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Simplified antimalarial therapeutic monitoring: using the day-7 drug level?

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The blood concentration profiles of most antimalarial drugs vary considerably between patients. The interpretation of antimalarial drug trials evaluating efficacy and effectiveness would be improved considerably if the exposure of the infecting parasite population to the antimalarial drug treatment could be measured. Artemisinin combination treatments are now recommended as first-line drugs for the treatment of falciparum malaria. Measurement of the blood, serum or plasma concentration of the slowly eliminated partner antimalarial drug on day 7 of follow-up is simpler and might be a better determinant of therapeutic response than the area under the concentration–time curve. Measurement of the day-7 drug level should be considered as a routine part of antimalarial drug trials.

Antimalarial efficacy assessments

Behavioural, pharmacokinetic (PK) (see Glossary) and pharmacodynamic (PD) factors might all contribute to treatment failure (i.e. the failure to clear parasitaemia or the subsequent recrudescence of infection) following the administration of antimalarial drugs. In treating uncomplicated malaria, one or more of the following factors could be responsible for treatment failure: (i) low-quality drugs; (ii) incorrect or inadequate dosing; (iii) poor adherence (behavioural); (iv) vomiting and reduced absorption; (v) an expanded apparent volume of distribution; (vi) increased clearance (PK); or (vii) reduced parasite susceptibility (PD) [1]. In antimalarial drug assessments, it is essential to distinguish antimalarial drug resistance from the other host or drug factors that contribute to treatment failure. Many of the currently used antimalarial drugs are eliminated slowly, and most combination treatments contain a slowly eliminated component. Artemisinin-based combination treatment (ACT) is now recommended by the World Health Organization (WHO: http://www.who.int/en/) as the first-line treatment for falciparum malaria worldwide [1]. ACTs combine a rapidly eliminated artemisinin component with a slowly eliminated partner antimalarial. Methodologies have been developed to assay nearly all of the available antimalarial drugs using small volumes of blood or plasma. Several of these methods have been adapted to use dried filter paper samples, considerably facilitating drug measurement in large-community-based field studies. With the notable exception of artemisinin and its derivatives, all antimalarials can be measured in whole blood, although plasma or serum is preferable for some. Because prospective antimalarial efficacy assessments involve a clinical and parasitological assessment of the patient on the seventh day after treatment starts [2], we suggest that a routine blood sample for later measurement of the drug concentration should be taken during the day-7 assessment whenever a slowly eliminated antimalarial is being evaluated.

Assessing responses to combination treatments

When blood concentrations of antimalarial drugs exceed the minimum parasiticidal concentration (MPC) for the infecting parasites, there is a fixed fractional reduction in

Glossary

ACT: artemisinin-based combination treatment – a combination of artemisinin or derivative (usually artesunate, dihydroartemisinin or artemether) given for three days with a more slowly eliminated antimalarial drug.
AUC: the area under the whole-blood, serum or plasma concentration–time curve.
Distribution phase: the phase following drug administration during which the drug distributes to and exchanges with the tissues. During this phase, blood concentrations fall faster than during the elimination phase.
Elimination phase: the period during which the drug is eliminated following distribution. This might have one or more phases. The last is the terminal elimination phase, which is a first-order process for all antimalarial drugs, and therefore has a half-life (the terminal elimination half-life).
First-order kinetics: a reaction rate in which the rate is proportional to the concentration. In the case of drug (or malaria parasite) elimination, the rate of reduction in blood concentration at any time is proportional to the concentration at that time. The result is that a fixed fraction of the drug (or parasites) is cleared per unit time. When plotted on a semi-log scale, the plot is linear and, if the vertical axis is in natural logarithm ($\log_e$), the slope gives the first-order rate constant (k).
MIC: the minimum inhibitory concentration is the blood or plasma concentration of antimalarial at which the parasite multiplication factor per asexual cycle = 1.
MPC: the minimum parasiticidal concentration is the lowest concentration of antimalarial drug in the blood that provides maximal inhibition of parasite multiplication.
PRR: the parasite reduction ratio is the fractional reduction in parasite numbers per asexual cycle. Values typically vary between 10 and 10 000 per cycle.

