NEW DRUGS AND THEIR POTENTIAL USE AGAINST DRUG-RESISTANT MALARIA

D. C. Warhurst

PHLS Malaria Reference Laboratory, London School of Hygiene and Tropical Medicine, London

Summary.- Plasmodium falciparum resistant to currently used drugs is increasing throughout the malarious areas of the world. New drugs, particularly those with novel modes of action are needed to provide prophylaxis and treatment. This paper discusses the development and mode of action of a new generation of antimalarials such as the aryl amino alcohol mefloquine, the antifolate combination pyrimethamine-sulfadoxine, the 8-aminoquinoline WR 225,448, the naphthoquinone BW58C and the sesquiterpene lactone artemisinin (qing hao su).

Riassunto (Nuovi farmaci e loro possibile uso contro parassiti resistenti).- P. falciparum resistente ai farmaci di uso corrente è in aumento in tutte le aree malariche del globo. Nuovi farmaci, e in particolare farmaci con nuovi meccanismi d'azione, sono necessari per la profilassi e per il trattamento. Questo lavoro discute lo sviluppo e il meccanismo d'azione dei farmaci antimalarici di nuova generazione, come l'aryl amino alcool meflochina, la combinazione antifolato pirimetamina-sulfadossina, la 8-aminochinolina WR 225,448, il naftochinone BW58C e l'artemisinina (qing hao su).

There is no doubt that the world malaria problem is getting worse (1). In the United Kingdom we see this reflected on a small scale by the rise in imported malaria since the early 70's. Very recently we have seen chloroquine resistant Plasmodium falciparum malaria cases from Islamabad, Pakistan and from Gujerat state, India, imported into the United Kingdom.

The drugs we have depended on for prophylaxis and treatment become less and less effective over a yet larger area of the world. In particular resistance to the 4-aminoquinoline drugs like chloroquine, and more recently resistance to combinations like Fansidar and Maloprim, and even resistance to the old standby quinine have been seen.

In the region of 1/4 million new potential antimalarial compounds have been examined in the last 15 years and of these only one, mefloquine (Scheme 1-I), has reached a state where it can be used with confidence. In view of the relative ease with which resistance can be produced to mefloquine, especially in lines of experimental malaria already resistant to chloroquine, the availability of mefloquine is not enough. We need several such drugs, preferably with different modes of action. This is in addition to malaria control by more classical techniques, by attack upon the mosquito vector, or possibly by vaccines, when these become feasible.

Existing antimalarials can be grouped into three major classes based on their activity on different stages of the life cycle (2) and to some extent also their chemical structure (Fig. 1). The outer circle shows the life cycle, the dense lines indicate the activity of the three drug groups on particular stages.
Scheme 1. - Chemical structures.
Fig. 1. - Drug activity against different stages in the malaria life cycle. The outer ring, read anticlockwise, shows the malaria life-cycle (much simplified). The thick bands bordering the 3 inner rings show the range of activity of the drugs described as "blood schizontocides", "antimetabolites", and "8-aminoquinolines". Dashed lines indicate partial activity or activity against some species only.

Primaquine and other 8-aminoquinolines, which kill most stages of the parasite in the human host are too toxic to man for use against blood stages. Gametocytes and the liver stages are highly sensitive to these drugs, which are used to eliminate the dormant hypnozoite form of P. vivax responsible for relapses, and to interrupt transmission.

The antimetabolite drugs, that affect the production of 4H-folate cofactors in microorganisms, include sulphonamides, mimicking p-aminobenzoic acid, and pyrimethamine or cycloguanil which specifically inhibit plasmodial dihydrofolate reductase. These are effective on all growing stages but are relatively slow blood schizontocides. They are very effective causal prophylactics in non-resistant strains.

The blood schizontocides include the oldest antimalarial, quinine, still invaluable. Synthetic drugs based on this model include 4-aminoquinolines such as chloroquine and aryl amino alcohols like mefloquine. Blood schizontocides are effective only on actively growing forms in the erythrocyte. Mature gametocytes
and liver stages are unaffected.

For treatment of established infections the rapid action of the blood schizontocides and their relative lack of toxicity are invaluable.

Table 1 shows some aspects of malaria biochemistry. Points at which selective effects of drugs on the parasite can be achieved are on the requirement for pyrimidine synthesis, folate cofactor production from PABA as in bacteria, and the inability to detoxify haemin.

