A PHARMACOKINETIC AND PHARMACODYNAMIC STUDY OF ARTESUNATE FOR VIVAX MALARIA

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Abstract. To investigate the pharmacokinetic and pharmacodynamic properties of artesunate (ARTS) and its active metabolite dihydroartemisinin (DHA) in Plasmodium vivax infections, 12 male Vietnamese adults with slide-positive vivax malaria received either intravenous ARTS (120 mg; group 1) or oral ARTS (100 mg; group 2) with the alternative preparation given 8 hr later in a randomized, open, cross-over study. Following intravenous injection, ARTS had a peak plasma drug concentration \((C_{\text{max}})\) of 35.6 \(\mu\)M (13.7 mg/L), an elimination half-life \((t_{1/2})\) of 2.2 min, a clearance \((CL)\) of 3.0 L/hr/kg, and a volume of distribution \((V)\) of 0.16 L/kg. Dihydroartemisinin had a \(C_{\text{max}}\) of 7.7 \(\mu\)M (2.2 mg/L), a \(t_{\text{max}}\) of 8 min, a \(t_{1/2}\) of 37 min, an apparent CL of 1.1 L/hr/kg, and an apparent V of 0.9 L/kg. Following oral ARTS, the mean relative bioavailability of DHA was 85%, the \(C_{\text{max}}\) was 3.0 \(\mu\)M (0.85 mg/L), the \(t_{\text{max}}\) was 75 min, and \(t_{1/2}\) was 40 min. The mean time to 50% reduction in the parasite count \((PCT_{50})\) and median fever clearance time were 3 hr and 16 hr, respectively. Following intravenous ARTS (group 1), the \(PCT_{50}\) for total parasites, rings, trophozoites, and gametocytes was 3.3 hr, 3.2 hr, 4.0 hr, and 3.6 hr, respectively. This study confirms that ARTS is effective against \(P. vivax\), with rapid clearance of sexual and asexual forms of the parasite. Artesunate is a suitable initial treatment for vivax malaria, or when the plasmodial species cannot be reliably identified.

Pharmacokinetic data for artesunate (ARTS) and its active metabolite dihydroartemisinin (DHA) have been reported recently in Vietnamese children and adults with uncomplicated falciparum malaria. Studies in healthy volunteers have shown that peak plasma concentrations of DHA are significantly lower. There is, therefore, a need to determine the pharmacokinetic properties of ARTS in vivax and other benign human malarial species to ensure that relatively low drug concentrations do not result from administration of the recommended dosing regimen used in falciparum malaria, a situation that might contribute to high recrudescence rates.

Pharmacodynamic studies of ARTS in falciparum malaria demonstrate rapid parasite clearance, but are limited by the fact that only parasite forms in the first half of the life cycle are present in peripheral blood. All stages of development can be seen in vivax malaria. This provides an opportunity to assess the effects of antimalarial drugs across the 48-hr parasite life cycle.

The aims of the present study were to obtain pharmacokinetic data for ARTS and DHA in patients with vivax malaria and to make a preliminary assessment of the in vivo stage specificity of ARTS treatment.

PATIENTS AND METHODS

Patients. Twelve male patients with vivax malaria were recruited from Bao Loc Hospital, Lam Dong Province, Vietnam. The diagnosis was confirmed by microscopy and a complete clinical assessment including drug history was performed. Patients treated with ARTS or DHA in the previous 8 hr, artemisinin in the previous 12 hr, or artemether in the previous 24 hr were excluded. These criteria were based on available pharmacokinetic data at the time of the study and represented a period of at least five times the respective elimination half-life (40 min for DHA, 2.2–2.3 hr for artemisinin, and 4.2 hr for artemether). The study was approved by the Ministry of Health, Vietnam, and the University of Western Australia Human Rights Committee. All patients gave written informed consent to participate and were advised that they could withdraw from the study at any stage without prejudice to their continuing care.

