Eur J Clin Microbiol Infect Dis (2004) 23: 233–242
DOI 10.1007/s10096-004-1113-9

G. Edwards · S. Krishna

Pharmacokinetic and Pharmacodynamic Issues in the Treatment of Parasitic Infections

Published online: 17 March 2004

Abstract Despite increases in the incidence of many parasitic infections in recent years, the number of studies designed to improve the treatment of these infections has failed to keep pace with their huge impact on public health. Unfortunately, research and development in this field is not an economically attractive proposition for the pharmaceutical industry, and this neglect is exacerbated by the fact that many parasitic diseases have negligible profiles in countries that have the funds to research them. An absence of effective vaccines means that, for the foreseeable future, chemotherapy is likely to be the mainstay of disease management. This review describes the advances gained in our understanding of the relationship between pharmacokinetics and pharmacodynamics, with the aim of improving the way in which we use antiparasitic agents while at the same time highlighting those areas where there is an urgent need for further investigation. Unsurprisingly, much of our success has been in the chemotherapy of malaria, where the link between drug concentration and response is reasonably well characterised. For many other diseases, however, this link is poorly understood, in some cases because the mechanism of action of the drug has not been fully elucidated, or in other cases because a true pharmacodynamic endpoint may be unavailable. Overcoming these problems is critical if the clinician is to have the information necessary to enable optimal treatment of patients who may be severely ill and in need of immediate, life-saving attention.

Introduction

Parasitic infections are of immense global significance. Despite increases in the incidence of many parasitic infections in recent years, the number of studies designed to improve the treatment of these infections has not even come close to being sufficient to assess their huge impact on public health. This is mainly because research and development in this field is not an economically attractive proposition for the pharmaceutical industry, which has led to a neglect that is exacerbated by the fact that many parasitic diseases have negligible profiles in countries that have the funds to research them. Furthermore, there are no effective vaccines against any of the major parasitic infections in humans, and none are likely to be introduced into clinical use in the next few years, emphasising the importance of chemotherapeutic interventions to manage disease. The global burden of the principal diseases covered by the portfolio of the World Health Organisation's Special Programme of Research and Training in Tropical Diseases is shown in Table 1. These figures, accumulated by the World Health Organisation, are estimates, and often gross underestimates at that. To these data should be added those for gastrointestinal helminths. The global burden of disease caused by the three major intestinal nematodes is an estimated 22.1 million disability-adjusted life-years (DALYs) lost for hookworm, 10.5 million for *Ascaris lumbricoides*, 6.4 million for *Trichuris trichiura*, or 39 million for the three infections combined [1].

The burgeoning problem of resistance to hitherto effective antiparasitic agents in the last decade has added urgency to the need to discover new antiparasitic agents

Table 1 Global burden of major tropical parasitic diseases

Disease	Disease burden in DALYs (thousands)			Deaths (thousands)		
	Total	Male	Female	Total	Male	Female
African trypanosomiasis	1,598	1,029	568	50	32	18
Dengue	653	287	366	21	10	11
Leishmaniasis	2,357	1,410	946	59	35	24
Malaria	42,280	20,024	22,256	1,124	532	592
Schistosomiasis	1,760	1081	678	15	11	5
Chagas disease	649	333	316	13	7	6
Lymphatic filariasis	5,644	4,317	1,327	0	0	0
Onchocerciasis	987	571	416	0	0	0

DALYs, disability-adjusted life years (the number of healthy years of life lost due to premature death and disability)

and to make better use of existing ones. In human African trypanosomiasis, for example, there is now resistance to melarsoprol, the main treatment for stage II disease. Melarsoprol is an arsenical that is more than half a century old, and one that causes significant mortality itself (4–12%). For treatment of the West African form of disease, the only alternative to melarsoprol is eflornithine, a drug that is currently available because it has additional use in depilatory skin preparations in developed countries [2, 3]. Stage II disease requires hospitalisation and the parenteral route for treatment, and also demands careful and skilled nursing care for optimal management. There is only one drug (DB 289, a diamidine prodrug) in clinical development for treatment of human African trypanosomiasis. Fortunately, DB 289 can be given orally, but even if its clinical development proceeds rapidly, there is still a critical need for better treatment regimens, perhaps based on combinations of the few existing agents [3]. The evaluation of new treatment regimens will be hampered by the poor current understanding of the pathophysiology of human African trypanosomiasis as well as by other limitations such as the inability to diagnose the stage of disease with adequate sensitivity and the difficulties associated with ensuring long-term follow-up of patients in the difficult environments of much of rural Africa.

Treatment options for *Trypanosoma cruzi* infection are even more limited than those for the African trypanosomiases. Only benznidazole and nifurtimox are currently used, providing variable benefits depending on the stage of Chagas disease being treated. Neither drug can be used without the risk of important adverse effects. Apart from the notably recent development of miltefosine for oral treatment of visceral leishmaniasis, many treatments for some of the other protozoal diseases, such as giardiasis and amoebiasis, are still based on archaic formulations. The geohelminthic infections barely fare better, although they have the advantage of being "outside the body" inasmuch as treatments do not need to be absorbed to be effective. This may provide better therapeutic indices than for the more common protozoal infections, but this advantage cannot be extended to infections caused by tissue nematodes such as trichinellae, foodborne trematodes, and schistosomes.

For no parasitic disease, however, have the limitations of current treatments been more starkly illustrated than in the chemotherapy of falciparum malaria: in Southeast Asia, entire regions reside under the spectre of untreatable disease, and in Africa, ineffective cheaper drugs are usually used rather than more effective but unaffordable alternative drugs [4]. Scientific advances, particularly in molecular characterisation of parasitic pathogens, have the potential to make important contributions to the medical management of parasitic diseases. The genome of *Plasmodium falciparum* has recently been decoded [5], and the genomes of some kinetoplastids will be completely sequenced within the next few years. These advances have already yielded insights into novel drug targets and have identified fosfidomycin as a novel antimalarial agent that is now being developed rapidly [6], with more progress sure to follow [7]. Molecular advances will also assist in speciation, for example within the family of kinetoplastids, and may improve our understanding of mechanisms of drug resistance. The sequence of the human genome, together with those of parasitic pathogens, adds to the pharmacogenomic base of knowledge and should eventually influence treatment regimens much more than at present [8, 9].

For all these infections, the application of pharmacokinetic and dynamic principles is fundamental to optimising the therapeutic regimens already being used as well as to developing newer approaches. This short review is therefore necessarily eclectic, but it aims to show how far we have come and where we are going when applying pharmacokinetic principles to the management of some of the important parasitic infections.

