Reprint from

Handbook of Experimental Pharmacology, Vol. 68/I
Edited by W. Peters and W. H. G. Richards

Springer-Verlag
Berlin Heidelberg New York Tokyo

CHAPTER 9

Malaria Models in Simian Hosts

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A. Introduction

Recognition by Moore and Lanier (1961) and Young and Moore (1961) of *Plasmodium falciparum* strains resistant to chloroquine, coupled with the disease among United States service personnel caused by such resistant strains, required the finding of a small, readily obtainable animal susceptible to human plasmodia for the evaluation of candidate antimalarial drugs. At Gorgas Memorial Laboratory Young et al. (1966) demonstrated that the Panamanian *Aotus trivirgatus* (owl monkey) would support development of *Plasmodium vivax* obtained from a human patient. A year later, Geiman and Meagher (1967) reported the successful adaptation of an East African strain of *P. falciparum* in *A. trivirgatus* of Colombian origin. The Colombian *A. trivirgatus* model, infected with strains of *P. falciparum* of diverse susceptibilities and/or resistance to chloroquine, pyrimethamine, and quinine, afforded the basis for intensive investigations. Schmidt (1969) recognised the value of the owl monkey model for evaluating new antimalarials.

Fig. 1. The owl monkey or douroucouli (*Aotus trivirgatus*)

The spectrum of susceptibility of New World monkeys to human plasmodia has been the subject of various review articles by Young (1970) and Young et al. (1975, 1976). As will be discussed subsequently, only *A. trivirgatus* and, to a lesser extent, the squirrel monkey (*Saimiri sciureus*) have served as experimental hosts in drug evaluation studies. Prior to the establishment of the New World monkey model, the rhesus monkey, *Macaca mulatta*, infected with *P. cynomolgi* was most extensively used for chemotherapy trials. The literature through 1969, associated with such studies was reviewed by Peters (1970) and only later studies will be discussed here. Details of techniques and of the course of infection in some simian hosts are provided in Peters (1980).

B. Use in Blood Schizontocide Studies

I. Simian Plasmodia

1. *Plasmodium cynomolgi*

For more than 30 years, trophozoite- and sporozoite-induced *P. cynomolgi* infection in the rhesus monkey (*Macaca mulatta*) has served as a model for evaluating potential antimalarial agents for use against *P. vivax* in man. While the utility of the simian malaria system has been overshadowed by the development of the *P. vivax* – *A. trivirgatus* model, some reports were published during the past 11 years using *P. cynomolgi*.

a) Quinazolines

Infections of the B strain of *P. cynomolgi* were used to evaluate 2,4-diamino-6-(3,4-dichlorobenzylamino) quinazoline (PAM 1392) by Thompson et al. (1969). Doses of 100.0 mg/kg for 5 days, or 50.0 mg/kg for 10 days cured such infections.

A second quinazoline, 2,4-diamino-6-[(3,4-dichloro-benzyl)nitrosamino] quinazoline (CI 679) was first evaluated by Thompson et al. (1970). The base form of the drug cured infections of the B strain of *P. cynomolgi* when administered orally twice daily for 5 days at a dose of 2.5 mg/kg. A single intramuscular dose of 10.0 mg/kg also proved to be curative. The acetate form of CI 679, at a total oral dose of 50.0 mg/kg cured infections in 14 of 15 rhesus monkeys. This curative capacity was observed at a single 50.0 mg/kg dose or at a dose as low as 3.125 mg/kg, twice daily, for 8 days. When administered in a repository form, both the base and acetate of CI 679 protected monkeys for at least 105 days against repeated trophozoite challenge.

Schmidt and Rossan (1979) further evaluated CI 679 against pyrimethamine sensitive (Ro) and pyrimethamine-resistant (Ro/PM) strains of *P. cynomolgi*. The total amount of drug required to cure infections of the Ro/PM strain was ten times the amount needed to cure the Ro strain, indicating cross-resistance with the pyrimethamine strain. Resistance to CI 679 became evident rapidly in both strains.

b) Antibiotics

The schizontocidal activity of three chlorinated lincomycin analogues against the B strain of *P. cynomolgi* was assessed by Powers (1969). These analogues cured infections, but at doses of 50.0 and 100.0 mg/kg administered for 5 days and required 3–6 days for parasite clearance.

