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Activities of Various 4-Aminoquinolines Against Infections with Chloroquine-Resistant Strains of *Plasmodium falciparum*[1]

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The studies reported here stemmed from a personal report by Geiman on the capacity of the 4-aminoquinoline amodiaquin to inhibit in vitro maturation of ring stages of the chloroquine-resistant Monterey strain of *Plasmodium falciparum*. This observation, confirmed in owl monkeys infected with this strain, led to a comparison of the activities of chloroquine, amodiaquin, amopyroquin, and dichlorquinazine (12,278 RP) against infections with various chloroquine-susceptible and chloroquine-resistant strains. The results showed that: (i) these 4-aminoquinolines were essentially equally active against infections with chloroquine-susceptible strains and (ii) the activities of amodiaquin, amopyroquin, and dichlorquinazine were reduced significantly in the face of chloroquine resistance, but (iii) well-tolerated doses of these compounds would cure infections with strains that fully resisted treatment with maximally tolerated doses of chloroquine. Two other 4-aminoquinolines, SN-8137 and SN-9584, which also exhibited activity against chloroquine-resistant parasites in vitro, displayed curative activity in monkeys infected with a chloroquine-resistant strain. These observations show that there is cross-resistance among the 4-aminoquinolines, confirming earlier findings, but indicate that the dimensions of this phenomenon are sufficiently limited so that some derivatives are therapeutically effective against infections refractory to maximally tolerated doses of chloroquine.

In early 1961, Moore and Lanier (25) described the events associated with the first recognized infection with a strain of *Plasmodium falciparum* resistant to the 4-aminoquinoline chloroquine. Shortly thereafter, Young and Moore (49) provided unequivocal documentation of the resistance of this strain to the above drug via studies on blood-induced infections in a group of patients with neurosyphilis. Young (45) went on to demonstrate (i) that established infections with this parasite, of Colombian origin, were also refractory to treatment with recommended therapeutic doses of amodiaquin and hydroxychloroquine (two other 4-aminoquinolines) and (ii) that neither chloroquine nor amodiaquin, delivered in conventional suppressive regimens, would prevent evolution of clinical disease in patients bitten by mosquitoes infected with this plasmodium. Based on these observations, it was suggested that strains of *P. falciparum* resistant to chloroquine would probably be cross-resistant to other agents of the same chemical class. This suggestion has been strengthened by numerous observations on individual patients or small numbers of human volunteers infected with various chloroquine-resistant strains of *P. falciparum*, for the most part of southeast Asian origin (3-5, 8, 14, 19, 24, 29, 30, 31, 35, 46, 47). With few exceptions, infections with such strains were not eradicated and in some subjects were not controlled with respect to parasitemia and fever, when treated with the recommended curative doses of amodiaquin, amopyroquin, or hydroxychloroquine, the only 4-aminoquinolines other than chloroquine currently available for patient use. The concept of cross-resistance received further support from results of studies on mice infected with chloroquine-resistant strains of *P. berghei*, showing that infections with these parasites were in no way susceptible to treatment with maximally tolerated doses of amodiaquin and amopyroquin (17, 27, 39; personal communication, 1970, from the late Paul Thompson, University of Georgia, Athens).

The above observations on cross-resistance led those concerned with and responsible for development of new blood schizonticides to accord a very low priority to either synthesis and experimental and clinical evaluations of new 4-

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aminoquinoline derivatives or expanded studies on agents currently available. The results of substantial experimental studies, set forth in the current report, indicate clearly that these negative positions are not fully supportable and should be revised.

The investigations summarized here had their origins in late September 1969 after a discussion with Quentin Geiman (Department of Preventive Medicine, Stanford University, School of Medicine), who was then studying the cultivation of various strains of *P. falciparum* in vitro and the effects of diverse antimalarial drugs on parasite multiplication. Geiman related results of studies, published in detail 3 years later (38), that indicated that there were distinct differences in the capacities of chloroquine and amodiaquin to inhibit development of the chloroquine-resistant Vietnam Monterey strain. Briefly stated, he had found that chloroquine, added to culture medium at concentrations of 0.3 μg or less per ml, was without effect on maturation of parasites of this strain. In striking contrast, addition of amodiaquin, at a concentration of 0.12 μg/ml, inhibited development completely.