Drug Treatment of Parasitic Disease

Parasitic infections offer a seductively visual challenge to investigators. Their diagnosis frequently depends upon examination of the blood or other material from patients. The enumeration therein of parasites or products of infestation (ova) is routinely carried out in most laboratories that are equipped to diagnose their presence. It may appear, therefore, to be entirely logical to link parasite numbers (the apparent parasite "burden") to the severity of disease observed in an individual patient. Taken together with huge advances in our understanding of the pharmacokinetic basis of variation in drug response, we should be able to relate the treatment given and the responses to this treatment in a satisfyingly rigorous way,

using pharmacokinetic and pharmacodynamic analyses. This should then lead to rationalised, better treatment protocols for many common infections. Furthermore, these treatment protocols have the potential to expand when the harvest of novel drug targets first identified by genome sequencing of the pathogen eventually yields new drugs.

Ideally, drugs should have optimal antiparasitic activity and no host toxicity. They should also be able to be administered by the oral route and be required for only short periods of time (ensuring better compliance). If the patient's disease status or the drug formulation prevents oral administration, then there are, in many circumstances, advantages of the use of the intramuscular route of administration (or perhaps the intrarectal route for some drugs such the antimalarial artesunate [10]) over the intravenous route. Most antiparasitic agents, however, do not meet even these simple requirements.

The chemotherapy of parasitic disease also demands that factors relating to the presence of the parasite be considered in addition to the pharmacokinetics of the drug and the pharmacodynamic effects of the drug in the host [11]. For example, parasitic disease may profoundly influence drug disposition in the host. In malaria, there is a progressive contraction of the apparent volume of distribution of quinine as the severity of infection increases. This contraction in volume is associated with a reduction in the systemic clearance of quinine, a prolongation of the elimination half-life, and higher total plasma quinine concentrations (by up to 50%) in acute infection compared with convalescence [12]. Additionally, there is evidence that liver disease complicating schistosomiasis increases praziquantel's area under the curve. When classified by the degree of liver involvement, those with the most severe hepatic dysfunction had AUC and C_{max} values four times those of patients with mild cases of hepatic dysfunction. Elevated serum concentrations of praziquantel in this situation increased the risk of side effects [13]. Parasite populations also vary in their susceptibility to different treatments, which, in extreme cases, manifests as drug resistance. Furthermore, drug concentrations in the microenvironment of the parasite, rather than those measured in the host's circulation, may influence the pharmacokinetics of the drug and, subsequently, the host's clinical response. So, studies in patients are an essential adjunct to those in healthy subjects. However, there are important limitations in quantifying pharmacokinetic/pharmacodynamic relationships in parasitic infections or infestations, and appreciation of these limitations should modulate evaluations of the clinical value of antiparasitic agents. Examples of the usefulness and limitations of pharmacokinetic/pharmacodynamic approaches are given below for some different classes of parasitic diseases.

Principles of Antiparasitic Therapy

For a particular infection, the number of parasites infecting an individual should relate to the severity of disease experienced by that individual.

For the nonsequestering *Plasmodium* spp. (*vivax*, *ovale*, and *malariae*) enumeration of parasites on blood films is simple and allows calculation of the total patient parasite burden [14, 15]. However, each of these species has differing "fever thresholds" (parasitaemia level that causes fever), for reasons that are not fully elucidated but include host factors as important determinants of parasitaemia. These "fever threshold" values interested classical malariologists who first noted, for example, that a higher number (1,000–10,000/μl) of parasites was associated with fever in relapsing vivax malaria compared with the numbers (10–100/μl) associated with primary infections, a difference reflecting antiparasitic immunity in the former group of individuals [14]. On occasion, the diagnosis of vivax malaria is made more difficult also by the relatively low levels of parasitaemia in primary infections, and it may be still more difficult in malariae malaria, whose fever threshold rarely exceeds 5,000 parasites/μl [14]. These observations may be relevant today, for example, when comparing treatment studies of these parasites in geographically distinct regions with different transmission intensities or when comparing studies in which different methodologies were used to count parasites [16, 17]. The role of host immunity may be even more important in other protozoal infections. Babesiosis in humans due to *Babesia divergens*, for example, is often associated with patients whose reticuloendothelial defences have been crippled by splenectomy. The numbers of infected erythrocytes may increase to levels seen in falciparum malaria, requiring intervention with erythrocyte exchange transfusion as well as specific antiparasitic measures [18].

In asynchronous *Plasmodium falciparum* infections with organ involvement, there is a much larger parasite burden not visible by microscopy. In these circumstances, parasitaemia persists longer than that in uncomplicated infections, and circulating (peripheral) parasitaemia is a poorer way of assessing disease status and response to treatment [17]. Other laboratory values or clinical signs, such as indirect measures of parasite burden and organ involvement (e.g., hyperlactataemia) or the depth and length of coma or the occurrence of seizures, are better correlated with disease severity and outcome than is the simple measure of peripheral parasitaemia [19]. If quantification of both the stages of parasite development in a peripheral blood film and the amount of parasite pigment present is also attempted, then a more representative assessment of total parasite burden can be achieved, with useful prognostic implications [20, 21, 22, 23]. Despite the early recognition of the importance of staging parasite development and the pathophysiological importance of parasite pigment in erythrocytes and leucocytes, this type of analysis requires experience in the examination of blood films and is not carried out routinely in most laboratories.

The trypanosomiases illustrate further the complexities of understanding the contribution of parasite burden to disease processes. East African *Trypanosoma rhodesiense* infection generally produces a rapid course of illness with early cardiac involvement and progression to neurological complications within weeks or months after first exposure. Parasite numbers in blood are also higher than the West African (*Gambiense*) variety, aiding parasitological confirmation of the diagnosis. In *Gambiense* infection, which is more indolent in its clinical presentation, there are fewer parasites and diagnosis is harder. However, relating parasite numbers (the "overall burden") to the speed of development of symptoms and disease severity in both infections is less useful than with malaria because much of the pathophysiology of infection probably centres around host responses to the parasite and its products, rather than to tissue damage or impairment of function produced directly by the parasites themselves. In such infections, small numbers of infecting organisms may produce effects disproportionate to their absolute numbers over long periods of time, and the host response to infection may not only limit parasite multiplication but also contribute to morbidity.

