waters X-Bridge C18 3.5 µm guard cartridge was used for chromatographic separation under isocratic conditions, the mobile phase being 63.5:36.5 methanol/10 mM ammonium acetate [pH 4] (v/v) at a 0.2 mL/min flow rate. Dihydroartemisinin and the internal standard were monitored by an MS/MS detector in positive MRM mode. The single charged Q1/Q3 transitions were 302.3/163.0 amu for dihydroartemisinin

and 300.3/209.1 amu for the internal standard. The typical retention times were 2.95 and 2.84 min for dihydroartemisinin and the internal standard, respectively. The LLOQ was 10 ng/mL, and the calibration curve range was 10–1600 ng/mL. Independent quality-control samples had concentrations of 25.0, 250 and 1200 ng/mL of dihydroartemisinin. Samples were analysed for dihydroartemisinin determination in a total of seven analytical runs. The linearity and reproducibility of the calibration curves were evaluated from repeated analysis of the calibration curve samples. The inter-assay accuracy and precision were within 5.0 and 7.0 %, respectively. The mean  $R^2$  value was 0.9986. The accuracy and precision evaluated from repeated analysis of the quality-control samples were both within 6 %.

## 2.4 Pharmacokinetic and Statistical Methods

Plasma analyte concentrations were utilized for calculation of pharmacokinetic parameters, using a standard model-independent approach.

For piperazine, the area under the plasma concentration–time curve (AUC) from 0 to 168 h ( $AUC_{0-168\text{ h}}$ ) was calculated using the linear trapezoidal method. The maximum observed plasma concentration ( $C_{\max}$ ) and  $T_{\max}$  were taken directly from the data without interpolation. The mean transit time (MTT) over the sampling interval ( $MTT_{0-168\text{ h}}$ ) was calculated as  $AUMC_{0-168\text{ h}}/AUC_{0-168\text{ h}}$ , where  $AUMC_{0-168\text{ h}}$  is the area under the first moment of the plasma concentration–time curve (AUMC) from 0 to 168 h.

For dihydroartemisinin, the AUC from time zero to the time of the last quantifiable concentration ( $AUC_{0-\text{last}}$ ) was calculated using the linear trapezoidal method. The AUC from time zero to infinity ( $AUC_{0-\infty}$ ) was calculated as  $AUC_{0-\text{last}} + C_{\text{last}}/\lambda_z$ , where  $C_{\text{last}}$  is the last quantifiable concentration and  $\lambda_z$  is the terminal slope of the Ln-transformed concentration–time profile.  $C_{\max}$  and  $T_{\max}$  were taken directly from the data without interpolation. The MTT was calculated as  $AUMC_{0-\infty}/AUC_{0-\infty}$ . The terminal half-life ( $T_{1/2}$ ) was calculated as  $\ln(2)/\lambda_z$ .

A linear mixed-effects analysis of variance (ANOVA) model was used to analyse Ln-transformed AUC,  $C_{\max}$ , MTT and  $T_{1/2}$  parameters. The pooled variance was used to construct the 90 % confidence intervals (CIs) for the ratios of the fed and fasted treatment geometric least squares means. In constructing these 90 % vCIs, the fasted treatment was used as the reference. A non-parametric Mann–Whitney  $U$  test was used to assess treatment differences for the  $T_{\max}$  data. A Student's  $t$  test was used to determine differences in demographic parameters between the treatment groups.

Significance was set at an  $\alpha$  level of 0.05. Treatment equivalence was concluded if the 90 % CIs were within the limits of 80–125 %.

Phoenix<sup>®</sup> WinNonlin<sup>®</sup>, Version 1.3 (Pharsight Corporation, Mountain View, CA, USA) was used for pharmacokinetic and parametric statistical analyses. SPSS for Windows, Version 19 (SPSS Inc., Chicago, IL, USA) was used for non-parametric statistical analysis.