One of these agents, *N*-demethyl-4-pentyl-7-chlorolincomycin (U 24729A), was evaluated in a 7-day course of treatment by SCHMIDT et al. (1970) also against the B strain. Doses of U 24729A at 10.0 and 40.0 mg/kg cured infections, but the slow schizontocidal activity was again noted.

c) Pyrimidines

Trimethoprim, 2,4-diamino-5-(3',-4',5'-trimethoxybenzyl)-pyrimidine, was used by SCHMIDT et al. (1969) against two strains of *P. cynomolgi* – Ro (pyrimethamine susceptible) and Ro/PM (pyrimethamine resistant). Doses of 100.0 mg/kg cured infections of the Ro strain; two of three infections were cured at a dose of 50.0 mg/kg. In contrast, doses of up to 100.0 mg/kg had little or no activity against infections of the pyrimethamine-resistant strain. These data indicate a cross-resistance between the two folic acid antagonists –pyrimethamine and trimethoprim.

In the *P. cynomolgi*–rhesus system, SCHMIDT et al. (1977 b) examined the synergism of pyrimethamine and sulphadiazine. Against a pyrimethamine-sensitive strain (Ro), the curative activity of pyrimethamine was increased 16–32 times by the concomitant administration of sulphadiazine at 1%–2% of the dose which would produce a 50% cure rate when administered alone. The activity of pyrimethamine against a pyrimethamine-resistant strain (Ro/PM) was increased at least 30 times when the compound was given in combination with 1.56 mg/kg of sulphadiazine (a non-curative dose).

d) Dihydroacridinedione

The evaluation of floxacrine (HOE 991) against the B strain of *P. cynomolgi* was published by RAETHER and FINK (1979). Cure was achieved by oral administration at doses of 15.0 and 20.0 mg/kg for 7 days, or intramuscular administration of doses of 1.25–7.5 mg/kg again for 7 days. However, drug resistance was readily induced. The prophylactic/radical curative capacity of this compound is discussed in the appropriate section of this review.

e) 9-Phenanthrenemethanol and Quinoline Methanol

DAVIDSON et al. (1976) have described their use of the *P. cynomolgi* – rhesus monkey model for evaluating antimalarial drugs. Data were presented for standard compounds and two experimental compounds. WR 33063, a phenanthrenemethanol, had a slightly suppressive activity at a dose of 100.0 mg/kg administered for 7 days. A quinolinemethanol, WR 30090, cured trophozoite-induced infections, but at high doses, 100.0 and 316 mg/kg for 7 days.

2. *Plasmodium knowlesi*

The *P. knowlesi* – rhesus system has been used to a lesser extent than the *P. cynomolgi* model for chemotherapy studies.

a) Pyrimidines

Trimethoprim, in addition to its evaluation against *P. cynomolgi* by SCHMIDT et al. (1969), was tested against *P. knowlesi* by ROTHE et al. (1969). The latter group

showed that when trimethoprim was administered alone, doses of 50.0 mg/kg and lower only suppressed parasitaemias. The high sensitivity of *P. knowlesi* to sulphonamides was confirmed in that sulphalene (2-sulphanilamido-3-methoxypyrazine) cured infections in doses ranging from 50.0 to 0.5 mg/kg for 7 days. In combination, trimethoprim at a dose of 25.0 mg/kg with sulphalene at a dose of 0.5 mg/kg was curative, demonstrating a synergistic activity.

b) Quinazolines

Thompson et al. (1969) also used *P. knowlesi* to evaluate the quinazoline, PAM 1392. A dose of 100.0 mg/kg for 5 days cured the infection in two of three rhesus monkeys.

c) Antibiotics

Powers et al. (1976) examined the activity of clindamycin (U 21) and its *N*-demethyl-4-pentyl analogue (U 24) against trophozoite-induced infections of *P. knowlesi* in rhesus monkeys. A single 100.0 mg/kg dose of U 21 cleared parasitaemias, but did not cure the infection. When U 21 or U 24 were administered twice daily for 5 days at a dose of 50.0 mg/kg, infections were cured; a dose of 10.0 mg/kg administered in this regimen was also curative. In contrast, a single daily 10.0 mg/kg dose of chloroquine administered for 3 days cured these infections. While both forms of the antibiotic were curative, parasite clearance was slower than with chloroquine.

