

## PLASMODIUM: RESISTANCE TO ANTIMALARIAL DRUGS

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### SUMMARY

Quinine-resistant *Plasmodium falciparum* was first reported in 1910 from Brazil. Today this parasite is resistant in most endemic areas to the widely used blood schizonticide, chloroquine. Many strains are resistant also to antifolates (*e. g.* pyrimethamine, proguanil) and some are also no longer eliminated by quinine. These polyresistant parasites have an enhanced ability to resist also new drugs such as mefloquine and halofantrine. There are indications

that *P. vivax* is also becoming resistant to chloroquine in Papua-New Guinea where primaquine resistance of the hypnozoites also exists. The modes of action of antimalarials and mechanisms by which parasites become resistant to them are discussed. Future developments include the search for radically new compounds, for drugs that reverse chloroquine resistance and for new strategies to impede the progress of this problem.

### RÉSUMÉ : Résistance the *Plasmodium* aux antimalariques.

La première souche de *Plasmodium falciparum* résistante à la quinine a été signalée dès 1910 du Brésil. Aujourd'hui, ce parasite est résistant, dans la plupart des zones endémiques, au schizonticide sanguin le plus utilisé, la chloroquine. Beaucoup de souches sont également résistantes aux antifoliques et antifoliniques (pyriméthamine, proguanil) et quelques unes ne sont plus éliminées par la quinine. En outre ces souches polychimiorésistantes deviennent souvent résistantes aux nouveaux antimalariques comme la méfloquine et l'halofantrine. Par ailleurs, *P. vivax* deviendrait égale-

ment résistant à la chloroquine en Nouvelle-Guinée, où une résistance des hypnozoïtes à la primaquine a été signalée. Les modes d'action des antimalariques, et les mécanismes d'apparition de la résistance chez les parasites sont présentés. Les recherches futures sont orientées sur la découverte de substances complètement nouvelles, de médicaments qui inversent la résistance à la chloroquine, et sur les stratégies nouvelles pour empêcher la progression de ce problème.

### HISTORICAL ASPECTS

Half the world's population live in areas where one or more of the four, man-infecting species of *Plasmodium* are transmitted. One parasite, *P. falciparum* is estimated to kill, directly or indirectly, at least one million Africans, mainly infants and children, every year. Little impact has been made on the incidence of malaria on that continent, in spite of intense efforts to control the anopheline vectors with modern insecticides and the parasites with drugs. Elsewhere the frontiers of malaria have been drawn back only in those areas where the disease held a tenuous hold. Where transmission originally was more intense, the emergence of resistance to successive generations of insecticides and antimalarial drugs has led, partly for technical and partly for socio-economic reasons, to a significant resurgence of transmission, accompanied by increases in malaria morbidity and mortality (Grammicia & Beales, 1988).

Until World War II quinine was the only antimalarial

widely used for prophylaxis or for the treatment of established infections. Quinine acts primarily on the asexual intra-erythrocytic stages of the four *Plasmodium* species, and on immature gametocytes. In the late 1930's the first drug that would prevent relapses of *P. vivax* was introduced, pamaquine. It was replaced 20 years later by another 8-aminoquinoline, primaquine, which remains the only compound of practical value for this purpose. Mepacrine, a 9-aminoacridine, developed in Germany at about the same time as pamaquine, was superior to and less toxic to quinine which it replaced until a new blood schizonticide, chloroquine (also originally a German discovery), came into extended use early in the 1950's. During the massive search for new antimalarials during World War II, two other antimalarials with quite a different mode of action from the other compounds, proguanil and pyrimethamine, were produced in England. Unlike their predecessors, at extremely low doses they inhibited the growth also of the pre-erythrocytic hepatic stages and the sporogonic stages inside the mosquito vectors. Meanwhile it was shown that sulphonamides also had a somewhat similar activity.

In 1910 it was reported that *P. falciparum* infections in a number of German workers in Brazil were not responding as expected to quinine and this was later confirmed

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in some of the patients who survived the long boat journey back to Hamburg. For practical purposes, however, quinine resistance was insignificant although it was recognised that the amount of drug needed to cure a falciparum infection depended upon the geographical area in which the patient had been infected, *P. falciparum* in Africa, for example, being particularly responsive to this compound (Peters, 1987).

