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Pharmacology and Chemotherapy

VOLUME 16

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ADVANCES IN PHARMACOLOGY AND CHEMOTHERAPY, VOL. 16

New Experimental Antimalarial Drugs*

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I. Introduction

Malaria is a parasitic disease that has brought misery and death to many millions of people in tropical and subtropical regions. The social, economic, and political impact of malaria through the centuries has been severe. There was no effective treatment for the disease until quinine, the active principal of cinchona bark, was introduced into Europe over three centuries ago. This remarkable drug became accepted as the treatment of choice for malaria and its use was limited only by its availability.

Supplies of quinine were severely curtailed by World War I, and this shortage provided the impetus for an organized effort to develop synthetic antimalarial drugs in Germany. This effort led to the discovery of pamaquine, mepacrine, and chloroquine. However, the value of these synthetic agents was not fully realized and quinine remained the mainstay of antimalarial chemotherapy.

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It was not until World War II, when quinine supplies were again threatened, that a program to exploit synthetic antimalarial drugs was started in the United States. This led to the widespread use of mepacrine, the rediscovery of chloroquine, and the discovery of primaquine. The abrupt termination of this program following the war interrupted the development of many promising leads. However, some antimalarial drug synthesis by the drug industry continued during ensuing years, leading to the commercial development of drugs such as proguanil and pyrimethamine.

The malaria problem appeared to be solved. Chloroquine and these latter two drugs were rapidly effective against the erythrocytic stages of all species of *Plasmodium,* and primaquine was effective against exoerythrocytic stages. Complacency resulting from the widespread success of these drugs was abruptly shattered, however, when *Plasmodium falciparum* resistance to chloroquine was reported from widely separate geographic areas in the early 1960s. In addition, many strains of these parasites were also resistant to the other available drugs.

Because U.S. military personnel were involved in one of these areas of parasite resistance (Southeast Asia), the U.S. Army organized a new program in 1963 to develop drugs effective against these drug-resistant strains (Tigertt, 1969). It has proven to be the most massive antimalarial development program in history, with over 235,000 chemical compounds being examined to date.

From the inception of the program many thousands of compounds were shown to have good antimalarial activity in the animal test screens. A complex series of secondary tests successfully reduced the number of compounds for introduction into humans. These steps included evaluation of comparative efficacy and comparative toxicity in several animal models (Kinnamon and Rothe, 1975; Canfield and Rozman, 1974). Following introduction into humans, development of some drugs was discontinued because they were found to be poorly tolerated at doses necessary for antimalarial efficacy or they demonstrated less antimalarial activity when compared to the most active drugs. However, a number of highly active new antimalarial drugs emerged.

The scope of the present review is limited to the most promising of these newer antimalarial drugs. Many of the toxicological data and some of the clinical information on these drugs have had only limited distribution. All of the drugs to be discussed have undergone some clinical testing and have proven highly efficacious in man or are of sufficient interest in a primate model of human malaria to warrant clinical testing.

Older and well-established drugs will not be discussed at length. In addition, combinations of established drugs will not be covered in this review. For these and other antimalarial drugs of interest, attention is called to a number of recent reviews and summaries (Peters, 1970, 1974; Thomp-

son and Werbel, 1972; Steck, 1972; Rozman, 1973; Canfield and Rozman, 1974; Clyde, 1974; Strube, 1975; Hall, 1976).

II. Biology of the Parasite

A brief review of the protozoan parasite, with definitions of drug response, may help in understanding some of the results to be discussed in detail for each drug.

Four plasmodial species are responsible for naturally acquired human malaria. As shown in Fig. 1, *Plasmodium falciparum* does not have a persistent tissue form. *Plasmodium vivax* and *Plasmodium ovale* do have this secondary tissue schizont stage that can cause a long interval between clinical attacks. *Plasmodium malariae* persists as a latent erythrocytic infection. This lack of the secondary tissue phase by *P. falciparum* is of therapeutic importance; once the blood forms are destroyed, a radical cure is obtained.

