CHAPTER

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Antimalarial Drug Resistance

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Introduction

Malaria is a hematoprotozoan parasitic infection transmitted by certain species of anopheline mosquitoes. Four species of *Plasmodium* commonly infect humans, but *Plasmodium falciparum*, accounts for the majority of instances of morbidity and mortality. There has been a resurgence of interest in malaria in recent years because the efforts at control have foundered after the failure of the global eradication campaign in the 1960s. The control of malaria depends on two strong arms: the first is control of the anopheline mosquito vector through removal of breeding sites, use of insecticides and prevention of contact with humans via the use of screens and insecticide impregnated bed nets and the second is effective case management. A long hoped third arm, an effective malaria vaccine, has not materialized and is not expected for another decade. The antimalarials used in case management has largely relied on chloroquine, and sulfadoxine-pyrimethamine [SP], which are inexpensive and widely available and are eliminated slowly from the body. Antimalarials are among the most commonly used medications in tropical areas of the world and its misuse is also widespread. In many parts of the tropics, the majority of the population has detectable concentrations of chloroquine in the blood. The extensive deployment of these antimalarial drugs, in the past fifty years, has provided a tremendous selection pressure on human malaria parasites to evolve mechanisms of resistance. The emergence of resistance, particularly in *P. falciparum*, has been a major contributor to the global resurgence of malaria in the last three decades[1] and it is the most likely explanation for a doubling of malaria-attributable child mortality in eastern and southern Africa.[2]

Definition

Antimalarial drug resistance is defined as the ability of a parasite strain to survive and/or multiply despite the proper administration and absorption of an antimalarial drug in the dose normally recommended. Antimalarial drug resistance is not necessarily the same as malaria "treatment failure", which is a failure to clear malarial parasitemia and/or resolve clinical symptoms despite the administration of an antimalarial. Thus, while drug resistance may lead to treatment failure, not all treatment failures are caused by drug resistance. Treatment failure can also be the result of incorrect dosing, problems of treatment adherence (compliance), poor drug quality, interactions with other drugs, compromised drug absorption or misdiagnosis of the patient. Apart from leading to inappropriate case management, all these factors may also accelerate the spread of true drug resistance by exposure of the parasites to inadequate drug levels.

Global distribution of resistance

Resistance to antimalarials has been documented for *P. falciparum*, *P. vivax* and, recently, P. malariae. In *P. falciparum*, resistance has been observed to almost all currently used antimalarials (amodiaquine, chloroquine, mefloquine, quinine and sulfadoxine–pyrimethamine) except for artemisinin and its derivatives. The geographical distributions and rates of spread have varied considerably. Resistance to mefloquine is confined only to those areas where it has been used widely (Thailand, Cambodia, and Vietnam) but has arisen within six years of systematic deployment.[3] Chloroquine resistance is confined largely to Indonesia, East Timor, Papua New Guinea, other parts of Oceania and Peru. The epidemiology of resistance in *Plasmodium vivax* is less well studied. *P. vivax* remains sensitive to chloroquine in South-East Asia, the Indian subcontinent, the Korean peninsula, the Middle East, north-east Africa, and most of South and Central America.

Drug Resistance in India

As in other tropical countries malaria has been a problem in India also. Details of this disease can be found even in the ancient Indian medical literature like the 'Charaka Sanhita'. In the 30's the disease was rampant in the country and for the first time, to combat the menace of malaria, the government of India launched the National Malaria Control Programme in April 1953. The program proved highly successful and within five years the incidence of malaria dropped to 2 million per year. Encouraged by this, the central government started the National Malaria Eradication Program (NMEP) in 1958 and by 1961 the incidence dropped to 50,000 cases per year. Since then the program suffered repeated setbacks due to technical, operational and administrative reasons and the cases started rising again.[3,4] One of the important reasons for this was also development of chloroquine resistance in *P. falciparum.*