II. Human Plasmodia

1. *Plasmodium falciparum* and *P. vivax*

The establishment of human plasmodia in *A. trivirgatus* afforded an unique opportunity to evaluate the activity of experimental drugs directly against the parasites in a laboratory animal. Studies on the establishment in *A. trivirgatus* of diverse strains of *P. falciparum* and *P. vivax*, the courses of untreated infections, and the responses of these strains to chloroquine, pyrimethamine, and quinine have been detailed by Schmidt (1973, 1978 a, b, 1979 d), and drug susceptibilities are summarised in Table 1.

a) Pyrimidines

Schmidt et al. (1977 b) assessed the activity of combined treatment with pyrimethamine and sulphadiazine against two pyrimethamine-resistant *P. falciparum* strains, Malayan Camp-CH/Q and Vietnam Smith. Both strains are maximally resistant to 2.5 mg pyrimethamine/kg per day. Administration of this dose of pyrimethamine with 5.0 mg/kg of sulphadiazine cured five of seven infections of the Malayan Camp strain and one of five infections of the Vietnam Smith strain. In no case did the cure rate approach 100%. The authors argue that this failure is due to extremely high resistance of both strains to pyrimethamine, and project that failures of combined therapy in human cases may be the result of such resistance.

When both pyrimethamine and sulphadiazine were administered against infections of the pyrimethamine-resistant Vietnam Palo Alto strain of *P. vivax*, the ac-

Table 1. Antimalarial susceptibility/resistance of eight strains of *P. falciparum* and two strains of *P. vivax* in *Aotus trivirgatus*. Adapted from SCHMIDT (1978 b)

Strain	Response to		
	Chloroquine	Pyrimethamine	Quinine
P. falciparum			
Cambodian I	S	RIII	S
Malayan IV	RII	RIII	S
Malayan Camp-CH/Q	S(R-I)	RIII	S
Malayan Camp-Sadun	S	RIII	S
Uganda Palo Alto	S	RIII	S
Vietnam Monterrey	RIII	RII	RII
Vietnam Oak Knoll	RIII	S	RIII
Vietnam Smith	RIII	RIII	RIII
P. vivax			
New Guinea Chesson	S	S	RI(S)
Vietnam Palo Alto	S	RII	S

S, cured; RI, parasite clearance, with recrudescence; RII, only suppression of parasitaemia; RIII, no effect on parasitaemia or no more than marginal suppression. (S) and (RI) signify occasional response in group predominantly RI or S

tivity of the former compound was increased 16 times and that of the latter 64 times.

b) Quinazolines

WR 158122 [2,4-diamino-6-(2-naphthyl)-sulphonylquinazoline] and WR 159412 [2,4-diamino-6-(5-trifluoromethylphenyl)-thioquinazoline] were assessed against *P. falciparum* infections by SCHMIDT (1973, 1978 c, 1979 b). Cures of the pyrimethamine-susceptible Vietnam Oak Knoll strain were obtained with a low dose, 0.025 mg/kg for 7 days. Primary infections of the pyrimethamine-resistant Malayan Camp-CH/Q strain were cured also, although the dose required was at least 16 times that required for the Oak Knoll strain. Infections of the multiresistant Vietnam Smith strain were not uniformly cured at doses five to ten times those that consistently cured infections of the Malayan Camp-CH/Q strain. Thus, the activity of WR 158122 and WR 159412 proved to be inversely related to the level of pyrimethamine resistance exhibited by these three strains.

Additionally, subcurative treatment with WR 158122 and WR 159412 of the Malayan Camp and Oak Knoll strains rapidly produced strains highly resistant to these quinazolines. For example, quinazoline-resistant Oak Knoll strains could not be cured with a dose 500 times greater than that which cured the normal strain.

Cross-resistance between WR 158122 and pyrimethamine also occurred in *P. vivax* infections (SCHMIDT 1979 a). The calculated CD_{90} for cures of the pyrimethamine-resistant Vietnam Palo Alto strain was about 15 times greater than that required for the pyrimethamine-sensitive New Guinea Chesson strain. Repeated treatments with WR 158122 of infections of the Vietnam Palo Alto strain resulted in resistance to the compound.

Schmidt (1973, 1979b) then examined the activities of WR 158122 and WR 159412, both dihydrofolic acid reductase inhibitors, when administered in combination with a *p*-aminobenzoic acid inhibitor, sulphadiazine. Such combination therapy did prevent emergence of *P. falciparum* parasites resistant to WR 158122 and WR 159412 and the activities of both compounds were increased, so that small doses of WR 158122 cured infections of the highly pyrimethamine-resistant Vietnam Smith strain and the Vietnam Palo Alto strain of *P. vivax*.

