Antimalarial drugs and their actions

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Summary
New antimalarial drugs are required, partly because of the emergence of drug resistant strains of malaria parasites and partly because better compounds are needed to cure relapsing tertian malaria. In reviewing the diverse modes of action of currently used antimalarials, against a background of the pathogenesis of malaria, attention is drawn to deficiencies in our knowledge. Even less do we understand how the malaria parasite becomes resistant to certain drugs, in particular chloroquine. New approaches to the problem include the application of combinations of existing antimalarials, and the search for new drugs on an unprecedentedly vast scale. Out of over a quarter million compounds that have recently been screened, a handful are now in clinical trial and are showing great promise for the treatment of multiple resistant falciparum malaria. The paper concludes by summarizing current recommendations for the prophylaxis and therapy of malaria due to drug resistant parasites.

At the turn of the century malaria was still endemic in swampy areas of England such as the Thames estuary. With increasing urbanization, land utilization and swamp clearance, breeding of the anopheline mosquito vectors came under control and autochthonous malaria rapidly faded away. Today, however, we are witnessing a resurgence of malaria, not acquired within these shores, but imported by travellers returning from the many parts of the tropical world where the transmission of this disease continues on a vast scale. In spite of some 20 years of international campaigns to control or eradicate this threat, malaria still occurs in territory occupied by some 1,000 million people, nearly one third of the world’s population.

The scale of international travel to and from the world’s more wealthy countries is increasing on an unprecedented scale so that malaria, like other ‘exotic’ diseases, preventable as it may be, nevertheless is on the increase. In the U.K. an average of 120 cases of malaria a year have been reported between 1960 and 1970, with a mortality of 4.7% (Bruce-Chwatt, 1971). Most fatalities were due to the malignant tertian malaria parasite, Plasmodium falciparum. The mortality from falciparum malaria alone was 16%. This figure is appalling, especially so since (a) malaria is preventable and (b) treatable. Most fatalities occur because the diagnosis is missed, and the diagnosis is missed in most instances simply because the physician fails to ask the patient or his relatives the one question, ‘Where have you been, and when?’ This lesson is brought home by comparing falciparum mortality rates between civilian and military cases in the United States. Between 1966 and 1969, 10% of civilians with falciparum malaria died there (Neva et al., 1970). Among military personnel the fatality rate was only 0.3% (Canfield, 1972).

A massive drive to eradicate malaria was sponsored by the World Health Organization on the basis of guidelines laid down in 1957 (W.H.O., 1957). It was based primarily on the deployment of DDT and other chlorinated hydrocarbon insecticides to break the cycle of malaria transmission by killing vector anophelines as they rested in houses. Antimalarial drugs were given as a supplementary measure, partly for the relief of acute malaria and partly to speed the elimination of the parasite pool in the human population. Both prongs of this twoforked attack have been badly blunted by the development of drug resistance. By the end of 1968 some fifteen vector anopheline species had become resistant to DDT, thirty-six to dieldrin and thirteen of the fifteen to both. Already one species (Anopheles albimanus in Central America) is resistant to the new generation of insecticides, the organophosphates. On the parasite side the situation is no better. It has taken very little time from the large-scale introduction of a new drug to the appearance of parasites with the ability to survive it (Table 1). This has been a particularly severe problem in the case of P. falciparum.

New antimalarial drugs are needed, not only because of the resistance problem, but also because existing drugs do not include any that are able to produce a radical cure of relapsing tertian malaria due to Plasmodium vivax unless given in subtoxic doses for at least a week.
Pathogenesis of malaria and the place of antimalarial drugs in prevention and treatment

The malaria parasite undergoes two phases of asexual reproduction in man, the first in parenchymal cells of the liver and the second in red blood cells. From parasites of the latter phase are derived gametocytes which undergo further development culminating in sexual reproduction only after they are ingested by a suitable mosquito. The mosquito phase terminates in the production of infective forms, the sporozoites that enter the circulation of a new mammalian host together with secretions of the mosquito’s salivary glands. The cycle is represented in Fig. 1.

