Pharmacokinetics, Pharmacodynamics, and Allometric Scaling of Chloroquine in a Murine Malaria Model

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Chloroquine (CQ) is an important antimalarial drug for the treatment of special patient groups and as a comparator for preclinical testing of new drugs. Pharmacokinetic data for CQ in animal models are limited; thus, we conducted a three-part investigation, comprising (i) pharmacodynamic studies of CQ and CQ plus dihydroartemisinin (DHA) in Plasmodium berghei-infected mice, (ii) pharmacokinetic studies of CQ in healthy and malaria-infected mice, and (iii) interspecies allometric scaling for CQ from 6 animal and 12 human studies. The single-dose pharmacodynamic study (10 to 50 mg CQ/kg of body weight) showed dose-related reduction in parasitemia (5- to >500-fold) and a nadir 2 days after the dose. Multiple-dose regimens (total dose, 50 mg/kg CQ) demonstrated a lower nadir and longer survival time than did the single same dose. The CQ-DHA combination provided an additive effect compared to each drug alone. The elimination half-life (t1/2), clearance (CL), and volume of distribution (V) of CQ were 46.6 h, 9.9 liters/h/kg, and 667 liters/kg, respectively, in healthy mice and 99.3 h, 7.9 liters/h/kg, and 1,122 liters/kg, respectively, in malaria-infected mice. The allometric equations for CQ in healthy mammals (CL = 3.86 × W0.56, V = 230 × W0.94, and t1/2 = 123 × W0.2) were similar to those for malaria-infected groups. CQ showed a delayed dose-response relationship in the murine malaria model and additive efficacy when combined with DHA. The biphasic pharmacokinetic profiles of CQ are similar across mammalian species, and scaling of specific parameters is plausible for preclinical investigations.

Chloroquine (CQ) was introduced 50 years ago as an alternative to quinine (25, 70). It became the drug of choice for both prophylaxis and treatment of malaria, but resistance has caused a decline in the contemporary clinical use of chloroquine (25, 35, 40, 70, 72). Nevertheless, CQ remains one of the most important antimalarial drugs, especially in the treatment of vivax malaria and special patient groups, such as children and pregnant women (32, 37, 48, 51, 73), not least because it is inexpensive and well tolerated and has a rapid onset of action. Recent studies have clarified the mechanism of CQ resistance and shown that clinical efficacy of CQ may return at least a decade after it has been withdrawn from use, prompting suggestions that CQ could reemerge as an important therapeutic option for malaria, most likely in combination with artemisinin compounds or chemosensitizing agents (25, 28, 36, 42, 45, 70).

Notwithstanding its clinical status, CQ has a prominent role as a comparator for in vitro and in vivo preclinical testing of new antimalarial drugs (54, 56, 68, 74). Remarkably, there is a paucity of pharmacokinetic and pharmacodynamic data for CQ in preclinical animal models, particularly in murine malaria models (4, 11, 29).

The pharmacokinetic parameters for CQ exhibit wide interindividual variation, although limitations in study design and analytical procedures have been contributing factors (18, 64, 65). Recent clinical studies have provided more robust pharmacokinetic parameters for CQ, including data in children and pregnant women (32, 37, 51). CQ apparent clearance (CL/F) and apparent volume of distribution (V/F) data are matrix dependent, due to a high blood/plasma ratio (>5:1), but elimination rate constant (k) and half-life (t1/2) are comparable from blood and plasma pharmacokinetic profiles (18, 35, 65).

For example, the clearance of CQ from whole blood and plasma is approximately 0.1 to 0.25 liter/h/kg of body weight and 0.5 to 0.9 liter/h/kg, respectively, in healthy volunteers, and the t1/2 of CQ is 150 to 290 h (17–19, 32, 35, 47, 59). Approximately 30 to 50% of the dose of CQ is converted by hepatic metabolism to the active metabolite, monodesethylchloroquine (DCQ) (18, 59).

Pharmacokinetic studies of CQ in animal models are limited, but data from the more detailed investigations show biphasic elimination, long β-phase half-life, and large volume of distribution (1, 2, 50). As CQ is widely used in preclinical animal studies, we have conducted a three-component investigation, comprising (i) pharmacodynamic studies of single- and multiple-dose CQ, and a single-dose combination of dihydroartemisinin (DHA) plus CQ, in Plasmodium berghei-
infected mice; (ii) pharmacokinetic studies of CQ in control and *P. berghei*-infected mice; and (iii) an interspecies allometric analysis of pharmacokinetic parameters for CQ.