There are several reports of antimalarial drug resistance in India from different geographical regions. *Plasmodium falciparum* is resistant to chloroquine in most of the areas of north eastern states, Maharashtra and in central India. There are reports of multi drug resistance *P. falciparum* from north eastern states and of chloroquine resistance in *P. vivax* from some other regions. The resistance to sulfadoxine pyrimethamine has been reported both in *P. falciparum* and *P. vivax* in large number of areas in India.[5-11] A study was undertaken to generate data systematically on the efficacy of chloroquine in 287 patients from different epidemiological regions and the observed cure rates for 28 days were 100% and there was a rapid parasite clearance rate in all age groups from all study sites.[12]

The emergence and spread of antimalarial resistance

The development of resistance can be considered in two parts: the initial genetic event, which produces the resistant mutant; and the subsequent selection process in which the survival advantage in the presence of the drug leads to preferential transmission of resistant mutants and thus the spread of resistance.[13,14] In the absence of the antimalarial, resistant mutants may have a survival disadvantage. This "fitness cost" of the resistance mechanism may result in a decline in the prevalence of resistance, once drug pressure is removed. Resistance to one drug may select for resistance to another where the mechanisms of resistance are similar (cross-resistance). There are many parallels with antibiotic resistance, in particular the resistance to anti-tuberculosis drugs where, as for malaria, transferable resistance genes are not involved in the emergence of resistance. In experimental models, drug-resistant mutations can be selected without mosquito passage by exposure of large numbers of malaria parasites to sub therapeutic drug concentrations. Various factors determine the propensity for antimalarial drug resistance to develop and the important one are the intrinsic frequency with which the genetic changes occur and the degree of resistance conferred by the genetic change, the fitness cost of the resistance mechanism, the proportion of all transmissible infections that are exposed to

the drug (the selection pressure), the number of parasites exposed to the drug, the concentrations of drug to which these parasites are exposed, the pharmacokinetic and pharmacodynamic properties of the antimalarial, individual (dosing, duration, adherence) and community (quality, availability, distribution) patterns of drug use, the immunity profile of the community and the individual and the simultaneous presence of other antimalarials or substances in the blood to which the parasite is not resistant.

Selection and spread of resistance

The emergence of resistance is the product of the probabilities of its de novo emergence (a rare event) and subsequent spread. Resistant parasites, if present, will be selected when parasites are exposed to "selective" (subtherapeutic) drug concentrations. "Selective" in this context means a concentration of drug that will eradicate the sensitive parasites but still allow growth of the resistant parasite population such that it eventually transmits to another person. De novo resistance arises randomly among malaria parasites and the non-immune patients infected with large numbers of parasites who receive inadequate treatment (either because of poor drug quality, poor adherence, vomiting of an oral treatment, etc.) are the potent source of de novo resistance. This emphasizes the importance of correct prescribing, and good adherence to prescribed drug regimens, and also provision of treatment regimens that are still highly effective in hyperparasitaemic patients. The recrudescence and subsequent transmission of an infection that has generated a de novo resistant malaria parasite is essential for propagation of resistance.[15]