In addition to WR 158122 and WR 159412, five other 2,4-diamino-6-substituted quinazolines were tested by Schmidt (1979a) against infections of the chloroquine-resistant Vietnam Oak Knoll and pyrimethamine-resistant Malayan Camp-CH/Q strains of *P. falciparum*. Overall, these five compounds possessed less curative activity than WR 158122 and WR 159412.

c) 4-Aminoquinolines

A group of seven 4-aminoquinolines was evaluated by Schmidt et al. (1977c) for antimalarial activity against chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum*. The compounds were chloroquine, amodiaquine, amopyroquine, dichloroquinazine (12278 RP), SN 8137, SN 9584, and SN 10274. All of the compounds possessed similar activity against chloroquine-sensitive strains. As would be anticipated, the activities of amodiaquine, amopyroquine, and dichlorquinazine were less against the chloroquine-resistant strains; however, these compounds did cure, at well-tolerated doses, infections by such strains. Since the cross-resistance between certain 4-aminoquinolines and chloroquine-resistant falciparum strains in the *Aotus* model was limited, Schmidt et al. (1977c) cogently argued for the continued, targeted development of 4-aminoquinolines which could prove to be effective against chloroquine-resistant strains.

d) Aminoalcohols

α) *4-Quinolinemethanols*. The antimalarial assessment of some twelve 4-quinolinemethanols against chloroquine-sensitive and chloroquine-resistant *P. falciparum* strains and the Vietnam Palo Alto and New Guinea Chesson strains of *P. vivax* was reported by Schmidt (1973) and Schmidt et al. (1978a). One of these compounds, WR 142490 [α-(2-piperidyl)-2,8-(bis-trifluoromethyl)-4-quinolinemethanol hydrochloride] (subsequently named mefloquine), proved to be the most active of the 4-quinolinemethanols. The CD_{90}s to cure the pyrimethamine-resistant Malayan Camp-CH/Q strain and the chloroquine-resistant Vietnam Oak Knoll strain were identical (in 7-day administration, the CD_{90} was a total of 14.0 mg/kg). To cure infections of the multiresistant Vietnam Smith strain, the CD_{90} was a total of 28.0 mg/kg. Moreover it was shown that the curative activity of mefloquine is a function of the total amount of drug administered, either as a single dose, or the same total amount administered over a period of 3 or 7 days.

Mefloquine was highly active against vivax infections. The CD_{90}s, in a 7-day regimen, against the Palo Alto and Chesson strains of *P. vivax* were total doses of 8.0 and 14.0 mg/kg, respectively.

The activities of two other 4-quinolinemethanols were detailed by SCHMIDT et al. (1978d): WR 184086 [α-(*Tert*-butylaminoethyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol] and WR 226253 [α-(2-piperidyl)-2-trifluoromethyl-6,8-dichloro-4-quinolinemethanol]. The total curative dose of WR 184806 required for infections of the Vietnam Smith strain was two to three times that required for the Vietnam Oak Knoll strain. WR 184804 was about one-third as active as mefloquine against the Vietnam Smith strain. WR 226253 was five to ten times more active than WR 184806 against the Vietnam Smith strain. Moreover, WR 226253 was twice as active as mefloquine against infections with this multiresistant drug strain. The curative activities of both WR 184806 and WR 226253 were shown to be a function of the total dose administered, whether delivered as a single dose, or three or seven daily doses.

The antimalarial activity of the above 4-quinolinemethanols was greater against infections of the Vietnam Palo Alto *P. vivax* strain than against both *P. falciparum* strains.

β) 9-Phenanthrenemethanols. A total of 17 compounds in this chemical class was assessed by SCHMIDT (1978b) and SCHMIDT et al. (1978b) against *P. falciparum* infections. WR 122455 proved to be the most active compound, with a CD_{90} of 25.0 mg base/kg administered for 7 days against chloroquine-resistant and chloroquine-sensitive strains, as well as pyrimethamine-sensitive and pyrimethamine-resistant strains. Moreover, in comparison with chloroquine, WR 122455 was two to four times more active against chloroquine-susceptible falciparum strains. Cures were effected with single, 3-day, or 7-day doses, efficacy being related to the total dose administered.

A second, active compound in this class was WR 171669 (now also called halofantrine). While it was equally active against a chloroquine-resistant (Vietnam Oak Knoll) and a chloroquine-sensitive strain (Malayan Camp-CH/Q), it was less active against these strains when compared with WR 122455.

