

## EDITORIAL

### Rationale and prospects of combination therapy as a strategy for delaying antimalarial drug resistance

#### Introduction

Resistance to antimalarial drugs is an increasingly serious problem (1) and, apart from artemisinin derivatives, there is a dearth of new compounds on the horizon. There is therefore an urgent need to optimize the use of the currently available drugs, and there has been increasing advocacy for using them in combination (2-5). The Papua New Guinea Department of Health has recently changed the recommended therapy for uncomplicated malaria in adults from chloroquine (CQ) alone to a combination of chloroquine with Fansidar (sulfadoxine-pyrimethamine or SP). For children, amodiaquine (AQ), another 4-aminoquinoline, is to be used instead of chloroquine. In this article I will review the rationale for combination therapy as a way of delaying resistance, concentrating on CQ, AQ and SP, and make some suggestions for evaluating and monitoring the extent to which its predicted benefits are borne out in practice.

#### Rationale for combination therapy

Combination therapy is well established for the treatment of many illnesses such as HIV infection (6), tuberculosis and other bacterial infections (7), and leukaemia (8). To gain resistance to a drug combination, an organism must simultaneously develop multiple mutations (assuming the drugs have different modes of action). If these mutations arise independently, then the probability of a double mutation arising is the product of the individual probabilities. For example, if a resistance mutation for each of the two drugs arises once per billion divisions, then the double mutation arises once per billion billion. By contrast, if one drug is used alone until resistance develops and is then replaced by the second drug (ie, a policy of drug cycling) then only one billion divisions would be needed for resistance to arise to the first drug, and then only one billion more for resistance to the second drug. In this scenario, the singly-resistant parasites would initially be rare,

which would increase the time needed for them to generate the second billion divisions, but nevertheless the overall time to achieve double resistance would in theory be less. Mathematical models (9-12) predict that, under a variety of conditions, a policy of combination therapy is generally better — in terms of delaying drug resistance — than other policies such as cycling of single drugs. This applies to the spread of preexisting mutations, as well as those arising *de novo*. The only predicted exception occurs when multiple antibiotic resistance mutations are carried on the same plasmid (13), which should not be relevant for *Plasmodium*. Another benefit of combination therapy can be expected on pharmacological grounds. The combination increases the rate of reduction of the parasites, so the infection is brought under control while the drug concentrations are still relatively high. This reduces the opportunity for selection of resistance, since it exposes parasites to sublethal drug concentrations for a shorter time (14).

This account of combination therapy has implicitly made some simplifying assumptions, such as parasites not being exposed to either drug alone. In practice, differing pharmacological properties mean that this is not strictly true. In particular, the drug with the longer elimination half-life will be 'exposed' unless the other drug is given more than once. If the drugs do have well-matched half-lives, resistance should be hindered if they both have short, rather than long half-lives (other aspects, including treatment efficacy, being equal). This is because a long half-life increases the chances of the patient acquiring a new infection while the drugs remain at residual, sublethal concentrations. For the same reason a steep concentration-effect relationship should also hinder the development of resistance (15).

"Parasite reduction seems to be a first-order process throughout" (16), ie a constant proportion of the parasites are removed over

each asexual cycle. This means that the number of parasites decreases exponentially (as long as the drug concentration remains sufficiently high), so it takes as long to reduce the parasite density from 10 to 5 parasites per microlitre as it does from 10,000 to 5,000. Unless the immune system is invoked to postulate a level of parasitaemia “at which the host’s defences can remove the residuum” (16), a long treatment course may be needed, although this would have the disadvantage of increasing the selection pressure for resistance. Choosing the optimal duration of therapy may therefore not be totally straightforward (17).

The origin and spread of antimalarial drug resistance are thought also to depend on transmission intensity, although the exact relationship remains unclear (18). Resistance has often arisen in areas of low transmission, where infections are more likely to be symptomatic and parasites are more likely to be exposed to drugs. For example, resistance of *Plasmodium falciparum* to CQ was first detected in South-East Asia and South America (16). However, once multiresistant parasites have arisen, they may spread more quickly in areas of high transmission (assuming drug pressure is applied). The component genes of the multiresistant genotype will tend to be separated by genetic recombination, but it seems that this effect will usually be outweighed by the greater potential reproduction per generation (11).

### Combination therapy in practice

Despite its successes, combination therapy has not always proved as effective as hoped. For example, initial hopes that highly active antiretroviral therapy (HAART) could eradicate HIV from individuals have given way to a realization that available drugs should be used with caution (6,19). For malaria, the combination SP plus mefloquine (MQ) was introduced in Thailand in an attempt to halt the spread of resistance, but was unsuccessful. The failure was ascribed to the long half-life of MQ (Table 1) and the shallow slope of its concentration-effect relationship (15), as well as to the high initial prevalence of resistance to SP. By contrast, combining MQ with artesunate (an artemisinin derivative) in the same area has been followed by a stabilization

of resistance to MQ (20), although doubts have been expressed as to the causality of this association and its generalizability to other regions (18,21). Combinations with artesunate have several theoretical advantages such as the rapid reduction in parasite numbers ( $\geq 10,000$ -fold per asexual cycle, compared to  $\leq 1000$  for SP) (14) and its shorter half-life (Table 1), although this means that repeated doses are needed to clear parasites and prevent other drugs from being exposed. Possible drug interactions must of course also be taken into account — for example, artemisinin derivatives potentiate mefloquine but antagonize antifolates (22,23) — although weak antagonism in vitro may not have clinical significance.

