Drug resistance in malaria

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Drug resistance in malaria is now widespread and in many parts of the world is making treatment increasingly difficult. This article reviews current knowledge of the mechanisms and extent of resistance of Plasmodium falciparum and P. vivax to the available antimalarial drugs, and the recommendations for treating malaria in regions where resistance is prevalent.

Nearly 300 million people have malaria parasites in their blood as you read this article, and 0.5–2 million (mainly African children) will die from this parasitic infection each year. Of the four human parasites only P. falciparum regularly kills. This parasite has developed resistance to nearly all available antimalarial drugs\(^1\). Most important of these is chloroquine, a cheap, simply administered and relatively well-tolerated antimalarial that had become the mainstay of antimalarial treatment throughout the tropical world. In recent years P. vivax, particularly from Oceania and some parts of south-east Asia, has also developed resistance to chloroquine.

Antimalarial drugs

Dihydrofolate reductase inhibitors

Proguanil, chlorproguanil, and later pyrimethamine were developed shortly after the second world war. The antimalarial biguanides, proguanil and chlorproguanil, are metabolised to the active cyclic triazine metabolites, cycloguanil and chlorcycloguanil, respectively. These two metabolites, together with pyrimethamine, and the related antibacterial trimethoprim, all selectively inhibit plasmodial dihydrofolate reductase (DHFR). Their antimalarial activity is potentiated considerably by the addition of dihydropteroate synthase (DHPS) inhibitors (sulphonamides...
or sulphones) which block the conversion of p-amino benzoic acid to dihydropteroate and, thereby, provide sequential inhibition of the folate biosynthesis pathway. The sulphonamides used most commonly are the long acting sulphadoxine, and sulphalene. Although amplification of DHFR genes, and thus increased expression of the enzyme, can be induced experimentally, resistance in wild parasites is caused by mutations in the gene which confer reduced susceptibility of the enzyme to inhibitors. Unfortunately, single point mutations in the plasmodial DHFR and DHPS genes confer a significant reduction in affinity for these antimalarial drugs. For DHFR inhibitor resistance, the initial mutation is usually at position 108. Initially, this is usually Ser→Asn which confers pyrimethamine resistance, but only slightly reduced sensitivity to cycloguanil. Interestingly the Ser→Thr mutation at 108 combined with Ala→Val at position 16 confers cycloguanil but not pyrimethamine resistance. Additional mutations confer resistance to both classes of drugs. In areas such as south-east Asia, multiple DHFR mutations conferring high level pyrimethamine and cycloguanil resistance are now common. In clinical practice, resistance of both *P. falciparum* and *P. vivax* to proguanil, and later pyrimethamine, developed rapidly after the drugs were introduced in malaria endemic areas, and these drugs are no longer used alone in treatment. Resistance to the widely used sulphadoxine-pyrimethamine combination is now widespread in South America and East Asia. The combination is still effective in east and central Africa where it is steadily replacing chloroquine as first-line treatment for falciparum malaria. Unfortunately, resistance has developed already in a few areas. In west Africa, where pyrimethamine was used alone for many years and levels of resistance are often higher, sulphadoxine-pyrimethamine is unlikely to remain effective for long.