γ) 4-Pyridinemethanols. SCHMIDT et al. (1978c) and SCHMIDT (1979d) assessed the activities of 2,6-substituted-4-pyridinemethanols against *P. falciparum* strains of diverse drug susceptibility and resistance. Of ten compounds evaluated, three (two were diastereoisomers) were two to three times more active than chloroquine against infections of a chloroquine-sensitive strain; at the same doses they were similarly effective against the multiresistant Vietnam Smith strain.

Further assessment of two compounds, WR 172435 and WR 180409, against the Smith strain of *P. falciparum* and the chloroquine-sensitive Vietnam Palo Alto strain of *P. vivax* indicated the following: (a) with oral administration of the same total dose, 3-day and 7-day treatment schedules were similarly effective and somewhat better than a single-dose administration; (b) the activity of WR 172435 was somewhat greater than that of WR 180409; (c) the phosphate salt of WR 180409 could be administered intravenously and proved to be effective; (d) more rapid control of parasitaemia was obtained with either compound than any standard or new antimalarial compound; and (e) the therapeutic indices of the two drugs were four to eight times that of chloroquine against chloroquine-susceptible strains, with similar indices against drug-resistant strains.

e) Miscellaneous

A dihydro-acridinedione, floxacrine [7-chloro-10-hydroxy-3-(4-trifluoromethylphenyl)-3,4- dihydroacridine-1,9(2H, 10H)-dione], was evaluated by Schmidt (1979c) against two *P. falciparum* strains, Smith and Oak Knoll, and the Palo Alto strain of *P. vivax*. While floxacrine was equally suppressive against the three strains, it was less effective as a curative agent. From 6 to 64 times the dose required for parasite clearance was required for cure. To cure infections of the two falciparum and one vivax strain, more than a tenfold increase in doses was necessary. Floxacrine resistance occurred rapidly while treating the *P. vivax* infections. The prophylactic and radical curative activity of floxacrine against *P. cynomolgi* infections is discussed in a subsequent section.

Activities of two *o*-cresol derivatives, WR 194965 [2-(*t*-butyl-aminomethyl)-4-*t*-butyl-6-(4-chlorophenyl)-phenol] and WR 204165 [3,6-bis-(*t*-butyl)-8-(4-chlorophenyl)-2*H*, 4*H*-1,3-benzo-oxazine] were assessed against the Vietnam Smith strain of *P. falciparum* by Schmidt and Crosby (1978). This pilot evaluation showed that both compounds had similar CD_{90}s, total doses of 27.0 and 35.0, respectively, and that these values were similar to that of mefloquine (WR 142490). Further studies with WR 194965 against the Vietnam Smith strain of *P. falciparum* and Vietnam Palo Alto strain of *P. vivax* indicated that the antimalarial activity was similar whether the drug was administered in a single dose, or divided into three or seven daily doses. Parasitaemias were cleared rapidly. WR 194965 was about twice as active against infections of the Vietnam Palo Alto strain as against the Vietnam Smith strain.

Schmidt et al. (1977b), using the pyrimethamine-resistant Vietnam Palo Alto strain of *P. vivax*, showed that the curative activity of pyrimethamine was increased 16 times and sulphadiazine 64 times when these two compounds were administered in combination. The curative dose of pyrimethamine in the combined regimen proved to be equivalent to the dose in a single drug regimen against the pyrimethamine-sensitive New Guinea Chesson vivax strain.

Using the *P. falciparum* Camp strain, Voller et al. (1969) cured an infection with a single oral administration of sulfadoxine plus pyrimethamine, at doses of 20.0 mg/kg and 2.0 mg/kg, respectively. Intramuscular treatment of an infected monkey with 5.0–30.0 mg/kg doses, only cleared the parasitaemia, while the recrudescence was cured with a similar regimen.

The activity of two chlorinated lincomycin analogues against the chloroquine-resistant Vietnam Oak Knoll strain of *P. falciparum* was examined by Powers and Jacobs (1972). Seven-day oral administration of U 24 (*N*-demethyl-4-pentyl clindamycin hydrochloride) proved to be curative at doses of 10.0 and 50.0 mg/kg. Clindamycin hydrochloride (U 21) was curative at 75.0 mg/kg. Parasite clearance was slow, and they suggested that lincomycin should be combined with chloroquine or quinine.

Glew et al. (1978) reported on the experimental induction, by subcurative therapy, of RIII quinine resistance in the *P. falciparum* Panama II strain. This was the first such occurrence using a non-human primate model. The authors were concerned that, although RIII quinine resistance in the field is unusual, increased quinine resistance may appear in many areas.