Antimalarial drugs may affect one or more phases of this cycle. The compounds are given names that describe the type of action for which they are normally employed, e.g. chloroquine is known as a 'blood schizontocide', whereas primaquine is usually described either as an 'anti-relapse drug', a 'tissue schizontocide' or a 'gametocytocide'; the first two of these both describe the action of primaquine on the secondary exoerythrocytic liver schizonts that are responsible for relapses in benign tertian malaria due to P. vivax and ovale. (The existence of this phase in Plasmodium malariae is still in dispute.)

The pathogenic effects of malaria are all related to the asexual erythrocytic stages of the parasites (i.e. the direct damage they do to their host cells), though more distant effects due to the products of their metabolism, and the immunological response of the host. The direct damage consists of acute haemolysis, both of infected and uninfected red cells, with its attendant anaemia. The indirect damage is caused by various factors, such as the release of toxic materials of unknown nature (see review by Maegraith & Fletcher, 1972), which trigger a chain of events that include changes in capillary permeability, the production of pharmacologically active peptides, anoxic anoxia, and possibly some degree of disseminated intravascular coagulation. These events may occur to a lesser or greater degree in malaria caused by any of the species of Plasmodium that infect man, but are usually more severe in falciparum infection. This type of disease, justifiably known as 'malignant tertian malaria', is aggravated in addition by the tendency of the infected erythrocytes to adhere to capillaries of the deep circulation, and particularly those of the brain. In this situation interference with cerebral microcirculation can lead to the condition known as cerebral malaria.

Disturbances of renal vascular function may give rise to decreasing glomerular filtration and anuria, with all its consequences. Sudden haemolytic crises associated with falciparum malaria can produce the condition known as 'blackwater fever'. The pathogenesis of this syndrome is still incompletely understood. It is known that it used to be associated with the administration of quinine and that incidence of this complication has diminished greatly in recent years, but it is also possible that host genetic factors such as G-6-PD deficiency may play a role.

Treatment of malaria consists of the administration of specific drugs to destroy the intraerythrocytic parasites and of non-specific supportive measures aimed at correcting the general disturbances of host function. Certain highly effective blood schizontocides in fact possess general pharmacological properties that are of value also in a general manner. The anti-inflammatory action of chloroquine, for example, undoubtedly plays a role in the remarkably rapid response of a child with cerebral malaria to systemic administration of this compound.

A few drugs are of value, not only for their action on the asexual blood stages, but also as a means of blocking the transmission of the parasites through the anopheline vectors. Such drugs are pyrimethamine which has a potent sporontocidal action, and primaquine which appears to act upon the
The evolution of antimalarials

Ever since Pelletier & Caventou isolated the active principle of quinine in 1820, research workers have been endeavouring to produce a synthetic product with the properties of the original antimalarial but without its toxicity. One of the first compounds to be developed which proved to have antimalarial properties of a modest nature was methylene blue, and further work on this type of chemical structure led in the 1920's to the evolution of a number of 8-aminoquinolines. In the early 1930's pamaquine was developed by Schulemann and his collaborators of the I.G. Farbenindustrie. It proved to be a very poor blood schizontocide, although it was found to have considerable activity against exoerythrocytic stages. Later the 9-aminoacridine mepacrine was developed by other German workers and mepacrine proved, unlike its predecessor, to be a highly effective compound for the treatment of acute malaria. The mepacrine type of structure was later simplified and a series of 4-aminoquinolines resulted. The most active of these eventually proved to be chloroquine, which received its first clinical challenge during the Second World War. Other derivatives of the same series were introduced within a few years and these included such compounds as amodiaquine, which has come into very wide use, and the related derivatives amopyroquine and cycloquine. Further development of the 8-aminoquinolines led to a much less toxic analogue of pamaquine, namely primaquine. The structures of these compounds are illustrated below (Figs. 2 and 3).

The 8-aminoquinolines have an entirely different mode of action from quinine, mepacrine or the 4-aminoquinolines. It is now known that the 8-aminoquinolines suppress the mitochondrial functions of malaria parasites, at least in the erythrocytic stages, and possibly also the activity of structures equivalent to mitochondria in the erythrocytic forms. Moreover, it seems very likely that it is not the 8-aminoquinolines themselves that are active...
but 5,6-quinolinequinone derivatives formed by metabolization of the 8-aminoquinolines in the mammalian organism. This, however, remains to be proven.