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parasite number each asexual cycle (first-order kinetics). As concentrations fall below the MPC, the fractional reduction decreases until there is no inhibition of multiplication [3,4]. The blood concentration at which the multiplication factor per cycle is 1 can be called the minimum inhibitory concentration (MIC). Cure from falciparum malaria depends on providing antimalarial drug concentrations in blood (the target organ) that exceed the MIC for the infecting parasites in each successive cycle until the parasite biomass has either been eradicated from the body or been reduced sufficiently so that the host immune response can eradicate the remainder [3] (Figure 1). The precise PK determinants of treatment outcome in malaria remain uncertain but evidence indicates that, in uncomplicated malaria, the area under the plasma or blood concentration–time curve (AUC) is an important PK parameter [3,5]. This is because the AUC reflects both the time and the amount by which antimalarial drug concentrations are sufficient for parasite killing. The AUC comprises both the absorption and the elimination phases of a drug, and provides a measure of parasite exposure to the antimalarial but requires that multiple blood samples be assayed for adequate characterization. An alternative would be to express the time for which blood concentrations exceed the *in vivo* MPC or MIC, although these values are generally not known precisely and multiple samples would still be required. Now that combination treatments, usually with an artemisinin derivative (i.e. ACTs), are generally recommended in falciparum malaria, the PD importance of the early concentrations achieved with the less active, slowly eliminated partner diminishes (with the notable exception of resistance prevention). This is because the parasiticidal effect of the artemisinin derivative predominates and seems to be neither augmented nor reduced by the partner drug; parasite clearance rates are usually similar regardless of whether the artemisinin derivative is combined with a partner drug [6]. Parasite clearance is accelerated during the first two cycles exposed to the artemisinin derivative. Indeed, parasite clearance times following ACT administration are often less than one