**Aminoquinoline resistance**

Chloroquine is a remarkable antimalarial compound. It is rapidly active against the blood (asexual) stages of all four species of human malaria. It is cheap (total cost of treatment less than 10 cents), well tolerated, and requires only two or three days treatment for cure. Unfortunately, since 1959 resistance in *P. falciparum* has spread progressively from the two epicentres of Colombia and eastern Thailand to envelop most of the tropical world. Few areas are now unaffected; these are central America north of the Panama canal including Haiti, north Africa, some areas of the Middle East, and some parts of the east Asia. The most important loss to chloroquine resistance has been Africa where up to 90% of the global mortality from malaria occurs. Although chloroquine is still widely used across Africa, its efficacy is declining and it can no
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longer be relied upon in severe malaria\textsuperscript{13}. Resistance has been associated with an increase in the prevalence of severe anaemia and often an increase in mortality. Pyrimethamine/sulphadoxine has now replaced chloroquine as first line treatment in some areas of east and south Africa. Nevertheless, chloroquine is widely available and widely used throughout the tropical world and in many countries the majority of the population has chloroquine detectable in the blood at any time. The mode of action of chloroquine and related aminoquinolines is still not entirely clear, although it appears to be related to non-enzymatic inhibition of haem polymerisation\textsuperscript{14}, a necessary defence mechanism for the parasite. The parasite must detoxify free haem which is liberated during the digestion of haemoglobin. This is accomplished by the production of an insoluble and biologically inert polymer of \(\beta\)-haematin known as haemozoin or the malaria pigment\textsuperscript{15}. Chloroquine resistance is associated with reduced concentrations of chloroquine in the parasite digestive vacuole and this results from both reduced ingress and increased egress of chloroquine from this compartment. The association of chloroquine resistance with the P glycoprotein pump that mediates the multiple drug resistance (MDR) phenotype is still controversial\textsuperscript{13,16-18}. Two homologues of the MDR gene family, which are part of the ATP binding cassette gene superfamily, have been identified in \(P.\ falciparum\). The gene \(Pfmdr1\) encodes for a 162 kDa homologue of the ATP-dependent P glycoprotein pump (Pgh) that was identified originally in mammalian tumour cells. In general, the association with amplification of MDR genes (\(Pfmdr1\)) is closest with mefloquine resistance\textsuperscript{19}. In areas where there is reduced susceptibility to both drugs, mefloquine and chloroquine resistance in \(P.\ falciparum\) show an inverse relationship. Chloroquine resistance has been localised to a section of the parasite’s chromosome 7\textsuperscript{20}, but the precise molecular basis for resistance has yet to be identified. Chloroquine resistance can be reversed by a variety of structurally unrelated pump inhibitors such as verapamil, fluoxetine, amlodipine, cyproheptadine, desipramine, phenothiazines, etc\textsuperscript{21}. Unfortunately none of these has proved to be of clinical benefit in combination with chloroquine\textsuperscript{22}. Recently, chloroquine-resistance in \(P.\ vivax\) has been identified in Sumatra and Oceania\textsuperscript{23,24}. The molecular basis for resistance in \(P.\ vivax\) has not been identified. Chloroquine resistance is likely to be multi-genic and develops slowly over time, thus, whereas pyrimethamine resistant \(P.\ falciparum\) infections may not respond at all to therapeutic doses of pyrimethamine, parasites with low-grade chloroquine resistance still show partial responses to chloroquine treatment. For this reason, chloroquine is still a useful drug in many parts of the world where chloroquine resistant parasites are prevalent, although in general the full impact of antimalarial drug resistance in endemic areas in terms of morbidity and mortality is usually underestimated.
The Mannich bases

Amodiaquine and amopyroquine are closely related compounds with increased activity against moderately chloroquine-resistant isolates of *P. falciparum*. After oral administration, amodiaquine is almost entirely metabolised to a biologically active metabolite, desethylamodiaquine. Although resistance to these compounds and to chloroquine is correlated, in clinical practice cure rates in areas with low or intermediate grade chloroquine resistance (such as much of Africa) are significantly better with amodiaquine than with chloroquine. Amodiaquine should not be used in prophylaxis because it is associated with an unacceptably high incidence of agranulocytosis (approximately 1:2000) and drug-induced hepatitis. The risks of these serious adverse effects in treatment are considered to be much lower although definitive evidence is lacking. Pyronaridine is a Mannich base compound, distantly related to amodiaquine, which was developed in China, and is now being developed as a possible successor to chloroquine in Africa. It shows excellent activity against chloroquine and mefloquine resistant isolates of *P. falciparum*, though in general the multi-drug resistant parasites found in south east Asia are more resistant to pyronaridine than those in Africa. There is little information on cross resistance.