Step 1: de novo selection of resistance

In order to assess the factors determining the emergence and spread of resistance, we need to consider the numbers of malaria parasites likely to be exposed to the drugs, both within an individual and in the entire human population. Fortunately this estimate of parasite numbers is much more precise than for almost any other human pathogen. Malaria parasites are eukaryotes and meiosis occurs after a female anopheline mosquito has taken viable gametocytes in its blood meal. All the other 10^8–10^{13} cell divisions in the life cycle are mitotic and nearly all these divisions take place in the bloodstream of the human host. Usually, less than ten sporozoite parasites are inoculated by an infected mosquito in order to establish malaria infection.[16,17] These rapidly find their way to the liver. During *P. falciparum* infection, each infected hepatocyte liberates approximately 30,000 merozoites after 5–6 days of pre-erythrocytic schizogony. Thus approximately 100,000–300,000 merozoites are liberated into the bloodstream to begin the 48-hour asexual reproduction cycle. This is an important number, as it is the number of parasites that would encounter residual drug levels from a previous antimalarial treatment or drug levels during chemoprophylaxis.[8] The density of parasites in the blood at which symptoms and fever occur (the pyrogenic density), and thus the stage at which appropriate antimalarial treatment could be given, vary considerably.[17,18,19] In nonimmune people, nonspecific symptoms often occur a day or two before parasites are detectable on the blood smear (about 50 parasites per microliter of blood). This density corresponds to a total of between 10^8 and 10^9 asexual parasites in an adult with a red cell volume of about 2 litre. In areas of moderate- or high-intensity transmission, parasitemias considerably higher than this level may be tolerated without symptoms, although densities over 10,000 per microliter (between 10^{10} and 10^{11} parasites in the body of an adult, and correspondingly less in children) are usually symptomatic, even in very high-transmission settings.[20] Median or geometric mean parasite counts in malariometric surveys are usually below this value (i.e., most people with detectable parasitemias in these endemic areas are not obviously ill). It is estimated that approximately 300 million people in the world now have malaria parasites in their blood. Using current epidemiological data we have estimated that there must be less than 3×10^{16} malaria parasites in the world's asymptomatic carriers.[21]

Step 2: the spread of resistance

Resistance to one drug may be selected for by another drug in which the mechanism of resistance is similar (a phenomenon known as cross-resistance). Antimalarial drug resistance in malaria parasites spreads because it confers a survival advantage in the presence of the antimalarial drugs and therefore results in a greater probability of transmission for resistant than for sensitive parasites. Resistant infections are more likely to recrudesce, and eventually as resistance worsens, the infections with resistant parasites respond more slowly to treatment. Both increased rates of recrudescence and slow initial responses to treatment increase the likelihood of generating sufficient gametocyte densities to transmit the infection, as compared to drug-sensitive infections. Mathematically, it is this ratio of transmission probabilities in drug-resistant compared with drug-sensitive infections that drives the spread of resistance. The recrudescence and subsequent transmission of an infection that generated resistant malaria parasites de novo are essential for propagation of resistance.[22] If resistance is low grade or a highly effective combination treatment is given, then resistance may confer only a little increase in the treatment failure rate, and a correspondingly slow rate of spread. As resistance worsens, the failure rates rise and the rate of spread accelerates. In the rare but important infection in which resistance arises de novo, killing of the transmissible sexual stages (gametocytes) during the primary infection does not affect the spread of resistance because these gametocytes are derived from the drug-sensitive parasites. Gametocytes carrying the resistance genes will not reach transmissible densities until the resistant biomass has expanded to a population size close to that which is necessary to produce illness ($> 10^7$ parasites).[23] Thus, to prevent the spread of resistance, gametocyte production from the subsequent recrudescent-resistant infection must be prevented.

Chloroquine resistance in *P. falciparum* may be multigenic and is initially conferred by mutations in a gene encoding a transporter (PfCRT).[24] In the presence of PfCRT mutations, the mutations in a second transporter (PfMDR1) modulate the level of resistance in vitro. However the role of PfMDR1 mutations in determining the therapeutic response following chloroquine treatment remains unclear.[25] At least one other as-yet unidentified gene is also thought to be involved in this process. Resistance to chloroquine in *P. falciparum* has arisen spontaneously less than ten times in the past fifty years.[26] This suggests that the per-parasite probability of developing resistance de novo is on the order of 1 in 10^{20} parasite multiplications. The single point mutations in the gene encoding cytochrome b (*cytB*) which confer atovaquone resistance or in the gene encoding dihydrofolate reductase (*dhfr*) which confer pyrimethamine resistance, have a per-parasite probability of arising de novo of approximately 1 in 10^{12} parasite multiplications[22]. To put this in context, an adult with approximately 2% parasitemia has 10^{12} parasites in his or her body but in the laboratory much higher mutation rates than 1 in every 10^{12} are recorded.[27]

