Pharmacokinetics of Oral Doxycycline during Combination Treatment of Severe Falciparum Malaria

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The pharmacokinetics of oral doxycycline administered at 200 mg every 24 h were investigated in 17 patients recovering from severe Plasmodium falciparum malaria. The data suggest that the doses of doxycycline currently recommended (circa 3.5 mg/kg of body weight daily) may not be optimal.

Oral doxycycline, a lipophilic bacteriostatic antibiotic, is the recommended secondary oral antimalarial treatment during the recovery of nonpregnant adults from severe Plasmodium falciparum malaria. It is combined with primary parenteral treatments to ensure the clearance of all remaining parasites and to prevent the emergence of resistance to the primary antimalarial. There is no published information on the pharmacokinetics of doxycycline in patients with severe malaria, or indeed with any infectious disease, and the recommended dosage regimens currently used are empirical; they include 3 mg/kg of body weight/day, 100 mg every 12 or 24 h, and 200 mg every 24 h, with or without a loading dose (1, 2, 27, 28).

In healthy volunteers who have taken oral doxycycline, the maximum doxycycline plasma concentrations (Cmax) of 1.5 to 7.0 µg/ml are usually reached within 3 h, and the drug has a half-life of 14 to 24 h (16, 26). Concurrent ferrous sulfate, antacids, phenobarbital, heavy alcohol intake, and undernutrition are known to accelerate the clearance of doxycycline (11, 12, 13, 15, 16, 21). It is not known whether the pharmacokinetics of doxycycline are affected by same-day ferrous sulfate or malaria, and there is little information on the in vitro sensitivity of P. falciparum parasites to doxycycline (3, 8, 18, 23). It is not appropriate to assess the pharmacodynamic effects of doxycycline alone in vivo, as the drug should not be used alone. In combination, the antimalarial effect of the primary parenteral drugs predominates. The optimum dosage can be determined only from comparisons of the pharmacokinetics and by extrapolations from in vitro parasite sensitivity assessments.

The study was conducted from 1999 to 2002 at Mae Sot Hospital, Tak Province, western Thailand from May to July and November to December of each year. Patients aged 16 to 65 years were included if they or an attending relative were able and willing to give informed written consent and if they had slide-confirmed, single species P. falciparum parasitemia; no contraindications to doxycycline; a negative pregnancy test for females of reproductive age; no requirement for interacting drugs (warfarin, bismuth, antacids, aluminum, magnesium, calcium, iron, zinc salts, or sulfamate); and clinically severe malaria (14, 29). Ethical clearance was granted by the Ministry of Public Health, Government of Thailand. Patients were randomized to receive either intravenous artesunate (Guilin No. 2 Pharmaceutical Factory, Guangxi, People’s Republic of China) at 2.4 mg/kg of body weight, followed by 1.2 mg of artesunate/kg 12 h later and then 1.2 mg of artesunate/kg/day, or quinine dihydrochloride (Government Pharmaceutical Organization, Bangkok, Thailand) at 20 mg of salt/kg as a loading dose infused over 4 h, followed by 10 mg of salt/kg infused over 2 h three times a day. When patients were able to take tablets, either oral artesunate, to a total intravenous and oral dose of 12 mg/kg or quinine sulfate (Government Pharmaceutical Organization), at 10 mg of salt/kg every 8 h, was given for a total treatment course of 7 days. This regimen was supplemented with doxycycline (Vibramycin, 100-mg capsules; Pfizer) at 200 mg/day at 0800 h for 7 days.

Patients were allotted ferrous sulfate (200 mg) either at 1300 and 1800 h or every 12 h after the 7-day course of doxycycline. The first six patients were designated ad hoc, while the remaining 11 patients were randomly placed in one of the two ferrous sulfate treatment groups. The choice of treatment group was kept in an opaque envelope, which was opened only after the patient consented to the study. Patients were counseled to take all drugs as prescribed.

Blood samples for hematocrit and parasitemia tests and biochemistry were taken from the patients on admission. Axillary temperature, hematocrit, and parasite counts were measured every 6 h until parasite clearance (14). When the patient was able to eat, doxycycline was started. Four milliliters of venous blood was taken at 0, 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h after doxycycline administration. Plasma samples were stored at −30°C until analysis. After liquid-solid extraction with a Bond Elut C18 cartridge, the plasma doxycycline concentration was determined by reverse-phase high-performance liquid chromatography with UV detection (19), by using demeclocycline as the internal standard. The lower limit of
quantification was 25 ng/ml and the intra- and interassay coefficients of variation were lower than 3 and 6.7%, respectively.

Observed plasma doxycycline concentrations (C\text{max}) and time to C\text{max} (T\text{max}) were used, and the area under the curve from 0 to 24 h (AUC\text{0–24}) was calculated by using the linear trapezoidal rule, with log-linear extrapolation to infinity for AUC\text{0–\infty} (22) (Fig. 1). The AUC after the dose on day 7 was also adjusted by subtracting the AUC calculated from the day 7 trough and the elimination rate constant. The mean residence time, clearance, and volume of distribution for day 7 were calculated by using this adjusted estimate of AUC. A noncompartment model was used (Model 200, WinNonlin 3.1; Pharsight Corp., Cary, N.C.). Clearance was calculated as dose/AUC\text{0–\infty}. The antimalarial drug susceptibilities of fresh \textit{P. falciparum} isolates to doxycycline were determined by using the microdilution radioisotope method (incubation for 48 h in the presence of 5% O\textsubscript{2}, 5% CO\textsubscript{2}, and 90% N\textsubscript{2}), from a population similar to that from which the patients recruited to this study came, at the Shoklo Malaria Research Unit, Mae Sot, Thailand (4).