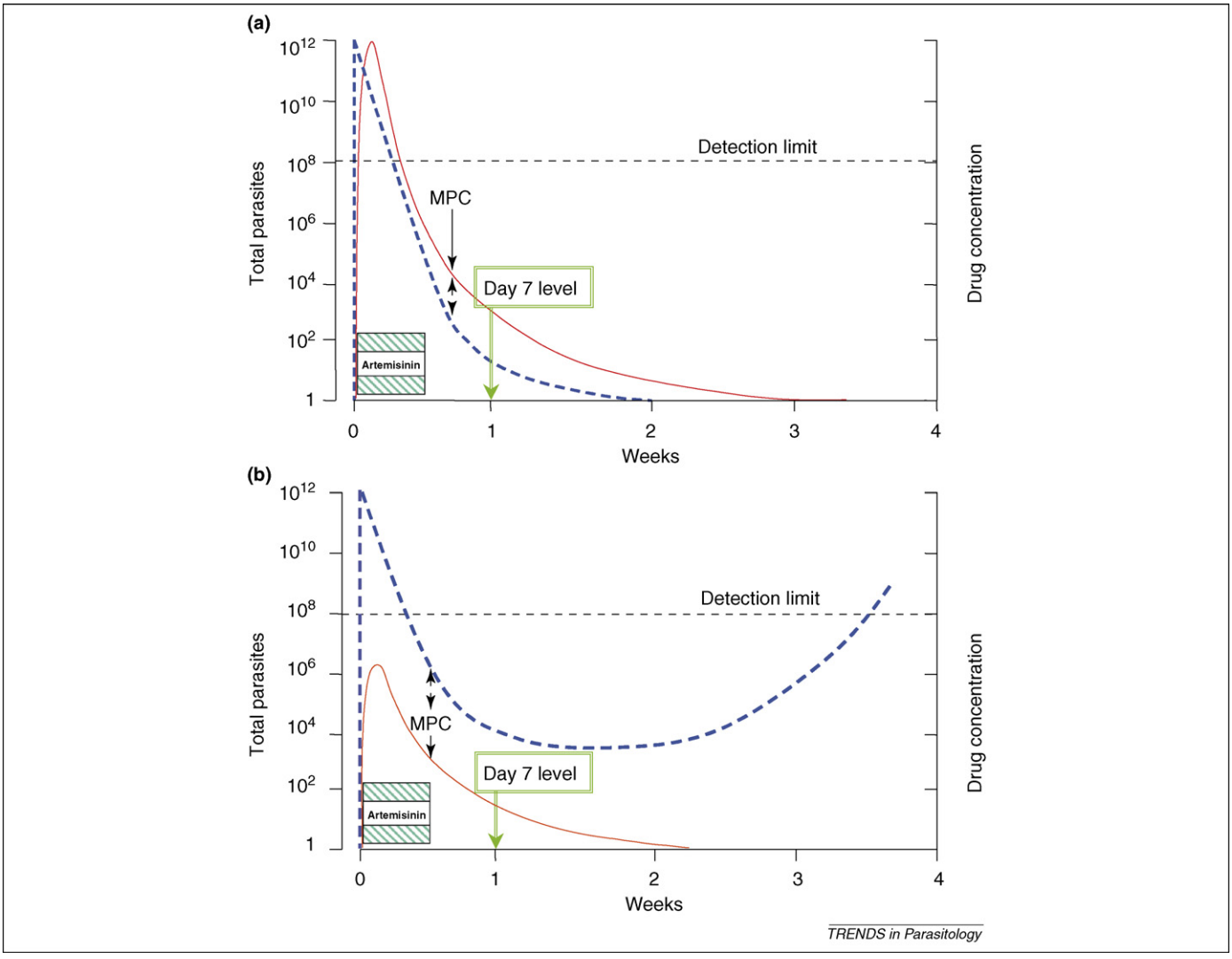

Figure 1. ACT PK–PD relationships. Shown are two hypothetical responses to ACT in falciparum malaria. The box containing artemisinin indicates the three-day treatment course with artemisinin or derivatives, which affects two asexual cycles (four days). Broken blue line denotes total parasite numbers in the body. The partner drug profile is shown in red in arbitrary concentrations. The partner drug has a half-life of approximately four days. Arrow denotes partner drug MPC. Below the MPC (i.e. to the right of the arrow), the decline in parasitaemia is no longer first-order. **(a)** Treatment success. The concentrations of partner antimalarial (red line) are adequate and the infection is cured. **(b)** Treatment failure. The concentrations of partner antimalarial are inadequate, falling below the MPC at the end of the second drug-exposed cycle, and the infection recrudesces by week 4.

cycle (48 h) in uncomplicated malaria. The main contribution of the partner drug to the therapeutic response is in the third and subsequent asexual cycles (i.e. four or more days), when the rapidly eliminated artemisinin derivative is no longer present in the blood [3,7].

Antimalarial pharmacodynamics

Even the most potent antimalarials – the artemisinin derivatives, which, at concentrations above the MPC, reduce parasite numbers ~10 000-fold per asexual parasite cycle (corresponding to a 99.99% reduction) – cannot clear all of the parasites from the entire blood volume in two asexual cycles (four days) [3,7,8]. Three-day regimens expose two asexual cycles to the artemisinin derivative [3]. If the maximum total parasite biomass possible in an adult patient is $\sim 10^{13}$ parasites, following three-day ACT regimens (providing parasite reduction of 10 000-fold per cycle for the first two drug-exposed cycles), there could be up to 100 000 residual parasites present in the body by the third cycle (4–6 days after the start of treatment). Consequently, with effective partner drugs, up to 1000 residual parasites could still be present in the blood during the fourth post-treatment cycle (third cycle for *Plasmodium malariae*) [7,8] (Box 1). It follows that blood concentrations of the partner drug at this time are an important determinant of cure because these residual parasites must be eliminated. In all prospective antimalarial drug assessments, all patients must be seen seven days after the start of treatment (even if they are not seen routinely before then), so a blood sample taken at this time reflects the drug exposure for these residual parasites [2].