Within a week of the above communication, our laboratories initiated an exploratory study aimed at determining whether the interesting observations of Geiman could be duplicated in vivo in owl monkeys infected with the Monterey strain. Ten subjects, used previously in evaluating a new agent for antimalarial activity, with parasitemias partially suppressed, but still readily measurable on thin films, were assigned to the experiment, one as an untreated control, three to treatment with chloroquine, and six to treatment with amodiaquin. Both drugs were administered once daily, via stomach tube, in doses of 20.0 mg of base equivalent per kg of body weight for 7 consecutive days. As in previous evaluations, administration of chloroquine had no significant effect on parasitemia. In contrast, clearance of parasitemia occurred in all six recipients of amodiaquin by the time dose 7 of this drug had been administered. The absence of recrudescences over a 90-day post-treatment follow-up period indicated that the infections had been cured. This in vivo confirmation of the in vitro observations of Geiman led to the sequence of studies summarized below. Some of the more relevant results were set forth previously (L. H. Schmidt, D. Vaughan, D. Mueller, and R. Hamilton, 1970 Annu. Meet. Am. Soc. Trop. Med. Hyg., San Francisco).

MATERIALS AND METHODS

Monkeys—acquisition, husbandry, and handling. Owl monkeys (*Aotus trivirgatus griseimembra*) of northern Colombian origin, subadults of both sexes ranging from 700 to 1,100 g in weight, were used exclusively. All were purchased through the offices of the Tarpon Zoo, Tarpon Springs, Fla., transported via air directly from Barranquilla, Colombia, to Birmingham, with a brief stopover in Miami for customs and quarantine clearance. The housing and caging of these subjects, dietary regimens, conditioning procedures, capture and handling practices, and methods used in obtaining blood specimens for various purposes, parasite inoculation, and drug treatment have been detailed elsewhere (36). All experimental procedures were carried out on hand-caught monkeys, neither tranquilized, sedated, nor anesthetized.

Prior to assignment to experiments, monkeys were screened for the presence of preexisting malarial or filarial infections via examination of thick blood films stained with Giemsa. Searches for plasmodia were uniformly negative. Monkeys with microfilaremias, sometimes infected with two or more filarial species (9), were uncovered. These animals were excluded from the current therapeutic experiments since systematic studies (results to be published) showed that the evolution of *P. falciparum* infections is either blocked completely or retarded markedly in owl monkeys with preexisting filarial disease.

Strains of plasmodia—origins, characteristics, and susceptibilities to standard antimalarial drugs. The strains of *P. falciparum* used in various experiments included the Uganda Palo Alto, Malayan Camp-CH/Q, Vietnam Monterey, Vietnam Oak Knoll, Vietnam Smith, and Malayan IV. The patient origins of these strains have been described by others (2, 6, 21, 38, 41). We are indebted to Quentin Geiman for providing owl monkeys actively infected with the Uganda Palo Alto, Vietnam Monterey, and Vietnam Oak Knoll strains (1968); to Peter G. Contacos (Laboratory of Parasite Chemotherapy. National Institute of Allergy and Infectious Diseases, Chamblee, Ga.) for a monkey infected with the Malayan IV strain (1968); and to Ronald Ward (Walter Reed Army Institute of Research, Washington, D.C.) for a monkey infected with the Vietnam Smith strain (1971). The procedures involved in establishing these strains in the owl monkey have been described by these investigators (7, 38, 41).

We are also indebted to Robin Powell (then of the Department of Medicine, University of Chicago) for providing blood from a volunteer in the Army Medical Research Project actively infected with the Malayan Camp-CH/Q strain (1968). Inoculation of two owl monkeys with this blood evoked low-level infections. Rapid serial transfers brought the strain to full virulence.

When first introduced to our laboratory, the Palo Alto strain was the only strain that produced fatal infections when passed in small numbers (about 10^2 parasites) to owl monkeys with intact spleens. The other five strains had been maintained in splenectomized monkeys and multiplied erratically in intact subjects. Via rapid serial transfers in monkeys with spleens, the virulence of each of these strains was enhanced to a level where intravenous inocula of 10^2 parasites invariably produced fatal disease.