**MATERIALS AND METHODS**

May-Grunwald-Giemsa stain was obtained from the Department of Microbiology, Royal Perth Hospital, Australia. CQ diphosphate (C₁₈H₂₈Cl₂N₂O₅, 2·H₂PO₄) was dissolved in water (pH 7.4) to make a 10 mg/ml stock solution. The solution was filtered prior to injection (Millex-HV 0.45-μm filter). The stock solution was then diluted to the required concentration for a standard 100-μl injection and prepared by dissolving CQ diphosphate in water (approximately 30 mg/ml) and sterilized and acidified with water (pH 2.5) to prevent bacterial infections (62).

**Parasites.** *Plasmodium berghei* ANKA parasites (Australian Army Malaria Research Institute, Enoggera, Australia) were maintained by continuous weekly passage of malaria parasites. Animals were housed at 22°C under a 12-h light-dark cycle with free access to sterilized commercial food pellets (Glen Forrest Stockfeeders, Perth, Australia) and sterilized, acidified water (HCl, pH 2.5) to prevent bacterial infections (62).

**Drug treatment and pharmacodynamic study.** CQ diphosphate is freely soluble in aqueous media, and solutions have a pH of about 4.5. CQ solutions were prepared by dissolving CQ diphosphate in water (approximately 30 mg/ml) and diluting it to the required concentration for a standard 100-μl injection volume. The solution was filtered prior to injection (MilliLIX-HV 0.45-μm filter; Millipore, Molsheim, France).

Based on previous reports (9, 11, 13) and a pilot study (data not shown), CQ doses of 0, 300, 600, 900, and 1,500 μg CQ (approximately 0.5, 1.0, 1.5, and 3.0 mg/kg, respectively, for a 30-g mouse) were selected for the dose ranging studies. The CQ solutions were administered to each mouse by i.p. injection, 6 h after inoculation with 10⁶ *P. berghei*-parasitized erythrocytes (anticipated parasitemia of 3 to 5%, confirmed by microscopy). Treatment groups comprised 9, 7, 9, and 7 mice for 10, 20, 30, and 50 mg/kg CQ, respectively, while the control group comprised 4 mice.

In the multiple-dose studies, two dosage strategies that delivered a total dose of 1,500 μg CQ (50 mg/kg) were compared (7 mice per group). The first regimen comprised three doses, 20 mg/kg, 20 mg/kg, and 10 mg/kg CQ, at 12-hour intervals. As *P. berghei* has a 24-h erythrocyte cycle (compared to 48 h in the principal human malaria), this dose interval had temporal similarity to a once-daily dose in humans. The second dose regimen comprised five doses of 10 mg/kg CQ at 12-hour intervals. In both multiple-dose regimens, the first dose was administered 64 h after parasite inoculation.

In the combination study, mice (*n* = 8) received single i.p. doses of 30 mg/kg DHA and 30 mg/kg CQ, 64 h after inoculation (DHA was dissolved in a 60:40 mixture of dimethyl sulfoxide and polysorbate 80). The doses of DHA and CQ were shown previously (23) and in the present study to be subtherapeutic, with peripheral parasitemia above detectable limits throughout the course of the study, thus facilitating a comparison of the single doses and the drug combination.

**Pharmacokinetic study.** Pharmacokinetic parameters for CQ and DCO were determined from 125 uninfected male Swiss mice (6 weeks old; mean weight, 33.8 ± 2.8 g) and 125 malaria-infected mice (31.7 ± 2.9 g; inoculated with 10⁶ *P. berghei*-parasitized and given CQ 64 h later, at which time the mean parasitemia was 3.2% ± 1.3%). CQ was administered i.p. at a dose of 1,500 μg/kg (approximately 50 mg/kg CQ). Mice were anesthetized with 50 to 100 mg/kg sodium pentobarbitone 5 to 10 min prior to blood collection. Blood was harvested from groups of mice (*n* = 5) by cardiac puncture at 10, 15, 20, 30, 45, 60, 75, and 90 min; 2, 2.5, 3, 4, 5, 8, 12, 18, 24, 30, 36, 48, and 6 h; and 3, 4, 5, and 7 days into 1-ml lithium heparin tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 3,000 × g for 10 min, and the plasma was separated and stored at −80°C until analyzed by high-performance liquid chromatography (HPLC) for CQ and DCO measurement (31). A peripheral blood film was prepared from each mouse prior to CQ dosing and at the time of euthanasia to confirm that the parasitemia data in the pharmacokinetic study mice were consistent with the pharmacodynamic study data (data not shown).