Mefloquine

Mefloquine is a fluorinated quinoline methanol compound with good activity against chloroquine resistant *P. falciparum*. Mefloquine has been used widely in south east Asia and south America. It has been the drug of choice for uncomplicated multi-drug resistant falciparum malaria in Thailand since 1984. This is the country with the greatest experience with the use of mefloquine. Although there is considerable heterogeneity in the *in vitro* sensitivity to mefloquine, and mefloquine resistant parasites have been identified even before exposure to the drug both in south east Asia and west Africa, clinical resistance did not appear in Thailand until 1989. Since then, there has been a remorseless decline in cure rates particularly on the Eastern and Western borders. Mefloquine was deployed initially in combination with sulphaadoxine-pyrimethamine in a single dose treatment containing 15 mg base/kg. This combination was employed to prevent the development of resistance but it failed, probably because *P. falciparum* in Thailand was already highly resistant to sulphaadoxine and pyrimethamine, and because the pharmacokinetic properties of the three drugs were not well matched. Mefloquine alone was then used and the dose was increased subsequently to the maximum tolerated regimen of 25 mg/kg. This provided only temporary respite.
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The cure rates with the high dose regimen have also decreased such that on the western and eastern borders of Thailand treatment with high dose mefloquine alone is associated with treatment failure rates of approximately 50%\textsuperscript{32,33}. Mefloquine resistance has been associated with amplification of \textit{Pfmdrl} genes and increased drug efflux from resistant parasites\textsuperscript{19}. Interestingly, this is reportedly blocked by penfluridol, but not by verapamil, whereas chloroquine resistance is reversed by verapamil, but not penfluridol.

\textbf{Halofantrine}

Halofantrine is a phenanthrene methanol compound metabolised \textit{in vivo} to an active metabolite, desbutylhalofantrine. Halofantrine resistance is also linked to \textit{Pfmdrl} amplification\textsuperscript{19}. Halofantrine and mefloquine resistance are positively correlated although halofantrine is intrinsically more active than mefloquine, and has proved superior against multi-drug resistant falciparum malaria when given in 3 day regimens\textsuperscript{34}. Unfortunately, these are associated with an increased risk of cardiotoxicity, and high dose halofantrine regimens are no longer recommended\textsuperscript{35}. The desbutyl metabolite does not induce changes in cardiac muscle repolarisation, and may be a safer alternative to the otherwise very well tolerated and effective parent compound.

\textbf{Quinine and quinidine}

The venerable Cinchona alkaloids still retain excellent activity against all species of human malaria parasites despite over 350 years of use. Although quinine resistance was first reported at the beginning of the century, it has developed slowly, particularly in south America and south east Asia, and there are still no well documented cases of high grade (R3) resistance to quinine treatment. Thus quinine can still be relied upon to treat malaria throughout the world. However, cure rates with quinine alone have declined progressively in south east Asia, and the drug is now usually combined with tetracycline or doxycycline in non-pregnant adult patients\textsuperscript{36-38}. This regime still gives cure rates of approximately 85–90% if compliance is good. Quinine has also been combined with other antibacterial drugs which have antimalarial activity (clindamycin, erythromycin, rifampicin and chloramphenicol). Most data are available for the quinine/clindamycin combination which has been used particularly in Brazil\textsuperscript{39,40}, although it is not yet clear whether this is as effective as the quinine/tetracycline combination in south east Asia. In general, quinine resistance and mefloquine resistance correlate well, although the precise
molecular basis for quinine resistance has not been fully elucidated. In animal models, it is difficult to induce quinine resistance although experimentally mefloquine resistance occurs relatively easily.

Lumefantrine

Lumefantrine is a recently introduced compound developed in China which has a mode of action similar to the quinoline antimalarials. There is little information on resistance although in combination with the artemisinin derivative, artemether, it has proved highly effective in the treatment of multi-drug resistant falciparum malaria.

Atovaquone

Atovaquone is a hydroxynaphthoquinone compound with activity against Pneumocystis carinii, Toxoplasma gondii, and Plasmodium species. These compounds interfere with parasite's cytochrome electron transport system. When atovaquone was used alone in the treatment of falciparum malaria, resistance developed in one-third of patients given 1–7 days' treatment. The drug is now available for the treatment of malaria only as a fixed ratio combination with the antimalarial biguanide proguanil. Atovaquone and proguanil are synergistic in vitro.

This combination in vivo has been associated with almost 100% cure rates even against the most multi-drug resistant P. falciparum parasites from south east Asia. Proguanil appears to be acting as the parent compound with an unidentified mechanism of antimalarial action, and not through biotransformation to the active triazine metabolite cycloguanil (i.e. it is not acting as a DHFR inhibitor). Atovaquone resistance is associated with single point mutations in the cytochrome b gene of P. falciparum, and these confer up to 10 000-fold reduction in sensitivity to the drug. These mutants occur in vivo at a frequency of at least 1 per 10^13 malaria parasites. Atovaquone resistant mutants are selected efficiently by treatment, and recrudescent infections with these parasites show no therapeutic response to repeat treatment. Unfortunately the atovaquone/proguanil combination does not entirely prevent emergence of resistance and this drug combination must be considered vulnerable if it is deployed alone.