## 3 Results

Complete concentration–time data were available for 36 participants (18 fasted, 18 fed) for assessment of piperazine pharmacokinetics, and for 37 participants (19 fasted, 18 fed) for assessment of dihydroartemisinin pharmacokinetics. One subject (in the fasted group) withdrew from the study prior to collection of the 168 h sample. Demographic information for the study population is summarized in Table 1; there were no statistically significant differences in demographic parameters between the two treatment groups.

Plasma concentration–time profiles in the fed and fasted states are displayed for piperazine in Fig. 1 and for dihydroartemisinin in Fig. 2. Pharmacokinetic parameters are summarized in Table 2, and the ratios of the fed/fasted data and associated 90 % CIs for the pharmacokinetic parameters are summarized in Fig. 3.

### 3.1 Piperazine

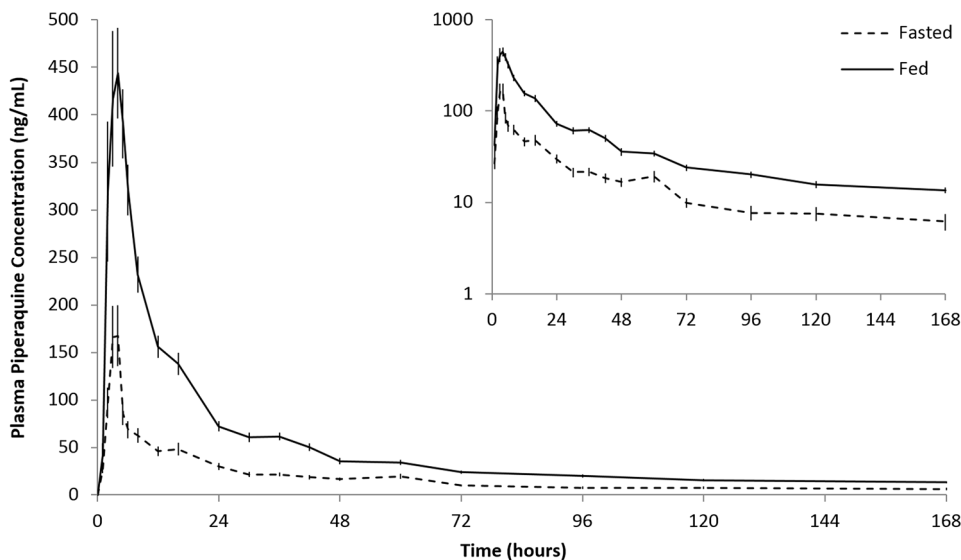
The study results indicated that there was a substantial increase in plasma piperazine concentrations after consumption of a high-fat, high-calorie meal (Fig. 1), with average AUC and  $C_{\max}$  parameters increasing approximately 3- to 4-fold ( $AUC_{0-168\text{ h FED}}/AUC_{0-168\text{ h FASTED}} = 99\%$ ;  $C_{\max\text{ FED}}/C_{\max\text{ FASTED}} = 395\%$ ). Equivalence assessment of the piperazine pharmacokinetic data indicated that the meal resulted in significantly higher  $AUC_{0-168\text{ h}}$  values ( $p < 0.001$ ) and  $C_{\max}$  values

