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Malaria and Resistance to Anti-Malarial Drugs

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Abstract.- The most important factor in the current rise of malaria morbidity is resistance to affordable and previously effective anti-malarial drugs. This is a massive threat to global health.

Key words: Malarial parasites, anti malarial drugs, drug resistance, global health

INTRODUCTION

Malaria, or ague as it was once commonly called, killed more people in the last century than were killed in both World Wars. It affects nearly five times as many people as TB, AIDS, measles and leprosy combined, and kills one person every 30 seconds. Two-fifths of the world's population is at risk.

Malaria parasites belong to the genus *Plasmodium*. There are four species of *Plasmodium* that affect man, of which *P. falciparum* is the most severe, taking responsibility for almost all of the mortality due to malaria. *P. vivax* is the most prevalent parasite outside tropical Africa, causing morbidity and economic losses, but is nowadays rarely lethal. Although there are malaria parasite species that affect many mammals, birds and reptiles, human malaria parasites infect only man and some non-human primates; the latter are not considered to be a significant animal reservoir of disease.

Human malaria parasites are believed to have arisen in Africa thousands of years ago, and moved with the spread of agriculture to nearly all of the tropical, sub-tropical and temperate regions of the world (Carter and Mendis, 2002).

During the first half of the twentieth century, improved living conditions, cheap and effective antimalarial drugs, and mosquito control programmes all helped to eliminate malaria from the United States and most of Europe. Continuing efforts after the Second World War, including extensive mosquito control programmes with insecticides such as DDT, reduced malaria-related mortality from the Mediterranean to the Western Pacific. At the dawn of the 21st century, malaria remains a major cause of death only in Africa. Despite progress elsewhere, sub-Saharan Africa has seen a steady rise in the number of cases and the number of deaths from malaria since the late 1980s. There are many reasons for this, including large population movements, environmental and climatic changes, war and civil disturbance, and uncontrolled development activities. However, by far the most important factor in the rise of malaria morbidity is the development by the parasite *P. falciparum* of resistance to the commonly used anti-malarial drugs, chloroquine and sulfadoxine-pyrimethamine (SP) (Hapuarachchi *et al.,* 2004; Khan *et al*., 2004; van den Broek IV *et al.,* 2004).

How does drug resistance arise and spread?

Antimalarial drug resistance is strictly defined as the ability of a parasite strain to survive and/or multiply, despite the administration and absorption of drug given in doses equal to or higher than those usually recommended, but within the limits of tolerance of the subject.

Resistance to antimalarial drugs has now been described for two of the four species of malaria parasite that naturally infect humans, *P. falciparum* and *P. vivax*. The situation is particularly grave for *P. falciparum*, which has developed resistance to nearly all of the antimalarials in current use.

Drug resistance arises as a result of spontaneously occurring changes in specific parasite genes. These alterations change the structure and or activity of the drug target in the malaria parasite. The access of the drug to the target can also be

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affected by genetic change, for example by alterations in drug transporters. Parasites carrying certain genetic alterations are able to survive in patients who have received an antimalarial drug, whereas the 'wild-type' parasites do not. Parasites that are resistant to the chemotherapeutic agent therefore increase in frequency in the population, a process known as drug selection.

Drug resistance can spread from one area to another in two ways. Mosquitoes carrying drug-resistant parasites can spread resistance on a small scale, but their flight ranges are restricted to a few kilometers. A more important method is the movement of infected people from an area of high drug resistance to one of low or no resistance. Human migration is probably an important factor in the spread of chloroquine resistance between different endemic regions of Asia and Oceania, and for the initial introduction of chloroquine resistance into East Africa.

Drugs with a long elimination time from the body (half-life), such as the commonly used prophylactic drug mefloquine (Lariam®), persist at sub-therapeutic concentrations in the plasma for many weeks after the initial *Plasmodium* infection has been cleared. If the person becomes reinfected during this period, parasites will be exposed to the drug at levels which will not kill them, but which may encourage the development of resistant parasites (Wernsdorfer, 1994).

The widespread use of antimalarial drugs is considered to be a major reason for the rapid spread of resistance. As the number of individuals in an area taking a particular antimalarial drug increases, so does the likelihood that parasites will be exposed to inadequate drug levels, and resistant mutants are more readily selected. The extent of drug use also influences the spread of resistant parasites once they have arisen since only resistant parasites will survive in individuals receiving the correct dose of drug. Generally, it has been observed that resistance rates are higher in urban areas than in rural areas, presumably reflecting the greater access to, and use of, drugs.

The distribution in the 1950s of ' medicated table salt' containing chloroquine along the Thai-Cambodian border is a good example of inappropriate drug use leading to the rapid expansion of drug-resistant parasites. The intense drug pressure, coupled with likely sub-therapeutic levels in some individuals, may have played a role in the emergence of chloroquine resistance in this area in the late 1950s.