During the search for new antimalarials in the Second World War it was discovered that many sulphonamides and sulphones have good antimalarial activity against avian malaria, but the promise of these compounds was not borne out by clinical studies against human malaria parasites. It is now known that this was partly because many of the compounds tested were rapidly excreted in man, and some modern, more long-acting sulphonamides have been shown now to have a far more satisfactory blood schizontostatic activity (Fig. 4).

Also during the Second World War research programmes a new type of chemical structure was evolved by chemists of I.C.I. from which the compound we know as proguanil emerged. Proguanil proved to be by far the most active compound known, and in addition it was the only truly causal prophylactic antimalarial ever discovered up until that time. Shortly afterwards pyrimethamine was produced by other British workers in the Wellcome research organization. It was only when the mode of action of proguanil was revealed that it was realized in retrospect how similar pyrimethamine was to the active metabolite of the earlier drug, which we now call cycloguanil (Fig. 5).

Chloroquine, together with amodiaquine, primaquine, proguanil and pyrimethamine provided an armamentarium of synthetic antimalarials that for a number of years following the Second World War appeared to provide all that could be desired in the field of malarial chemoprophylaxis and chemotherapy. It was, however, only a matter of a very few years before resistance began to appear in the field to proguanil and pyrimethamine. Nevertheless, this proved no cause for alarm, since parasites which appeared to be resistant to those compounds still responded to treatment with chloroquine or mepacrine, and in certain cases (when it was still used) quinine. It was indeed only in about 1959 when the first cases of *P. falciparum* infection resistant to chloroquine were found, that serious concern began to be shown about a possible breakdown in drug weapons against malaria. Since this happened to coincide with a period in history when considerable numbers of non-immune troops were being deployed in a highly malarious part of Southeast Asia, namely the Republic of South Vietnam, the coincidence of the emergence of chloroquine resistance and of a major war set in train a series of research programmes which, up to the present time, have culminated in the study of well over 200,000 new compounds in the search for better antimalarials (Peters, 1970; Kinnamon, 1972). Furthermore, considerable effort was devoted to studying the mode
of action of even the older compounds, since this topic had been largely ignored up to this time. Fortunately a new and very useful laboratory model had been developed during the interim period. This was a malaria parasite of rodents which was readily adaptable to ordinary laboratory mice, namely *Plasmodium berghei*. This has proved extremely useful for large-scale screening of drugs for antimalarial activity and has proven to be a far better model for human malaria than the avian parasite *Plasmodium gallinaceum* that was the basis of most drug screening during the Second World War programme. Still more recently an even better model has been discovered in a combination of human *P. falciparum* in the little South American monkey known as *Aotus trivirgatus*. This is the only simian species in which human malaria parasites will fairly readily develop.

**Mode of action of chloroquine and quinine-type compounds**

*P. berghei* has been used extensively for the study of the mode of action of chloroquine. It has long been known that this drug is highly concentrated in parasitized erythrocytes, and it is now clear that the concentration takes place very rapidly in the parasites themselves. The immediate effect of exposure to chloroquine is clumping of the malarial pigment, which is maximal after a period of about 60 min. The malarial pigment that forms within vesicles in which the parasites digest host cell haemoglobin is probably the end product of the breakdown of the haemoglobin protein chains. Interference with this process by chloroquine must quite clearly also interfere with the production of the basic amino acid building blocks required by the parasites. At a later stage of its action chloroquine induces a breakdown of the larger RNA particles of the malaria parasites. Electron micrographs show that chloroquine leads to the development of large cytolysosomes which contain all the haemozoin together with a considerable number of malarial ribosomes. A number of compounds have been shown by Warhurst et al. (1971) to interfere with this process of cytolysosome
formation (Type B). On the other hand, several compounds can be seen to have the same type of action as chloroquine, namely the induction of haemoglobin clumping and cytolysosome formation (Type A) (Table 2). Drugs that produce such an effect are other 4-aminoquinolines and mepacrine. Quinine does not induce clumping of haemoglobin but inhibits the clumping which itself is produced by exposing the parasites to chloroquine. It is now known that the inhibition of chloroquine-induced clumping follows exposure of the parasites (at a suitable interval before their exposure to chloroquine), to inhibitors of protein synthesis, of RNA synthesis and inhibitors of parasite respiration. Quinine itself is a competitive antagonist of chloroquine in this clumping system. Other chemical structures, such as quinoline methanols and phenanthrene methanols, that have certain structural similarities to quinine, exert a similar inhibition of chloroquine-induced clumping of malarial pigment to quinine itself (Warhurst et al., 1972). Thus the study of the action of other agents on chloroquine-induced haemoglobin clumping in malaria parasites has proved to be an extremely useful tool, both for the study of the mode of action of new drugs and indeed for the study of the physiological processes of the malaria parasite itself (Homewood et al., 1972). This technique has the advantage that it enables us to examine what is happening inside the intact host-parasite complex, whereas most other biochemical techniques necessitate the separation of the parasite from the host, which in turn produces an artificial situation, if not death of the parasites, in the process. As a final stage in its mode of action chloroquine probably intercalates with the nucleic acids of the parasite, and in so doing prevents the normal replication of plasmodial nucleic acids.