**Table 1** Participant demographic information

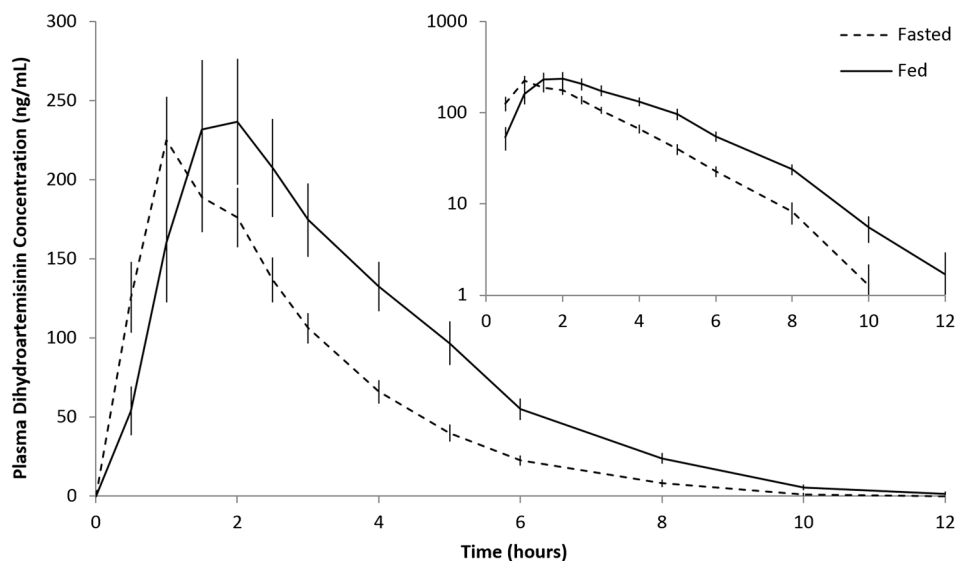
Parameter	Fasted population	Fed population
Sex: male ( <i>n</i> )	19	18
Ethnicity: Caucasian ( <i>n</i> )	19	18
Age (years)	24.4 ± 7.70	25.8 ± 8.55
Weight (kg)	81.6 ± 5.11	82.2 ± 4.05
Height (cm)	182.9 ± 5.98	182.7 ± 4.47
Body mass index (kg/m <sup>2</sup> )	24.5 ± 2.29	24.7 ± 1.32

The data are expressed as mean ± standard deviation, unless stated otherwise

**Fig. 1** Plasma piperavaquine concentration–time profiles after administration of a single oral dose of 160 mg of dihydroartemisinin/1280 mg of piperavaquine in the fed (*solid line*) and fasted states (*dashed line*). The data are expressed as mean  $\pm$  standard error of the mean



**Fig. 2** Plasma dihydroartemisinin concentration–time profiles after administration of a single oral dose of 160 mg of dihydroartemisinin/1280 mg of piperavaquine in the fed (*solid line*) and fasted states (*dashed line*). The data are expressed as mean  $\pm$  standard error of the mean



( $p < 0.001$ ), with 90 % CIs well above the 80–125 % limits (Fig. 3). On the other hand, no treatment differences were detected for  $MTT_{0-168\text{ h}}$  ( $MTT_{0-168\text{ h FED}}/MTT_{0-168\text{ h FASTED}} = 87\%$ ; 90 % CI 80–95 %). No significant differences in  $T_{\max}$  values ( $p = 0.309$ ) were found.

### 3.2 Dihydroartemisinin

Statistical analysis of the plasma dihydroartemisinin data indicated that consumption of a high-fat, high-calorie meal resulted in significantly higher  $AUC_{0-\text{last}}$  values ( $p = 0.022$ ),  $AUC_{0-\infty}$  values ( $p = 0.013$ ) and  $MTT$  values ( $p = 0.005$ ) [ $AUC_{0-\text{last FED}}/AUC_{0-\text{last FASTED}} = 142\%$ ;  $AUC_{0-\infty FED}/AUC_{0-\infty FASTED} = 142\%$ ;  $MTT_{\text{FED}}/MTT_{\text{FASTED}} = 135\%$ ). In addition, the 90 % CIs for the ratios of the fed/fasted data extended beyond the 80–125 %

limits, indicating that the treatments could not be considered equivalent with respect to  $AUC_{0-\text{last}}$ ,  $AUC_{0-\infty}$ ,  $C_{\max}$  and  $MTT$  (Fig. 3). The meal was also shown to result in significantly later maximum plasma concentrations ( $2.47 \pm 1.37\text{ h}$  vs  $1.16 \pm 0.448\text{ h}$ ;  $p < 0.001$ ). No significant differences in  $C_{\max}$  values ( $p = 0.239$ ) and  $T_{1/2}$  values ( $p = 0.598$ ) were found.