Several terms directly relating to drug action and the developmental forms of the parasite may be defined as follows.

Causal prophylactic—a drug producing complete prevention of erythrocytic infection by destroying either the sporozoites or the primary tissue forms of the parasite.

Suppressive prophylactic—a drug that prevents or eliminates clinical symptoms and/or parasitemia by early destruction of the erythrocytic forms without affecting the establishment of the exoerythrocytic forms.

Clinical cure—relief by drugs of symptoms of a malaria attack without necessarily complete elimination of the infection.

Radical cure—complete elimination of the malaria parasite from the body by drugs so that relapses cannot occur.

Relapse—renewed manifestation of malarial infection separated from previous manifestations of the same infection by an interval greater than those due to the normal periodicity of the paroxysms.

Recrudescence—renewed manifestation of infection believed due to survival of erythrocytic forms.

As already stated, it can be seen that since *P. falciparum* does not have the persistent tissue forms, complete suppression of the asexual erythrocytic parasites will result in a cure. This is not the case with *P. vivax*, where a tissue reservoir exists.

In evaluating the results of drug therapy both in subhuman models and in human trials, the method of infecting the host is important. As Fig. 1 indicates, mosquito (and thus sporozoite)-induced infection occurs in the liver before the peripheral blood cycles begin. On the other hand, blood (and thus trophozoite)-induced infections bypass the liver and will not