Box 1. Reduction of *Plasmodium* parasite numbers following antimalarial treatment

Artemisinin derivatives are extremely potent and have parasite reduction ratio (PRR) values of $\sim 10^4$-fold (or 99.99%) per cycle. Artesunate exposure in two asexual parasite cycles corresponds to a 10^8-fold (or 99.999999%) reduction in parasite numbers over four days if all parasites are susceptible. Because the maximum possible total parasite biomass is 10^{13}, this leaves a maximum of 100 000 remaining parasites.

The slowly eliminated antimalarials at ≥ MPC concentrations have PRR values ≥100 per asexual cycle. Thus, assuming a continued first-order process, if blood concentrations remain above the MPC, the infection will be eradicated within three further cycles (six days) after the fourth day of treatment, or the tenth day after the start of treatment. Some treatment outcome possibilities are as follows.

(i) If the PRR is 10^3, all residual parasites should be eradicated in two cycles.
(ii) If the PRR is 10^2, all residual parasites should be eradicated in three cycles.
(iii) If the PRR is 10, all residual parasites should be eradicated in six cycles.

If the PRR is still ≥100 by day 7 (the fourth cycle), then – assuming that there are ≥100 000 parasites in the third asexual cycle following the start of treatment – there would be a maximum of 10^3 parasites on day 7.

The day-7 level is predictive of outcome because it reflects the concentrations of drug to which low numbers of residual parasites are exposed. If the concentrations of the slowly eliminated antimalarial drug [all have terminal elimination half-lives ($t_{1/2}\beta$) of at least three days] by day 7 are ≥ twice the MPC, all infections should be eradicated. The elimination half-life does not affect this prediction, provided that it is more than three days. Even if parasite numbers after the three-day artemisinin course are higher than 10^5 because of dormancy, the day-7 level is still a critical determinant of outcome.

Antimalarial pharmacokinetics

Although several antimalarials have multiphasic elimination profiles, most are in the exponential (i.e. log–linear) terminal elimination phase (sometimes termed β phase) seven days after the start of drug administration (i.e. the fourth day after finishing a three-day ACT course). The AUC after day 7 is obtained by dividing the blood or plasma concentration at this time (C_7) by the first-order terminal elimination rate constant (k_e) (Equation 1).

$$AUC_{7-\infty} = C_7/k_e \quad \text{[Eqn 1]}$$

The $AUC_{7-\infty}$ is easier to characterize than the total AUC ($AUC_{0-\infty}$). Variance in $AUC_{7-\infty}$ is less than that in $AUC_{0-\infty}$ because it is determined only by the variability in C_7 and k_e, whereas variance in $AUC_{0-\infty}$ is determined by variability in absorption rate, extent of absorption, initial distribution rates and disease-related changes in apparent volume of distribution and elimination (Figure 2). By day 7, most patients are no longer febrile and ill, so disease effects are lessened and the distribution phase is usually complete. For very slowly eliminated ACT partner drugs, much of the elimination phase (and, therefore, much of the total $AUC_{0-\infty}$) could occur after parasite clearance (Box 1). For example, with slowly eliminated drugs such as piperaquine and chloroquine, up to half of the $AUC_{0-\infty}$ could be contributed by blood concentrations that occur after complete clearance of the infection [9–11]. Changes in the terminal elimination phase affect total AUC much more than they do the day-7 level.