The pharmacokinetic properties for CQ and DCO were also determined in a multidose study of 125 uninfected male Swiss mice (30.8 ± 3.1 g) and 125 malaria-infected mice (29.2 ± 3.1 g; inoculated with 10⁶ *P. berghei* parasites and given CQ 64 h later; mean parasitemia = 2.8% ± 1.4%). Five doses of 1,500 μg/kg (50 mg/kg CQ) i.p. were administered at 24-h intervals (these doses were used to extend survival of the mice). Blood was harvested from groups of mice (*n* = 5) by cardiac puncture at 4, 8, 12, and 24 h after the first dose; 24 h after the second, third, and fourth doses; and then 1, 2, 4, 6, 8, 12, and 18 h after the fifth dose and at 5, 5.25, 5.5, 6, 6.5, 7, 8, 10, 15, 21, and 30 days after commencement of the dosage regimen. The blood was processed for CQ and DCO measurement by HPLC for peripheral blood films as described above. In addition, a peripheral blood film was prepared each day from mice in the last two cohorts (21 and 30 days; *n* = 10) to assess the pharmacodynamic response to the five-dose 50-mg/kg/dose regimen.

**HPLC analysis.** Stock solutions of CQ and DCO (l-glutaric acid) were prepared separately, and two internal standards (AQ and PQ) were used, to counter any interference in the analysis. A 5-point linear calibration curve was prepared, using blank human plasma, and quality-control samples (5 μg/ml and 20 μg/liter) were included for each analytical batch. CQ and DCO extraction from plasma was based on our established method (31). Briefly, plasma standards and samples (500 μl) were spiked with internal standard (PQ, 200 ng, and AQ, 200 ng), mixed with 5 ml of r-butylmethyl ether and 200 μl of 5 M NaOH, and then manually shaken for 10 min. All tubes were centrifugated (1,500 × g) for 10 min, and 4.5 ml of the organic phase was transferred into clean tubes and back-extracted into 0.1 ml of 0.1 M HCl by shaking for 5 min. This procedure was repeated for 3 centrifugations, (500 × g) for 10 min each, and the organic phase was aspirated to waste. The HCl layer was transferred to a clean glass tube and centrifuged (1,500 × g) for 20 min, after which 60-μl aliquots were injected onto the HPLC column.

Separation was performed on a Gemini C₁₈-phenyl 110A (150- by 4.6-mm, 5-μm) HPLC column connected to a Gemini C₁₈-phenyl (4- by 3-mm) guard column (Phenomenex, Lane Cove, Australia) at 30°C. The mobile phase comprised 0.05 M KH₂PO₄, (pH 2.5) and 13% (vol/vol) acetonitrile, with a flow rate of 1 ml/min, and analytes were detected by UV absorbance at 343 nm. The approximate retention times for PQ, DCO, QA, and AQ were 2.5, 4.2, 5.2, and 6.8 min, respectively.

The intraday relative standard deviations (RSDs) for CQ were 7.8, 4.2, and 3.6% at 5 μg/liter, 500 μg/liter, and 3,000 μg/liter, respectively (*n* = 5), while interday RSDs were 8.3, 5.8, and 5.2% at 5 μg/liter, 500 μg/liter, and 3,000 μg/liter, respectively (*n* = 25). The intraday RSDs for DCQ were 6.9, 3.4% at 5 μg/liter, 100 μg/liter, and 1,000 μg/liter, respectively (*n* = 5), while interday RSDs were 7.9, 6.3, and 5.4% at 5 μg/liter, 100 μg/liter, and 1,000 μg/liter, respectively (*n* = 25). The limits of quantification and detection were 1.2 μg/liter and 0.6 μg/liter for CQ and 1 μg/liter and 0.5 μg/liter for DCQ, respectively.