In addition to their specific antimalarial effect, chloroquine, mepacrine and quinine have marked anti-inflammatory properties, which are perhaps attributable to their ability to stabilize lysosomal membranes, although it may prove that they also have a further action, such as the inhibition of prostaglandin production by certain target tissues in the host organism. Current work will be directed to investigating this possible mode of action of anti-malarials. The anti-inflammatory effects of such drugs as chloroquine are invaluable as a non-specific measure in the treatment of acute malaria. For this reason the threatened loss of chloroquine from our armamentarium was a particularly serious blow.

Mode of action of antimetabolites

Sulphonamides and sulphones exert their effect on malaria parasites in the same way as they do on bacteria, namely by inhibiting the utilization of para-aminobenzoic acid. It appears that their effectiveness lies in the pharmacokinetic properties of the individual compounds in the host (Tables 3 and 4).

<table>
<thead>
<tr>
<th>Approximate 'half-life' in man (hrs)</th>
<th>'Medium-acting'</th>
<th>'Long-acting'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphadiazine</td>
<td>17</td>
<td>120</td>
</tr>
<tr>
<td>Dapsone</td>
<td>25</td>
<td>84</td>
</tr>
</tbody>
</table>

* Sulphormethoxime.
Antimalarial drugs

Table 4. Half-life of repository drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mouse</th>
<th>Man</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycloguanil embonate</td>
<td>6–8</td>
<td>24</td>
</tr>
<tr>
<td>Acedapsone*</td>
<td>6–13</td>
<td>7</td>
</tr>
</tbody>
</table>

* Dads.

For example, sulphonamides with a prolonged half-life in man are proving to be remarkably effective antimalarial agents, as indeed are such newer sulphone derivatives as the diformyl analogue of dapsone.

Proguanil and its active metabolite cycloguanil, pyrimethamine and trimethoprim inhibit the conversion of dihydrofolate to tetrahydrofolate in the malaria parasite. Their selectively toxic action against malaria parasites is due to the fact that they are more strongly bound to the enzyme of the parasites than to the enzyme of the host (Ferone, Burchall & Hitchings, 1969). If sulphonamides or sulphones are given at the same time as one of these antifols, a marked potentiation is observed. This is found not only in systems employing the blood forms of malarial parasites but also in the mosquito stages, and indeed even in the liver stages in the few cases where this has been examined. The same situation applies not only in experimental laboratory models (Peters, 1968) but also in the treatment of human malaria and particularly in *P. falciparum*.

One of the problems, apart from any question of drug resistance, has been the desirability of having antimalarial compounds which can be given at irregular intervals under field conditions where regular chemoprophylaxis is an impracticable proposition. In their search for such long-acting compounds, the late Paul Thompson and his colleagues of the Parke-Davis organization developed poorly soluble salts of cycloguanil and of dapsone. These were found to have extremely long activity, both in mice and in man, when given in an injectable repository formulation (for formulae of cycloguanil embonate and acedapsone, see Figs. 5 and 4 respectively). Moreover, it was hoped that by giving a combination of cycloguanil pamoate and acedapsone one could avoid stimulating the development of parasite strains resistant to one or other of the individual components. Unfortunately this did not prove to be the case when the mixture of these two compounds was applied in extensive field trials. Nevertheless, even cycloguanil pamoate alone proved in human volunteers to protect against sporozoite challenge with both *P. falciparum* and *P. vivax*, in some cases for more than 1 year. Under field conditions the duration of protection was far less, usually only about 3 months, but even this could have offered a considerable advantage for the control of malaria if drug resistance had not appeared within a very short time.