This level of pathogenicity has been maintained by regular passage of each strain from intact monkey to intact monkey approximately every 2 weeks, utilizing an inoculum of 5×10^4 parasites, intravenously.

The strains referred to above were selected for use in the current study because of their diverse responses to standard antimalarial drugs such as chloroquine, quinine, and pyrimethamine. These responses, determined preliminarily to utilizing the strains in the search for new and more effective antimalarial drugs, have been detailed elsewhere (36). Stated briefly, infections with the Uganda Palo Alto and Malayan Camp-CH/Q strains are susceptible to treatment with well-tolerated doses of chloroquine and quinine and fully resistant to treatment with maximally tolerated doses of pyrimethamine. Infections with the Vietnam Monterey strain are resistant to treatment with chloroquine and quinine and susceptible to treatment with high doses of pyrimethamine. Infections with the Vietnam Oak Knoll strain are resistant to treatment with chloroquine and quinine and fully susceptible to treatment with pyrimethamine. Infections with the Vietnam Smith and Malayan IV strains are resistant to treatment with maximally tolerated doses of each of the above drugs. As indicated previously, the responses of infections in owl monkeys to the above drugs paralleled responses in patients from whom the strains were isolated and/or in human volunteers (36).

Experimental drugs and administration. The structures of the seven compounds used in these studies are shown in Fig. 1. The group included six 7-chloro-4-aminoquinoline derivatives, with diverse substituents at position 4, and a single 6-methoxy-4-aminoquinoline (SN-10274), with a 4-substituent identical to that of amodiaquin. All were available as bulk crystalline preparations. Chloroquine diphosphate was provided by Sterling Winthrop Research Institute (1968), amodiaquin dihydrochloride and amopyroquin hydrochloride by Parke Davis Research Laboratories (1969), and dichlorquinazine base (12,278 RP) by Rhone Poulenc (1965). SN-8137 diphosphate, SN-9584 diphosphate, and SN-10274 dihydrochloride were drawn from a stock of active antimalarial drugs that had been maintained in our laboratories since the end of the World War II Malaria Program (1945). The first two agents were prepared (1944) by the late Nathan L. Drake, University of Maryland. The third compound was synthesized (1944) by J. H. Burckhalter, then of Parke, Davis & Co. Prior to use in the current investigations, these old compounds were checked for purity via countercurrent distribution to determine whether they had deteriorated during protracted storage. Results indicated no change in purity. All agents were water soluble, except dichlorquinazine (our personal designation for "12,278 RP"). At the time of use, the latter compound, a base, was converted to the water-soluble dihydrochloride salt by grinding the requisite quantity with the theoretical volume of 1.0 N hydrochloric acid prior to dilution to the appropriate volume.

Stock solutions of all compounds, 5 mg of base equivalent per ml, were prepared weekly and stored at 4°C. Just prior to delivery to infected monkeys, the volumes of these stock drugs required for treatment were measured into Erlenmeyer flasks and diluted to 10 ml with distilled water. These working solutions, followed by 5 ml of distilled water rinses, were administered via stomach tubes to the designated monkeys, utilizing procedures described elsewhere (36). Except as indicated below, drugs were administered once daily for 7 consecutive days, between 8:00 and 9:00 a.m., 3 to 4 h prior to the regular feeding period (probably on a fasting stomach). In situations where doses were utilized that might provoke acute toxic reactions, the total daily dose was administered in two equal fractions at 8:00 a.m. and 8:00 p.m.

Parasitological procedures. All assignees to therapeutic studies were inoculated intravenously with 5×10^6 parasites. This inoculum was derived from a