**Allometric scaling.** A detailed literature search for pharmacokinetic studies of CQ in mammals was performed. Where necessary, estimates of the parameters of interest (α, β, and clearance) were determined using model-independent pharmacokinetic calculations. Regression analysis of the log-transformed data was used to determine the coefficients (α and exponent (β) for the simple allometric equation *Y* = *a* × *W*β, where *Y* is the pharmacokinetic parameter and *W* is body weight (20, 41, 60). Interspecies scaling of half-life included all available data from malaria-infected and control subjects. However, due to the high blood/
plasma ratio of CQ (18, 65), scaling of clearance and volume of distribution comprised only the plasma data.

Statistical and pharmacokinetic analyses. Data analysis and representation were performed with SigmaPlot version 11 (Systat Software Inc., San Jose, CA). Data are means ± standard deviations (SDs) unless otherwise indicated. The Student t test or one-way analysis of variance (ANOVA) was used for comparison of groups as appropriate, with a significance level of P < 0.05.

For pharmacokinetic modeling, measured plasma concentrations were normalized to a dose of 50 mg/kg CQ, according to the weight of each mouse at the time of dosing. Consistent with the principles of destructive testing (5, 75), the mean normalized plasma concentration for each group of mice was used to estimate pharmacokinetic parameters. Pharmacokinetic analysis was performed using Kinetica version 5.0 (Thermo Fisher Scientific, Inc., Wal- tham, MA). Noncompartmental analysis of the plasma concentration-time data was used to estimate area under the curve (AUC0–t). Symbols: control (n = 4); ▲, 10 mg/kg CQ (n = 9); ▲, 20 mg/kg (n = 7); △, 30 mg/kg (n = 9); ●, 50 mg/kg (n = 7).

RESULTS

Pharmacodynamic study. Administration of a single dose of CQ resulted in a decline in parasitemia at all doses tested (Fig. 1). At 10, 20, 30, and 50 mg/kg CQ, the parasitemia fell to a nadir of 1%, 0.04%, 0.008%, and 0.004%, respectively, approximately 2 days after the dose. The parasitemia then increased until the experimental endpoints were reached, with median (range) survival times of 6 (5 to 8), 4 (3 to 5), 6 (5 to 11), and 8 (6 to 11) days for the 10, 20, 30, and 50 mg/kg CQ dose groups, respectively.

Mice receiving the 3-dose regimen (20, 20, and 10 mg/kg CQ, 12 h apart) showed a significant decrease in parasitemia, falling below the limit of detection approximately 3 days after the initial dose and remaining undetected for at least 12 h (Fig. 2). Parasite nadir was estimated by extrapolation to occur 74 h after the initial dose of CQ, at a parasitemia of 0.0005%, and the median (range) survival time was 7 (6 to 10) days. The five-dose 10-mg/kg/dose series also showed a sustained decrease in parasitemia, falling below the limit of detection approximately 66 h after the first dose and remaining undetectable for more than 2 days (Fig. 2). The median survival time in this group was 9 (7 to 16) days.

Single-dose DHA (30 mg/kg) and CQ (30 mg/kg) and the DHA-CQ combination showed a prompt decrease in parasitemia to parasite nadirs at 25 h (0.8%), 49 h (0.16%), and 49 h (0.08%), respectively, (Fig. 3). The nadir parasitemia for DHA-CQ was significantly lower than that for DHA alone (P = 0.004; t test) and suggested an additive effect.

In mice that received five 50-mg/kg doses of CQ (from the pharmacokinetic study), a prompt decline in mean parasitemia was observed, falling below the limit of detection after the third dose (Fig. 4). Parasites remained undetectable for a further 7 days, and recrudescence was observed 10 days after the initial dose. From 14 to 21 days, the mean parasitemia remained relatively stable (0.37 to 0.59%) and then steadily decreased until becoming undetectable 24 days after the initial CQ dose.

Pharmacokinetic study. The plasma CQ and DCQ concentration-time profiles following a single dose and multiple doses of CQ are shown in Fig. 5 and 6. The $C_{\text{max}}$, $t_{\text{max}}$, elimination $t_{1/2} \text{CL/F}$, and $V/F$ of CQ were 1,708 μg/liter, 20 min, 46.6 h, 9.9 liters/h/kg, and 667 liters/kg, respectively, in healthy mice and 1,436 μg/liter, 10 min, 99.3 h, 7.9 liters/h/kg, and 1,122 liters/kg in malaria-infected mice (50-mg single-dose data; noncompartmental analysis). The $C_{\text{max}}$, $t_{\text{max}}$, and $t_{1/2}$ of DCQ were 614 μg/liter, 4 h, and 32.6 h, respectively, in healthy mice