Seventeen patients were recruited to the study during their recovery from severe malaria (Table 1). The median interval between admission and start of oral doxycycline was 4 days (range, 0 to 7 days). Phenobarbital (a 200-mg intramuscular dose) was given for a median of 4 days (range, 0 to 6 days) before the first dose of doxycycline for nine patients. Two patients vomited within 30 min of the first dose of doxycycline and were successfully redosed 24 h later. All patients completed the full course of doxycycline. The median parasite clearance time was 66 h (range, 36 to 84 h). All patients survived, and 10 were again in attendance at the hospital on the morning of the seventh doxycycline dose.

Median serum bicarbonate levels were 21 (range, 20 to 27) and 25 (range, 22 to 28) mmol/liter, creatinine levels were 64 (range, 48 to 680) and 64 (range, 48 to 528) µmol/liter, urea levels were 12.7 (range, 5.3 to 64.1) and 9.6 (range, 5.5 to 45.3) mmol/liter, and total bilirubin levels were 8.9 (range, 2.2 to 18.9) and 7.8 (range, 2.2 to 9.0) µmol/liter on the first and last days, respectively. Serum bilirubin, creatinine, and urea levels were significantly higher, and serum bicarbonate levels were significantly lower, on admission than on the day of the first doxycycline dose (P < 0.02). After acute administration, the median elimination half-life was 10.5 h (range, 6.9 to 17.9 h) (Table 2). Although the sample size was small, there were no significant differences in the pharmacokinetic parameters between those who did and did not have ferrous sulfate ≥5 h after the doxycycline. Before the seventh doxycycline dose was administered, the median trough plasma doxycycline was 0.56 µg/ml (range, 0 to 2.28 µg/ml). The actual and adjusted C\text{max}, AUC\text{0–24}, and AUC\text{0–\infty} after the last dose were not significantly larger than these measures after the first dose (P > 0.05). The median ratio of unadjusted AUC\text{0–24} between that after the seventh doses and first doses was 1.2 (range, 0.5 to 2.8). The 24-h accumulation index (22) was 1.26.

Of 633 \textit{P. falciparum} isolates from primary infections collected in 2001 to 2004, the geometric mean (95% confidence interval [CI]) of the 50% effective concentration (EC\text{50}) was 4.86 µg/ml (range, 4.58 to 5.15 µg/ml) (equivalent to 10.1 µmol/liter [range, 9.5 to 10.7 µmol/liter]). After the first and last dose of doxycycline, three and four patients, respectively, had a C\text{max} exceeding the estimated EC\text{50}. In comparison to previous reports on the pharmacokinetics of doxycycline, the C\text{max}, T\text{max}, and volume of distribution described here are similar, but the half-life was relatively short at 10.5 h (16, 26). Neither acute malaria nor concurrent ferrous sulfate given ≥5 h after doxycycline apparently altered doxycycline pharmacokinetics. Potential problems with the study include the small sample size and potential suboptimal adherence to doxycycline and ferrous sulfate regimens. However, pill counts on day 7 suggested that all medication had been taken and that the study would reflect a real-life situation without observed therapy.

The geometric mean for the \textit{P. falciparum} doxycycline EC\text{50} of 10.1 µmol/liter described here is similar to those in previous reports from Senegal (18) and western Thailand (23) but double those described for isolates from Cambodia and West Af-
on thick film assuming a white cell count of 8 × 10⁹/liter, to mmol/liter, multiply by 2.08. To convert doxycycline concentration (µg/ml) to mmol/liter, multiply by 2.08.

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Normal range, 2.5 to 6.7 mmol/liter.

Normal range, 70 to 150 µmol/liter.

Normal range, 3.5 to 5.5 mmol/liter.

Normal range, <4 mmol/liter.

Normal range, 10 to 17 µmol/liter.

Normal range, 3 to 17 µmol/liter.

Normal range, 21 to 28 mmol/liter.

Normal range, 3 to 17 µmol/liter.

Normal range, 3.5 to 5.5 mmol/liter.

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with a doxycycline half-life of 10.5 h, twice daily dosing may be more appropriate. A loading dose of twice the maintenance dose (i.e., 400 mg) would allow plasma doxycycline concentrations to rise above a therapeutic level quickly (22). Although doses of up to 600 mg of doxycycline may be well tolerated when taken with food and water (9), patients recovering from severe disease frequently have difficulty eating.

Without reliable information on the relationship between plasma concentration of doxycycline and the therapeutic response, and thus on which pharmacokinetic variable is most strongly associated with pharmacodynamic response, doubt will remain as to the optimum regimen. There is limited clinical trial evidence that doxycycline is an effective companion drug to quinine or the artemisinin derivatives in the treatment of uncomplicated or severe malaria (5, 6, 10, 17, 20, 24, 25). Longitudinal monitoring of the sensitivity of *P. falciparum* to doxycycline and further investigation of doxycycline pharmacokinetic-antimalarial pharmacodynamic interrelationships, the tolerability of higher doses, adherence, and standardization of methods will be important to ensure that the correct dose of doxycycline is used in combination therapy.

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