Resistance to the antifols appears to be due to the development by the parasites of a mutant enzyme with a decreased affinity for the drug (Ferone, 1970). Moreover, the mutant enzyme is produced in a larger quantity than the normal enzyme, thus compensating for the presence of the inhibitory drug. Nevertheless, if such a resistant strain, or indeed a strain which is resistant both to an antifol such as pyrimethamine and to a sulphonamide, is exposed to a combination of these two compounds, the combination proves to be effective. This has been shown not only by ourselves and others in experimental situations (Peters, 1971), but also by our American colleagues in human volunteers infected with multiple-resistant strains of *P. falciparum*.

Experimental investigation of resistance to chloroquine

The main laboratory model used up to 1948 for the investigation of antimalarial drugs was the avian parasite *P. gallinaceum* in the chick. In this model it proved very easy to produce resistance to the sulphonamides and to the antifols. It was, however, by no means the same case with the newly developed compounds of the chloroquine type. Numerous attempts to develop strains of *P. gallinaceum* resistant to mepacrine or to chloroquine failed. This induced a false sense of security in the potential value of these modern synthetic antimalarials. This sense of security was abruptly shattered, however, when chloroquine-resistant strains of *P. falciparum* were demonstrated first in South America, and then, within a year to two, in parts of Southeast Asia. Investigations were started rapidly to try to discover how malaria parasites could become resistant to chloroquine. One of the first experiments revealed that chloroquine-resistant *P. berghei* concentrated chloroquine to a much lower degree than did normally chloroquine-sensitive parasites (Macomber, O'Brien & Hahn, 1966). Peters, Fletcher & Stäubli (1965) showed that chloroquine-resistant *P. berghei* failed to produce normal malarial pigment and that the parasites lived in immature rather than in mature red cells. While investigating the respiration of normal and resistant parasites, Howells et al. (1970) found that there is a cyclical change in the mode of respiration of *P. berghei* between the mammalian and mosquito-stages.

While the forms in the red blood cells utilized anaerobic respiration of the Embden–Meyerhof type, the mosquito stages more fully utilized glucose by opening up pathways of the aerobic Krebs cycle. It was shown that blood stages of the highly chloroquine-resistant parasites also utilized Krebs cycle pathways. The hypothesis was put forward that the
parasites were chloroquine-resistant by virtue of their ability to 'switch on' the Krebs cycle enzymes prematurely, that is, in the red cells rather than waiting to go into the mosquito. It was suggested that this would enable the parasites to make up for any loss of amino acids, which may be caused by the interference in their digestive processes by chloroquine, by producing Krebs cycle intermediates which they could then transaminate to produce their own amino acids. Unfortunately, however, we were unable to demonstrate the presence of Krebs cycle enzymes in the malaria parasites themselves in electron microscope cytochemical studies.

Howells et al. (1972) have been able to show that there is indeed an increase in Krebs cycle enzymes in red cells inhabited by malaria parasites, but that the increase is an increase in the host cell enzymes and not in the parasite enzymes. It is relevant, however, that this increase is much more marked in red cells inhabited by chloroquine-resistant P. berghei than in red cells inhabited by drug-sensitive organisms. We believe now that, while this may be the way in which parasites are able to survive once they are resistant to chloroquine, the chloroquine resistance itself is due to a decrease in high affinity binding sites for chloroquine, as suggested originally by Fitch (1969, 1970). This is probably associated with a simple mutation which is inherited in the normal manner and which is stable through mosquito passage. It is interesting to note that we found in our experimental model that exposure of parasites of chloroquine-resistant strains of P. berghei in the mouse to chloroquine led to the production of higher infection rates in mosquitoes subsequently feeding on those mice than would appear if the mice had not received chloroquine prior to the mosquito feed (Ramkaran & Peters, 1969). This situation, if it applies also to chloroquine-resistant P. falciparum in the field, may facilitate the spread of such resistant mutants. This perhaps is one way in which chloroquine resistance has become so rapidly extended from what were probably relatively limited foci in South America and Southeast Asia.