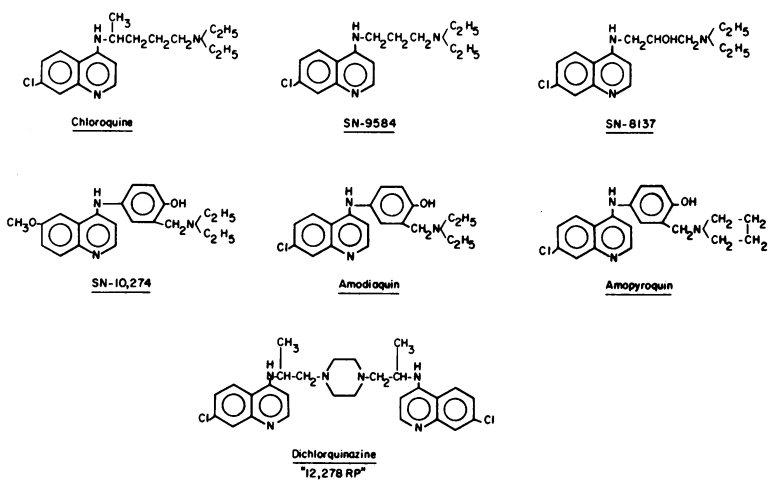

FIG. 1. *Chemical structures of chloroquine and other 4-aminoquinoline derivatives employed in various studies.*

monkey of the appropriate passage series in the midcourse of disease when 8 to 12% of the erythrocytes were parasitized. After precise measurement of parasitemia, via methods described previously (37), 0.3 to 0.5 ml of blood was drawn from the midsaphena of the donor into a 1.0-ml glass tuberculin syringe coated with diluted heparin and transferred to a volume of sterile iced saline such that each milliliter contained 5×10^6 parasites. One-milliliter portions of this diluted blood were injected immediately into the midsaphena of each monkey committed to a given therapeutic study, using a different glass syringe and hypodermic needle for each inoculee. Strict attention was given to maintenance of asepsis during these inoculation procedures. The sterility of the inoculum was always established by culture of 0.2-ml volumes in beef heart infusion broth and liquid thioglycolate medium.

To protect against rare but potentially disastrous transfer of small numbers of β-hemolytic streptococci, *Streptococcus pneumoniae*, *Pasteurella multocida*, and *Haemophilus influenzae* from the blood of donor monkeys to recipients, the latter were routinely injected intramuscularly with procaine penicillin (200,000 U) and streptomycin sulfate (50 mg) once daily for 5 consecutive days, beginning immediately after parasite inoculation. Critical studies have shown that the evolution of parasitemia is not altered by this antimicrobial drug regimen.

Parasitological studies were initiated 48 h after inoculation and repeated daily throughout the pretreatment and treatment intervals. Thick and thin films were prepared with blood obtained from the marginal ear vein and stained with Geimsa, and the numbers of parasites thereon were measured by established procedures (37). Drug treatments were started when parasite densities of 20 to $100/10^4$ erythrocytes were attained. The response to the initial treatment regimen determined the course of subsequent studies.

When treatment had no effect on parasitemia, the monkey was usually transferred out of the current experiment and assigned to evaluation of an agent of a chemical class different from the 4-aminoquinolines. When parasitemia was held near pretreatment levels or persisted at reduced levels, the infection was retreated with either twice the dose of the compound used initially or the same dose of a second 4-aminoquinoline. If such retreatment did not result in parasite clearance, the monkey was reassigned to another ongoing experiment as described above. If retreatment resulted in parasite clearance, parasitological examinations followed the pattern described below.

If blood films became parasite negative during the period of drug delivery or immediately thereafter and remained so for at least 5 consecutive days after treatment had been completed, parasitological examinations were reduced to a schedule of three times weekly (Monday, Wednesday, and Friday or Tuesday, Thursday, and Saturday) for the ensuing 3 weeks. If the infection did not recrudesce in this interval, parasitological examinations were further reduced to a schedule of once weekly and maintained there for 10 to 12 additional weeks. If blood films were consistently negative throughout the total 13- to 15-week post-treatment period, the infection was considered cured and the monkey was transferred to a pool of previously used monkeys reserved for evaluating the activities of new agents against infections with *P. vivax* (36). If the infection recrudesced subsequent to a blood film-negative interval of 7 to 14 days, retreatment was undertaken according to the pattern described above for subjects whose parasitemias had been suppressed by primary treatment. In this situation the schedule of parasitological studies, carried out subsequently to retreatment, was identical with that pursued after primary treatment. In the event of retreatment failure, monkeys were eliminated from the current series of experiments and assigned to pilot evaluations of newly submitted antimalarial agents of diverse chemical classes. This procedure avoided the complications of acquired immunity in the current study, but at the same time made qualified use of the infected subject in our malaria chemotherapy program.