Mutations may be associated with fitness disadvantages (i.e., in the absence of the drug they are less fit and multiply less well than their drug-sensitive counterparts). Another factor that may explain the discrepancy between in vitro and much lower apparent in vivo rates of spontaneous mutation is host immunity. Even a previously nonimmune individual develops a specific immune response to a malaria infection. This response is systematically evaded by the parasite population through programmed antigenic variation of the main red cell surface–expressed epitopes. In *falciparum* malaria, *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), which is encoded by the var multigene family, changes its behavior in 2–3% of parasites in each asexual cycle.[28] The untreated infection is characterized by successive waves of parasites, each comprising largely one antigenically distinct surface phenotype. It is likely that this specific immune response directed against the immunodominant surface antigens will reduce

the probability of the usually single mutant parasite ever multiplying sufficiently to transmit as for *P. falciparum*. There is only a 2–3% chance that the genetic event causing resistance would arise in the antigenically variant subpopulation that will expand to reach transmissible densities.

The cause of chloroquine resistance in *P. vivax* has not been found. Resistance to mefloquine and other structurally related arylaminoalcohols in *P. falciparum* results from amplifications (i.e., duplications, not mutations) in *Pfmdr*, which encodes an energy-demanding p-glycoprotein pump (*Pgh*).[29–32] This is a more common genetic event. It is tempting to speculate that the relatively poor fidelity in mitotic duplication of this sequence allows the parasite populations to respond to environmental stresses like alterations in human diet while causes reduced intracellular concentrations of the antimalarial drugs.

P. falciparum and *P. vivax* resistance to antifols (pyrimethamine and cycloguanil) results from the sequential acquisition of mutations in *dhfr*.[33] Each mutation confers a stepwise reduction in susceptibility. Resistance to the sulfonamides and sulfones, which are often administered in synergistic combination with antifols can also result from mutations in the gene *dhps*, which encodes the target enzyme dihydropteroate synthase.[34] Resistance to atovaquone results from point mutations in the gene *cytB*, coding for cytochrome b. Atovaquone is deployed only in a fixed combination with proguanil (chloroguanide). In this combination, it is proguanil itself acting on the mitochondrial membrane, rather than the dhfr-inhibiting proguanil metabolite cycloguanil, that appears to be the important factor, however the exact mechanism of proguanil's mitochondrial action are not known.[35] Although the target for the artemisinins has recently been identified (*PfATPase6*), preliminary studies have not so far shown polymorphisms in the gene encoding this enzyme which can cause reduced susceptibility to artemisinins.[31]

If we assure that probability of spontaneous occurrence of genetic event resulting in resistance is equally distributed in the parasites life cycle then it is likely to take place in only a single parasite at the peak of infection. These genetic events may result in moderate changes in drug susceptibility and the drug still remains effective (e.g., the serine-to-asparagine mutation at position 108 in Pfdhfr) or less commonly very large reductions in susceptibility making the drug completely ineffective (e.g., the mutations in cytB responsible for atovaquone resistance).[29,35,36] It had been thought that resistance to some antimalarial compounds (notably pyrimethamine and SP) in human malaria parasites emerged relatively frequently. This suggested that prevention of the emergence of resistance would be very difficult, and control efforts would be better directed at limiting the subsequent spread of resistance. Recent remarkable molecular epidemiological studies in South America, southern Africa, and Southeast Asia have challenged this view. By examination of the sequence of the regions flanking the *Pfdhfr* gene, it has become apparent that, even for SP, multiple de novo emergence of resistance has not been a frequent event and a single parasite mutation (in *Pfdhfr* at positions 51, 59, and 108) has swept across each of these continents.[37–39] The ability of these resistant organisms to spread has been phenomenal and may well relate to the apparent stimulation of gametocytogenesis that characterizes poor therapeutic responses to SP.[40] Gametocyte carriage is considerably augmented following SP treatment of resistant infections. Studies to date do not suggest reduced infectivity for these gametocytes. There is a sigmoid curve relationship between gametocyte densities in blood and infectivity, which in volunteer studies was shown to saturate at gametocyte densities above 1,000 per microliter (a relatively high density in field observations). Thus it is the relative transmission advantage conferred by increased gametocyte carriage that drives the spread of resistance.[21,22]