Helminths are more complicated than protozoa, not only because they are multicellular organisms but also partly because they do not necessarily multiply inside their hosts. Exceptions include disseminating strongyloidiasis rising from retroinfection with *Strongyloides stercoralis*. In the case of gastrointestinal nematodes, one egg can only produce one infective larva that develops into one adult worm. Therefore, the development of clinical disease in hosts infected with nematodes depends almost entirely on the actual number of larvae infecting a susceptible host. Thus, the outcome of such an infection is more dependent on the parasite burden. Generally speaking, there is a direct correlation between the number of infecting larvae and the severity of any disease produced; a small number of infective larvae will produce minor pathological changes and no obvious clinical disease. It will often take many larvae (thousands or even tens of thousands) to incite pathological changes severe enough to produce serious clinical signs. In schistosomal infections, there is considerable variation in the quantitation of eggs, due to methodological factors as well as to diurnal periodicity in the release of eggs [24]. However, the pathophysiology of infection is clearly related to egg burden [25] and may, in the acute phase of disease, be reversible with antischistosomal drugs. Established infections cause symptoms due to prolonged inflammatory responses against ova that result in fibrosis and scarring, sometimes requiring surgical as well as medical treatment.

The rate of disappearance of parasites should relate to the effectiveness of an antiparasitic agent.

If parasite burden is related to disease severity, then eliminating parasites should cure the disease, providing there are no residual sequelae of infection. It seems to follow that the faster parasites can be eliminated, the "better" the antiparasitic treatment. However, two important considerations modulate this presumption. The first is that cure from infection is not always related to immediate response to treatment, because there is a risk of relapse from undetectable but persistent infections in some cases. The second consideration is that symptomatic relief by rapid reduction of parasite numbers may not be the most important endpoint in assessing clinical response: reductions in other endpoints such as mortality or incidence of neurological or other sequelae may be even more important goals to achieve than seeing parasites disappear. In these instances, the role of adjunct therapies may play an important role in reducing mortality from parasitic infection. For example, prednisolone pretreatment of patients with stage II *Gambiense* infection reduces complications associated with using melarsoprol [26] and also suggests that immunological factors are of pathophysiological significance in producing complications such as encephalopathy. Cure is then defined not only as an early improvement in host responses to infection (for example in clinical status, inflammatory markers, etc.) but also by an absence of indices of infection after an appropriate length of follow up assessments. Steroids (and anticonvulsants) are often more useful as adjunct therapy than is anthelminthic intervention in acute management of neurocysticercosis. Immunological factors are also likely to be important in the Mazzotti reaction [27].

For the intraerythrocytic protozoal infections, the major limitations to assessing pharmacodynamic responses are related to establishing the total parasite burdens with accuracy. In parasites that also have latent hepatic stages of infection (*Plasmodium vivax* and *Plasmodium ovale*), later relapse will not be related to the efficacy of antimalarial agents against asexual-stage parasites alone, necessitating the use of antimalarial agents appropriate for the hepatic stage of development to achieve complete cures. Bearing this in mind, the usefulness of measures of parasite clearance in predicting efficacy of treatments becomes obvious when certain antimalarial regimens (chloroquine, primaquine, and mefloquine) are used to treat *Plasmodium vivax* infections in Thailand [28]. In this detailed study of a model protozoal infection, the more rapidly acting artemisinins used in 5 days of monotherapy achieved the most rapid reductions in parasite numbers, or parasite reduction ratios (the fall in parasitaemia after one cycle [48 h] compared with admission parasitaemia was >1,500 for artemisinins, compared with 449 for mefloquine and 438 for chloroquine and only 97 for quinine), but subsequent reappearance of parasites soon after cessation of artemisinin or quinine therapy was attributed to relapse caused by maturation of hypnozoites rather than recrudescence of the primary infection (Fig. 1A and B). Molecular refinements should allow this hypothesis to be tested.

In *Plasmodium falciparum* infections, parasitaemia may rise, be sustained, or fall rapidly after a particular treatment, changes that reflect either release of merozoites and invasion from sequestered schizonts, sustained circulation of young rings, or parasite sequestration [29]. If the sequestered parasite load is likely to be only a small fraction of the total parasite burden, for example in

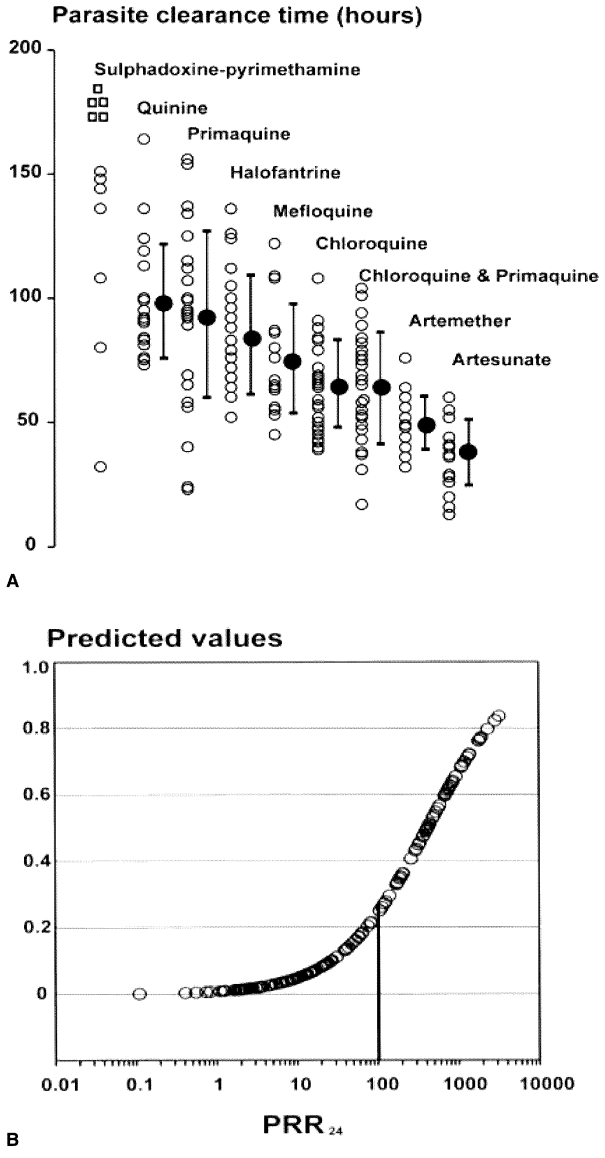

Fig. 1 (**A**) Parasite clearance times for various groups of patients treated for *Plasmodium vivax* infection. Data are means and standard deviations. (**B**) Parasite reduction ratio (PRR24)-dependent predictive value for parasite clearance time (PC_T) of <48 h in patients with *Plasmodium vivax* infection (from Pukrittayakamee et al. [28], with permission)