Categorization of therapeutic responses. Impacts of treatment on the course of infection have been categorized in conventional terms. "No effect" implies that evolution of parasitemia in a treated subject differed little, if at all, from the parasitic courses of untreated controls. "Suppressed" implies that parasitemia persisted under treatment, either at approximately the starting level or at a significantly lower level. "Cleared" implies that thick blood films were consistently negative for at least 10 days after completion of treatment. Such a response could be an antecedent to either recrudescence of infection or cure. "Cure" implies the absence of parasitemia for 90 to 105 days after delivery of the last drug dose.

RESULTS

Comparative activities of chloroquine and amodiaquin against infections with the Vietnam Monterey strain of *P. falciparum*. Twenty owl monkeys infected with the chloroquine-resistant Vietnam Monterey strain were used in this comparison. Two of these monkeys served as untreated controls; two subgroups, of three monkeys each, were assigned to treatment with chloroquine in daily doses of 5.0 or 20.0 mg of base equivalent per kg of body weight; four similarly sized subgroups were treated with amodiaquin in daily doses of 2.5, 5.0, 10.0, or 20.0 mg per kg of body weight. The responses of these subjects to the initial treatment course are shown in Fig. 2. Responses of four owl monkeys infected with the chloroquine-susceptible Uganda Palo Alto strain to treatment with this 4-aminoquinoline (experiment of 13 November 1969) have been included in Fig. 2.

A comparison of the data in charts 1 through 3, upper section of Fig. 2, shows that chloroquine, administered in doses of 5.0 mg/kg, had

