Pharmacokinetics and Ex Vivo Pharmacodynamic Antimalarial
Activity of Dihydroartemisinin-Piperaquine in Patients with
Uncomplicated Falciparum Malaria in Vietnam

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ABSTRACT

Compared with healthy subjects, malaria causes a reduction in the mean oral clearance (1.19 versus 5.87 liter/h/kg) and apparent volume of distribution (1.47 versus 8.02 liter/kg) of dihydroartemisinin in Vietnamese patients following treatment with Artekin for uncomplicated *Plasmodium falciparum*. Dihydroartemisinin is responsible for most of the ex vivo antimalarial activity of dihydroartemisinin-piperaquine.

**Key words** pharmacokinetics · ex vivo antimalarial activity · dihydroartemisinin · piperaquine · Artekin · malaria

Artekin (dihydroartemisinin-piperaquine) is an artemisinin-based combination treatment (ACT) drug that is well tolerated and highly effective in the treatment of *Plasmodium falciparum* malaria in Southeast Asia and Africa, with cure rates typically greater than 95% following a standard 3-day course (8, 9, 15, 17). Despite its extensive use over the past 5 years, no data are available on the clinical pharmacokinetics of dihydroartemisinin in malaria patients following treatment with the ACT. Few studies have investigated the pharmacokinetics of piperaquine in malaria patients. The highly lipophilic drug exhibits biphasic disposition kinetics, with a large apparent volume of distribution, low oral clearance and a lengthy elimination half-life of about 3 to 4 weeks in malaria patients treated with Artekin (6, 18).

The present study investigated the clinical pharmacokinetic properties of dihydroartemisinin and piperaquine after a 3-day course of Artekin in the treatment of uncomplicated *P. falciparum* malaria in Vietnamese patients. In addition to assessing the in vivo response of the ACT, the ex vivo pharmacodynamic antimalarial activity of dihydroartemisinin-piperaquine in the patients’ plasma was investigated against two lines of
P. falciparum. The study was conducted at Military Hospital 175 in Ho Chi Minh City, Vietnam in 12 adult Vietnamese patients. The volunteers had to satisfy the following inclusion criteria: male, aged 17 to 55 years old, parasite density between 500 and 100,000 per µl of blood, axillary temperature ≥ 37.5ºC or a history of fever in the previous 24 h, written informed consent and willing to be followed-up for 35-days. The exclusion criteria were as follows: antimalarial treatment within the preceding 2 weeks, mixed plasmodial infection, and history of another serious medical disease. The patients acquired their infections in Binh Phuoc Province, about 120 km from Ho Chi Minh City. The patients stayed at the hospital for the entire 35-day follow-up period and because Ho Chi Minh City is free of malaria transmission the possibility of reinfection was avoided. Ethical approval for the study was obtained from the Review and Scientific Board of Military Hospital 175 and the Australian Defence Human Research Ethics Committee (ADHREC No. 379/05).

The patients were administered a weight-based 3-day course of Artekin (Holleykin Pharmaceuticals, China, each tablet contained 40 mg dihydroartemisinin and 320 mg piperaquine phosphate) at 2.4 mg/kg of dihydroartemisinin and 19.2 mg/kg of piperaquine of body weight per day, rounded up or down to the nearest half tablet, with day 0 being designated the first dose. Artekin was administered within 15 min of having a standard Vietnamese breakfast of rice, noodles and meat to enhance the absorption of piperaquine(13). Parasitemia and axillary temperature were measured before commencement of treatment and then every 8 h afterwards to determine parasite and fever clearance times. Blood samples (7 ml) were collected at 0, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, and 12 h using an indwelling cannula kept patent with heparinized saline after the last dose of Artekin on day 2. Subsequent heparinized blood samples were collected by venipuncture at days 3, 4, 7, 14, 21, 28 and 35 after commencement of treatment. Blood samples were centrifuged, and the separated plasma
samples were stored at –25°C until transported on dry ice to Australia for drug analysis, which was within 12 months of collection.