C. Use in Tissue Schizontocide Studies

I. Simian Plasmodia

1. *Plasmodium cynomolgi* in *Macaca mulatta* (Rhesus Monkey)

a) Aminobenzene

RC 12 [1,2-dimethoxy-4-(bis-diethhylaminoethyl)-amino-5-bromo-benzene] was the subject of two reports. SCHMIDT et al. (1966) found that RC 12 acted as a prophylactic against *P. cynomolgi* when administered 1 day before sporozoite inoculation, on the day of inoculation, and for 7 days after. Radical cures were achieved when RC 12, in conjunction with chloroquine, was administered daily for 14 days.

In a series of experiments, SODEMAN et al. (1972) confirmed that RC 12 does possess causal prophylactic capability against *P. cynomolgi* in rhesus monkeys. Following a single weekly administration of a 25.0 mg/kg dose for 6 weeks, a group of rhesus was challenged with sporozoites and treatment continued for an additional 3 weeks. No patent infections developed in the treated animals. To test for radical curative activity, patent parasitaemias were cleared by quinine or chloroquine, and then groups of five rhesus each were treated with 25.0 mg/kg of RC 12, for 5, 6 or 7 days. No regimen proved able to eradicate completely the tissue stages, as relapses occurred in animals in each group.

b) Quinoline Ester

RYLEY and PETERS (1970) examined the causal prophylactic activity of a quinoline ester (ICI 56780) in the rhesus model. A total of ten daily doses at 20.0 mg/kg did protect monkeys from infection, no infections developed in primaquine-treated comparison subjects and patent parasitaemias developed in untreated controls.

c) 8-Aminoquinolines

While primaquine is the only drug currently available for the radical cure of *P. vivax* infections, it is sometimes toxic in individual people who have glucose-6-phosphate dehydrogenase (G6PD) deficiency. Primaquine is a racemic mixture. Recently SCHMIDT et al. (1977a) were able to compare the radical curative activity of the *d* and *l* isomers with the racemate against *P. cynomolgi* infections in the rhesus monkey. All three forms were equivalent in their curative capacity when administered for 7 days with chloroquine. The therapeutic index of *d*-primaquine proved to be at least twice that of primaquine. Based upon their observations, the authors suggested that human trials with the *d* isomer should be carried out and projected that a 7-day dosage regimen might not cause haemolytic reactions in patients with G6PD deficiency.

In a report covering the period 1946–1975, SCHMIDT et al. (1977d) dealt with the total dose concept for radical cure by various 8-aminoquinolines. They showed that 7-day and 14-day dosage regimens of pamaquine, pentaquine or isopentaquine (with quinine as a blood schizontocide) were equally effective in radically curing infections with the M strain of *P. cynomolgi*. Primaquine (with chloroquine) was as effective in a 7-day dosage regimen as in a 14-day regimen in curing infections of the B strain of *P. cynomolgi*. Primaquine or 4-methyl primaquine (with

chloroquine) proved to be equally effective in single dose, 3-day and 7-day dosage regimens as curative agents for B strain infections. Thus, duration of treatment with 8-aminoquinolines is not the critical factor in achieving radical cure, the total dose administered being the essential element. This supports the concepts of seeking new radical curative agents, less toxic than primaquine, which will produce radical cure in 1- or 3-day dosage regimens.

d) 4-Quinolinemethanol

The blood schizontocidal properties of mefloquine were discussed in a previous section. Mefloquine does not possess the capacity to act as a prophylactic or radical curative agent against sporozoite-induced *P. cynomolgi* infections, as shown by Schmidt et al. (1978a). However, when mefloquine was used in conjunction with primaquine for radical cure, it proved to be as effective as chloroquine.

e) Dihydroacridinedione

The activity of floxacrine against trophozoite-induced infections of human plasmodia was indicated in a previous section. Evaluation of floxacrine by Schmidt (1979c) for prophylactic activity showed that, when administered after inoculation of *P. cynomolgi* sporozoites, it afforded complete protection in rhesus monkeys. Of particular interest is that floxacrine does not act as a radical curative agent. No previous compound has exhibited such divergent activity against tissue schizonts. As the compound produced a haemorrhagic syndrome in some treated monkeys, its potential usefulness as a prophylactic agent may be limited.

f) 2,4-Diamino-6-Substituted Quinazolines

The evaluation by Schmidt and Rossan (1979) of CI 679, 2,4-diamino-6- [(3,4-dichlorobenzyl)-nitrosoamino]-quinazoline, against pyrimethamine-sensitive (Ro) and pyrimethamine-resistant (Ro/PM) strains of *P. cynomolgi* showed that the compound possesses neither causal prophylactic nor radical curative activity against either strain. There was, however, a dose-related delay in the onset of patency in both strains, as well as extensions of relapse intervals in the radical curative activity component.

g) Antibiotics

Two reports were concerned with the effect of antibiotics on exoerythrocytic stages.