Table 1. - Special Features of the Biochemistry of Plasmodium in the Mammalian Host

1. MAJOR ENERGY PATHWAY IS GLYCOLYSIS, GIVING LACTATE.
2. USES EXOGENOUS PURINES.
3. SYNTHESISES ITS OWN PYRIMIDINES.
4. MICROBIAL TYPE OF FOLATE CO-FACTOR SYNTHESIS, DEPENDENT ON p-AMINO BENZOIC ACID.
5. DIGESTION OF HAEMOGLOBIN (BLOOD STAGES).
6. CANNOT CLEAVE THE HAEM RING.

Pyrimidine synthesis for nucleic acids depends on the availability of reduced folate cofactors, which in malaria parasites, as in many other microorganisms, are synthesised from PABA, pteridine and glutamate unlike in the host, and this renders the organism susceptible to analogues of PABA such as sulphonamides and sulphones. The dihydrofolate reductase enzyme of plasmodia is exquisitely sensitive to the folate analogues pyrimethamine and cycloguanil, sensitivity being a factor of 1000 higher than that of enzyme from the host.

In addition, pyrimidine synthesis apparently depends on the availability of oxidised ubiquinone 8, from electron transport (Scheme 1-V). This is probably affected by metabolites of primaquine and the naphthoquinones (Fig. 2). So there are 3 points of attack based on pyrimidine synthesis in plasmodia.

Malaria parasites may also suffer from oxidative stress because of the action of quinone-type metabolites of primaquine.

When it is in the red cell the malaria parasite digests haemoglobin. In mammalian cells which do this, the iron-containing haem-ring (Scheme 1-II), which is released in an oxidised free toxic form, is broken down using the enzyme haem-oxygenase, to the less toxic bilirubin: iron released is utilised. Malaria parasites do not possess haem-oxygenase (3), but toxic haemin released is sequestered with protein to form crystalline malaria pigment (4) (Table 2). Apparently no iron is available to the parasite from haemin.

![Fig. 2. - Linkage of respiration to Pyrimidine Synthesis.](X: blockage by naphthoquinones & other quinones)
Cultures of *P. falciparum* are very sensitive to iron chelators, and it has been shown that the iron needs of the parasite are obtained from the iron carrier, serum transferrin (5). It is probable that at least some of the blood schizontocides act by binding to haem in released during haemoglobin digestion and preventing its sequestration (6,7). This would explain the inactivity of such drugs as chloroquine, quinine and mepacrine on liver stages where the parasite does not digest haemoglobin.

<table>
<thead>
<tr>
<th>Table 2. - Characteristic of malaria pigment</th>
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<tr>
<td>CRYSTALLINE</td>
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<tr>
<td>OPTICALLY ACTIVE (POLARISING)</td>
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<tr>
<td>LATTICE SPACING FOR IRON ATOMS OF c. 10 Å</td>
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<tr>
<td>UP TO 70% HAEMIN: 30% PROTEIN (DRY WEIGHT)</td>
</tr>
<tr>
<td>ABSORPTION PEAK 650nm (VARIES WITH SPECIES?)</td>
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<tr>
<td>NON-TOXIC</td>
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Screening for antimalarial activity was originally done on bird malarias, but the introduction of tests in mice on the rodent malaria *P. bergheii* in the 50's led to tests of greater relevance and wider utility. A more recent modification of *P. bergheii* testing is that of Rane and coll., where a single dose of drug is tested for its efficacy in extending the time to death of infected animals. The 4-day suppressive test refined by Peters is ideal for accurate measurements of the relative sensitivity of strains to new drugs. Mouse tests using mosquito transmission are used for causal prophylactic screening.

The simian malaria *P. cynomolgi* is used for testing drugs aimed at the hypnozoites for radical cure of *P. vivax*. Blood schizontocide tests in mice may be followed up in Aotus monkeys using sensitive and resistant strains of human malaria (8).

Preliminary drug screening using cultured *P. falciparum* is now a routine procedure (9). This is a very economical system and avoids having to use animals. It is also advantageous in that the human parasite is used.

New drugs

In the antimetabolite group the introduction into field use of combinations of sulphonamides or sulphones with pyrimethamine has been a major step taken in recent years (10). These potentiating combinations have been valuable in prophylaxis of antimetabolite-resistant malaria as they are effective against strains resistant to pyrimethamine alone. Strains of *P. falciparum* resistant to such combinations are however known to occur in SE Asia, E. Africa and S. America.

Combinations of pyrimethamine with the long-acting sulphonamide sulfadoxine as in Fansidar, are theoretically superior to dapsone-pyrimethamine combinations, because of the similarity in plasma half life of sulfadoxine and pyrimethamine: over 100 hours in both.

Half life of dapsone is 17-33 hrs, and pyrimethamine is unprotected by dapsone for a significant period of time in combinations of these drugs (11).