Melding pharmacokinetics and pharmacodynamics

As the initial therapeutic responses following ACTs are determined primarily by the artemisinin component, the

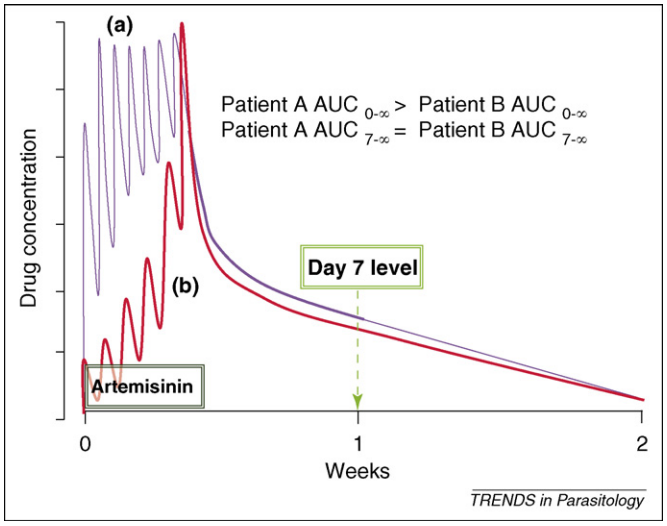

Figure 2. The day-7 level varies less than the AUC. This example illustrates that, for a variably absorbed drug (e.g. lumefantrine), the $AUC_{0-\infty}$ can vary considerably – mainly because of absorption variability – yet the elimination phases are similar. Two hypothetical patient profiles are shown, A and B, with different drug absorption profiles but similar elimination profiles. The therapeutic response in these two cases should be similar because the three-day course of artemisinin (or derivative) shown in the box would determine the reduction in parasite numbers in the first two cycles (four days).

plasma concentrations of the partner drug during the first three days of treatment are relevant mainly because: (i) they are needed to kill any spontaneously arising artemisinin-resistant mutant parasites (i.e. resistance prevention); and (ii) they determine the blood concentrations after three days that will eradicate the residual parasites after artemisinin treatment (a maximum of 100 000 parasites) [3] (Box 1). There is also considerable variability in the blood concentration profiles of the artemisinin derivatives, although – with current treatment regimens – the levels are usually in excess of the MPC required for maximum parasite killing [12]. If the artemisinin derivative does exert maximal parasite-killing effects during the first two treatment cycles (days 1–4) (i.e. it contributes a 'fixed effect'), so the partner drug is the main source of variation in treatment response. This indicates that the day-7 blood concentration of the partner drug might be a better determinant of cure than is the $AUC_{0-\infty}$ because the former reflects the concentrations of antimalarial drugs present as the infection is being eliminated from the body. Relationships between PK variables and cure rates are not evident when cure rates are very high. Such relationships are apparent only when resistance develops or doses are inadequate. This was first shown when a four-dose regimen of artemether–lumefantrine was recommended; the day-7 lumefantrine level provided an excellent predictive value for treatment outcome [13,14] (Figure 3). More recently, this has been shown for pyrimethamine, sulfadoxine and piperaquine [15,16].

Sampling methods for monitoring *in vivo* responses to antimalarial drugs

In vivo responses to antimalarial drugs must be monitored on a regular basis to ensure that cure rates are adequate and to provide an early warning of the development of resistance [2]. Antimalarial treatment is reliably efficacious only if antimalarial drug concentrations in the blood are adequate. Treatment failure results from inadequate blood concentrations of the drug or from resistance (i.e. inadequate drug activity), or both. In the past, the measurement of antimalarial drugs in blood, serum or plasma has been relatively difficult and confined to only a small number of research centres. In the past ten years, high-throughput, highly sensitive, low-sample-volume assays have been developed (although assay availability is still limited and costs are high). Concentrations of capillary blood samples often correlate well with those of venous blood samples [14,17–20], although variable mixing of tissue fluid with blood squeezed from the finger is a confounder, and the samples are vulnerable to contamination if the person sampling also handles the drugs. Accurate measurement of antimalarial drug concentrations in small volumes of blood pipetted onto filter paper enables large-population-based PK–PD studies to be conducted in which the clinical and parasitological therapeutic responses, and any dose-related adverse effects, can be characterized adequately. It is essential that each assay is validated correctly and that precise volumes of blood are dispensed. Some filter paper assays require specially pretreated paper.