Schmidt et al. (1970) showed that both 7-chlorolincomycin (U 21251F) and *N*-demethyl-4-pentyl-7-chloro-lincomycin (U 24729A) delayed the onset of parasitaemia during causal prophylactic studies of *P. cynomolgi* (B strain) in rhesus monkeys. Similar delays ensued when the compounds were tested against the pyrimethamine-susceptible (Ro) and -resistant (Ro/PM) strains of *P. cynomolgi*. U 24729A effected a longer delay in the onset of patency than did U 21251F and examination of liver sections showed that the former's activity was directly against the developing pre-erythrocytic stages. Radical cures of *P. cynomolgi* infections were not achieved with U 21251F, but U 24729A administered at a dose of 10.0 mg/kg for 7 days cured one of eight infections, and at a dose of 40.0 mg/kg for 7 days cured two of three infections.

On day 3 and 4 following inoculation of *P. vivax* sporozoites into a chimpanzee, GARNHAM et al. (1971) administered oxytetracycline (Terramycin) because of secondary illness in the animal. No exoerythrocytic stages could be demonstrated in sections from a liver biopsy obtained on day 8 and patency was delayed 12 days. This observation prompted a further study, using sporozoites of *P. cynomolgi ceylonensis* in the rhesus monkey. An untreated control had normal exoerythrocytic schizonts in an 8-day liver biopsy, and a patent infection beginning the same day. A second rhesus, treated with oxytetracycline on days 3 and 4 postinoculation, had small and abnormal exoerythrocytic schizonts on days 8 and 11. The prepatent period was 12 days.

h) Cyclopentane

WR 14997 (1-aminocyclopentane-carboxylic acid) was shown by OMAR and COLLINS (1974) to have no prophylactic activity against *P. cynomolgi*.

i) 9-Phenanthrenemethanol

Assessment by SCHMIDT et al. (1978 b) of the tissue schizontocidal activities of WR 122455 showed that administration of the drug during the incubation period following inoculation of *P. cynomolgi* sporozoites had no effect on the developing preerythrocytic forms. Patent parasitaemias in the treated animals occurred on the same day as the untreated controls. While retreatment of the patent infections cleared the parasites, subsequent relapses indicated that WR 122455 has no activity against the secondary tissue schizonts.

2. *Plasmodium fieldi*

COLLINS and CONTACOS (1971) reported the first chemotherapeutic studies with this malaria species in rhesus monkeys. Sporozoite-induced infections of three *P. fieldi* strains were treated with curative blood schizontocidal doses of quinine (300 mg × 5 or 7 days) or chloroquine (50 mg × 3 days or 150 mg × 2 days). Relapses occurred in seven of seven monkeys, multiply treated, during 1 year's observation. In one rhesus, 15 such relapses were observed, following administration of 300 mg quinine for 7 days.

The authors concluded that this model would be suitable for additional chemotherapy studies.

II. Human Plasmodia

A definitive non-human primate model for the testing of causal prophylactic and/or radical curative agents has yet to be established, the issue being more critical for *P. vivax* than for *P. falciparum* as there are no persisting exoerythrocytic forms for the latter species. An ideal system would require the following:

1. Production, in a vertebrate host, of infectious gametocytes during relatively defined periods
2. An efficient vector, in which the salivary glands become heavily infected with sporozoites
3. A consistently reproducible prepatent period in monkey recipients and all sporozoite-inoculated, untreated animals should develop patent parasitaemias

No mosquito–New World monkey system has been found to satisfy all of these criteria, as the data summarised in Table 2 show. When sporozoites of *P. vivax* were inoculated in *A. trivirgatus*, the per cent of animals in which patent infections developed varied from 7 to 75, while the prepatent period ranged from 8 to 48 days. Fewer trials have been reported for *P. falciparum* transmission attempts, but Collins et al. (1977, 1979) were relatively successful in obtaining patent infections in *A. trivirgatus*, 74% and 86%, respectively, of those inoculated. Prepatent periods, however, ranged from 17 to 67 days. None of these models could be considered useful for examining the prophylactic potential of compounds, according to the third desiderata stated above. The *P. vivax* system might serve a limited use in evaluating radical curative efficacy. Such a system was developed for the squirrel monkey (*Saimiri sciureus*) infected with *P. vivax*.