Extrinsic factors affecting emergence of drug resistance

Availability

Most developing nations give license to only those

antimalarial drugs, which are provided through their national health programs.[41] This approach often excludes relatively expensive or risky therapies, even for patients who may be able to afford a given drug and have access to medical supervision. The main factor affecting availability is economic – the ability or inability to purchase a drug for broad distribution and the potential reluctance to use the drug because of an inability either to screen users or to monitor its quality.

Adherence

Most people taking antimalarial drugs live in rural regions of the developing world and are not supervised by health professionals. A study conducted among 1640 febrile patients with malaria in Burkina Faso[42] showed that 69 per cent were self-treated, and in a study in Ethiopia, among 630 febrile patients with malaria, 67 per cent were self-treated.[43] Complex, inconvenient or poorly tolerated antimalarial regimens carry a substantial risk of inadequate adherence.[44] Among 414 Brazilian patients, the risk of recurrent malaria correlated with self-reported poor adherence,[45] whereas among 632 Nigerian children strict adherence correlated with clinical recovery.[46] Convenient and easily understood packaging and education of the patients alleviate poor adherence. Owing to lengthy and complex regimens, currently used therapies such as quinine and primaquine and new combined therapeutic strategies challenge the ease of adherence.[47]

Counterfeit and Substandard Drugs

Counterfeit antimalarial drugs pose a serious threat in regions where the trade in pharmaceuticals is not rigorously regulated. A survey conducted in Cameroon found insufficient or inactive ingredients in 38 per cent of preparations labeled chloroquine, 78 per cent of those labeled quinine, and 12 per cent of tablets labeled as an antifolate agent.[48] A survey in Southeast Asia involving 104 purchases of artesunate tablets found that 38 per cent of the tablets contained no drug.[49] In some countries the trade in counterfeit drugs undoubtedly results in many deaths.[50] The inadvertent marketing of substandard pharmaceuticals poses another threat. In a survey of eight authorized wholesalers in Tanzania selling combined sulfadoxine–pyrimethamine tablets, 11 per cent of the tablets failed industry standards for content, and 44 per cent failed dissolution testing.[51]

Intrinsic factors affecting emergence of drug resistance

Plasmodia pass through distinct stages of form, function, location, clinical consequence, and susceptibility to antimalarial drugs. Drug activity ranges from narrow (e.g., the activity of quinine against asexual blood stages) to broad (e.g., the activity of primaquine against sexual and asexual forms in the blood and liver). Stage specific susceptibility differs among species of plasmodia – for example, chloroquine kills the gametocytes of P. vivax but exerts no effect against those of P. falciparum. These intrinsic properties define the recommended uses of antimalarial drugs. Species specific innate resistance (e.g., asexual blood stages of P. falciparum lack susceptibility to primaquine, whereas those of P. vivax appear to be sensitive to it);[52] strain-specific innate resistance (e.g., that of asexual liver stages of P. vivax from the island of New Guinea against primaquine)[53] and acquired resistance which is most important, because failure may occur even in the presence of complete adherence to the recommended therapies.