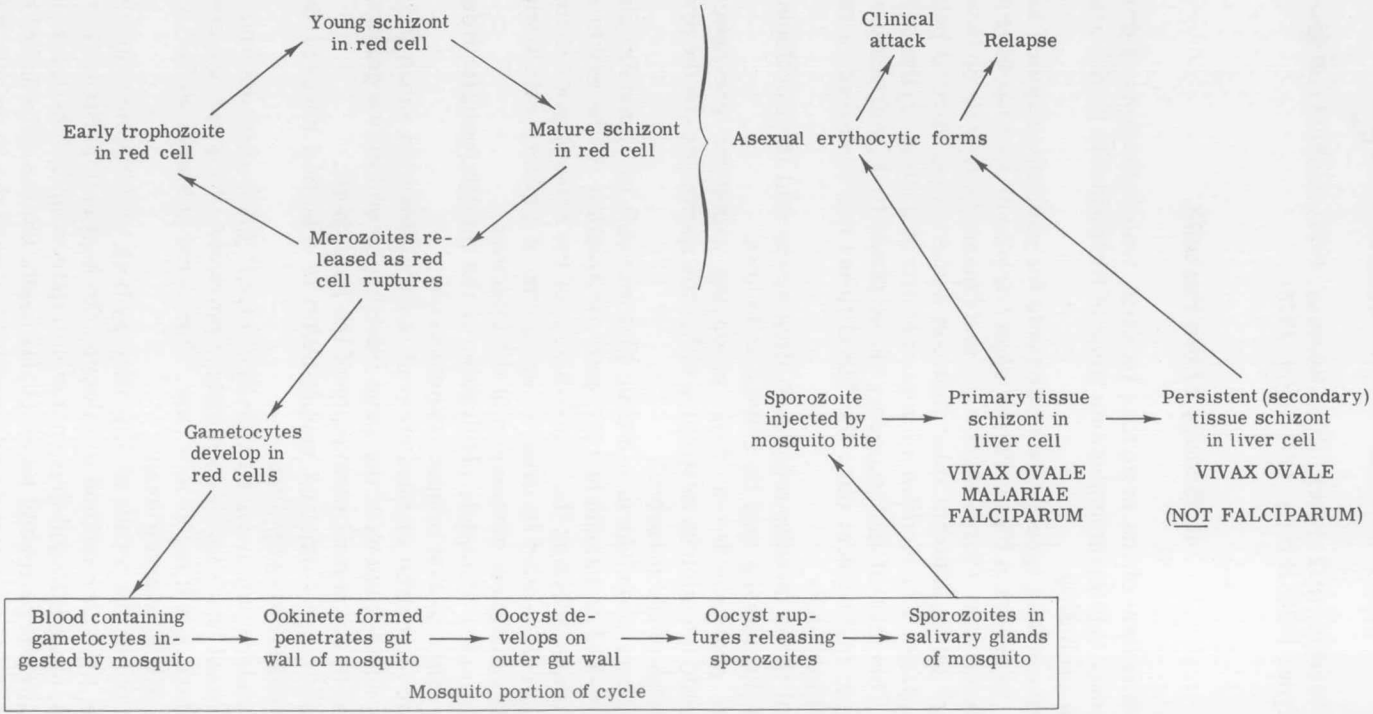

FIG. 1. Life cycle of human plasmodia.

show an effect from a pure causal prophylactic drug. Conversely, the drug may be an effective suppressive agent, but not produce a radical cure in *P. vivax*.

The primary initial animal test for blood schizonticidal activity used in the Army program is a blood-induced *Plasmodium berghei* infection in mice, developed by Dr. Leo Rane (Osdene *et al.*, 1967). The most important subhuman primate screens are blood-induced *P. falciparum* and *P. vivax* infections in the *Aotus* monkey (Schmidt, 1973). Since these systems test drugs against established erythrocytic parasitemias, causal prophylactic effects cannot be determined without modifications. Drugs being evaluated for this effect are initially tested in a sporozoite-induced mouse system (Gregory and Peters, 1970) and confirmed in rhesus monkeys with sporozoite-induced *Plasmodium cynomolgi* infections (Davidson *et al.*, 1976).

The same points must be made about Phase II studies in human volunteers. Some of the infections in volunteers were blood-induced and some were mosquito-induced, depending on the type of drug activity being tested. In both man and animals, different strains of parasites are used. Resistance to drugs in animal parasites is often artifically induced and is well characterized in terms of sensitivity to various drugs. In man, some infections are produced with naturally occurring strains that have developed a resistance to one or more of the antimalarial drugs; these strains originate in different areas of the world. Most of these human strains have had their drug-resistance patterns detailed in relation to degree of resistance to the standard antimalarial drugs. Studies in volunteers with several of these characterized strains are thus effective predictors of response to naturally acquired infections in different areas of the world. These human strains can also be used to infect the *Aotus* monkey, and thus this test system is used to evaluate new drugs against specific drug-resistant strains of the target organism.

Among the classes of compounds that produced effective drugs against resistant strains were the quinolinemethanols. They show considerable promise and will be discussed first.

III. Quinolinemethanols

A. WR 30,090

1. *Chemistry*

Synthesis of WR 30,090 (Fig. 2) was first reported by Lutz *et al.* (1946). This compound was originally synthesized as a part of the World War II antimalarial program, and was assigned Survey Number (SN) 15,068. Be-

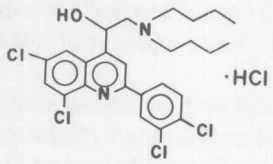

FIG. 2. Drug WR 30,090. α-[(Dibutylamino)methyl]-6,8-dichloro-2-(3,4-dichlorophenyl)-4-quinolinemethanol hydrochloride.

cause the compound was developed late in that war, it was not tested in humans (Wiselogle, 1946) until reactivation of the antimalarial drug development program by the U.S. Army.

Partitioning studies between organic solvents and aqueous buffered solutions showed that WR 30,090 has a very high lipophilicity, with Kp for a number of organic solvents in the general magnitude of 10^3 (Mu *et al.*, 1975).

In vitro studies have shown the hydrochloride salt form to be stable in solution for several days in organic solvents. The free base of WR 30,090 was also relatively stable in these solvents when not exposed to sunlight or to ultraviolet irradiation. When irradiated at ~3000 Å, however, the free base very rapidly decomposed to produce mainly 6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinecarboxyaldehyde (Okada *et al.*, 1975).