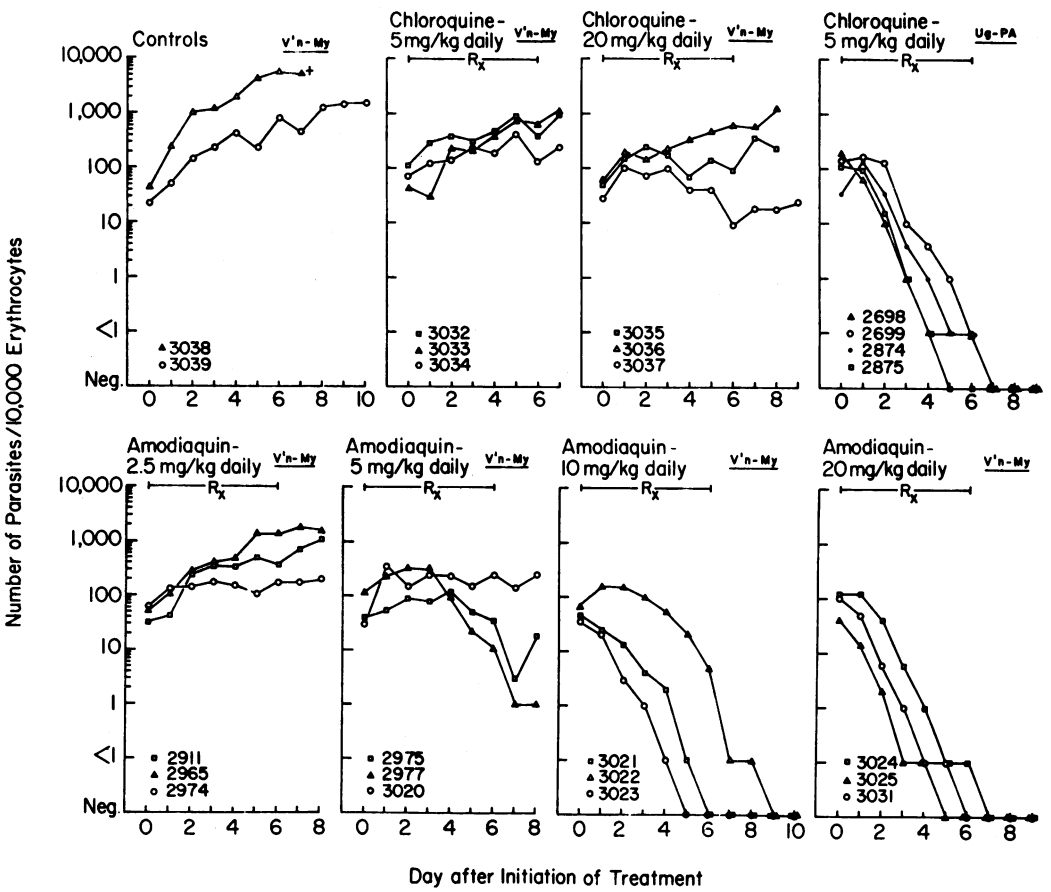

FIG. 2. *Comparative activities of chloroquine and amodiaquin in owl monkeys infected with the chloroquine-resistant Vietnam Monterey (V'n-My) strain of P. falciparum. The response to chloroquine of infections with chloroquine-susceptible Uganda Palo Alto strain (Ug-PA) is set forth for comparison.*

no more than a marginal effect on the course of infection with the Monterey strain and that the accomplishments of chloroquine in doses of 20.0 mg/kg in Atr 3035 and Atr 3036 were no more impressive. The response of Atr 3037, the third recipient of the 20.0-mg/kg dose, was slightly more favorable, parasitemia being maintained at the level that prevailed at the start of treatment.

The responses of infections to amodiaquin in doses of 5.0, 10.0, or 20.0 mg/kg (charts 2 through 4, lower section of Fig. 2) were clearly superior to the responses to 20.0-mg/kg doses of chloroquine. Parasitemias of all three recipients of 5.0-mg/kg doses of amodiaquin were suppressed; parasitemias of the six recipients of 10.0- and 20.0-mg doses were cleared. The infections of Atr 3021 and Atr 3023, both treated with 10.0-mg doses, subsequently recrudesced. The infections of Atr 3022, the third recipient of such doses, and all three recipients of 20.0-mg/kg doses were cured.

Attention should be focused on the responses of infections with the chloroquine-susceptible Uganda Palo Alto strain to treatment with 5.0-mg/kg doses of this 4-aminoquinoline (chart 4, upper section of Fig. 2). Parasitemias of each recipient were cleared promptly; the customary follow-up showed that these infections had been cured. Thus, as would be anticipated from the strain classification, chloroquine has a wholly different level of effectiveness against infections with the Uganda Palo Alto strain than against infections with the Vietnam Monterey strain. It is essential to emphasize, however, that, on a comparable dose basis, the activity of chloroquine against infections with the above chloroquine-susceptible strain is clearly superior to the activity of amodiaquin against infections with the chloroquine-resistant strain. Since amodiaquin and chloroquine have closely comparable activities against infections with susceptible strains, one is forced to conclude that the effectiveness of the former 4-aminoqui-

noline, although not lost entirely, is compromised by preexisting chloroquine resistance. Thus, there is clear evidence of the existence of cross-resistance insofar as amodiaquin and chloroquine are concerned; however, this cross-resistance is not of a sufficient dimension to destroy the utility of amodiaquin.

Comparative activities of chloroquine, amodiaquin, amopyroquin, and dichlorquinazine (12,278 RP) against infections with various strains of *P. falciparum*. The provocative observations summarized above encouraged an expanded study of the activities of amodiaquin against infections with a variety of strains of *P. falciparum* possessing diverse levels of susceptibility or resistance to chloroquine. Amopyroquin and dichlorquinazine (see structures in Fig. 1) were included in this investigation. The inclusion of amopyroquin was suggested by the late Paul Thompson (University of Georgia, Athens), who had found that this compound was as active as the structurally related amodiaquin against infections with blood schizonts of *P. cynomolgi* in rhesus monkeys, but was better tolerated by these animals (40). Dichlorquinazine, a conjugate of 7-chloro-4-(2-diethylamino-1-methylethyl)-aminoquinoline, was included at the suggestion of the late Jean Schneider (Rhone-Poulenc, Vitry, France), who directed attention to both the published (1) and unpublished (personal communication) results of Benazet that showed that this compound had essentially the same activity against infections with chloroquine-susceptible and chloroquine-resistant strains of *P. berghei* in mice.

Side-by-side comparisons of the activities of amodiaquin, amopyroquin, dichlorquinazine, and chloroquine were carried out in varying sized groups of owl monkeys infected with the chloroquine-susceptible Uganda Palo Alto and Malayan Camp-CH/Q strains and the chloroquine-resistant Vietnam Monterey and Vietnam Oak Knoll strains. Due to restrictions on the availability of owl monkeys, experiments with the chloroquine-resistant Malayan IV and Vietnam Smith strains were limited to a comparison of the activities of amopyroquin and chloroquine.