FIG. 1. Parasitemia-time profile in Swiss mice following adminis-
istration of single i.p. doses of CQ (†) administered 64 h after inocu-
lation with 10⁷ P. berghei-parasitized erythrocytes. Data are shown as
maximum plasma concentration (time of maxi-
mum plasma concentration), $t_{\text{max}}$, and $t_{1/2}$ of DCQ, for the single-dose dataset. The metabolic ratio of DCQ to CQ in control and malaria-infected mice was calculated from the time of CQ administration. Consistent with the principles of destructive testing (5, 46), the mean normalized plasma concentration for each group of mice was used to estimate pharmacokinetic parameters. Pharmacokinetic analysis was performed using Kinetica version 5.0 (Thermo Fisher Scientific, Inc., Wal-
tham, MA). Noncompartmental analysis of the plasma concentration-time data was used to estimate area under the curve (AUC0–t). Symbols: control (n = 4); ▲, 10 mg/kg CQ (n = 9); ▲, 20 mg/kg (n = 7); △, 30 mg/kg (n = 9); ●, 50 mg/kg (n = 7).

FIG. 2. Parasitemia-time profile in Swiss mice following adminis-
tration of either a single i.p. dose of 50 mg/kg CQ (†; 0 days), a
three-dose regimen (20, 20, and 10 mg/kg at 0, 0.5, and 1 day,
respectively; †), or a five-dose regimen (5 doses of 10 mg/kg at
12-hour intervals; ††) with the first dose administered 64 h after
inoculation with 10⁷ P. berghei-parasitized erythrocytes. Data are
shown as total parasitemia (log scale; mean percentage of erythro-
cytes infected ± SD). Symbols: control (n = 4); ▲, 50-mg/kg CQ
single dose (n = 7); ▲, 3-dose regimen (n = 8); △, 5-dose regimen
(n = 8).
and 345 μg/liter, 2.5 h, and 74.4 h in malaria-infected mice. The metabolic ratio of DCQ to CQ was 1.08 and 0.62 for healthy and malaria-infected mice, respectively.

Based on the two-compartment model, $t_{1/2}$ and $t_{1/2,\text{Formation}}$ of CQ were 3.3 and 53 h, respectively, in healthy mice and 4.7 and 163 h in malaria-infected mice (Fig. 5). CQ and DCQ data were incomplete (analytes not detected) after 7 days in the multiple-dose study; hence, detailed pharmacokinetic data were not obtained from the two-compartment model. However, combining control and malaria-infected data from the single- and multiple-dose studies, the mean rate of formation of DCQ from CQ ($k_F$) was $0.63 \pm 0.55$ h$^{-1}$ and the formation half-life ($t_{1/2,\text{Formation}}$) was $1.7 \pm 1.0$ h.

**Allometric scaling.** Data for allometric scaling were obtained from 6 animal studies (mice, rats, rabbits, monkeys, and dogs) and 12 human studies (Tables 1 and 2). The allometric plots for CQ plasma clearance in controls and malaria-infected mammals are shown in Fig. 7, and the allometric equations were $CL = 3.86 \times W^{0.56}$ ($r^2 = 0.96$) and $CL = 2.16 \times W^{0.65}$ ($r^2 = 0.99$), respectively. The allometric equations for CQ half-life were $t_{1/2} = 123 \times W^{0.2}$ ($r^2 = 0.58$) and $t_{1/2} = 134 \times W^{0.11}$ ($r^2 = 0.61$) for controls and malaria-infected groups, respectively. The allometric equations for CQ volume of distribution were $V = 230 \times W^{0.94}$ ($r^2 = 0.92$) and $V = 245 \times W^{0.68}$ ($r^2 = 0.9$) for controls and malaria-infected groups, respectively.

Using allometric scaling principles and the equation for CQ plasma clearance in malaria infection ($CL = 2.16 \times W^{0.65}$), a dose of 35 to 40 mg/kg CQ base would be required in children weighing 10 to 20 kg in order to achieve the same plasma concentrations as the adult dose of 25 mg/kg CQ base (73). By comparison, a simple weight-based equation ([dose$_{\text{child}}$ [mg] = dose$_{\text{adult}}$ [mg] × [weight$_{\text{child}}$/weight$_{\text{adult}}$]$^{0.75}$] [30]) would estimate a similar dose range of 30 to 35 mg/kg for children of 10 to 20 kg in body weight.
Our study provides a novel perspective on the pharmacokinetics and pharmacodynamics of CQ in a murine malaria treatment model, and we report the first detailed allometric scaling of CQ pharmacokinetic parameters. As we have demonstrated that CQ efficacy is dependent on both the total dose and the regimen design, our pharmacodynamic data have direct relevance to clinical researchers testing novel dosage strategies in trials (6, 34, 67) and to drug discovery investigators using CQ as a comparator drug.