### Prospects for the chloroquine-sulfadoxine-pyrimethamine combination

Chloroquine is believed to act by blocking detoxification by the parasite of haemin, which is produced by digestion of human haemoglobin. Resistance is associated with mutations in the *pfmdr1* and *pfcr1* genes, which code for a P-glycoprotein homologue and a transmembrane protein respectively (24-26), although the mutual roles of these and possibly other genes have yet to be fully clarified. The first report of *Plasmodium falciparum* resistant to CQ in Papua New Guinea was in 1976 by Grimmond et al. (27). Resistance was widespread by 1983 (28), with in vitro resistance now being almost universal in at least one province (29). Amodiaquine resistance is also present (30-32). The first ever report of *Plasmodium vivax* resistance to CQ was in 1989, and referred to an infection acquired in Papua New Guinea (33), with additional cases being reported shortly afterwards (34).

The components of SP have synergistic modes of action on different stages of the synthetic pathway to tetrahydrofolate, which is essential for DNA synthesis (26). More specifically, sulfadoxine and pyrimethamine compete for the enzymes dihydropteroate synthetase (DHPS) and dihydrofolate reductase (DHFR) respectively. Resistance develops rapidly to SP despite the fact that it is a combination of drugs. Various point mutations in the genes for DHFR and DHPS are

associated with decreased parasite susceptibility (16). The genes are on different chromosomes, but field isolates are usually resistant to either both drugs or neither (35,36), and “no satisfactory explanation exists for the rapid development of resistance to the combination” (37). In Papua New Guinea, resistance of *Plasmodium falciparum* to SP was reported in 1980 (38). In vivo and in vitro resistance to SP has been found (36,39), but there is less information than for the 4-aminoquinolines.

In a systematic review of the efficacy of 4-aminoquinolines combined with SP, McIntosh and Greenwood (40) identified only five trials but found some evidence favouring the combinations in terms of control of symptoms (especially fever) and of cure rate, without apparent serious adverse events. Since then, at least three more trials have been published (41-43), with similar conclusions.

There is not a great deal of published evidence on which to base expectations for the success of CQ+SP (or AQ+SP in children) in Papua New Guinea. In Colombia, CQ+SP was the first-line treatment for uncomplicated malaria from 1986 to 1999, when increasing resistance to CQ prompted a change to AQ+SP (44). Here, the emergence of resistance to SP may have been delayed by the addition of primaquine to reduce the transmission of gametocytes of surviving strains, although the magnitude of such a benefit is uncertain. Venezuela used CQ+SP in a similar way for four years (Leopoldo Villegas, personal communication). Vanuatu has been using CQ+SP for *Plasmodium falciparum*, and CQ alone for *P. vivax* since 1991 (45). Other countries which have recently decided to implement a policy of CQ+SP include Ethiopia, Uganda and Zimbabwe (Sylvia Meek, personal communication) but little information is currently available on the success of these policies. The mode of action of the 4-aminoquinolines is independent of the antifolate drugs, although the discrepancy between the half-lives of the antifolates and CQ is greater than it is with MQ that was used in the unsuccessful Thai policy of the 1980s (Table 1). Moreover, resistance to 4-aminoquinolines is already common in Papua New Guinea and, despite the smaller amount of

data, the same may well also be true for SP. In addition, the wide range of transmission intensities offers a variety of opportunities for resistance to emerge and spread. Hence there are reasons to fear that resistance to CQ+SP may not take long to develop.

**TABLE 1**

TERMINAL ELIMINATION HALF-LIVES OF  
SELECTED ANTIMALARIAL DRUGS

Chloroquine	1-2 months
Mefloquine	2-3 weeks
Sulfadoxine	10 days
Pyrimethamine	3 days
Quinine	16 hours
Artesunate	1 hour

Data from N. White (16)

### **The future of antimalarial combination therapy**

The failure of previous policies, which seemed well-founded when initiated, emphasizes the need to closely monitor the progress of new drug combinations. This applies to the combinations of 4-aminoquinolines with SP, as well as to combinations with artemisinin derivatives, since they are the remaining class of currently available drugs to which in vivo resistance has yet to be observed. This monitoring should include sentinel sites at which resistance is measured according to standardized (WHO) protocols, along with genetic markers, and key covariates such as transmission intensity and treatment-seeking behaviour. Such a scheme is operating effectively in East Africa, called the East African Network for Monitoring Antimalarial Treatment (EANMAT). In addition, steps should be taken to ensure that compliance is as high as possible. To guide future policy decisions, there is a need for studies specifically designed to have drug resistance as an endpoint. Such trials are starting to appear for antibiotics, for example the crossover study of intensive care units by de Man et al. (46). Cluster randomization is another suitable methodology (47) which would be more applicable to malaria. Despite

the practical difficulties in conducting such trials, they are capable of providing crucial evidence on which to base vital decisions on the best use of the few remaining antimalarial drugs.

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