Study design and procedures. Patients were randomized to receive intravenous (iv) ARTS (120 mg in 10 ml of 5% [w/v] dextrose) given as a bolus injection over a 2-min period (group 1) or oral ARTS (100 mg composed of 2 × 50 mg tablets; group 2). The alternative preparation was given 8 hr later in an open cross-over design. Both ARTS formulations were obtained from the Guilin No. 2 Pharmaceutical Factory (Guangxi, People’s Republic of China). A single 750-mg dose of mefloquine was administered 24 hr after admission to the study.

Venous blood samples were obtained from the arm opposite to that used for drug administration. In the case of iv ARTS, sampling was done at 0, 5, 7, 9, 12, 15, 20, 30, 45, 60, and 90 min and 2, 3, 4, and 8 hr and for oral ARTS at 0, 15, 30, 45, 60, 75, 90, and 105 min and 2, 3, 4, 6, and 8 hr. Blood was collected into fluoride-oxalate tubes and chilled immediately to prevent ARTS degradation by plasma esterases. After centrifugation within 30 min to minimize hemolysis, separated plasma was stored at −25°C until analyzed. Thick and thin blood films were prepared from the hourly samples, and every 4 hr thereafter, until parasite...
clearance. Vital signs were monitored every 4 hr. Patients were discharged when afebrile and aparasitemic.

**Pharmacokinetic and pharmacodynamic analysis.** The ARTS powder for injection and tablets were assayed by high-performance liquid chromatography (HPLC), as reported previously. Since the mean content of the ARTS injection was 59.2 mg, pharmacokinetic analysis assumed the nominal strength (60 mg). The mean content of the ARTS tablets was 44.8 mg; thus, pharmacokinetic parameters were calculated assuming the assayed potency of 45 mg per tablet.

Plasma samples were assayed by HPLC. The between-run coefficients of variation for ARTS were 19.9% and 8.3% at 1,090 nM and 4,380 nM, respectively, and 21.2% and 10.6% for DHA at 920 nM and 3,780 nM, respectively (n = 12). The stability of both ARTS and DHA in plasma frozen at −25°C for at least eight months has been confirmed previously.

Pharmacokinetic parameters were determined from the plasma concentration-time data using noncompartmental analysis, and included the total area under the plasma concentration-time curve (AUC₀→∞), elimination rate constant (k), elimination half-life (t₁/₂), mean residence time (MRT), clearance (CL), volume of distribution (V), the observed maximum plasma drug concentration (Cₘₓ), and the time after the dose to achieve the observed maximum plasma drug concentration (tₘₓ). Bioavailability was calculated as F = (AUC₀→∞/AUC₀→∞) × (Doseₚ/DoseGRAL), with correction for tablet potency. Pharmacokinetic parameters derived for DHA assume complete bioconversion from ARTS.

Stained blood films were examined by a single microscopist (NPT). Thick films were used to determine parasite density in all patients. The number of asexual parasites per microliter of whole blood was determined by counting white blood cells (WBC) in high-power microscopic fields containing a total of 500 parasites where the ratio of parasites to WBC was more than one, or the number of parasites per 1,000 WBC where the ratio of parasites to WBC was less than one. The parasitemia was calculated as the product of the parasite to WBC ratio and the WBC count.

Thin films were used for counting of parasite stages that were differentiated using conventional descriptions. Young trophozoites were small rings with even, regular cytoplasm and no scattered pigment. Mature trophozoites were larger than rings and had an irregular, fragmented cytoplasm and scattered pigment. Schizonts were segmented forms with clear definition of merozoites and associated Schüffner’s dots. Gametocytes were nonsegmented with multiple malaria-pigment granules in an enlarged, stippled erythrocyte. The proportion of each stage at each time point was determined from examination of > 100 parasites per film. The absolute density of each stage was calculated from this proportion and the total parasite count.