The results of these comparative studies, compartmentalized with respect to the level of therapeutic achievement (capacity to suppress, clear, or cure), have been detailed in Tables 1 through 3. Since the numbers of infections treated with chloroquine in the current study were not large and the range of doses employed was quite limited (2.5 and 5.0 mg/kg against infections with susceptible strains and 10.0 and 20.0 or 20.0 and 40.0 mg/kg against infections with resistant strains), the results of this comparative study have been supplemented with results obtained in unrelated experiments in which the activity of chloroquine was evaluated over a broader range of doses. This reinforcement was deemed acceptable since both groups of studies were pursued with identical techniques in the same general time period.

Tables 1 through 3 show that the capacities of amodiaquin, amopyroquin, or dichlorquinazine to suppress, clear, or cure infections with the chloroquine-susceptible Uganda Palo Alto or Malayan Camp-CH/Q strains were remarkably similar to the corresponding capabilities of chloroquine. There are suggestions that the curative activity of amopyroquin against infections with the Camp strain is slightly superior to that of chloroquine and that the curative activity of dichlorquinazine is slightly inferior. The dimensions of the current experience would have to be expanded substantially to make these suggestions fully supportable.

As the data in Tables 1 through 3 show, the activities of amodiaquin, amopyroquin, and dichlorquinazine against infections with chloroquine-resistant strains were markedly different from the activities of chloroquine. The former three agents were uniformly and significantly superior to chloroquine in the capacity to suppress and clear parasitemia or cure infections with the chloroquine-resistant Vietnam Monterey and Oak Knoll strains. Amopyroquin exhibited a similar level of superiority over chloroquine against infections with the resistant Malayan IV and Vietnam Smith strains.

By utilizing the data in Tables 1 through 3 and the conventional graphic log of the dose-probit analysis procedure, rough calculations were made of the doses of each compound required to suppress, clear, or cure 90% of the infections with each of the chloroquine-susceptible and chloroquine-resistant strains. The results of these calculations (Table 4) show, as would be expected, that doses required for cure are systematically greater than those required for parasite clearance, which in turn are larger than doses required to suppress parasitemia. Since dose-drug-strain relationships are roughly parallel for these various end points, comments will be limited to data on the 90% effective dose (ED_{90}).

Table 4 shows that the ED_{90} values for chloroquine, amodiaquin, amopyroquin, and dichlorquinazine against infections with the chloroquine-susceptible Uganda Palo Alto strain were essentially the same (7.0 to 7.5 mg/kg). The ED_{90} values of the first three agents against infections with the susceptible Malayan Camp-CH/Q were also essentially identical (8.0 to 8.5 mg/kg). Dichlorquinazine ap-

TABLE 1. *Capacities of various 4-aminoquinolines to suppress parasitemia in established infections with diverse chloroquine-susceptible and chloroquine-resistant strains of P. falciparum*

Strain		Response to treatment				
Designation	Response to chloroquine[a]	Daily dose[b] (mg of base/kg of body wt)	No. of infections suppressed[c]/no. treated			
			Chloroquine	Amodiaquin	Amopyroquin	Dichlorquinazine
Uganda Palo Alto	S	1.25	8/22			
		2.5	18/21	3/3	2/2	2/3
		5.0	70/71	4/4	4/4	5/5
		10.0	17/17	6/6	4/4	3/3
Malayan Camp-CH/Q	S	1.25	1/3			
		2.5	3/3	3/3	8/8	0/3
		5.0	15/15	6/6	12/12	4/4
		10.0	12/12	4/4	6/6	5/5
Vietnam Monterey	R	2.5	0/6	4/5		
		5.0	1/15	9/9	2/2	9/9
		10.0	0/4	12/12	9/9	
		20.0	22/35	24/24	12/12	10/10
		40.0	30/30		8/8	
Vietnam Oak Knoll	R	1.25	0/6			
		5.0	2/6	3/3	7/7	5/5
		10.0		3/3	15/15	6/6
		20.0	15/19	9/9	18/18	6/6
Vietnam Smith	R	5.0	0/4			
		20.0	10/11		22/22	
Malayan IV	R	1.25	1/18			
		5.0	12/28		2/2	
		10.0	1/2		5/5	
		20.0	66/66		6/6	

[a] S, Susceptible to chloroquine; R, resistant to chloroquine.