## 4 Discussion

A comprehensive understanding of the pharmacokinetics of drugs and the factors contributing to inter- and intra-individual variability is critical in order to develop evidence-based guidelines for appropriate prescription of these drugs in clinical practice. This study was conducted to examine

**Table 2** Derived piperazine and dihydroartemisinin pharmacokinetic parameters in participants administered 1280 mg of piperazine phosphate/160 mg of dihydroartemisinin in the fed or fasted state

Parameter	Fasted treatment	Fed treatment
<b>Piperazine</b>		
Subjects ( <i>n</i> )	18	18
AUC <sub>0–168 h</sub> (ng·h/mL)	3054 ± 1376	8327 ± 1784 <sup>†</sup>
C <sub>max</sub> (ng/mL)	188 ± 142	596 ± 201 <sup>†</sup>
MTT <sub>0–168 h</sub> (h)	45.6 ± 9.16	39.1 ± 3.92 <sup>†</sup>
T <sub>max</sub> (h)	3.39 ± 0.850 (3.50)	3.83 ± 1.30 (4.00)
<b>Dihydroartemisinin</b>		
Subjects ( <i>n</i> )	19	18
AUC <sub>0–last</sub> (ng·h/mL)	649 ± 239	933 ± 340 <sup>†</sup>
AUC <sub>0–∞</sub> (ng·h/mL)	686 ± 244	971 ± 330 <sup>†</sup>
C <sub>max</sub> (ng/mL)	252 ± 106	324 ± 154
MTT (h)	2.80 ± 0.703	3.90 ± 1.47 <sup>†</sup>
T <sub>1/2</sub> (h)	1.49 ± 0.470	1.53 ± 0.388
T <sub>max</sub> (h)	1.16 ± 0.448 (1.00)	2.47 ± 1.37 (2.00) <sup>†</sup>

The data are expressed as mean ± standard deviation (median), unless stated otherwise

AUC area under the plasma concentration–time curve, AUC<sub>0–168 h</sub> AUC from 0 to 168 h, AUC<sub>0–last</sub> AUC from time zero to the time of the last quantifiable concentration, AUC<sub>0–∞</sub> AUC from time zero to infinity, C<sub>max</sub> maximum observed plasma concentration, MTT mean transit time, MTT<sub>0–168 h</sub> MTT from 0 to 168 h, T<sub>1/2</sub> terminal half-life, T<sub>max</sub> time to reach C<sub>max</sub>

<sup>†</sup> *p* < 0.05 in comparison with the fasted treatment data

potential food–drug interactions for piperazine and dihydroartemisinin in healthy adult volunteers.