uncomplicated infections (where by definition there is no major organ involvement), which are caused by synchronous populations of parasites, then initial responses may reflect both the stage specificity and the rapidity of action of an antimalarial agent. Since the elimination of parasites from the blood of patients with malaria can be represented by first-order kinetics [30], increasing drug concentrations above a threshold value will not augment clearance beyond the numbers fixed by the first-order kinetic term [31]. Drug concentrations below the minimum inhibitory concentration are associated with net growth of parasites. Therefore, it is essential that drug concentrations exceed the minimum inhibitory concentration until the last parasite has been cleared from the blood, if complete cure (i.e. eradication of the parasites causing the disease) is to be achieved. If the parasite reduction ratio, the slope of the concentration-response curve, and the pharmacokinetic properties of the drug are known, then the time for which antimalarial drugs need to be sustained in the blood to achieve a clinical cure can be estimated [31]. Most effective antimalarial agents have parasite reduction ratios greater than 100. For those drugs that are rapidly eliminated (i.e. where $T_{1/2}$ is less than 24 h), then, with a parasite reduction ratio of 1,000, four asexual cycles must be covered to ensure cure of infections in which the burden of parasites is greater than 10^9. Resistance (a rightward shift in the concentration-response curve) is associated ultimately with a reduction in the parasite reduction ratio. These pharmacodynamic models have proved to be very useful in increasing our understanding of the action of antimalarial agents, particularly in population-based studies, although there may be limitations in applying such approaches to certain combination therapies (such as quinine with tetracycline [31]).

Rapidly acting antimalarial agents used to treat severe malaria act most potently on the large ring/early trophozoite stage of infection, the very stages of *Plasmodium falciparum* development that are invisible under the microscope because they are sequestered in capillaries and venules [31, 32]. Parasites presumably die in situ. Artemisinins have efficacy against small rings as well as maturing schizonts, stages that are less susceptible to quinolines or quinine [32]. Thus, in many individuals who receive quinine treatment, parasitaemia rises after treatment, since the onset of antimalarial activity takes some hours and mainly affects the early trophozoite stages of parasite development; in contrast, earlier stage ring forms are immediately cleared (within 6–12 h) after exposure to artemisinins [33]. These differences in early parasite clearance kinetics reflect mainly the pharmacodynamic properties of antimalarial agents rather than other modulators such as the immune status of the host, and allow us to translate some results from in vitro pharmacodynamic actions to those observed in vivo [32]. Treatment of onchocerciasis with ivermectin may result in a reduction in parasitaemia as assessed by measurement of microfilariae in skin snips. However, ivermectin has no effect on *Onchocerca* adult females, who may continue to produce microfilariae for many years once treatment has stopped. In the absence of a true macrofilaricide, treatment of onchocerciasis with ivermectin must continue for the lifespan of the adult worms if a successful cure is to be achieved.

Pharmacokinetic analysis of antiparasitic agents should relate to pharmacodynamic (response) measures.

Measurement of antimalarial drug concentrations, particularly for drugs with relatively long elimination half-lives, is useful when attempting to differentiate high-level resistance from pharmacokinetics as a reason for therapeutic failure [29]. Some patients will have relatively low drug concentrations despite having taken the drug, and others may have vomited, failed to absorb the drug, or have been misdosed. In the case of recurring parasitaemia

following the administration of more slowly eliminated drugs, measurements of blood concentration are useful as they indicate that the parasite population could expand, despite drug being present. So, drug concentrations measured at recrudescence must be below the minimum inhibitory concentration of that drug for the infecting parasite in order for the parasite to be considered still sensitive to the antimalarial agent.

For artemisinin derivatives, monitoring of blood concentration provides information useful in a different context, for example, regarding the overall variability in absorption by different routes [10, 34]. Dihydroartemisinin, the active metabolite of many artemisinin derivatives, has a relatively short elimination half-life (around 1 hour), so concentration-time measurements may not accurately reflect previous exposure or help to differentiate parasite "resistance" from inadequate treatment regimens.

In stage II trypanosomiasis, a detailed pharmacokinetic analysis of melarsoprol has produced a much shorter and less unwieldy treatment regimen in comparison to classical treatment protocols [35, 36], underscoring the value of pharmacokinetic approaches to optimising use of scarce resources.

Apart from antiprotozoal chemotherapy, there have been relatively few studies of the relationships between pharmacodynamics and pharmacokinetics. While one reason for this may be an absence of appropriate analytical methodology, it may simply be that the relationship between circulating drug concentrations and the pharmacological response is not a simple one. For example, it may not be necessary for the drug to be present in the blood for a pharmacological effect to be sustained. In the case of diethylcarbamazine, it was demonstrated that the ability of the drug to clear skin microfilariae in onchocerciasis was related to the circulating concentrations of diethylcarbamazine. Moreover, the adverse effects observed in patients with onchocerciasis treated with diethylcarbamazine (Mazzotti reaction) were related not only to the total burden of microfilariae but also to the plasma concentration of diethylcarbamazine. Although reactions to diethylcarbamazine are reduced in proportion to the circulating plasma concentrations, so too is the antiparasitic effect of the drug [37]. While much about the Mazzotti reaction remains to be understood [27], the emergence of ivermectin as a preferred treatment for onchocerciasis means efforts to moderate the adverse effects of diethylcarbamazine take on less importance. By contrast with diethylcarbamazine, the pharmacokinetic-pharmacodynamic relationship for ivermectin in onchocerciasis is less clear. Ivermectin has microfilaricidal activity without any adulticidal effect on the viable female worm of *Onchocerca*. This effect on filarial body load persists for up to 12 months in the absence of reinfection, even though for the majority of this time there is no drug present in the host [38], suggesting that the drug exerts an effect on the parasite for which continued drug presence is unnecessary [11].

Resistance of parasites to antiparasitic treatment should relate to the parasite's biochemical and molecular biological characteristics.

Recent advances in understanding the molecular basis of drug resistance are now being developed into tools that are useful in epidemiological terms (for example, in aiding decisions as to when to change drug policies) and that also have the potential to aid in determining individual patient treatment regimens in the future. In *Trypanosoma brucei* infections, melarsoprol resistance is in major part associated with defective uptake of the drug. Uptake is mediated by a purine transporter (P2), and whilst the molecular basis for the resistant phenotype may vary, there is a common impeded pathway.

