Split dosing of artemisinins does not improve antimalarial therapeutic efficacy

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It has been suggested recently, based on pharmacokinetic-pharmacodynamic modelling exercises, that twice daily dosing of artemisinins increases malaria parasite killing and so could “dramatically enhance and restore drug effectiveness” in artemisinin resistant P. falciparum malaria infections. It was recommended that split dosing should be incorporated into all artemisinin combination regimen designs. To explain why parasite clearance rates were not faster with split dose regimens it was concluded that splenic malaria parasite clearance capacity was readily exceeded, resulting in the accumulation of dead parasites in the circulation, that parasite clearance was therefore an unreliable measure of drug efficacy, and instead that human immunity is the primary determinant of clearance rates. To test these various hypotheses we performed a logistic meta-regression analysis of cure rates from all falciparum malaria treatment trials (n = 40) with monotherapy arms containing artemisinin or a derivative (76 arms). There was no evidence that split dosing enhanced cure rates.

When artemisinin and its derivatives were first evaluated in the treatment of malaria a variety of doses and dosing schedules were assessed. Following single or multiple doses, rates of parasite clearance in falciparum and vivax malaria were faster than observed previously with other classes of antimalarial drug, but satisfactory cure rates with artemisinins alone in falciparum malaria required dosing for more than five days⁴⁻¹⁰. The artemisinins are eliminated rapidly (t₁/₂~1 hour) but giving them twice or even three times in one day did not appear to provide additional benefit over once daily administration, so this became the norm⁴⁻¹⁴. There were a few exceptions. In uncomplicated malaria artether-lumefantrine required twice daily administration because of the readily saturated oral absorption of lumefantrine. In severe malaria there was concern that in a highly synchronous infection in which mature schizonts predominated, sub-maximal effects might result from the first parenteral administration so a second dose was given at 12 hours as an “insurance policy”.

Recently, based on pharmacokinetic-pharmacodynamic (PK-PD) modelling studies, it has been suggested that twice daily dosing of artemisinins could increase parasite killing¹⁴⁻¹⁷ and one study went as far as to claim that it could “dramatically enhance and restore drug effectiveness” in artemisinin resistant infections¹⁸. The authors further recommended “that twice-daily dosing should be incorporated into all artesinin combination treatment (ACT) regimen design considerations as a simple and effective way of ensuring the continued long-term effectiveness of ACTs”. The Liverpool group’s strong recommendation for a major change in dosing, which would have a profound effect on current and future practices if followed, was based on PK-PD modelling and was not supported by clinical trial data¹⁹. The modelling predicted that splitting current once daily ACT doses into twice per day administration would increase parasite killing enormously (by a factor of 10⁸)¹⁶⁻¹⁸. So have treatment recommendations been wrong all these years - or is there something wrong with the modelling?

PK-PD modelling

Standard PK-PD models of antimalarial drug concentration-effect relationships generally parameterise the ‘PD’ component (parasite killing) as a sigmoid – Emax relationship driven by plasma concentrations in which parasite numbers decline as a first-order process for a given drug concentration. From observed 48-hour cycle parasite reduction ratios (PRR) and measured plasma concentration profiles in artemisinin treated patients, these models imply that parasite killing rates are very high for a few hours following drug administration, and then decline rapidly as concentrations of artesunate (or artether) and their main metabolite dihydroartemisinin all fall. It

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It was conjectured that this “saturation” of artemisinin but they fail to predict the observed PRR when more than one dose is given per day. Recent modelling claims to have ‘solved’ this model misfit by hypothesising that the splenic clearance capacity for infected erythrocytes is exceeded by parasite killing by artemisinin antimalarials. It was concluded unreservedly that “human immunity is the primary determinant of clearance rates, unless or until artemisinin killing has fallen to near-infective levels” and more recently that “the impact of human immunity in clearing erythrocytes containing dead or dying parasites makes parasite clearance rates highly insensitive and non-specific diagnostics of resistance”. If indeed these conjectures are all true they would deal a serious blow to current epidemiological assessments of artemisinin resistance, which rely heavily on this metric for phenotyping and for validating the parasite genotyping used in molecular surveillance. This study examines whether evidence from previous clinical studies of the efficacy of artemisinin and its derivatives supports this hypothesized model structure and the derived therapeutic recommendations.