Further developments of the active metabolite of proguanil, cycloguanil, and dihydrotriazines are of interest. Proguanil itself is still widely used as a prophylactic, and McLarty et al. have recently (12) reported its successful use as a prophylactic in Tanzania (200 mg/day) an area where multiresistant strains of *P. falciparum* are common.

Clociguanil, an arylalkoxy derivative of cycloguanil is highly antimalarial in mice, and had a very high binding constant for malarial dihydrofolate reductase of $10^{12}$, better than pyrimethamine. It also shows potentiation with
sulphonamides (13).

When tested in man it showed no advantages over pyrimethamine or proguanil. Its rapid excretion is apparently a problem.

There has been some progress on new hypnozoitocidal drugs (radical cure for P. vivax).

It was early shown that pamaquine was less active in vitro against P. gallinaceum and P. lophurae than the quinoline diquinone metabolite extracted from faeces of birds fed the drug. The metabolites were also more toxic to erythrocytes.

The probable 5,6 quinoline quinone and quinone imine metabolites of 8-aminoquinolines are considered to be responsible for toxic, and at least some of the antiparasitic effects.

It was reported that in primates the l-enantiomer of primaquine was less toxic than, although equally antimalarial to, the d-enantiomer. 4-methyl primaquine was found to be more active than primaquine, but to be unacceptably toxic.

Five, and 4,5 substituted primamaquines were more promising and WR225448 (Scheme 1-III) was the best, being 5 times as active as primaquine and also had good blood schizontocidal activity (14). Morphological studies showed it had similar effects to primaquine on the parasite mitochondria (15).

It is known that some naphthoquinones which structurally resemble the metabolites of primaquine and ubiquinone, have similar effects to primaquine on malarial mitochondria causing swelling. Menoctone and some other 2-hydroxy naphthoquinones were active in rodents.

Very disappointing results were found in causal prophylaxis and therapy in man. Hudson (16) points out that an alicyclic (e.g. cyclohexane) substituent in the 3 position affects the metabolic fate of naphthoquinones, leading to production on an alcohol, still antimalariaIlly active, rather than a carboxylic acid which is inactive. It is also known that the cyclohexyl group should be attached close to the ring for optimum activity in vitro.

An extensive synthesis and in vitro testing programme at Wellcome Laboratories has now identified compound BW58C (Scheme 1-IV) with an IC_{50} of $10^{-16}$ mol/l against P. falciparum in vitro. It is active in rodent and simian malaria, causally prophylactic in mice.

It was shown to inhibit respiration and the ubiquinone-linked dihydroorotate dehydrogenase of P. yoelii at a concentration 200 times lower than that active on host enzyme (17). This is an extremely promising compound, although at an early stage in development.

**Blood schizontocides**

There has been significant progress here since the 60's.

Quinine is a quinoline amino methanol drug, i.e. it has a quinoline heterocyclic nucleus with an aminoalcohol side chain. Quinine is rapidly inactivated in the body by oxidation at the 2 position (Scheme 1-VI).

Analogue of quinine were synthesized in the 1940s with the 2 position blocked with a phenyl group. They were much more active, but had phototoxic side effects. However a 3,4 dichlorophenyl group eliminated phototoxicity, when added at the 2 position.

Following up this lead, in the more recent American Army Programme, the phenyl group was replaced with a tri-fluoromethyl group, and further substituted with a similar group in the 8 position. This very active compound WR 142490, now mefloquine (Scheme 1-I), was 8 times as active as chloroquine, 64 times as active as quinine in the Kain test.

Evidence from P. berghei shows some cross resistance of highly chloroquine resistant strains, but sensitivity of NS P. yoelii. Resistance production in the laboratory was delayed when the drug was used in combination with pyrimethamine-sulfadoxine (18). In vitro it showed the same activity against sensitive and resistant P. falciparum and it was the most active compound tested.
in the pigment clumping inhibition test (19) (Table 3).

The mode of action may be via interaction with haemin (7), like quinine and chloroquine, but mefloquine also has very strong binding to membrane phospholipids (20).

In man, a 1500 mg dose is curative, with few side effects.

The biological half life is 2 weeks. It was more effective than pyrimethamine-sulfadoxine in multiresistant infections.