In *Plasmodium falciparum* infections, chloroquine resistance is again associated with a phenotype that takes up less drug than do chloroquine-sensitive parasites. Recent advances have established that mutations in PfCRT, the chloroquine resistance transporter protein, are critical to the development of the resistance phenotype [39]. The *Plasmodium falciparum* orthologue of the mammalian multidrug resistance protein may also modulate the chloroquine resistance phenotype, which is associated with variability in both particular single amino acid residues and gene copy number. Assays for changes in PfCRT [40] or Pfmdr1 [41] therefore provide a potentially convenient method for monitoring drug resistance without the necessity of culturing parasites and determining MIC50 values. These molecular methods are currently undergoing validation, although studies may have to be carried out locally in different geographic areas with different patterns of resistance before their applicability to particular populations is established. Once a particular drug target has been identified, then molecular approaches are even more useful. The identification of the mutations in the DHFR-DPS enzyme complex in *Plasmodium falciparum* is a good example of a case in which the molecular target for antifolates has been identified, and susceptibility of parasites can therefore be monitored.

Alarms have been sounded concerning the reduced sensitivity of schistosomes to praziquantel and the reduced sensitivity of filarial nematodes to ivermectin. Patients from villages in Egypt and Senegal have yielded isolates that can tolerate higher dosages of praziquantel than other control isolates when passaged and subjected to drug treatment in mice. In vitro tests on these and the laboratory-selected isolate support the view that a degree of resistance to praziquantel can occur in *Schistosoma mansoni*, but the level of drug resistance found so far is low. Work has begun to identify those genetic, physiological, and morphological characteristics associated with praziquantel resistance, and some of these characteristics may find use as markers for monitoring whether resistance is developing in endemic areas where the drug is used. More intensive application of praziquantel can be expected, particularly in other parts of Africa, and vigilance will be needed to ensure that it continues to be useful as a drug for treatment of schistosomiasis [42].

In the control of onchocerciasis, ivermectin is used either as an adjunct to vector control or as the sole agent. Ivermectin is supplied free of charge by the Mectizan Donation Programme to all residents of onchocerciasis-endemic areas for as long as they need it [43]. The success of ivermectin-based control programmes requires that ivermectin remains effective, however long it takes to control or eliminate the disease. The possibility that *Onchocerca volvulus* could develop resistance to ivermectin has been considered, and a constant surveillance for this phenomenon has been advocated [44]. The World Health Organization (WHO) has urged that methods be established to detect the development of this resistance and to study the underlying mechanisms [45]. Thus, the development of probes to detect ivermectin resistance is an important objective of the WHO's Product Research and Development Unit for Filariases (Macrofil). There is at present no firm evidence of populations of *Onchocerca volvulus* that are resistant to ivermectin. However, whenever a single agent bears the responsibility of disease control and is to be used on a long-term basis in differing dosages in many countries, it is prudent to continuously examine for any differences between the observed and the expected effects of treatment. Should resistance to ivermectin develop and become widespread, the known effects of *Onchocerca volvulus* infection described above may recur, with time.

The use of drugs in combinations may overcome resistance.

Combination chemotherapy is well established in the treatment of mycobacterial infection, HIV infection, and cancer. Should drug resistance develop by spontaneous point mutation or gene amplification, then the probability of resistance developing to two structurally unrelated drugs with different modes of action is a product of the two mutation frequencies. Since these frequencies are very low in malaria parasites, it is most unlikely that a parasite would exist that is spontaneously resistant to two unrelated drugs [4, 31]. For three drugs, the likelihood of a spontaneously resistant viable mutant is the product of three mutation frequencies.

Sulfadoxine and pyrimethamine were used originally in a triple combination with mefloquine when it was deployed in Thailand in 1984. This was done specifically to retard the development of resistance to mefloquine. While theoretically sound, this strategy did not work in practice because *Plasmodium falciparum* in Thailand in 1984 was already highly resistant to both sulfadoxine and pyrimethamine. Moreover, by the time that circulating concentrations of mefloquine had become subtherapeutic, both pyrimethamine and sulfadoxine had disappeared from the blood because of their more rapid systemic clearance. There was no protection of mefloquine from exposure to parasites during the elimination phase. Thus, in order for antimalarial drug combinations to be effective, either the two or three components must be pharmacokinetically well matched, or the parasite biomass must be reduced sufficiently by one of the drug components so that the chances of mutation to the other, more slowly eliminated drug are greatly reduced [4]. This is the rationale behind the combination of artemisinin derivatives with mefloquine and other drugs. The artemisinin derivatives are the most active of the available antimalarial compounds and produce a fractional reduction in parasite biomass of approximately 10^4 per asexual cycle [33]. So, 3 days of treatment, which involves two cycles, usually produces a 10^8-fold reduction in biomass, leaving a maximum of 10^5parasites for the other antimalarial drug (usually mefloquine or lumefantrine) to clear. This reduces considerably the exposure of the parasite population to mefloquine or lumefantrine, thereby reducing the chance of an escape-resistant mutant arising from the infection [4]. The artemisinin derivatives also have the advantage of reducing gametocyte carriage and thus transmission [46]. So, there is a case to be made for no longer using single antimalarial drugs but instead always using a combination of an artemisinin derivative with an existing or newly introduced antimalarial compound. In areas of low malaria transmission, this may be a very effective malaria control measure, although again, such artemisinin-based combinations are not the only ones that are useful in different geographic areas [47].

Artemether-lumefantrine is the first fixed combination of an artemisinin derivative and a second unrelated antimalarial compound. Lumefantrine (formerly benflumetol) is an aryl amino-alcohol in the same general group as mefloquine and halofantrine. It was discovered, as many of our currently useful antimalarial agents, in the People's Republic of China and has been used there for several years. Lumefantrine is active against all the human malaria parasites, including multidrug resistant *Plasmodium falciparum* (although there is some cross-resistance with halofantrine and mefloquine). Artemether-lumefantrine has been used mainly at an adult oral dose of 80/480 mg given at 0, 8, 24, and 48 hours. This has given satisfactory cure rates in semi-immune subjects but has proved inferior to artesunate-mefloquine in nonimmune individuals. Pharmacokinetic-pharmacodynamic studies have indicated that the principal pharmacokinetic determinant of cure was the area under the plasma lumefantrine concentration-time curve (AUC), or its surrogate, the day 7 lumefantrine level. Day 7 levels over 500 ng/ml are associated with >90% cure rates [48]. Lumefantrine absorption (like that of atovaquone and halofantrine) is critically dependent on coadministration with fats, and thus plasma concentrations vary markedly between patients. To increase the AUC and thus the cure rate, a six-dose regimen (adult dose 80/480 mg at 0, 8, 24, 36, 48, 60 hours) was evaluated. This has proved highly effective and remarkably well tolerated. Against multidrug-resistant falciparum malaria, the six-dose regimen of artemether-lumefantrine was as effective and was better tolerated than artesunate-mefloquine [49] Artemether-lumefantrine is becoming increasingly available in tropical countries, despite its cost. The rapid and reliable therapeutic response, the high level of efficacy, and the theoretical mutual protection provided by each of the drugs against

resistance selection makes combinations such as this attractive antimalarial treatments.