Artemisinin derivatives

The artemisinin derivatives artemether, artesunate, and dihydroartemisinin, are all derived from the qinghao or sweet wormwood plant. They are peroxidic antimalarials with a unique mode of action.
which involves intraparasitic haem catalysed production of carbon centred free radicals. These damage the mitochondria-like organelles and alkylate parasite proteins\textsuperscript{44}. Although small decreases in sensitivity in \textit{P. falciparum} can be induced in vitro, true stable resistance to this group of compounds has not been described either in the laboratory or in clinical practice.

**Drug usage**

**Clinical implications**

The main factor determining antimalarial drug usage in the tropical world is cost\textsuperscript{45}. For this reason, drugs to which malaria parasites have already become resistant, are still widely used because of their low cost. Chloroquine is still the most widely used antimalarial drug in the world, and it remains first line treatment for falciparum malaria throughout most of Africa despite widespread resistance. Fully sensitive parasites respond rapidly to chloroquine, whereas with low grade resistance the parasitaemia clears and the patient defervesces, but the infection returns within 1–4 weeks. This is associated with poor haematological recovery and an increase in the prevalence of anaemia as a presenting feature of malaria\textsuperscript{13}. As resistance increases further, infections are encountered in which parasitaemia does not clear within 7 days (R2 resistance). With further increases in resistance, infections are encountered which show no parasitological or clinical response at all (high grade or R3 resistance). In the last situation, chloroquine should no longer be used as deaths occur from unresponsive falciparum malaria. This is the current situation in many parts of Africa; both morbidity and mortality are increasing. The conventional dose of 25 mg of base/kg given over 3 days can be increased, but this does not increase cure rates significantly in areas with low grade resistant parasites. Although \textit{in vitro} sensitivity data are useful epidemiological tools to track the emergence and spread of chloroquine resistance, they are not useful in individual cases as they do not predict well the response to treatment. In many areas of the tropics, background immunity against malaria augments the therapeutic effect of the antimalarial drugs and resolution of the infection may occur despite \textit{in vitro} resistance. In this context it is the youngest children (who have the least immunity), non-immune travellers, and pregnant women who have the highest incidence of recrudescent infections. It is generally recommended that an antimalarial drug should not be used when more than 25% of patients treated experience a symptomatic recrudescence of the infection, although in practice, purchasing power is a more powerful determinant of drug policies.
When chloroquine fails the next choice is usually pyrimethamine/sulphadoxine. In south east Asia and south America this combination enjoyed a relatively brief period of usefulness (approximately 5 years) before highly resistant parasites emerged. The transition from sensitivity to resistance, being mediated by single point mutations, was abrupt and complete failures to respond to treatment occurred early in the evolution of resistance. This meant that pyrimethamine/sulphadoxine could no longer be relied upon. Amodiaquine is increasingly being used as an alternative to pyrimethamine/sulphadoxine in areas with low grade chloroquine resistance. The evolution of amodiaquine resistance parallels that of chloroquine resistance. After resistance has developed to these compounds the usual next choice is either quinine or mefloquine. Increasing treatment failure rates with quinine are not usually improved by prolonging the course of treatment to 10 or 12 days, but the combination with tetracycline or doxycycline provides cure rates which reliably remain above 85%.\textsuperscript{36-38} Minor adverse effects with chloroquine (pruritus in dark skinned patients, nausea, abdominal discomfort, and dysphoria) and pyrimethamine/sulphadoxine (rare serious sulphonamide hypersensitivity) are not usually limiting, whereas quinine is generally poorly tolerated. Quinine is extremely bitter (more so than chloroquine) and reliably induces the symptom complex of cinchonism (nausea, tinnitus, dysphoria, and high tone deafness). Patients are usually loath to take a full 7 day course and compliance with unsupervised regimens is poor. Thus overall recrudescence rates are usually higher in general practice than those reported in carefully supervised clinical trials. Mefloquine has the advantage that a full treatment can be given in a single or split dosage, but it also commonly produces nausea and vomiting (particularly in young children) and central nervous system effects including dysphoria, dizziness and disturbed sleep are common\textsuperscript{46}. More serious neuropsychiatric reactions occur in between 1:200 and 1:1300 patients treated for acute falciparum malaria. The incidence of these serious effects (psychosis, encephalopathy, convulsions) rises to approximately 1:20 when mefloquine is used following recovery from cerebral malaria\textsuperscript{47}. Nevertheless, mefloquine is generally well accepted where it is needed. Provided attention is given to the method of administration to young children (i.e. they are calmed and cooled before drug administration), the drug is usually well retained. Halofantrine is considerably better tolerated, with only rare cases of diarrhoea reported. Unfortunately, it induces a concentration dependent prolongation in atrioventricular and ventricular repolarisation which manifests on the electrocardiogram as prolongation of the PR and QTc intervals\textsuperscript{35}. QT prolongation is a risk factor for ventricular tachyarrhythmias and halofantrine has been associated with sudden death. The risk of cardiotoxicity has severely curtailed the deployment of halofantrine. It
Drug resistance has narrowed the therapeutic options in severe malaria. Chloroquine should no longer be used for severe malaria outside the few areas where sensitivity is retained. For nearly all the tropical world, the choice now lies between quinine (or quinidine) and either artemether or artesunate. The artemisinin derivatives are more rapidly parasiticidal than quinine, and safer in severe malaria (they do not cause hypoglycaemia), but to date large randomised trials have largely involved artemether which is relatively slowly absorbed particularly in severe malaria. These have not shown significant benefit in terms of mortality for artemether$^{50-52}$. Although there has been some evidence of a decline in the efficacy of quinine in severe malaria in southeast Asia, these recent large randomised comparative trials with artemether have provided reassuring evidence that the mortality with quinine treatment is not rising significantly.