2. *Preclinical Efficacy and Biology*

Efficacy studies published by Wiselogle (1946) showed that orally administered WR 30,090 effectively suppressed *Plasmodium lophurae* in ducks, with a twenty-fold greater potency than quinine. Coatney *et al.* (1953) reported efficacy against *Plasmodium gallinaceum,* with a therapeutic index of 186.5.

Drug WR 30,090 when administered subcutaneously as single doses of 160 mg/kg suspended in peanut oil produced radical cures of all mice infected with *Plasmodium berghei* (Strube, 1975). Temporary suppression of parasitemia was observed at single doses as low as 10 mg/kg. The oral dose of WR 30,090 required to produce 90% suppression of *P. berghei* parasitemia in mice when administered daily to sensitive strains ranged from 0.8 (Thompson, 1972) to 2.7 mg/kg/day (Peters *et al.*, 1975).

The minimum daily oral dose required to cure rhesus monkeys infected with blood-induced *Plasmodium cynomolgi* was 100 mg/kg/day for 7 days (Davidson *et al.*, 1976). Schmidt (1973) found no evidence of cross-resistance with chloroquine in *Plasmodium falciparum*-infected *Aotus* monkeys which were given WR 30,090 orally for 7 days (Strube, 1975).

By contrast, Peters *et al.* (1975) found cross-resistance with the highly chloroquine-resistant RC strain of *P. berghei*. However, they saw little cross-resistance to their moderately chloroquine-resistant NS strain. Essentially the same pattern of cross-resistance was observed by Thompson (1972).

A number of drugs that competitively inhibit chloroquine binding to high-affinity drug receptors (Fitch, 1972) have been shown to have an apparent K_i similar to that of chloroquine.* The apparent K_i for the high-affinity receptor of chloroquine-susceptible *P. berghei* was 5×10^{-7} *M* for chloroquine and 2×10^{-6} *M* for WR 30,090 (Fitch, 1972). This ability to compete for high-affinity binding sites is thought to be a reflection of chloroquine cross-resistance for this parasite.

Another area that was investigated in attempts to predict cross-resistance was the clumping of hemozoin pigment caused by chloroquine in *P. berghei*-infected red blood cells. A number of drugs, including WR 30,090, inhibited chloroquine-induced pigment clumping in *P. berghei* (Warhurst *et al.*, 1972; Warhurst and Thomas, 1975; Einheber *et al.*, 1976). This implied that these drugs compete for the same binding site as chloroquine. Further evidence that WR 30,090 was competing for the chloroquine binding site was found by Einheber *et al.* (1976) who showed disaggregation of fully formed chloroquine-induced hemozoin clumps by WR 30,090.

Warhurst and Thomas (1975) indicated that, in comparison with Fitch's high-affinity binding site, the clumping binding site was more structure specific. The possibility exists that modifications of these several types of sites in chloroquine-resistant *P. berghei* may help explain the inconsistent cross-resistance patterns observed in these studies.

3. *Preclinical Toxicology*

Twenty-day toxicity studies were carried out in rats (Powers, 1968a). Four groups of 5 rats each received 0, 500, 1000, or 2000 mg/kg/day by oral intubation. Appearance and behavior of the rats were normal, but food consumption and body weight gain at the two higher doses were depressed. No other drug-related effects were seen.

An 84-day toxicity study was carried out in rats (Lee *et al.*, 1971a). Four groups of 6 rats each received 0, 250, 500, or 1000 mg/kg/day by oral intubation. No toxicity was associated with the 250-mg/kg/day group. Slight toxic signs were seen in the animals receiving the two higher doses, with a slight depression of food consumption and growth rate.

* The apparent K_i as used here is the association constant for a drug to binding sites in *P. berghei*-infected erythrocytes estimated graphically from Lineweaver–Burk plots.