Can studies necessary to optimise drug therapy of parasitic diseases be performed in the tropics?

Drugs should be given via the most suitable route in the right amount for an appropriate period. Sensitive and selective methods can guarantee precise and accurate monitoring of drug concentrations in plasma, but determination of pharmacodynamic endpoints and/or surrogate markers of drug response have proven more elusive. Pharmacokinetic models can provide a mathematical representation of the processes by which a drug enters the body, is distributed throughout the body, is enzymatically transformed to another species, and is finally eliminated. Such models can be combined with a mathematical description of the concentration-response relationship (where this information is available) to create a combined pharmacokinetic-pharmacodynamic model to aid the clinician in optimising drug use.

Conventional pharmacokinetic studies involve multiple blood sampling in relatively small numbers of patients and/or healthy volunteers. Such studies are labour-intensive and inconvenient to both subject and investigator, and the findings may not be representative of the target population. Larger studies should be performed in which the influence of certain patient characteristics (age, sex, weight, ethnicity, disease state, etc.) on drug disposition can be explored. The principal drawback to such studies is the intensity of blood sampling that is required to characterise a complete pharmacokinetic profile. An alternative approach is to use pharmacokinetic data from relatively few blood samples per individual and to analyse the information using population-based (Bayesian) methods of analysis. Key pharmacokinetic parameters can be determined, and those patient characteristics (covariates) that explain the majority of the interindividual variability can be identified. [50, 51, 52]. Dedicated software is available commercially to handle such operations, and most of these analyses can be performed on a simple desktop or laptop computer. The studies with artemether-lumefantrine referred to above used such methodology. Additionally, pharmacokinetic-pharmacodynamic relationships involving mefloquine were also explored using a population approach [53]. Looking at a number of trials conducted over a 5-year period, it was demonstrated that mefloquine pharmacokinetics were not significantly influenced by age, sex, or measures of acute malaria severity. Dose splitting was associated with increased oral bioavailability of mefloquine, which in turn was associated with more rapid clearance of parasites (Fig. 2). It has been possible to successfully use a retrospective population-based pharmacokinetic analysis of sparse data to prove the therapeutic benefit of split-dose mefloquine in uncomplicated falciparum malaria.

For treatment of severe malaria, a prospective population-based pharmacokinetic study of intramuscular quinine examined a loading dose and showed that it is reliably absorbed. Demographic variables or other measures of disease severity do not influence pharmacokinetic profiles,

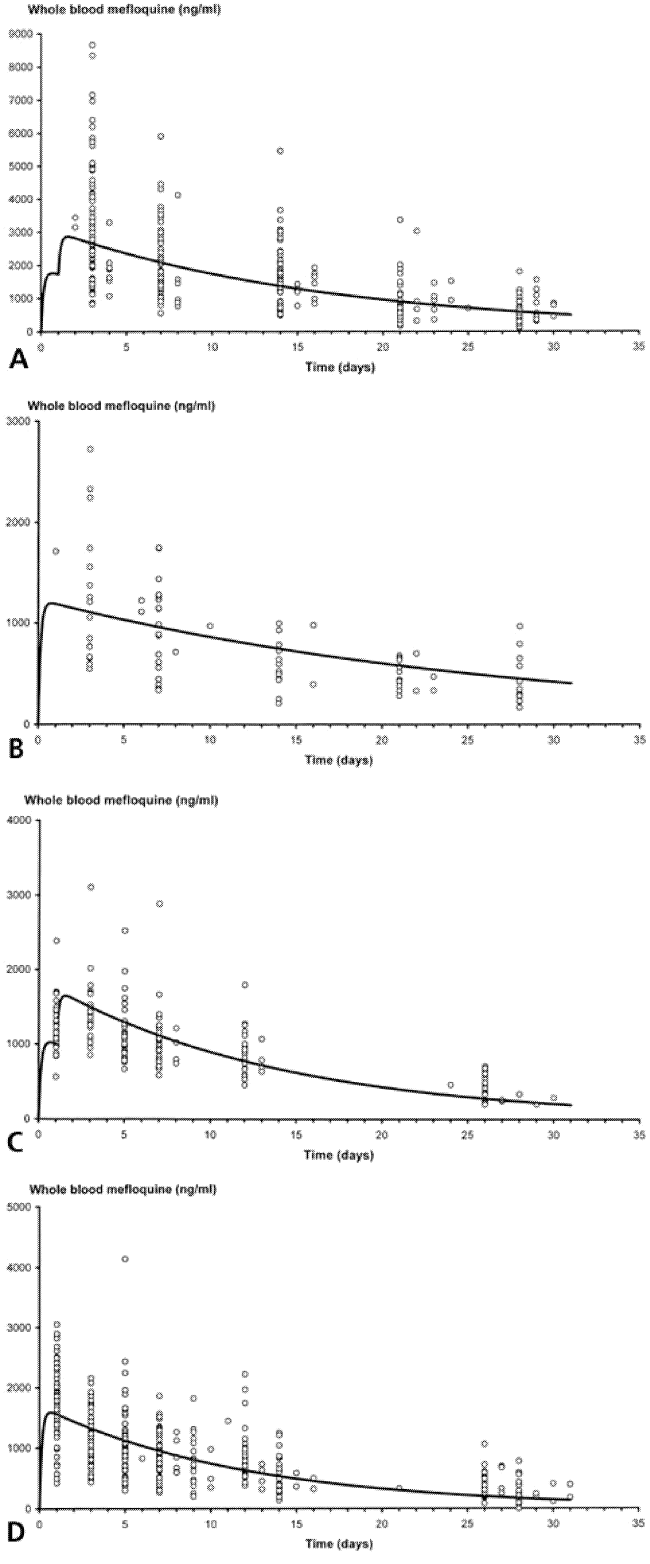

Fig. 2 Whole blood mefloquine concentrations in severe malaria. The solid line shows the fit of the data and represents the predicted concentration profile for the population mean. **A** is mefloquine with split dosing of 15 and 10 mg/kg; **B** is mefloquine in a single dose of 25 mg/kg; **C** is mefloquine and artesunate with split dosing of 15 mg/kg, 10 mg/kg; and **D** is mefloquine and artesunate in a single dose of 25 mg/kg (from Simpson et al. [53], with permission)

providing reassurance that this route of administration is appropriate for children [54]. Population modelling may not be possible for all drugs, but where successfully applied, it is a powerful methodology.