#### EARLY EXPERIENCE WITH RESISTANCE

In the few animal models available up to 1948 when a rodent parasite was discovered (*e. g.* *P. knowlesi* in the rhesus monkey, *P. cathemerium* in the canary), intense efforts were made to discover whether the parasites had the ability to develop resistance to quinine, pamaquine or mepacrine, with somewhat equivocal results that have been extensively reviewed elsewhere (Peters, 1987). When the early sulphonamides were demonstrated to be antimalarial, as well as antibacterial, it was readily shown that the parasites could develop (as could bacteria) high levels of resistance. Subsequently the same was shown for proguanil and pyrimethamine, not only in animal models both *in vitro* and *in vivo*, but also in man. It was, therefore, a considerable relief to be able to claim that neither the animal nor human parasites investigated up to 1957 appeared to have the ability to become resistant to chloroquine which, by then, had come to dominate the antimalarial field because of its efficacy, safety and cheapness.

Very rapidly following their introduction, proguanil and pyrimethamine which had initially shown such promise, started to fail. Clearcut resistance to them was proven in *P. falciparum*, *P. vivax* and *P. malariae*. Still they continued to be used, the latter compound particularly together with chloroquine on the assumption that parasites which did not succumb to pyrimethamine would be killed by the chloroquine. In 1958 Indian workers showed that the avian parasite, *P. gallinaceum* could, if put under drug selection pressure for a sufficiently long time, develop a fairly high level of resistance to chloroquine. Prior to this a new parasite was discovered in an African tree rat. This parasite, *P. berghei*, could be passaged by blood transfer in laboratory mice, thus providing, for the first time, a convenient model of a malaria parasite in a small mammal. It was soon shown that *P. berghei* too could develop resistance to proguanil, pyrimethamine and to sulphonamides. In 1957 the Indian investigators reported that *P. berghei* could also become resistant to chloroquine and thus commenced a new era of experimental malaria chemotherapy.

In 1961 appeared the first report that *P. falciparum* acquired by two non-immune field workers in Colombia was not responding normally to chloroquine. The next year chloroquine-resistant *P. falciparum* was reported also from Thailand. In fact it is probable that the first of such cases were occurring as early as 1957 but not yet being recognised.

#### THE SPREAD OF CHLOROQUINE AND POLYRESISTANT *P. falciparum*

##### I — Established compounds

It is most fortunate that, at least until very recently, chloroquine resistance has been limited to *P. falciparum* but, at the same time, a misfortune in that this is the parasite that kills people. (It now seems likely that chloroquine-resistant *P. vivax* is beginning to appear in Papua-New Guinea). The emergence of chloroquine resistance coincided with two major events. The first of these was the impending failure of the malaria eradication campaign during which enormous amounts of chloroquine had been used throughout the world (WHO, 1984). The second major event was the prolonged war in Vietnam during which large numbers of non-immune servicemen were exposed to heavy transmission of both *P. falciparum* and *P. vivax* in areas where chloroquine-resistant strains of the former parasite were obviously already circulating. The war and the tragic disruption of civil life in all the countries of Indochina that followed it, associated with mass population movements, poverty and the significant reduction in many areas of the anti-malaria control measures, have ensured that the intensity of falciparum transmission has continued, for the most part, unabated.