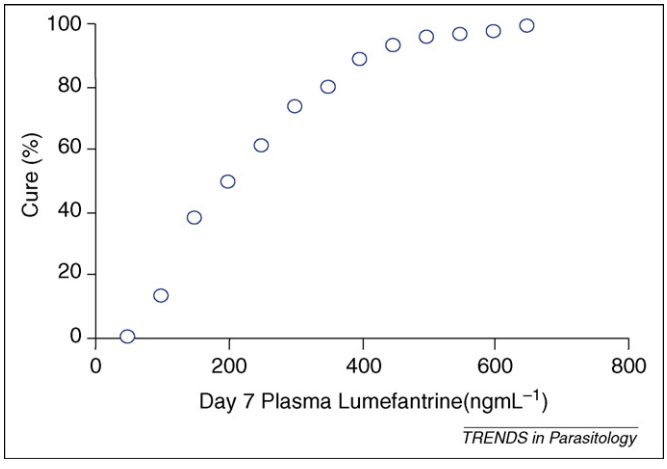

Figure 3. Plasma lumefantrine day-7 levels and cure rates. Shown is the relationship between the day-7 lumefantrine concentrations in plasma and artemether–lumefantrine cure rates obtained during clinical trials conducted as the drug was being developed (when four-day regimens were evaluated). Reproduced, with permission, from Ref. [3].

Informing policy and practice

The efficacy and safety of ACTs and their successors should be monitored regularly to inform policy recommendations. Because treatment failure results from low blood concentrations of the antimalarial drug, from resistance or from both, it is important that drug exposure be assessed. With the exception of *P. malariae* and *Plasmodium knowlesi* infections, the day-7 antimalarial blood level is a measure of parasite exposure in the fourth asexual cycle after the start of treatment. Blood levels of most antimalarials are usually within the assay limits of detection at this time [4,14,15,21–25]. Once the terminal phase of drug elimination has been reached (i.e. distribution is complete), the remaining AUC after that point is a simple function of the blood concentration at a given time. The terminal elimination phase for most antimalarials starts before day 7, but even if it does not the day-7 level is still likely to be a good determinant of outcome because it reflects the concentrations that occur when relatively few residual parasites are present. This is a crucial period that determines whether the residual parasite population is eliminated (cure) or re-expands to cause recrudescence. Clearly, levels of antimalarial drugs several weeks later are irrelevant to treatment outcome because they occur well after parasite elimination or, in the case of recrudescence, the nadir of parasite numbers. Further studies of individual drugs are needed to determine which single measurement best predicts the treatment response. The final choice also needs to take into account feasibility and potential loss to follow-up. Available data favour the day-7 measurement as a simple and practicable predictor of treatment outcome [4,13,16,26].

After the population profile for day-7 concentrations has been characterized, day-7 levels could help to determine whether treatment failures result from low levels of partner drug or from drug resistance. They are valuable in the interpretation of adverse effects and they can be used to identify subgroups that are in need of dose adjustment [21]. Conventional PK studies would then still be needed to optimize dose regimens [26]. In trials evaluating the efficacy or effectiveness of antimalarial

drugs, all patients are seen on day 7, and a blood sample is taken for parasite count and often haematocrit. If suitable low-volume assays are available, an additional 50–200 μl of blood can easily be taken. If the drug and any active metabolites are chemically stable at room temperature on drying, the blood can be transferred onto appropriate filter paper and stored for later measurement of drug concentration.

Concluding remarks and future directions

The interpretation of results from antimalarial drug trials, and the optimization of drug dosage would be facilitated by measuring individual drug exposure. However, taking multiple samples in large numbers of treated patients is seldom feasible. Routine measurement of day-7 antimalarial drug levels could considerably improve the assessment of both efficacy and effectiveness. The value of this simple measurement should be compared with more-complex PK parameters in both retrospective and prospective studies. The challenge now is to make antimalarial drug assays affordable and available.

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