The single-dose study (Fig. 1) showed a delay of 6 to 12 h before the decline in parasite density and a nadir parasitemia approximately 2 days (i.e., two erythrocytic cycles in P. berghei) after CQ administration. It is therefore likely that the period of maximum efficacy for CQ in this model is on the order of 24 to 36 h posttreatment. By comparison, DHA produces a prompt decline and parasite nadir 24 h after single doses, and the maximum antimalarial effect appears to occur about 12 h post-treatment (23). These observations suggest that characterizing the pharmacodynamic profile of new chemical entities and comparator antimalarial drugs has the scope to improve the study design and interpretation of data in antimalarial drug development research. Although it may not be feasible to compare novel antimalarial compounds to similar drugs (54, 68, 74), comparisons would be assisted by using a range of comparator drugs (53) with established pharmacodynamic profiles.

Direct extrapolation of dosage strategies from animal studies to clinical trials is problematic; however, our multiple-dose data demonstrate that investigations of treatment options can be informative (Fig. 2). The same total dose (50 mg/kg) was shown to be significantly more effective when given as five 10-mg/kg doses over two erythrocytic cycles, compared to a one-cycle (24-h) or single-dose strategy. For each regimen, the nadir parasitemia occurred approximately 2 days after the final dose of CQ, thus providing evidence of a delayed dose-response relationship.

The multiple-dose pharmacodynamic study also produced an interesting finding that would be relevant to investigations of the immune system and malaria infection (Fig. 4). Consistent with our previous reports of piperaquine in the murine malaria model (43, 44), high-dose CQ led to a curative response. This outcome occurred at very low plasma concentrations several weeks after dosing (the value was unable to be extrapolated from the present study but was likely <1 μg/liter) (Fig. 6). Therefore, we can conclude that resolution of the recrudescence parasitemia was a combination of drug efficacy (to reduce the initial parasite bioburden) and immunological mechanisms. Further investigations of this finding were beyond the scope of the present study.

This multiple-dose, murine malaria treatment model could be applied to investigations that complement recent clinical studies of high-dose antimalarial drugs (6, 67). For example, a total dose of 50 mg/kg CQ as a 5-dose regimen over 3 days was given to children for treatment of falciparum malaria (67). This dose is twice the WHO recommendation (for adults and children) of 25 mg/kg CQ as a 3-dose regimen over 3 days (73). Although it was not specifically tested in the present study, we have shown that the murine model could be used for pharmacodynamic comparisons of standard and high-dose regimens, including variable dose intervals. However, as noted previously, the murine studies provide a conceptual understanding and direct extrapolation to human malaria may require prudent consideration (23, 43).

Extending our study to evaluate the combination of CQ and an artemisinin drug (DHA) provided a valuable comparison to previous studies. In vitro and in vivo efficacy studies have shown paradoxical findings for a range of quinoline and artemisinin combinations (12, 13, 15, 21, 26). CQ is reported to be antagonistic and additive in combination with artemisinins in vitro (12, 21, 26) and additive in the Peters 4-day suppressive test in mice (13). Results from our murine treatment model suggest a weak additive effect of CQ and DHA in vivo (Fig. 3). There are few clinical studies of CQ in combination with artemisinin drugs (48), but reports of CQ-artsunate treatment in West Africa and Vietnam suggest that beneficial outcomes can be achieved (24, 33, 49, 63).