When resistance to an antimalarial such as chloroquine first surfaces, it does so in non-immune people, *i. e.*, indigenous infants and young children who have still to acquire immunity (if they survive) from repeated malaria attacks, or adult foreign visitors to endemic areas. The very first such infections, when further studied in non-immune human volunteers (prior to the development of *in vitro* methods of drug sensitivity testing), proved to be normally responsive to the antifols (proguanil and pyrimethamine) as well as to quinine. They were cross-resistant to mepacrine and usually also to amodiaquine. It was soon recognised that the continuing transmission of such parasites under natural conditions was followed by an increasing level of resistance such that even partially immune, indigenous patients developed *P. falciparum* parasitaemia that was poorly or non-responsive to chloroquine in the dosage normally curative in such individuals. An *in vivo* method was introduced by WHO in 1967 to measure the chloroquine response and it was recorded as S (sensitive), RI, RII or RIII (fully resistant) according to set criteria. The *in vivo* tests were soon to be supplemented by *in vitro* tests, in 1968 a macrotest requiring the use of venous blood and, in 1978, a microtest using capillary (finger-prick) blood that has now been adopted also for a range of additional antimalarial compounds including pyrimethamine, sulfadoxine, quinine and mefloquine. Both in Latin America and Southeast Asia it was found that chloroquine-resistant *P. falciparum* failed to respond progressively to antimalarials other than chlo-

roquine. Pyrimethamine was an early example, followed by proguanil (resistance to these was already common in the other human *Plasmodium* species) and, more recently, a decrease in the sensitivity to quinine has become apparent in widely dispersed foci, the most notorious of which are in Southeast Asia (Peters, 1989). RIII quinine resistance has now been established in Thailand on the Kam-puchean border area and in the south of Vietnam. However, even strains that were resistant both to chloroquine and pyrimethamine, for a long time responded readily to the synergistic combination of pyrimethamine with the long-acting sulphonamides, sulfadoxine or sulphalene. This situation, unfortunately, has changed in the past few years and « polyresistance » now includes, very often, a high level of resistance also to these potent combinations.

## II — Development of new antimalarials

With the advent of resistance to chloroquine, until then the most widely used and one of the safest antimalarials, it became evident that new drugs would be required. Quinine, an expensive plant product, was both scarce and toxic, even though it could, by and large, be depended upon to abort an acute parasitaemia. In the absence of any conspicuous enthusiasm on the part of the pharmaceutical industry, the US Army Research and Development Command launched its own antimalarial development programme in 1963. This has evolved into the largest drug screening and development programme ever undertaken in the chemotherapeutic field. It has, to date, included over 300,000 compounds in primary screening and has resulted in the development, in collaboration with WHO and industry, of two new compounds, aminoalcohols with a mode of action akin to that of quinine, namely mefloquine (a very long-acting 4-quinoline-methanol) and halofantrine (a short-acting 9-phenanthrene-methanol). Other target compounds have appeared but most have failed to survive early preclinical or clinical testing. One exception is an 8-aminoquinoline (WR 238,605) which is analogous to primaquine but shows promise of providing good blood schizontocidal activity. In addition it is likely to have marked hypnozoitocidal activity against *P. vivax* (Gutteridge, 1989). To date mefloquine has, on the whole, only been deployed under relatively strict governmental control in countries where polyresistant *P. falciparum* poses a practical problem. Even so, increasing reports are appearing of the identification, mainly so far by *in vitro* sensitivity testing, of isolates of this parasite that show a reduced sensitivity to mefloquine, even in localities where the drug has never been used. In at least one area where it is being administered fairly regularly, eastern Thailand, a significant number of clinical failures are being reported and a parallel failure of response to quinine is emerging as a conspicuous feature of such infections. As must be anticipated from its structural affinity to mefloquine, cross-resistance

between mefloquine and halofantrine has also been documented, both in animal models and in man, even though the use of this compound to date has been restricted for therapy and it has been deployed, so far, on only a very limited scale in clinical trials.

Polyresistant *P. falciparum* infections still respond, as a rule, to a combination of quinine with tetracycline or clindamycin although both these antibiotics have serious limitations, and recrudescences to such combined therapy are by no means uncommon. Fortunately an entirely novel series of blood schizontocides is becoming available thanks to the skill of Chinese medicinal chemists in identifying the active principle of a traditional herbal remedy, renowned for its antipyretic properties for at least 2,000 years. The plant, *Artemisia annua* (Compositae) (« Qing Hao ») has yielded a sesquiterpene lactone now named artemisinin (« Qing Hao Su ») from which have been derived several semi-synthetic analogues, artemether, arteether, sodium artesunate and sodium arteminate, all of which promise to be somewhat more effective and as safe as artemisinin itself (Wernsdorfer & Trigg, 1988). All these compounds have a very short half-life and are rapidly acting blood schizontocides. Although they are now fully active against polyresistant *P. falciparum* as well as against the other species of human malaria parasites, recrudescences are common and it is clear that more knowledge is required of the pharmacokinetics of the artemisinin series in order to make the best use of them. The same has been found even of chloroquine and quinine, recent pharmacokinetic studies having demonstrated the optimal ways in which even these compounds can be used to our best advantage (White *et al.*, 1988).