Plasma dihydroartemisinin concentrations were measured by liquid chromatography-tandem mass spectrometry, with a lower limit of quantification (LLOQ) of 1 ng/ml (2). The overall precision of analysis for dihydroartemisinin, as defined by the percent coefficient of variation of spiked samples was 6.3% at 1 ng/ml, 5.7% at 20 ng/ml, 4.6% at 200 ng/ml, and 6.6% at 750 ng/ml. The corresponding inaccuracy values were 1.3%, 1.5%, 0.7%, and 3.2%.

Plasma piperaquine concentrations were measured by a validated high-performance liquid chromatography method, with a LLOQ of 5 ng/ml (11). The precision of the assay was 10.3% at 10 ng/ml, 6.8% at 100 ng/ml, 6.7% at 500 ng/ml and 6.5% at 1,000 ng/ml. The corresponding inaccuracy values were 11.1%, 2.8%, 0.8% and 0.5%.

Pharmacokinetic parameters (peak concentration \([C_{\text{max}}]\), time to reach maximum concentration \([T_{\text{max}}]\), area under the concentration-time curve from day 2 to last data point \([\text{AUC}_{\text{day 2→last}}]\) and day 2 to infinity \([\text{AUC}_{\text{day 2→∞}}]\), terminal half-life \([t_{1/2}]\), apparent oral clearance \([CL/F]\), and apparent volume of distribution \([V/F]\)) were determined from the plasma concentration-time data using noncompartmental methods.

The ex vivo pharmacodynamic antimalarial activity of dihydroartemisinin-piperaquine was assessed by culturing malaria parasites in vitro in the presence of patients’ plasma samples collected after the last administration of Artekin using the method of Kotecka et al. (10), with minor modifications. Briefly, patients’ plasma samples (50 µl) were serially diluted twofold on microtiter plates with drug-free human plasma. The in vitro drug susceptibility of two lines of \(P. falciparum\) (chloroquine-sensitive D6 and chloroquine-resistant K1) to dihydroartemisinin and piperaquine were determined in parallel with the patients’ plasma samples. Fifty microlitres of spiked drug solutions prepared in human plasma were serially diluted twofold on microtiter plates using drug-free plasma. Inoculum (50 µl) was added to
each well containing either the patient’s plasma samples or spiked drug solutions, so that the total cell suspension (100 µl) contained 50% plasma in hypoxanthine-free plain LPLF-RPMI1640, with a final hematocrit of 2% and a parasitemia (>95% rings) of 1%. Tritiated-hypoxanthine incorporation was used to determine the extents to which parasite growth was inhibited by different drug concentrations or dilutions of the patients’ plasma during 48 h of incubation. The inhibitory concentration (IC$_{90}$ for spiked drug samples) and inhibitory dilution (ID$_{90}$ for patient plasma samples) were defined as the drug concentrations and the number of dilutions of the patient plasma sample, respectively, that produced a 90% inhibition of uptake of tritiated hypoxanthine by intraerythrocytic malaria parasites compared to drug-free plasma samples (controls).

The mean (± standard deviation) age of the patients was 26.3 (10.4) years, with a mean weight of 56.5 (7.8) kg. The patients had an admission geometric mean parasitemia of 15,198 parasites/µl of blood (range, 738 to 79,310 parasites/µl) and a mean temperature of 37.7 (1.3)ºC, with 42% (5 of 12) of patients with fever. Treatment with Artekin promptly reduced fever, with a median fever clearance time of 24 h (range, 16 to 48 h) and led to a rapid reduction in parasite density, with a median parasite clearance time of 28 h (range, 16 to 56 h). Over the 35-day follow-up period there was no recurrence of infection in the patients. The mean content of 5 Artekin tablets was 105.4 ± 4.8% for dihydroartemisinin and 109.0 ± 1.1% for piperaquine. Although none of the patients reported treatment with antimalarial drugs two weeks before commencing Artekin, no dihydroartemisinin or piperaquine were detected in their pre-dose samples, which confirmed no recent treatment with either artesunate or CV8 (dihydroartemisinin-piperaquine-primaquine-trimethoprim).