By comparison, we have recently shown an additive effect when DHA and piperaquine were administered concurrently

FIG. 6. Concentration-time profile of CQ (A) and desethylchloroquine (B) in mice given five doses of 50-mg/kg i.p. CQ at 24-hour intervals. Data are mean ± SD (n = 5) plasma CQ concentrations in healthy (○) and malaria-infected (●) mice and plasma desethylchloroquine concentrations in healthy (□) and malaria-infected (●) mice. The lines represent the best fit of a two-compartment model to the respective data sets.
in the murine malaria treatment model (43), a finding that is consistent with the clinical efficacy of this combination (7, 46, 58) but contrasts with in vitro data suggesting mild antagonism (15). As suggested by these reports, the selection of antimalarial partner drugs is complex, requiring consideration of the

![FIG. 7. Allometric plot for CQ plasma clearance (CL/F) in healthy (\(\Delta\); CL = 3.86 \(\times\) W\(^{0.56}\)) and malaria-infected (\(\bullet\); CL = 2.16 \(\times\) W\(^{0.65}\)) species (log scale on both axes).](http://aac.asm.org/)

### TABLE 1. Chloroquine pharmacokinetic data from healthy mammalian species

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<td>71</td>
</tr>
<tr>
<td>Human, adults</td>
<td>6</td>
<td>45</td>
<td>67</td>
<td>0.83</td>
<td>82.6</td>
<td>17</td>
</tr>
<tr>
<td>Human, adults</td>
<td>5</td>
<td>58</td>
<td>70.5</td>
<td>0.62</td>
<td>63.5</td>
<td>17</td>
</tr>
<tr>
<td>Human, adults</td>
<td>30</td>
<td>52</td>
<td>291</td>
<td>0.46</td>
<td>129.5</td>
<td>32</td>
</tr>
<tr>
<td>Total no. of human adults(^d)</td>
<td>102</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td></td>
<td>62 ± 3</td>
<td>304 ± 31</td>
<td>0.63 ± 0.04</td>
<td>211 ± 22</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 2. Chloroquine pharmacokinetic data from mammalian species with malaria infection

<table>
<thead>
<tr>
<th>Matrix and species</th>
<th>n</th>
<th>Wt (kg)</th>
<th>Half-life (h)</th>
<th>CL(^a) (liters/h/kg)</th>
<th>V(^a) (liters/kg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>0.03</td>
<td>99.3</td>
<td>7.9</td>
<td>1,122</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbits(^c)</td>
<td>8</td>
<td>2.0</td>
<td>223</td>
<td>0.31</td>
<td>86</td>
<td>2</td>
</tr>
<tr>
<td>Dogs, beagle</td>
<td>2</td>
<td>11.8</td>
<td>302</td>
<td>0.13</td>
<td>53</td>
<td>2</td>
</tr>
<tr>
<td>Human, adults</td>
<td>5</td>
<td>72</td>
<td>1,248</td>
<td>0.091</td>
<td>81</td>
<td>22</td>
</tr>
<tr>
<td>Human, adults</td>
<td>7</td>
<td>59</td>
<td>150</td>
<td>0.21</td>
<td>45.3</td>
<td>47</td>
</tr>
<tr>
<td>Human, adults</td>
<td>17</td>
<td>66.5</td>
<td>162</td>
<td>0.25</td>
<td>57.8</td>
<td>59</td>
</tr>
</tbody>
</table>

### Notes:

\(^a\) CL is apparent clearance (CL/F), and V is apparent volume of distribution (V/F).

\(^b\) Pharmacokinetic parameters determined from concentration-time profile of 24 h after dose.

\(^c\) Pharmacokinetic parameters determined from concentration-time profile of 4 h after dose.

\(^d\) Pharmacokinetic parameters determined from concentration-time profile of 48 h after dose.

\(^e\) Pharmacokinetic parameters determined from concentration-time profile of 24 h after dose.

\(^f\) Pharmacokinetic parameters determined from concentration-time profile of 4 h after dose.

\(^g\) Present study.
pharmacology, pharmacokinetics, and pharmaceutical properties of each drug, alone and in combination. Our studies indicate that this murine model can be a valuable preclinical tool in antimalarial drug discovery and therapeutic refinement (8, 43).

An essential component of in vivo antimalarial efficacy tests is determining the pharmacokinetic properties of the drug(s). However, pharmacokinetic data for CQ in mice were notably deficient. The only specific murine study of CQ pharmacokinetics was an investigation in healthy and malaria-infected (Plasmodium chabaudi) mice (11). Whole-blood CQ concentration was measured for the pharmacokinetic determinations, but the sampling period was only 4 h postdose, therefore limiting the findings to the distribution phase of the pharmacokinetic profile. Nevertheless, Cambie et al. (11) found that malaria infection (particularly high parasitemia of >20% in mice) increased the half-life and V/F, which suggests a decreased CL/F (Tables 1 and 2). One other investigation of CQ pharmacokinetics in healthy mice has been reported, in the context of grapefruit juice interaction (4). However, the data were incomplete and implausible and could not be compared to the present study.