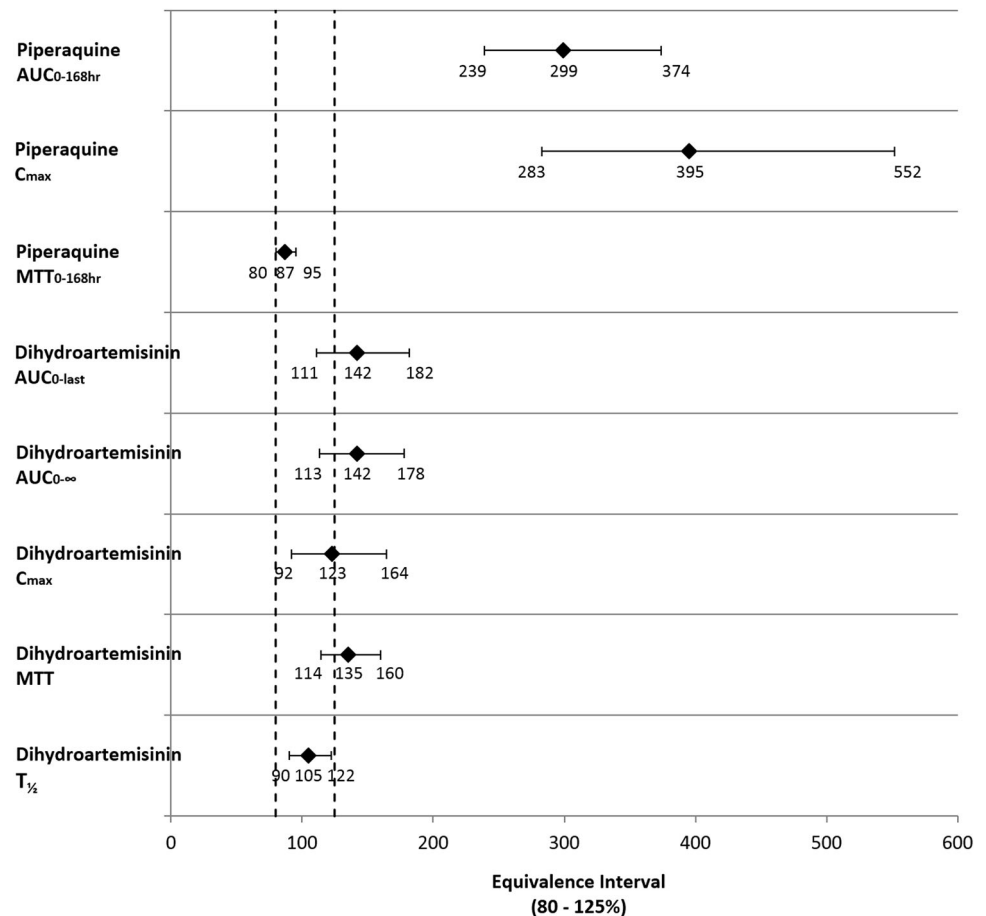
Given the poor water solubility and likely low oral bioavailability of piperazine [22], it is proposed that administration with food results in enhanced bioavailability and increases in drug exposure. A number of studies have examined the influence of food on piperazine pharmacokinetics, with varying results. In a small (*n* = 8), randomized, crossover study, Sim et al. [14] demonstrated significant increases in both the rate of absorption (213 % increase in C<sub>max</sub>) and the extent of absorption (107 % increase in AUC<sub>0–last</sub>) of piperazine after administration with a high-fat, high-calorie meal (53.4 g/900–1000 kcal), in comparison with the values observed in the fasted state. Similarly, Chinh et al. [13] reported significant increases in C<sub>max</sub> and AUC parameters after consumption of a standard Vietnamese noodle meal (with 16.7 g of fat) in comparison with fasting values in 26 healthy subjects. On the other hand, Hai et al. [16] reported no significant impact of a similar Vietnamese meal (with 17 g fat) on the pharmacokinetics of piperazine; however, it should be noted that the reported C<sub>max</sub> values and AUC from 0 to 24 h (AUC<sub>0–24</sub>) values were higher in the fed group (*n* = 16) than in the fasted group (*n* = 16) [C<sub>max</sub> 212 vs 130 µg/L; AUC<sub>0–24</sub> 2.2 vs 1.7 mg·h/L]. Recently, Annerberg et al.

[15] investigated the impact of consumption of a small amount of fat (chocolate milk containing 6.7 g of fat) on piperazine pharmacokinetics in malaria patients and were unable to detect any significant differences in pharmacokinetics parameters between the fasted group (*n* = 14) and the fed group (*n* = 15).

In the present study, a significant increase in plasma piperazine concentrations was demonstrated after consumption of a high-fat, high-calorie meal (Fig. 1), in keeping with the findings reported by Sim et al. [14]. Given the 3- to 4-fold increases in AUC and C<sub>max</sub> values, it can be concluded that the meal resulted in increases in the extent of exposure to piperazine. Assuming that the meal had no effects on the clearance or volume of distribution of piperazine, these findings are likely to reflect a substantial increase in the oral bioavailability of the drug. Although no difference in the MTT values for piperazine was observed, this is not unexpected given that the mean residence time (MRT) far exceeds the mean absorption time (MAT) for piperazine and, as such, it is unlikely that small differences in the absorption time could be detected. As a result, limited conclusions can be drawn with regard to the effect of food on the rate of absorption of piperazine. It should be noted that because of the considerably long half-life of piperazine, the sampling schedule employed for this study was unable to characterize the full piperazine concentration–time profile; consequently, the terminal phase and associated pharmacokinetic parameters could not be determined. However, the sampling schedule was considered adequate to ensure complete absorption of the drug; therefore the C<sub>max</sub> and AUC<sub>0–168 h</sub> values were considered sufficient to characterize the peak and total drug exposure as per the FDA guidelines.