Deane et al. (1966) first reported the susceptibility of *S. sciureus* to *P. vivax* by the inoculation of infected human blood. Young et al. (1971) and Rossan et al. (1972) showed that squirrel monkeys could be infected with blood containing an *Aotus*-adapted *P. vivax* strain and extended this observation by establishing sporozoite-induced infections. The model was then used by Rossan et al. (1975) to evaluate the activity of standard antimalarial compounds. Trophozoite-induced infections were cured by a single 20.0 mg base/kg dose of chloroquine, or by three daily doses of 10.0, 10.0, and 5.0 mg base/kg. The 3-day regimen of chloroquine, plus primaquine given for 14 days at a dose of 1.0 mg base/kg daily cured sporozoite-induced infections.

When sporozoite-induced infections in *S. sciureus* were treated with chloroquine at a single 10.0 mg base/kg dose or a total 25.0 mg base/kg dose administered in 3 days, relapses occurred in three of seven surviving monkeys and also in un-

Table 2. Summary of attempts to obtain sporozoite-induced infections of *P. vivax* and *P. falciparum* in *Aotus trivirgatus*

Monkeys No. positive/ No. inoculated	Prepatent period (days range)	Reference
P. vivax		
3/44	18–26	Baerg et al. (1969)
21/207	8–42	D. C. Baerg, R. N. Rossan, M. D. Young (unpublished data)
12/22	14–48	Collins et al. (1973 a)
2/11	30–32	Collins et al. (1980)
3/4	12–42	Ward et al. (1969)
P. falciparum		
1/1	36	Collins and Contacos (1972)
6/9	19–67	Collins et al. (1973 b)
20/27	17–46	Collins et al. (1977)
6/7	17–19	Collins et al. (1979)
1/2	33	Hayes and Ward (1977)
1/2	18	Ward and Hayes (1972)

treated control animals. Infections relapsed in *A. trivirgatus* inoculated with vivax sporozoites and treated only with chloroquine in three daily doses of 10.0, 10.0, and 5.0 mg base/kg. These results were the first chemotherapeutic evidence for the persistence of exoerythrocytic stages of *P. vivax* in New World monkeys.

D. Conclusion

During the past decade, the development of the *Aotus*–human *Plasmodium* model for the testing of experimental antimalarial drugs proved to be an invaluable asset in the field of chemotherapy. Evaluations of numerous drugs in at least eight chemical classes have been reported, resulting in a wide spectrum of efficacy against the blood stages of *P. falciparum* and *P. vivax*. The most promising new agent is mefloquine, a 4-quinolinemethanol, possessing significant activity against the multidrug-resistant Vietnam Smith strain of *P. falciparum*.

SCHMIDT et al. (1977c) showed that the cross-resistance between chloroquine and amodiaquine in resistant strains of *P. falciparum* was not absolute, thus arguing for the development of other, potentially effective, 4-aminoquinoline analogues.

No single outstanding new drug was evaluated against the trophozoite stages of *P. cynomolgi* in the rhesus monkey model. Of interest, however, is that an antibiotic, lincomycin, proved to have both blood schizontocidal and some causal prophylactic activity. In the latter instance, the activity was manifested by a delay in the onset of patent infections following inoculation of *P. cynomolgi* sporozoites.

The problems associated with primaquine as a radical curative agent may be obviated based upon studies by SCHMIDT et al. (1977a, d). The use of the *d* isomer of primaquine in a 7-day dosage regimen may cure naturally acquired infections of *P. vivax* and not produce a haemolytic crisis in glucose-6-phosphate dehydrogenase-deficient patients, since this isomer is less toxic than the normally used drug.

The search for effective new antimalarial drugs must continue as new geographical areas with drug-resistant *P. falciparum* strains are continually being identified.

Dedication. This chapter is dedicated to Dr. LEON H. SCHMIDT, who pioneered the development of non-human primate models, using simian and human plasmodia, for the assessment of experimental antimalarial drugs. His work has contributed significantly to the advancement in chemotherapy of human malarias.

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