The prevention of resistance

Resistance develops most rapidly when a population of parasites encounters sub-therapeutic concentrations of antimalarial drug. These act as a selective pressure filtering out the more resistant parasites within
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the infecting population. Selection is most efficient when single point mutations confer high level resistance. The mutations which confer reduced drug susceptibility within an infecting parasite population are thought to occur independently of drug pressure. Drugs with long terminal elimination phases, such as mefloquine, are particularly vulnerable because sub-therapeutic concentrations may occur for weeks or months after a single therapeutic dose. Selection for resistance in microorganisms is greatest at ‘intermediate’ levels of drug activity (generally between 20% and 80% of maximum effect). Although chloroquine has the longest of all the elimination phases (terminal half-life 1–2 months), the blood concentrations during the terminal phase are very low and may lie below the ‘sensitive’ part of the concentration effect relationship where selective pressure is greatest.

The pharmacological characteristics which predispose a drug to the development of resistance are weak intrinsic activity with a ‘flat’ dose-response or concentration effect relationship, single or double point genetic mutations which confer marked reductions in susceptibility, and a long terminal elimination phase during which blood concentrations fall slowly down the concentration effect curve for the infecting population of parasites.

Initially at low levels of resistance selection occurs only from newly acquired infections, but, as resistance worsens, some of the primary infections are able to survive the initial therapeutic onslaught and to recrudesce subsequently. These are by definition the most resistant parasites, and they are preferentially transmitted because gametocyte carriage is more likely during the recrudescent infection. The chances of a resistant mutant parasite surviving can be reduced considerably if a second drug, with an independent locus of antimalarial action, is added. Escape would now require two simultaneous but independent mutational events. Mutations are rare events and the chance that two independent mutations would occur in the same parasite is the product of their individual mutation frequencies. This rationale for combination chemotherapy in malaria was applied originally to mefloquine/sulphadoxine/pyrimethamine but it did not work because of the pharmacokinetic mismatch between the three compounds, and because where it was introduced in Thailand in 1984, *P. falciparum* was already highly resistant to both pyrimethamine and sulphadoxine. Combinations of artemisinin derivatives with slower acting and more slowly eliminated antimalarials are particularly effective because of the considerable biomass reduction achieved by artemisinin compounds with a treatment course as short as 3 days (circa 10^8-fold reduction in parasite numbers). This ensures that there are relatively few parasites (maximum 10^5) remaining for the second, weaker drug to eliminate. Furthermore, these parasites are exposed to maximum concentrations of
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the second drug. The artemisinin derivative is also ‘protected’ by the second drug. This would argue for combining an artemisinin derivative with all slowly acting antimalarial drugs.

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