The time to 50% reduction in the parasite count (PCTₜ₀) was determined by linear interpolation of the parasite density-time data for total parasites, young trophozoite rings, mature trophozoites, schizonts, and gametocytes. Fever clearance time (FCT) was the time at which the oral temperature decreased to less than 37.5°C.

**Statistical analysis.** Differences between paired samples were analyzed by the Student’s t-test. Multiple linear regression was used to assess associations between variables.

### RESULTS

**Clinical course.** Biochemical variables were within laboratory reference ranges in all patients (Table 1). The mean PCTₜ₀ and median FCT were 3 and 16 hr, respectively (Table 1). There were no side effects after either iv or oral ARTS. Since there were no significant differences in demographic, clinical, or laboratory variables between the two groups, data were pooled (Table 1). Two patients who had taken antimalarial drugs at least 24 hr prior to admission (ARTS and quinine in one case and Fansidar® [sulfadoxine-pyrimethamine]; [E Hoffmann La Roche, Basel, Switzerland] in the other) were excluded from the pharmacodynamic analysis.

**Pharmacokinetic analysis.** Pharmacokinetic data are summarized in Table 2 and presented in Figure 1. Following
iv administration, ARTS had a mean extrapolated peak concentration ($C_{\text{max}}$) of 35.6 μM (13.7 mg/L) at the end of the 2-min injection. The median $C_{\text{max}}$ for DHA was 7.7 μM (2.2 mg/L), and mean elimination $t_{\text{1/2}}$ was 37 min (Table 2 and Figure 1A). Mean values of CL (1.1 L/hr/kg) and V (0.9 L/kg) for DHA assume complete conversion of ARTS to DHA.11 Following oral dosing, ARTS concentrations above the assay limit of sensitivity (130 nM or 50 μg/L) were observed in only five of 12 patients. The mean bioavailability of ARTS in these five patients was estimated to be 23%. Interpretable pharmacokinetic data for DHA were obtained from 11 of the 12 patients after oral ARTS (Table 2 and Figure 1B). Plasma concentration-time data for the other patient were insufficient for comprehensive analysis. Mean relative bioavailability of DHA (n = 11), which also assumes complete conversion of ARTS to DHA before subsequent metabolism, was 85%.

**Pharmacodynamic analysis.** There were no correlations between either the PCT$_{50}$ for total parasite densities or FCT and the AUC, $C_{\text{max}}$, or MRT for DHA. Geometric mean parasite densities for total parasites, rings, trophozoites, and gametocytes from the patients who received iv ARTS as the first dose (group 1) are presented in Figure 2. Schizonts were observed at only one time point in two patients; thus, these data are not shown. Since two patients from group 2 were excluded due to prior antimalarial therapy, and because there were marked differences in MRT, $C_{\text{max}}$, and $t_{\text{max}}$ for DHA following iv and oral ARTS therapy (Table 2), data from the patients in group 2 were not analyzed.

The baseline geometric mean parasite count in group 1 patients was 2,650/μL, comprising 72% rings, 16% trophozoites, and 12% gametocytes (Figure 2). Parasite clearance was rapid, with mean (95% confidence interval) PCT$_{50}$ values of 3.3 (2.6–4.0) hr for total parasites, 3.2 (2.9–3.5) hr for rings, 4.0 (0.5–7.5) hr for trophozoites, and 3.6 (2.0–5.4) hr for gametocytes (n = 6). At 8 hr after iv ARTS, the geometric mean parasite densities for total parasites, rings, trophozoites, and gametocytes were ≤ 13% of the original
counts. The total parasitemia was generally less than 400/μL beyond 8 hr, a density below which the precision of counts is low (coefficient of variation > 20%).

FIGURE 2


discussion

Our study provides novel pharmacokinetic data for ARTS in vivax malaria and confirms that this drug is rapidly effective against Plasmodium vivax when given either orally or by iv injection. The pharmacokinetic properties of ARTS and its active metabolite DHA in our patients were similar to those reported in uncomplicated falciparum malaria.2,3,7 These results suggest that dosage regimens used currently for initial treatment of falciparum malaria can be adopted in cases of vivax malaria.