Resistance to Chloroquine in P. falciparum

Chloroquine was introduced for treatment and control of malaria in the 1940s and quickly became the drug of choice for most malaria-endemic countries. The drug causes very few side-effects, mostly mild in nature, and is safe for use, even in children and pregnant women. Importantly it is also cheap in comparison to most modern drugs.

Chloroquine treatment failure was first reported in Colombia and, as mentioned above, at the Cambodia-Thai border in the late 1950s, approximately 12 years after it was first introduced. Resistance spread rapidly (Wongsrichanalai *et al*., 2002) and is now widespread in most malaria-endemic countries (Table I). In East Africa, the drug fails to clear parasitaemia in more than 50% of treated patients.

Table I.- Geographical variations in drug-resistance of *P. falciparum*

Drug	Geographical distribution of resistance
Chloroquine	widespread resistance, thought to a varying extent-except Parts of Central America (north-west of the Panama Canal), The island of Hispaniola (Haiti and the Dominican Republic), and very limited areas of the Middle East and Central Asia.
Sulfadoxine pyrimethamine	High levels in the Amazon basin and throughout Southeast Asia. Low levels in the Indian subcontinent, Central and Southern Africa and in the coastal areas of South America. Increasing levels in East Africa.
Mefloquine	Common in Thailand, Myanmar and Cambodia. Very low levels in South America.
Quinine	Parts of Southeast Asia, and parts of South America.

Chloroquine works by interfering with the detoxification processes in the parasite. Malaria parasites live within red blood cells and obtain some

nutrients by breaking down haemoglobin. Haemoglobin is digested within the parasite's food vacuole, releasing haem in its poisonous haematin form, in large amounts. Haematin is usually detoxified by polymerization to form insoluble crystals of haemozoin, also known as malaria pigment.

Chloroquine accumulates in the food vacuole, and interrupts this haematin detoxification. It is thought that the drug binds to the haematin and also to the haemozoin crystals themselves, preventing polymerization and forming toxic complexes that cause parasite death. Less chloroquine accumulates in the food vacuole of drug-resistant than of drug-sensitive parasites.

Mutations in parasite gene called (*P. falciparum* chloroquine resistance transporter) *Pfcrt* have been associated with chloroquine resistance (Wellems and Plowe, 2001). *Pfcrt* encodes a transporter-like protein that localises to the food vacuole membrane, and which may be involved in drug flux and or pH regulation. However, the presence of the mutated form of *Pfcrt* cannot be used to predict chloroquine treatment failure. In some areas, mutant *Pfcrt* is found in all or nearly all parasites, yet chloroquine can still be effective in some patients. In Mali, for example, chloroquine treatment cured 60% of adults and older children who had parasites with the mutant form of *Pfcrt*. The ability of people to eliminate resistant parasites increased with age, implying that immunity plays an important role in the clearance of chloroquine resistant parasites.

Mutations in another gene called *Pfmdr* 1 may play a modulatory role in chloroquine resistance (Wongsrichanalai *et al*., 2002). Pfmdr 1 encodes an ABC-type membrane transporter, which may be involved in altering flux of the drug into or out of the food vacuole. This is akin to mammalian tumour cells exhibiting a multidrug-resistance (*mdr*) phenotype, responsible for expulsion of a wide range of inhibitors (Hayton and Su, 2004).

Resistance of P. falciparum *to other commonly used anti-malarial drugs*

Quinine and mefloquine

Resistance to the chloroquine-like compounds mefloquine and quinine is restricted to Southeast Asia (Table I). Mefloquine was first introduced in 1977, and resistance was reported from the Thai-Myanmar and the Thai-Cambodian borders in 1982 (Smithuis *et al.,* 2004) Quinine has been in use since the 17th century, and resistance was first reported in 1910.

The mode of action of quinine and mefloquine are believed to be similar to that of chloroquine, but the mechanisms of resistance are different. Some *in vivo* studies have shown associations between resistances to these drugs probably involve alterations in the sequence and copy number of the *Pfmdr1* gene.

Antifolates

Antifolates are antimalarial drugs that target enzymes in the folate pathway of the parasite. Antifolates, such as the drug combination sulfadoxine-pyrimethamine (SP), act by sequential and synergistic blockage of two important enzymes in the folate synthesis pathway. Pyrimethamine inhibits dihydrofolate reductase (DHFR), and sulfadoxine inhibits dihydropteroate synthetase (DHPS). These enzymes are essential for the provision of nucleotides for DNA synthesis and for amino acid metabolism. Antifolate drugs, therefore, act to prevent DNA replication, and hence inhibit parasite multiplication (Wongsrichanalai *et al*., 2002).

P. falciparum resistance to SP is primarily conferred by successive single-point mutations in parasite *dhfr*, which encodes DHFR, and by additional mutations in *dhps*, which encodes DHPS (de Pecoulas *et al*,, 2004; Talisuna *et al.,* 2003) These mutations directly affect the binding of the inhibitory drugs to the enzyme targets, with little effects on the binding of the natural substrate (Hyde, 2002).