The mean plasma concentration-time profiles of dihydroartemisinin and piperaquine after a 3-day course of Artekin are shown in Fig. 1 and the pharmacokinetics of the two drugs are summarized in Table 1. Because dihydroartemisinin is rapidly eliminated with a $t_{1/2}$ of...
about 1 h in healthy Vietnamese subjects (2) and does not accumulate with daily administration, we were able to compare the pharmacokinetics of dihydroartemisinin in the Vietnamese patients after the last daily dose of the 3-day course of Artekin with values obtained in healthy Vietnamese subjects given a single-dose of Artekin (2). The mean $C_{\text{max}}$ and AUC of dihydroartemisinin were markedly higher in the Vietnamese patients than in healthy Vietnamese subjects ($C_{\text{max}}$ 698 versus 176 ng/ml; $AUC_{\text{day 2→∞}}$ 1,949 versus 398 ng·h/ml). Although the difference was not as large, Binh et al. (1) reported approximately a twofold higher mean $C_{\text{max}}$ (1,045 versus 480 ng/ml) and AUC (2,401 versus 932 ng·h/ml) of dihydroartemisinin in Vietnamese patients compared with healthy subjects administered a single-dose of dihydroartemisinin (120 mg) alone. Dihydroartemisinin was rapidly eliminated in the patients with a $t_{1/2}$ of 0.85 ± 0.15 h. The apparent oral clearance and apparent volume of distribution of dihydroartemisinin were 4.9-fold (1.19 versus 5.87 liter/h/kg) and 5.5-fold (1.47 versus 8.02 liter/kg), respectively, lower in the Vietnamese patients than in healthy subjects (2). A reduction in clearance and contraction in the apparent volume of distribution has also been reported for other antimalarial drugs such as mefloquine (7) and quinine (21) during the acute phase of malaria. A likely explanation for the increase in bioavailability of dihydroartemisinin in patients compared with healthy subjects is a decrease in hepatic clearance of dihydroartemisinin due to malaria (1). Alpha-acid glycoprotein levels also increase during acute malaria (16) and, similar to quinine, this may cause an increase in binding of the protein-bound dihydroartemisinin, with a reduction in the apparent volume of distribution of the drug (12).

In contrast to dihydroartemisinin, no data are available on the pharmacokinetics of piperaquine given as a 3-day course of Artekin in healthy volunteers, and thus a comparison between patients and healthy subjects could not be made to elucidate whether malaria affects the disposition of piperaquine after Artekin administration. In the present study, the mean
plasma concentration of piperaquine immediately before the last dose of Artekin was 131 ng/ml (range, 48 to 261 ng/ml). The mean $C_{\text{max}}$ of piperaquine was 568 ng/ml, which was reached at 5.7 h after the final dose. The mean $t_{1/2}$ of piperaquine of 17.8 days in the Vietnamese patients was less than 23 days in Cambodian adult patients (6) and 28 days in Burmese and Karen patients (18). However, the estimated elimination half-life in the present study might have been underestimated since piperaquine exhibits multiphasic elimination (19), with blood sampling limited to 35 days after starting Artekin treatment. It has been previously reported that the day 7 piperaquine concentration is an important determinant of therapeutic response to Artekin and patients with levels below 30 ng/ml are more likely to have a recurrence of malaria (14). All the patients had day 7 piperaquine concentrations greater than 30 ng/ml, with a range of 37 to 118 ng/ml.

In vitro drug susceptibility testing revealed that dihydroartemisinin was 15-times [mean $IC_{90}$ of 4.50 ± 0.25 versus 67.51 ± 1.87 nM (n=3)] and 25.2-times [(IC$_{90}$) of 4.85 ± 1.51 versus 122.16 ± 23.69 nM (n=7)] more active than piperaquine in inhibiting the D6 and K1 lines of *P. falciparum* malaria, respectively. The IC$_{50}$ values for dihydroartemisinin (2.57 ± 1.27 nM) and piperaquine (82.06 ± 35.25 nM) were about twofold higher than previously published data using the K1 line (3, 4). A likely explanation for this discordance in IC$_{50}$ values is the higher human plasma concentration (50% versus 10%) used in the present study compared with others using standard in vitro methods (M. Chavchich, unpublished data).