Table 3.- The activity of a series of aryl amino alcohols in vitro (inhibition of chloroquine induced pigment clumping in P. berghei) and in vivo.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Activity in vitro (clumping inhibition)</th>
<th>Activity in vivo against P. berghei in mice (drug administered s.c.)</th>
<th>Activity against chloroquine-resistant P. falciparum in man</th>
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<tr>
<td></td>
<td>gmK_{I} n</td>
<td>ED_{90}</td>
<td>I_{90}^*</td>
</tr>
<tr>
<td>Quinine</td>
<td>410 2.3</td>
<td>87</td>
<td>0.8</td>
</tr>
<tr>
<td>9-epiquinine</td>
<td>10^{-5} ND</td>
<td>Inactive</td>
<td>ND</td>
</tr>
<tr>
<td>Ro 21-0960</td>
<td>53 1.7</td>
<td>3.7</td>
<td>2.6</td>
</tr>
<tr>
<td>WR 142 490</td>
<td>4 1.0</td>
<td>8.5</td>
<td>1.3</td>
</tr>
<tr>
<td>WR 30 090</td>
<td>25 ND</td>
<td>2.7</td>
<td>1.2</td>
</tr>
<tr>
<td>WR 122 455</td>
<td>11 1.3</td>
<td>10</td>
<td>1.9</td>
</tr>
<tr>
<td>WR 165 355</td>
<td>7 ND</td>
<td>2.6</td>
<td>1.5</td>
</tr>
<tr>
<td>WR 33 063</td>
<td>29 ND</td>
<td>18.5</td>
<td>ND</td>
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* Resistance factor.

For prophylactic and therapeutic use the combination of one part by weight of pyrimethamine to 20 parts of sulfadoxine and 7 parts of mefloquine is being tested. Clinical trials are giving encouraging results. Another of the aryl amino alcohols, this time a phenanthrene amino alcohol, halofantrine (21), is also in clinical trial after successful studies against chloroquine-resistant P. falciparum in vitro and in Aotus monkeys. A strain of rodent malaria made resistant to this drug showed appreciable cross-resistance to chloroquine (22).

The new blood schizontocide, qing hao su (artemisinin) was isolated by Chinese scientists from a wormwood plant, Artemisia annua, which had been in use for over 1000 years as a febrifuge. Artemisinin is a sesquiterpene lactone with an endoperoxide group, quite unlike any other antimalarial. The reduced form, dihydroartemisinin (Scheme I-VII), is more active in vitro (23), and the substituted reduced forms are more active in vitro and in vivo (24). Activity is lost with the loss of one of the oxygen atoms of the peroxide group.

Artemisinin has a higher chemotherapeutic index than chloroquine and is active in chloroquine-resistant strains of rodent and human malaria. Although toxicity at high dose (> 1 g kg) is manifested in bone marrow, liver and heart, there is significant fetotoxicity in rats and mice at much lower doses (25). The drug has a very rapid effect therapeutically, superior to mefloquine, and has been proposed for use in acute malaria, especially cerebral malaria. Obviously the decision to use the drug in women of child bearing age must be taken with caution.
Mode of action appears to be different from that of the other blood schizontocides. One of the earliest ultrastructural effects is damage to membranes (26). Incorporation of amino acids into protein is halted within 30 minutes (27). Tritiated reduced drug is concentrated by infected red cells. The K is 10 nmol/l. Uptake experiments indicate that when the close analogue artemether and the cold drug are used they will inhibit uptake if added before or at the same time as the radiolabelled drug. Chloroquine, interestingly, also has some effect when added before the radiolabelled drug (28).

Ultrastructural autoradiography shows that radiolabel associates with parasite membranes (26).

Although it would seem that the peroxide bridge might be actively involved in the mode of action of the molecule, it has not yet been possible to confirm this. Activity is lost if any of the rings is broken (29), and this indicates that the integrity of the rings is important. Building the molecule we find that it is remarkably rigid. The polar oxygen groups are all along one edge, the rest of the molecule being lipophilic. This type of structure will associate well with membrane, the non-polar part with the hydrocarbon phase, and the polar with the aqueous phase, like cholesterol or a phospholipid (26).

We have produced resistance to artemisinin in P. yoelii (30) and the resistant strain is cross-resistant to mefloquine, chloroquine and quinine.

Recalling now the observation that chloroquine had some effect on dihydroartemisinin uptake, it appears that though the mode of action may not be like that of chloroquine, the mechanism of resistance is linked, possibly through membrane changes.

It is recommended that artemisinin be protected in potentiating combinations, to avoid development of resistance in the field, and to allow minimal doses of the drug to be used. Mefloquine and primaquine both potentiate with artemisinin.

Chinese workers reported antagonism with antifolate drugs, and we confirm this for pyrimethamine and sulfadoxine. This would be a contraindication for use of Fansidar and artemisinin together in the field (31).

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