Parasite Burden

Most patients with malaria carry a burden of 10^8 to 10^{13} parasites.[54] Effective chemotherapy induces a constant fractional decline with each asexual cycle,[55] at a rate that varies according to the susceptibility of the parasite to a given drug. For example, artemisinin derivatives induce reductions of 10^4, whereas tetracycline achieves a reduction by only a factor of 10 with each cycle. The duration of exposure to a drug that is needed to eliminate infection hinges on the intrinsic rate of decline and on initial parasite burden.[55] High levels of parasitemia, as compared with a low burden,

require longer exposure to effective drug levels and have a relatively higher risk of treatment failure.[56]

Prevention of resistance by antimalarial combination therapy

The theory underlying combination drug treatment of tuberculosis, leprosy, and HIV infection is well known and is now generally accepted for malaria.[21,22,57–60] If two drugs are used with different modes of action, and therefore different resistance mechanisms, then the per-parasite probability of developing resistance to both drugs is the product of their individual per-parasite probabilities. This is particularly powerful in malaria, because there are only about 10^{17} malaria parasites in the entire world. For example, if the per-parasite probabilities of developing resistance to drug A and drug B are both 1 in 10^{12}, then a simultaneously resistant mutant will arise spontaneously every 1 in 10^{24} parasites. As there is a cumulative total of less than 10^{20} malaria parasites in existence in one year, then such a simultaneously resistant parasite would arise spontaneously roughly once every 10,000 years – provided the drugs always confronted the parasites in combination. Thus the lower the de novo per-parasite probability of developing resistance, the greater the delay in the emergence of resistance.

Stable and therapeutically significant resistance to the artemisinin derivatives has not yet been identified and cannot be induced yet in the laboratory, which suggests that it may be a very rare event. But it would be unwise to bank on its not happening, and should it arise, it would be a global disaster. For mutual protection against the emergence of drug resistance, these drugs should be used only in combination with other antimalarials.

Artemisinin derivatives are particularly effective in combinations because of their very high killing rates (parasite reduction ratios 10,000-fold per cycle), lack of adverse effects, and absence of significant resistance.[61] The ideal pharmacokinetic properties for an antimalarial drug have been greatly debated. From a resistance-prevention perspective, the combination partners should have similar pharmacokinetic properties. Rapid elimination ensures that the residual concentrations do not provide a selective filter for resistant parasites, but these drugs (if used alone) must be given for 7 days, and adherence to 7-day regimens is poor. Even 7-day regimens of artemisinin derivatives are associated with approximately 10% failure rates. In order to be highly effective in a 3-day regimen, terminal elimination half-lives of at least one drug component need to exceed 24 hours. Combinations of artemisinin derivatives (which are eliminated very rapidly) given for 3 days, with a slowly eliminated drug such as mefloquine (artemisinin combination treatment) provide complete protection for the artemisinin derivatives from selection of a de novo resistant mutant if adherence is good (i.e., no parasite is exposed to artemisinin during one asexual cycle without mefloquine being present). But this does leave the slowly eliminated "tail" of mefloquine unprotected by the artemisinin derivative. The residual number of parasites exposed to mefloquine alone, following two asexual cycles, is a tiny fraction (less than 0.00001%) of those present at the peak of the acute symptomatic infection. Furthermore, these residual parasites are exposed to relatively high levels of mefloquine, and, even if susceptibility is reduced, these levels are usually sufficient to eradicate infection. This strategy would be expected to be effective at preventing the de novo emergence of resistance at higher levels of transmission, where high-biomass infections still constitute the major source of de novo resistance. Various combination therapy used presently are Artemether + Lumefantrine, Artesunate + Amodiaquine, Artesunate + Mefloquine and Artesunate + Sulfadoxine Pyrimethamine.

The national drug policy for malaria in high-risk areas is advocating the full dose of chloroquine in chloroquine sensitive areas and a combination of artesunate and sulfadoxine–pyrimethamine in chloroquine resistant areas in uncomplicated malaria, whereas all patients of severe malaria irrespective of chloroquine sensitivity of the region should be treated by I.V. quinine or artesunate.

Since malaria is changing its facet from time to time; all the knowledge of this phenomenon is highly essential for every level healthcare provider for administering effective management to the suffering humanity.

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