While the effects of food on the pharmacokinetics of orally administered dihydroartemisinin have not been previously investigated, studies have examined the impact of food on the pharmacokinetics of other artemisinin-derived compounds, with varying results. Fitoussi et al. [20] reported a significant reduction in artesunate and dihydroartemisinin (metabolite) C<sub>max</sub> and AUC<sub>0–last</sub> values and increased dihydroartemisinin T<sub>max</sub> values after administration of artesunate in combination with a meal, in comparison with doses administered in the fasted state. On the other hand, Tan et al. [21] identified food as a significant covariate for the absorption rate constant in a population pharmacokinetic model of artesunate, with an associated increase in the absorption half-life of artesunate from 10.8 min (fasted) to 67.5 min (fed); however, the extent of absorption was not significantly affected. Dien et al. [19] investigated the impact of food on artemisinin pharmacokinetics and concluded that consumption of a meal had no effects on AUC, C<sub>max</sub>, T<sub>max</sub>, apparent oral clearance or MTT values.

**Fig. 3** 90 % confidence intervals of piperavaquine and dihydroartemisinin pharmacokinetic parameters obtained after administration of a single oral dose of 160 mg of dihydroartemisinin/1280 mg of piperavaquine in the fed and fasted states. The data are expressed as the 90 % confidence interval around the ratio (%fed/fast) of the geometric least squares mean.  $AUC$  area under the plasma concentration–time curve,  $AUC_{0-168\text{ h}}$  AUC from 0 to 168 h,  $AUC_{0-last}$  AUC from time zero to the time of the last quantifiable concentration,  $AUC_{0-\infty}$  AUC from time zero to infinity,  $C_{max}$  maximum observed plasma concentration,  $MTT$  mean transit time,  $MTT_{0-168\text{ h}}$  MTT from 0 to 168 h,  $T_{1/2}$  terminal half-life



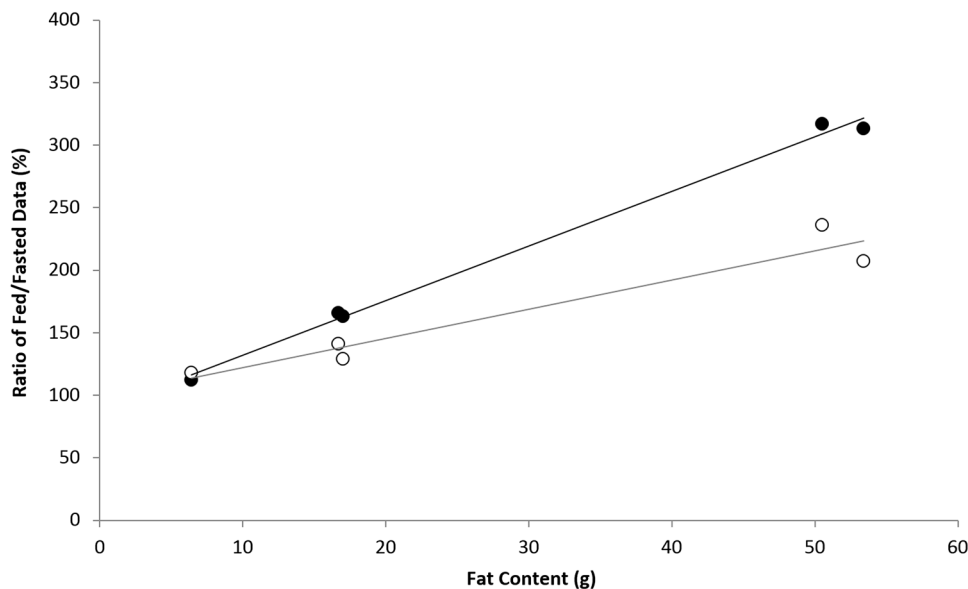
The present study was able to demonstrate that administration of dihydroartemisinin in combination with a high-fat, high-calorie meal resulted in significant increases in  $AUC_{0-last}$ ,  $AUC_{0-\infty}$  and  $MTT$  values. As  $MTT$  comprises  $MRT + MAT$ , and given that  $MRT$  was likely to be similar in the two treatment groups (as the clearance and volume of distribution would not be expected to be affected by the meal, and  $T_{1/2}$  values were not significantly altered), it can be assumed that the increases in  $MTT$  values were reflective of increased  $MAT$ . The observed increases in  $AUC_{0-last}$ ,  $AUC_{0-\infty}$  and  $MTT$  values therefore demonstrate that consumption of a high-fat, high-calorie meal results in delayed and enhanced absorption of orally administered dihydroartemisinin in comparison with absorption in the fasted state.