Reports of CQ pharmacokinetics in other nonhuman species also are limited (Tables 1 and 2), but there is general consistency in the findings of a long half-life, large V/F, and low CL/F for CQ. There is a paucity of animal data in malaria infection, but the available human studies suggest modest changes in pharmacokinetic properties of CQ (Tables 1 and 2).

Against this background, our study was designed to provide detailed pharmacokinetic profiles for CQ and the active metabolite, monodesethylchloroquine (DCQ), following single and multiple doses in healthy and malaria-infected (P. berghei) mice. The pharmacokinetic data were consistent with expectations (Figs. 5 and 6), despite incomplete results from the multiple-dose study. As evident in Table 1, the longer t1/2 and lower CL of CQ in malaria infection, compared to healthy controls, were in accordance with other controlled studies (17, 19, 47, 57). The estimated t1/2 of DCQ in malaria-infected and control mice was shorter than the t1/2 of CQ, suggesting that elimination of DCQ is formation rate dependent (61). Further data to support this conclusion were the metabolic ratios (DCQ to CQ) in malaria-infected (0.6) and control (1.08) mice, both of which were comparable to those of malaria-infected patients (47) and healthy volunteers (ratio, 0.78 to 0.87), respectively (22, 32, 59).

Comparison of the pharmacokinetic data from our study was best represented by allometric scaling, although limitations included the paucity of nonhuman data and the matrix used in the studies. The majority of CQ pharmacokinetic studies have used plasma, and all of the applicable human data were represented separately, as this did not alter the allometric equations (Fig. 7). The allometric scaling indicated that the differences in pharmacokinetic parameters between healthy controls and malaria infection were modest (based on confidence intervals [data not shown]), but the power of these comparisons was limited by the small number of species available for the scaling. Although interspecies scaling is well established in antimicrobial chemotherapy (16, 38, 41, 60), there are few reports of scaling for antimalarial drugs (10, 14). Interspecies comparison of halofantrine using data from uninfected rats and dogs, and malaria-infected humans, suggested a predictive relationship for CL and V (10). Allometric scaling has also been applied to predict human clearance of a novel 4-aminoquinoline from pharmacokinetic data in uninfected mice, rats, dogs, and monkeys (14). Therefore, our analysis of CQ suggests that interspecies scaling data could be applied to phase I studies of new antimalarial drugs, particularly for estimating the dose in first-in-human studies.

Despite the potential value of allometric scaling, clinical limitations of this technique have been identified and cautious interpolation of mammalian data to predict pediatric dosing is warranted (20, 30). Nevertheless, weight-based doses for numerous drugs are higher in children than in adults (30) and recent reports indicate that pediatric doses of antimalarials should be reevaluated (6, 66, 67). In relation to the reports of double-dose CQ (50 mg/kg over 3 days [66, 67]), application of a simple weight-based pediatric equation (30) or our allometric equation to the standard WHO adult dose of 25 mg/kg (73) demonstrates that 35 mg/kg and 40 mg/kg, respectively, would be appropriate. Hence, it could be concluded that 50 mg/kg CQ is only 25 to 40% higher than the scaled equivalent dose.

Our study contributes to the pharmacokinetic and pharmacodynamic knowledge of CQ, but limitations associated with animal studies necessitate prudent conclusions. We have evaluated only a small range of dosage strategies, and the pharmacodynamic outcomes were dose dependent, although some generalizations are credible. Our pharmacokinetic results were limited by the incomplete data in the multiple-dose study, and we did not investigate the possibility of dose-dependent pharmacokinetics for CQ. Further pharmacokinetic studies of CQ could include a detailed evaluation of blood/plasma ratio, as well as the effect of severity of malaria infection on CQ pharmacokinetic properties.

We conclude that the biphasic pharmacokinetic profiles of CQ are similar across mammalian species and that scaling of specific parameters is plausible. CQ was shown to have a delayed dose-response relationship and to have additive efficacy when combined with DHA. In addition to demonstrating the scope of preclinical data that can be generated using a murine malaria treatment model, our study provides pharmacokinetic and pharmacodynamic information for investigators who use CQ as a comparator in drug discovery research programs.

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REFERENCES