One of the few other novel antimalarials is a naphthoquinone, BW566C (Gutteridge, 1989). This compound which is just commencing in clinical trials is active in experimental models and against *P. falciparum in vitro* at extremely low dose levels and retains its activity not only against a wide spectrum of drug-resistant strains, but also against all the stages of the life cycle. Further data on BW566C will be awaited with great interest.

## FUTURE RESEARCH STRATEGIES

To identify and develop novel antimalarials is costly in time and resources. A more concerted effort must, therefore, be made to (1) demonstrate how existing antimalarials function, (2) discover how the parasites become resistant to them, (3) if possible, reverse resistance and (4) find ways to prevent such resistance developing (or at least slow down this process).

### I — Mode of action of antimalarials

Limitations on time and space prohibit a detailed presentation here of this topic which has been discussed at

length elsewhere (Peters, 1987; Wernsdorfer & Trigg, 1988). Suffice it to say that, while the mode of action of antifolates is well known, that of chloroquine is still the subject of great controversy. In a recent paper the argument has reverted full circle back to the role of chloroquine as an intercalator in parasite nucleic acid. What is clear is that quinine, mefloquine and halofantrine function in a different manner and that artemisinin has yet another mode of action related to its possession of an unusual peroxide structure. Other papers in this Congress will no doubt give further details of current hypotheses.

## II — Mechanisms of drug resistance

While simple genetic mutations have been shown to be responsible for resistance to the antifolates and sulphonamides, there is still controversy over the underlying basis of resistance to chloroquine. Although there is now evidence that *P. falciparum* possesses a gene with affinities to the multiple drug-resistance (mdr) gene of human cancer cells, and that this gene is moderately amplified in at least one isolate of a polyresistant parasite, the same is not true of all such strains and it now seems doubtful if the mdr gene alone is responsible for resistance to this compound (Peters, 1989). Current investigations suggest that, as in rodent malaria parasites, chloroquine resistance in *P. falciparum* is caused by the selection of a mutant gene. Experimental and epidemiological evidence indicate that such a mutation is likely to be dominant, thus accounting for the rapid dissemination of resistance to chloroquine in nature. The genetic basis and mechanisms of resistance to quinine and mefloquine have not yet been established.

## III — Reversal of drug resistance

The demonstration that chloroquine resistance which is associated with a decreased drug concentration inside parasitised erythrocytes (compared with that in cells containing drug-sensitive parasites) has been followed by the discovery that this is due to an increased drug efflux. The efflux can be reversed in *P. falciparum in vitro* by a calcium channel blocker, verapamil. It can also be reversed by desipramine which does not possess this property and by cyproheptadine which has quite different pharmacological properties. Although these observations have opened a new avenue of research both into the mechanism of drug action and resistance (calmodulin functions are very much in fashion at the present time —see Scheibel *et al.*, 1989) and

ways of reversing such resistance, great prudence is advisable before launching into clinical trials of such drug combinations which may very readily enhance the toxicity of the individual compounds. It is vital to investigate this thoroughly in advance of clinical testing.

## IV — Prevention of drug resistance

Several fundamental principles should govern the deployment of antimalarials in order to impede the emergence of drug resistance, especially to new compounds. These are (1) establish a regular monitoring of the response of parasites to antimalarials, (2) define the indications for their use (especially new drugs) and limit their deployment through strict national governmental and international control, (3) search for combinations of drugs that can be shown experimentally to impede the emergence of resistance to the individual components and (4) do not depend on drugs to control malaria in the community but identify what role they can play in an integrated, custom-designed plan that incorporates other measures such as vector control, health education and, if and when possible, the use of appropriate vaccines.

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