Previous studies have shown that ARTS is cleared rapidly (t½ = 2-3 min) and has a low volume of distribution (< 0.2 L/kg).3,12,13 The pharmacokinetic parameters for DHA following ARTS administration have been reported in 10 volunteers,4,12 and in 10 children4 and 32 adults5,7 with uncomplicated falciparum malaria. The present study also demonstrates that high plasma concentrations of ARTS and DHA are achieved following iv ARTS and that the elimination t½ of ARTS and DHA are comparable with those found in previous studies,2,3,7 in uncomplicated falciparum malaria.

Compared with iv administration, the disposition of oral ARTS is more variable. Our data are consistent with previous reports that plasma ARTS concentrations are both transient and near or below assay detection limits3,4 and suggest extensive first-pass metabolism by esterases in the gut wall and/or liver. Nevertheless, because of the high relative oral bioavailability of DHA after oral ARTS (> 80%), this form of treatment given in an appropriate mg/kg dose should be adequate for either uncomplicated falciparum5 or vivax malaria.

Using the same formulation and a similar mg/kg oral dose of ARTS in children to that in our patients, Bethell and others2 found AUC and Cmax values for DHA consistent with those in the present study and in a previous study from our group.1 In contrast, Benakis and others4 and Navaratnam and others5 used ARTS tablets (Plasmodtrim®-Lactab®) obtained from Mepha Pharmaceuticals (Basel, Switzerland) in healthy volunteers and found AUC and Cmax values that were less than 50% of those in our patients. This disparity might reflect malaria infection per se and/or ethnicity. However, differences in formulation and analytical techniques are alternative explanations, and should be evaluated before direct comparisons between pharmacokinetic studies are made.

There was a rapid decrease in total parasitemia after ARTS administration. The mean PCT50 of 3 hr in our patients with vivax malaria was shorter than the mean PCT50 of 6.5 hr seen with the same treatment regimen in falciparum malaria.1 This may have been due to the different sensitivities of the plasmodial species or to the lower baseline parasitemia in the present series. The clearance of both rings and trophozoites after ARTS administration generally ran in parallel to post-treatment changes in the total parasitemia. Furthermore, the mean PCT50 for young trophozoite rings (4 hr) was much shorter than the average time for maturation of a young trophozoite ring to a mature trophozoite (12–16 hr). This suggests that the clearance of rings and trophozoites after treatment with ARTS was due primarily to common reticuloendothelial removal processes rather than through continued maturation. However, there was a shift in the ratio of rings to trophozoites 6–10 hr after ARTS dosing, with the latter becoming predominant. Although counts by stage were close to the limit of quantitation, the change was consistent within each subject. One explanation for this observation is that a small proportion of young rings (< 5%) remained unaffected by the first dose of ARTS and subsequently matured to trophozoites. This hypothesis is consistent with in vitro studies that found young rings to be the stage least affected by exposure to artemisinin.14,15

The present data demonstrate that the pharmacokinetic properties of ARTS and its active metabolite DHA in Vietnamese patients with vivax malaria are similar to those reported previously in uncomplicated falciparum malaria.2 Our preliminary in vivo stage-specific data also indicate that rings and trophozoites of P. vivax are cleared rapidly from the blood. Artemisinin derivatives have been shown to clear P. falciparum gametocytes in humans16 and may reduce transmission of the infection.17 Our data show that ARTS treatment is associated with a rapid decrease in P. vivax gametocyte density, a finding that may also have important epidemiologic implications.

Vivax malaria provides a robust model for pharmacodynamic evaluation since all stages of parasite development are present in the peripheral blood and the unquantifiable contribution of sequestered trophozoites and schizonts to post-treatment clearance curves in falciparum malaria is avoided. Further studies examining differential clearance of parasite stages with extended sampling periods may help in our understanding of the mechanisms of both parasite clearance and recrudescence after artemisinin treatment.

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