Clinical resistance to the drug combination SP *in vivo* seems to require mutations in both *dhfr* and *dhps*, although the correlation between treatment failure and the presence of different mutant parasites is contentious. Parasites that have three specific point mutations in *dhfr*, combined with at least two in *dhps*, are highly correlated with treatment failure with SP. However, the association of treatment outcome for parasites with lower levels of mutation in these genes is much less certain.

Malarone ®

The synergistic combination of the Antifolate drug, proguanil, and another agent called atovaquone is known as Malarone®. Malarone is commonly used prophylactically for the prevention of malaria in travelers. Resistance to the antifolate, cycloguanil, the active metabolite of proguanil, is the result of mutation in *dhfr*.

Atovaquone inhibits electron transport at the cytochrome bc_1 complex in the parasite mitochondrion, but does not affect the host mitochondrial function at the doses used. Resistance to atovaquone develops very rapidly when it is used alone, but more slowly when used in combination with other drugs. Resistance is conferred by singly or doubles point mutations in the parasite cytochrome b (*cytb*) gene. There have been very limited reports of Malarone resistance in Africa.

Drug resistant P. vivax

P. vivax causes 75-90 million cases of non-fatal disease annually (Mendis *et al*., 2001), most of which are outside Africa. Infections are usually treated with chloroquine and primaquine, the latter is used specifically to treat hypnozoites, a dormant stage in the liver that is responsible for the relapses observed in *P. vivax.*

Chloroquine-resistant *P. vivax* was first reported in Papua New Guinea (PNG) in 1989, nearly 30 years after the discovery of chloroquine-resistant *P. falciparum*, despite similar levels of chloroquine use for treatment of the two species. By the early 1990s, treatment with chloroquine was unsuccessful in 22% of patients in a field trial in PNG. More recently, nearly 50% of infections in this area showed reduced susceptibility to chloroquine. Resistance has also been reported in Southeast Asia and South America (Whitby, 1997). There are also reports of resistance to primaquine.

What is the impact of drug resistance on malaria mortality?

The increase in chloroquine resistance in Africa has coincided with a doubling (or more) of malaria mortality, notably amongst children (Trape *et al*., 2002). Antimalarial drug resistance has also been implicated in the increasing frequency and severity of epidemics.

One dramatic study highlights the impact of chloroquine resistance on malaria mortality (Trape *et al*., 2002). The first cases of chloroquine resistance were noted in 1990, and by the following year, half of the *P.falciparum* infections were not responding to chloroquine. Malaria mortality increased eleven-fold in children under the age of five children died because they were treated first with chloroquine, and when this failed, they progressed too rapidly to severe disease. Over 70% of deaths occurred within one week of treatment failure.

CONCLUSIONS

Prompt and effective treatment of malaria is critical for the control of malaria. Antimalarial drug resistance is one of the biggest challenges of malaria control today. Currently less than 45% of children fewer than five years receive the treatment they require, and many of these receive chloroquine, which is rapidly losing its effectiveness. It is likely that the replacement drug, sulfadoxine-pyrimethamine, will not retain its usefulness for much longer.

Despite the seriousness of the malaria problem, there are few new drugs in development. Many of the chemotherapeutic agents in use today date back when the research was begun during the Second World and the Vietnam Wars. The most recently introduced antimalarial drugs, the artemisinin derivatives, are also in some senses the oldest. Artemisinins are based on the antimalarial *qinghaosu*, an extract of Artemisia annua (Wormwood). The drug has been used for more than 2000 years in China, but only came to the attention of the Western world in the 1970s. These drugs have enormous potential. They are already saving lives, but they are not currently widely available, and are expensive- unaffordable for many afflicted.

Experience with drug treatment of TB and HIV infection highlights the importance of combination therapy to delay the development of resistance. The parasite has to mutate in several separate sites simultaneously to become resistant to a combination. For malaria, new combinations of old drugs are being used (chlorproguanil-dapsone), as well as combinations of new drugs such as the

artemisinins. However, the efficiency of the old drug combinations may be short-lived, as the resistance mutations already exist in the parasite population.

It is obvious that new drugs are needed. Unfortunately, malaria is a disease of poverty, and despite much scientific knowledge, there is an insufficient market incentive to interest the 'big Pharma'. Recognising this, the Medicines for Malaria Venture (MMV) was established to bring together academic and industrial scientists to identify suitable targets and develop new drugs (Ridley, 2002). MMV currently has 15 projects developing new drugs in various stages of development, several of which are in clinical trials.

The future for malaria control does not lie solely with antimalarial chemotherapy, and other methods of control are proving highly effective in some areas. Insecticide-treated bednets and curtains protect against malaria, reducing the number of deaths in young children by as much as 20%. Much scientific research has concentrated on vaccine development, and some candidate vaccines have been tested in clinical trials, some with promising results (Richie and Saul, 2002). Realistically an effective vaccine is still some way off. Until that time chemotherapy will remain the mainstay of malaria control programmes, and drug resistance an unfortunate fact of life.

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