The ex vivo pharmacodynamic antimalarial activity profile (ID$_{90}$ values) of dihydroartemisinin-piperaquine corresponded with the plasma concentration-time data of dihydroartemisinin from 0.5 h to 10 h after dosing (Fig 1). Dihydroartemisinin’s superior potency, rapid onset of action and broader blood stage specificity (5, 20) compared to piperaquine appears to provide the major contribution to the rapid clearance of parasites and fever in the patients. At the $T_{\text{max}}$ of dihydroartemisinin, the mean number of dilutions of
patients’ plasma samples required to produce an ID$_{90}$ were 639 and 513 against the D6 and
K1 lines, respectively. However, by 12 h after the last dose of Artekin, most of the antimalarial activity in the patients’ plasma was solely due to piperaquine. At day 7 after commencement of treatment, the mean number of dilutions of patients’ plasma samples required to produce an ID$_{90}$ had declined to <2 for both D6 and K1 lines. At day 28, the patients’ piperaquine concentrations (mean 24.9 ng/ml, range, 9.8 to 41.8 ng/ml) were insufficient to completely kill either the D6 or K1 parasites. The clinical significance of the ex vivo pharmacodynamic antimalarial activity profile of dihydroartemisinin-piperaquine is that any mutant parasite that might survive physiological dihydroartemisinin and piperaquine concentrations during the 3-day treatment period of Artekin will only have to engage the less active piperaquine. Furthermore, because of piperaquine’s prolong terminal half-life and diminishing parasiticidal concentrations, selection pressure will be of concern for the potential emergence of resistant parasites to piperaquine.

In conclusion, malaria infection affects the disposition of dihydroartemisinin, with a reduction in both the apparent volume of distribution and apparent oral clearance of the drug. During the first 10 h after Artekin administration, the highly active and rapidly eliminated dihydroartemisinin contributes most of the ex vivo pharmacodynamic antimalarial activity of the dihydroartemisinin-piperaquine combination. After this period the less active and slowly eliminated piperaquine will lead to selection pressure for the potential development of drug resistance, which may limit the future effectiveness of dihydroartemisinin-piperaquine, particularly in non-immune patients.
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FIG. 1. Mean (standard deviation) plasma dihydroartemisinin (○) and piperaquine (●) concentration-time profiles after the last dose of a 3-day course of Artekin (2.4-mg/kg of dihydroartemisinin and 19.2-mg/kg of piperaquine daily) for the treatment of uncomplicated *Plasmodium falciparum* malaria in 12 Vietnamese patients. The inset shows the piperaquine concentrations from day 2 to day 35 after commencement of treatment. The ex vivo pharmacodynamic antimalarial activity profile (mean ID<sub>90</sub> values, ▲) of patients' plasma samples after Artekin treatment derived from the K1 line of *P. falciparum*.
<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Dihydroartemisinin</th>
<th>Piperaquine</th>
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<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>698 ± 169</td>
<td>568 ± 288</td>
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<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>2.8 ± 1.1</td>
<td>5.7 ± 1.9</td>
</tr>
<tr>
<td>$\text{AUC}_{\text{day 2-last}}$ (ng·h/ml)</td>
<td>1,946 ± 445</td>
<td>44,430 ± 17,435</td>
</tr>
<tr>
<td>$\text{AUC}_{\text{day 2-}}$ (ng·h/ml)</td>
<td>1,949 ± 445</td>
<td>56,418 ± 20,144</td>
</tr>
<tr>
<td>$AUC_{\text{extrap}}$ (%)</td>
<td>0.14 ± 0.17</td>
<td>29.0 ± 22.1</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>0.85 ± 0.15</td>
<td>427 ± 128</td>
</tr>
<tr>
<td>$CL/F$ (liter/h/kg)</td>
<td>1.19 ± 0.28</td>
<td></td>
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<tr>
<td>$V/F$ (liter/kg)</td>
<td>1.47 ± 0.46</td>
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Data are presented as mean ± SD.

$AUC_{\text{extrap}}$ (%) = $\frac{(\text{AUC}_{\text{day 2-}} - \text{AUC}_{\text{day 2-last}})}{(\text{AUC}_{\text{day 2-}})} \times 100$
REFERENCES