Clinical observations
The radical suggestions of the Liverpool group are not supported by clinical observations. If immunity is the primary determinant of parasite clearance rates it cannot explain why parasite clearance rates are twice as slow in patients of similar age and geographic origin, who have K13 mutant artemisinin-resistant compared with K13 wild type artemisinin-sensitive parasites. In studies where the effects of immunity, or age as a surrogate of cumulative exposure, on parasite clearance rates have been quantitated, the effects are much smaller than the effects of artemisinin resistance. In assessing treatment responses in artemisinin resistant falciparum malaria the hypothesis that human immunity is the primary determinant of parasite clearance rate does not fit the facts.

Cure rates following single versus split dosing
If parasite killing substantially exceeds splenic clearance capacity, and giving artemisinins twice daily is so much better than once daily as claimed, then irrespective of effects on parasite clearance there should be substantial differences in cure rates with twice daily versus once daily administration.

To test this hypothesis we performed a logistic meta-regression analysis of all trials with monotherapy arms containing artemisinin or a derivative. The dependent variable was cure rate, and the independent variables were duration of treatment (in days) and number of artemisinin doses. If the Liverpool group’s hypothesis was correct then the number of doses given should have been a significant covariate. A further meta-regression model was run on all studies of oral artesunate with the mg/kg dose as an independent variable. The logistic meta-regressions were run with a random effect for each study (random intercept term) and fixed effects for the number of doses and duration of treatment (and dose). We fitted the model in R (version 3.1.1) using the function glmer from the lme4 package. The database of extracted study meta-data can be found in the supplementary materials.

Search strategy
We searched the WorldWide Antimalarial Resistance Network (WWARN) Clinical Trials Publication library for eligible studies. This online resource contains all antimalarial clinical efficacy trials conducted and published since 1960. Studies were eligible if once daily or more frequent administration of artemisinin or a derivative was used as monotherapy to treat uncomplicated falciparum malaria and if cure rates at 28 days were reported for each treatment group (PCR adjusted or unadjusted). For two studies the numbers of failures by day 28 were not reported and were requested from the corresponding author.

In early studies where patients were kept in hospital for 28 days to assess cure rate (i.e. reinfection was not possible) the result was combined with later community based studies with PCR adjusted estimates.
the maximum parasiticidal effect a logistic meta-regression was performed using only studies with the most frequently evaluated drug, oral artesunate, (n = 39). This found that the dose in mg/kg was not a significant predictor of efficacy (lowest doses were 1.6 mg/kg). This confirms that ~2 mg/kg gives almost maximum antiparasitic effects in artemisinin-sensitive *P. falciparum* infections.

**Discussion**

For antimalarial treatments with artemisinin or its derivatives there is no evidence that split dosing either accelerates parasite clearance or augments cure rates significantly. In those studies where cure rates were sub-maximal, and the conjectured “dramatic enhancement of drug effectiveness” should have been evident, none was found. An effect of dose frequency on treatment efficacy was observed in a small study of 43 patients in which once daily artemether-lumefantrine was compared with standard twice daily dosing. In that study PCR adjusted cure rates were 85.1% and 94.4% respectively but this difference was explained entirely by 30% lower lumefantrine levels in the former group since lumefantrine exposure is the principal determinant of cure following treatment with this ACT. In studies of artemisinin monotherapies where twice or thrice daily administration has been evaluated, and in contemporary or sequential comparisons with once daily administration there is no evidence that cure rates are substantially higher with more than once daily dosing. Furthermore there is no evidence that splenic clearance functions are as low as the values needed to sustain this hypothesis. Taken together there is no clinical support for these PK-PD modelling predictions, which appear to be wrong. The fundamental problem is probably the modelling of parasite killing and clearance in *falciparum* malaria as a simple first order process. Whilst the decline in parasite densities is log linear, and can therefore be described as a first order process, it seems that once daily exposures to artemisinin or its derivatives produce maximum effects in the majority of patients. The kinetics of malaria parasite killing and parasite recovery are more complex than currently modelled.

**Data availability statement.** The data are uploaded as a supplementary file.

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E.A. performed the literature search, J.W. analysed the data, N.J.W. conceived the investigation and wrote the first draft of the manuscript. All authors contributed to the design, data interpretation and content of the submitted version.

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