Conclusions

Our successes in understanding the pharmacokinetic-pharmacodynamic relationships among antimalarial agents are yet to be matched in other parasitic diseases. While this may be because there is a lack of appropriate pharmacokinetic information, it might also be because the relationship between the circulating drug concentrations and the antiparasitic effect is poorly understood or because the measurement of a true pharmacodynamic endpoint is impossible. Unless these problems can be overcome, the clinician will not have the information necessary to enable optimal treatment of patients who may be severely ill and in need of immediate, life-saving attention.

References

1. Stephenson LS, Latham MC, Ottesen EA (2000) Malnutrition and parasitic helminth infections. Parasitology 121 (Suppl): S23–S38
2. Stich AH, Abel P, Krishna S (2002) Human African trypanasomiasis. Brit Med J 325:203–206
3. Stich AH, Barrett M, Krishna S (2003) Waking up to sleeping sickness. Parasitol Today 19:195–197
4. White NJ (1999) Antimalarial drug resistance and combination chemotherapy. Phil Trans R Soc Lond B 354:739–749
5. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen IT, et al (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature 419:498–511
6. Missinou MA, Borrmann S, Schindler A, Issifou S, Adegnika AA, Matsiegui PB, Binder R, Lell B, Wiesner J, Baranek T, Jomaa H, Kremsner PG (2002) Fosmidomycin for malaria. Lancet 360:1941–1942
7. Ridley RG (2002) Medical need, scientific opportunity and the drive for antimalarial drugs. Nature 415:686–693
8. Evans WE, McLeod HL (2003) Pharmacogenomics—drug disposition, drug targets, and side effects. N Engl J Med 348:538–549
9. Weinshilboum R (2003) Inheritance and drug response. N Engl J Med 348:529–537
10. Krishna S, Planche T, Agbenyega T, Woodrow C, Agranoff D, Bedu-Addo G, Owusu-Ofori AK, Appiah JA, Ramanathan S, Mansor SM, Navaratnam V (2001) Bioavailability and preliminary clinical efficacy of intrarectal artesunate in Ghanaian children with moderate malaria. Antimicrob Agents Chemother 45:509–516
11. Edwards G, Winstanley PA, Ward SA (1994) Clinical pharmacokinetics in the treatment of tropical disease: some applications and limitations. Clin Pharmacokinet 27:150–165
12. Supanaranond W, Davis TME, Pukrittayakamee S, Silamut K, Karbwang J, Molunto P, Chanond, L, White NJ (1991) Disposition of oral quinine in acute falciparum malaria Eur J Clin Pharmacol 40:49–52
13. Watt G, White NJ, Padre L, Ritter W, Fernando MT, Ranoa CP, Laughlin LW (1998) Praziquantel pharmacokinetics and side effects in *Schistosoma japonicum*-infected patients with liver disease J Infect Dis 157:530–535
14. Field JW, Sandosham AA, Yap LF (1963) Technical miscellanea. The microscopical diagnosis of human malaria. I. A morphological study of the erythrocytic parasites in thick blood films, 2nd edn. Institute of Medical Research, Federation of Malaya, Kuala Lumpur
15. Field JW, Shute PG (1956) The microscopic diagnosis of human malaria—a morphological study of the erythrocytic parasite. Government Press, Kuala Lumpur
16. White NJ, Krishna S (1989) Treatment of malaria: some considerations and limitations of current methods of assessment. Trans R Soc Trop Med Hyg 83:767–777
17. Planche T, Krishna S, Kombila M, Engel K, Faucher J, Ngou-Milama E, Kremsner P (2001) A comparison of laboratory methods for the assessment of children with malaria. Am J Trop Med Hyg 65:599–602
18. Chiodini P (2003) Babesiosis. In: Cook G, Zumla A (eds) Manson's tropical diseases, 21st edn, vol. 1. Elsevier Science, pp 1297–1301
19. Krishna S, Waller D, Kuile F ter, Kwiatkowski D, Crawley J, Craddock CFC, Nosten F, ChapmanD, Brewster D, Holloway PA, White NJ (1994) Lactic acidosis and hypoglycaemia in children with severe malaria: pathophysiological and prognostic significance. Trans R Soc Trop Med Hyg 88:67–73
20. Silamut K, White NJ (1993) Relation of the stage of parasite development in the peripheral blood to prognosis in severe malaria. Trans R Soc Trop Med Hyg 87:436–443
21. Amodu OK, Adeyemo AA, Olumese PE, Gbadegesin RA (1998) Intraleucocytic malaria pigment and clinical severity of malaria in children. Trans R Soc Trop Med Hyg 92:54–56
22. Metzger WG, Mordmüller BG, Kremsner PG (1995) Malaria pigment in leucocytes. Trans R Soc Trop Med Hyg 89:637–638
23. Phu NH, Day N, Diep PT, Ferguson DJP, White NJ (1995) Intraleucocytic malaria pigment and prognosis in severe malaria. Trans R Soc Trop Med Hyg 89:200–204
24. Braun-Munzinger RA, Southgate BA (1992) Repeatability and reproducibility of egg counts of *Schistosoma haematobium* in urine. Trop Med Parasitol 43:149–154
25. Cheever AW, Kamel IA, Elwi AM, Mosimann JE, Danner R (1997) *Schistosoma mansoni* and *S. haematobium* infections in Egypt. II. Quantitative parasitological findings at necropsy. Am J Trop Med Hyg 26:702–716
26. Pepin J, Milord F, Guern C, Mpia B, Ethier L, Mansinsa D (1989) Trial of prednisolone for prevention of melarsoprol-induced encephalopathy in Gambiense sleeping sickness. Lancet i:1246–1250
27. Cooper PJ, Guderian RH, Prakash D, Remick DG, Espine I, Nutman TB, Taylor DW, Griffin GE (1996) RANTES in onchocerciasis: changes with ivermectin treatment. Clin Exp Immunol 106:462–467
28. Pukrittayakamee S, Chantra A, Simpson JA, Vanijanonta S, Clemens R, Looareesuwan S, White NJ (2000) Therapeutic responses to different antimalarial drugs in vivax malaria. Antimicrob Agents Chemother 44:1680–1685
29. White NJ (2002) The assessment of antimalarial drug efficacy. Trends Parasitol 18:458–464
30. Day NP, Pham TD, Phan TL, Dinh XS, Pham PL, Ly VC, Tran TH, Nguyen TH, Bethell DB, Nguyan HP, Tran TH, White NJ (1996) Clearance kinetics of parasites and pigment-containing leukocytes in severe malaria. Blood 88:4694–4700
31. White NJ (1997) Assessment of the pharmacodynamic properties of antimalarial drugs *in vivo*. Antimicrob Agents Chemother 41:1413–1422
32. Kuile FO ter, White NJ, Holloway PA, Pasvol G, Krishna S (1993) *Plasmodium falciparum*: *In vitro* studies of the pharmacodynamic properties of antimalarials used for the treatment of severe malaria. Exp Parasitol 76:85–95
33. Watt G, Shanks GD, Phintuyothin P (1992) Prognostic significance of rises in parasitaemia during treatment of falciparum malaria. Trans R Soc Trop Med Hyg 86:359–360