The effects of food on the absorption of piperavaquine and dihydroartemisinin are likely to reflect a number of factors, including delayed gastric emptying, increased bile salt secretion, increased solubility and dissolution of the drugs and/or changes in gastric pH [12]. While the exact mechanism is unknown, it is interesting to note that the absorption of piperavaquine appears to be directly related to the fat content of the meal. In fact, combining our findings with those of other studies, it can be seen that there is a

significant correlation between the fat content of the administered meal and the ratio of the fed/fast  $C_{max}$  and  $AUC$  data (Fig. 4).

Despite the reported significant alterations in the pharmacokinetics of piperavaquine and dihydroartemisinin with food, the clinical relevance of these findings is questionable. Given that the fat content of standard meals within endemic areas is relatively low in comparison with Western countries [23], and patients with malarial infection are typically anorexic [1], these results are unlikely to impact on the clinical utility of these drugs. While it has been suggested that piperavaquine–dihydroartemisinin should be co-administered with a high-fat meal to improve treatment exposure (as is recommended for artemether–lumefantrine [20, 24]), given the established efficacy of piperavaquine–dihydroartemisinin [2–6], this practice would be unlikely to improve treatment effectiveness—rather, it would impact on the safety profile of these drugs. In particular, as a potential risk of corrected QT (QTc) interval prolongation has been noted for piperavaquine–dihydroartemisinin, concurrent consumption of food with treatment administration would likely result in increased drug exposure and consequently an increased risk of cardiac adverse effects [11]. It should also be noted that while the standard diets of

**Fig. 4** Relationship between the fat content of the administered meal and the ratio of the piperazine fed/fasted data in terms of the maximum observed plasma concentration (black circles;  $R^2 = 0.995$ ,  $p < 0.001$ ) and the area under the plasma concentration–time curve (white circles;  $R^2 = 0.930$ ,  $p = 0.008$ ). The data are taken from the present study, Chinh et al. [13], Sim et al. [14], Annerberg et al. [15] and Hai et al. [16]



populations within malaria-endemic regions are not expected to impact significantly on the safety profiles of these drugs, co-administration of food with these anti-malarials by populations consuming typical Western diets should be avoided, especially given that food has been shown to have no impact on the efficacy of piperazine–dihydroartemisinin [17]. It is therefore a general recommendation that piperazine–dihydroartemisinin not be administered within  $\pm 3$  h of food consumption.

## 5 Conclusion

This study demonstrated that administration of piperazine–dihydroartemisinin combination therapy with a high-fat, high-calorie meal results in a substantial increase in the extent of exposure to piperazine and both delayed and enhanced absorption of dihydroartemisinin. The mechanism of the food–drug interaction for piperazine and dihydroartemisinin is unknown; however, for piperazine, the data indicate that the effect is directly correlated with the fat content of the meal. Although malaria patients in endemic regions are unlikely to consume a high-fat diet, given that piperazine–dihydroartemisinin has been associated with a potential risk of QTc interval prolongation, it is prudent to recommend that these drugs not be administered within  $\pm 3$  h of food consumption.

### Compliance with Ethical Standards

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**Research involving human participants** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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