What is perhaps a little more difficult to understand is why chloroquine resistance in P. falciparum has never yet been proven to exist anywhere on the African continent. We believe that the different host response to P. falciparum by African people may play a significant role in this phenomenon. Fortunately, the potentiating combinations of sulphonamides or sulphones with antifols are effective even against the vast majority of strains of P. falciparum which are resistant to chloroquine, to antifols alone, or indeed to sulphonamides and sulphones alone. There is, however, already some indication that certain strains are showing up which do not respond to these potentiating combinations as one would hope. What is not clear at the moment is whether these failures are due to resistance of the parasite or to some inherent difference in the host, such as more rapid excretion or a failure of absorption or utilization of the compounds themselves. This clearly requires further investigation. It may reveal, for example, a genetic difference, such as a rapid acetylation and excretion of sulphonamides or failure to metabolize certain compounds to their active derivatives.

Summary of recommendations for prophylaxis and therapy

Where there is no question of drug resistance being present, prevention is simple. The regular consumption of chloroquine or of proguanil or pyrimethamine will provide adequate protection against all the human malarias. In the case of an acute attack of malaria developing because of the failure to take adequate prophylaxis, malaria is simply too cruel to treat, provided that it is diagnosed rapidly and accurately. Details of the dosage of the various drugs will be found in any medical textbook as well as in the specialized monographs listed below (e.g., Ross Institute, 1972; Covell et al., 1955; Peters, 1970).

The problem arises where there is suspicion that drug resistance may be present. It is essential that the physician who is concerned with giving advice on this matter should make himself familiar with the situation regarding antimalarial drug resistance in the areas to which his patients are going.

If it is known that resistance to proguanil and/or pyrimethamine is present, then the drug of choice for malaria prophylaxis is one of the 4-aminoquinolines, such as chloroquine or amodiaquine. In parts of South America and Southeast Asia where chloroquine resistance is known to occur, the regular consumption of 200 mg of proguanil per day, or even 25–50 mg of pyrimethamine once per week may provide adequate protection in spite of the fact that some measure of resistance may also be present to these antifols. It is, however, not easy to prescribe malarial prophylaxis with confidence in areas such as these where multiple drug resistance is known to occur. Some take a sulphonamide–antifol combination, but it is probably wiser not to do this, since such potentiating combinations are still extremely useful drugs on which to fall back if one needs to for the treatment of an acute attack. It is perhaps wiser to recommend simply a double dose of one of the antifols and to reserve the combination for treatment in the event of a breakthrough.

Most multiple-resistant strains of P. falciparum will still respond, at least in the first instance, to intensive treatment with quinine. Fortunately, several new antimalarial drugs of the phenanthrene...
and quinoline-methanol series are currently being tested which also are very effective against multiple-resistant strains of *P. falciparum*. So far it is only in this species of parasite that resistance to chloroquine has been clearly shown to occur. Chloroquine is still a very effective drug for the prevention or treatment of malaria due to the other three parasites of man.

An exception, however, is in the radical cure of infection due to relapsing *P. vivax* or *ovale* malaria. Here it is essential, in addition to chloroquine, to provide a 14-day course of a tissue schizontocide, such as primaquine, in order to exclude the possibility of relapses due to secondary exoerythrocytic schizonts. Primaquine is also extremely valuable in the treatment of multiple drug-resistant malaria, not so much from the point of view of the patient as of the community. Primaquine has been shown clearly to retain a marked gametocytocidal action even against strains of this parasite which are highly resistant to chloroquine, and to most other drugs as far as the asexual erythrocytic infection is concerned. An
excellent statement of current recommendations for malaria chemoprophylaxis and chemotherapy will be found in the publication of the Ross Institute listed below.

Acknowledgments
I should like to thank Mr S. N. McDermott for preparing the figure and Mrs D. Steedman for the careful preparation of the manuscript.

References

Fig. 8. Distribution of chloroquine-resistant strains of *P. falciparum* in Southeast Asia. Stipple, chloroquine resistance proven; vertical hatching, chloroquine resistance suspected.


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