34. Nealon C, Dzeing A, Muller-Romer U, Planche T, Sinou V, Kombila M, Kremsner PG, Parzy D, Krishna S (2002) Intramuscular bioavailability and clinical efficacy of artesunate in Gabonese children with severe malaria. Antimicrob Agents Chemother 6:3933–3939
35. Burri C, Baltz T, Giroud C, Doua F, Welker HA, Brun R (1993) Pharmacokinetic properties of the trypanocidal drug melarsoprol. Chemotherapy 39:225–234
36. Burri C, Nkunku S, Merolle A, Smith T, Blum J, Brun R (2000) Efficacy of new, concise schedule for melarsoprol in treatment of sleeping sickness caused by *Trypanosoma brucei gambiense*: a randomised trial. Lancet 355:1419–1425
37. Awadzi K, Adjepon-Yamoah KK, Edwards G, Orme ML'E, Breckenridge AM, Gilles HM (1996) The effect of moderate urine alkalinisation on low-dose diethylcarbamazine therapy in patients with onchocerciasis. Br J Clin Pharmacol 21:669–676
38. Edwards G, Dingsdale A, Helsby NA, Orme ML'E, Breckenridge AM (1988) Relative systemic availability of ivermectin after administration as capsule, tablet and oral solution. Eur J Clin Pharmacol 35:681–684
39. Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT, Ursos LM, Sidhu B, Naude B, Deitsch KW, Su XZ, Wootton JC, Roepe PD, Wellems TE (2000) Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Mol Cell 6:861–871
40. Djimde A, Doumbo OK, Cortese JF, Kayentao K, Doumbo S, Diourte Y, Dicko A, Su XZ, Nomura T, Fidock DA, Wellems TE, Plowe CV, Coulibaly D (2001) A molecular marker for chloroquine-resistant falciparum malaria. N Engl J Med 344:257–263
41. Price R, Cassar CA, Brockman A, Duraisingh M, van Vugt M, White NJ, Nosten F, Krishna S (1999) *Pfmdr1* gene amplification and multidrug-resistant *Plasmodium falciparum* on the north west border of Thailand. Antimicrob Agents Chemother 43:2943–2949
42. Doenhoff MJ, Kusel JR, Coles GC, Cioli D (2002) Resistance of *Schistosoma mansoni* to praziquantel: is there a problem? Trans R Soc Trop Med Hyg 96:465–469
43. Dull HB (1990) Mectizan donation and the Mectizan Expert Committee. Acta Leiden 59:399–403
44. Awadzi K (1993) Drug surveillance: international cooperation past, present and future. In: Proceedings of the XXVIIth CIOMS (Council for International Organisation of Medical Sciences) Confererence, Geneva,14–15 September 1993, pp 36–140
45. World Health Organisation (1995) Onchocerciasis and its control. Report of a WHO expert committee on onchocerciasis control. Tech Report Ser 852:1–104
46. Price RN, Nosten F, Luxemburger C, Kuile F ter, Paiphun L, Chongsuphajaisiddhi T, White NJ (1996) The effects of artemisinin derivatives on malaria transmissibility. Lancet 347:1654–1658
47. Kremsner PG, Krishna S (2002) Antimalarial cocktails—tropical flavours of the month. Lancet 360:1998–1999
48. Ezzet F, Vugt M van, Nosten F, Looareesuwan S, White NJ (2000) The pharmacokinetics and pharmacodynamics of lumefantrine (benflumetol) in acute uncomplicated falciparum malaria. Antimicrob Agents Chemother 44:697–704
49. Vugt M van, Brockman A, Gemperli B, Luxemburger C, Gathman I, Royce C, Slight T, Looareesuwan S, White NJ, Nosten F (1998) Randomised comparison of artemether-benflumetol and artesunate-mefloquine in the treatment of multi-drug resistant falciparum malaria. Antimicrob Agents Chemother 42:135–139
50. Aarons L (1993) Sparse data analysis. Eur J Drug Metab Pharmacokinet 18:97–100
51. Simpson JA, Aarons L, White NJ (2001) How can we do pharmacokinetic studies in the tropics? Trans R Soc Trop Med Hyg 95:347–351
52. Vozeh S, Steimer JL, Rowland M, Morselli P, Mentre F, Balant LP, Aarons L (1996) The use of population pharmacokinetics in drug development. Clin Pharmacokinet 30:81–93
53. Simpson JA, Price R, Kuile F ter, Teja-Isavatharm P, Nosten F, Chongsuphajaisiddhi T, Looareesuwan S, Aarons L, White NJ (1999) Population pharmacokinetics of mefloquine in patients with acute falciparum malaria. Clin Pharmacol Ther 66:472–484
54. Krishna S, Nagaraja NV, Planche T, Agbenyega T, Bedo-Addo G, Ansong D, Owusu-Ofori A, Shroads AL, Henderson G, Hutson A, Derendorf H, Stacpoole PW (2001) Population pharmacokinetics of intramuscular quinine in children with severe malaria. Antimicrob Agents Chemother 245:1803–1809