[b] Doses of 1.25 to 20.0 mg/kg were administered once daily between 8:00 and 9:00 a.m. for 7 consecutive days; doses of 40.0 mg/kg were administered in two equal fractions at 8:00 a.m. and 8:00 p.m. over the same time period.

[c] Infections were categorized as suppressed when parasitemias were consistently positive throughout the course of treatment and for 5 days thereafter, but at no time exceeded twice the levels that existed when therapy was started.

peared to be slightly less active than these compounds against infections with the latter strain. In contrast to the above findings, the ED_{90} values of amodiaquin, amopyroquin, or dichlorquinazine against infections with the chloroquine-resistant Vietnam Monterey and Oak Knoll strains and the ED_{90} values of amopyroquin against infections with the Vietnam Smith and Malayan IV strains were significantly less, by a factor of 2 to 5, than the ED_{90} values of chloroquine. However, the doses of amodiaquin and amopyroquin required for cure of 90% of the infections with these resistant strains were two to three times larger than the doses required for a similar result in infections with the chloroquine-susceptible strains. Surprisingly, the data indicated that dichlorquinazine was equally active against infections with the chloroquine-resistant Vietnam Monterey and Oak Knoll strains and the susceptible Malayan Camp strain. This provocative observation merits confirmation before it is accepted without qualification.

These observations are fully supportive of the conclusion reached during study of the activity of amodiaquin against infections with the Vietnam Monterey strain. They show that there is significant cross-resistance among different 4-aminoquinolines, but that cross-resistance is not solid. As a result, infections that are refractory to treatment with maximally tolerated doses of chloroquine may still be cured by larger-than-normal, but well-tolerated, doses of amodiaquin, amopyroquin, and dichlorquinazine.

Comparative activities of selected 4-amino-

TABLE 2. *Capacities of various 4-aminoquinolines to clear parasitemia in established infections with diverse chloroquine-susceptible and chloroquine-resistant strains of P. falciparum*

Strain		Response to treatment				
Designation	Response to chloroquine[a]	Daily dose[b] (mg of base/kg of body wt)	No. of infections cleared[c]/no. treated			
			Chloroquine	Amodiaquin	Amopyroquin	Dichlorquinazine
Uganda Palo Alto	S	1.25	0/22			
		2.5	13/21	3/3	2/2	1/3
		5.0	69/71	4/4	4/4	5/5
		10.0	16/17	6/6	4/4	3/3
Malayan Camp-CH/Q	S	1.25	0/3			
		2.5	2/3	2/3	6/8	0/3
		5.0	15/15	6/6	12/12	3/4
		10.0	12/12	4/4	6/6	5/5
Vietnam Monterey	R	2.5	0/6	0/5		
		5.0	0/15	4/9	0/2	9/9
		10.0	0/4	10/12	8/9	
		20.0	6/35	24/24	12/12	10/10
		40.0	27/30		8/8	
Vietnam Oak Knoll	R	1.25	0/6			
		5.0	0/6	1/3	3/7	4/5
		10.0		3/3	15/15	6/6
		20.0	5/19	9/9	18/18	6/6
Vietnam Smith	R	5.0	0/4			
		20.0	3/11		22/22	
Malayan IV	R	1.25	0/18			
		5.0	6/28		0/2	
		10.0	0/2		5/5	
		20.0	66/66		6/6	

[a] S, Susceptible to chloroquine; R, resistant to chloroquine.

[b] Doses of 1.25 to 20.0 mg/kg were administered once daily between 8:00 and 9:00 a.m. for 7 consecutive days; doses of 40.0 mg/kg were administered in two equal fractions at 8:00 a.m. and 8:00 p.m. over the same time period.

[c] Infections were categorized as cleared when thick blood films became parasite negative during the treatment interval or 3 days thereafter and remained so for a minimum of 10 days after therapy had been completed.

quinolines against infections with the chloroquine-susceptible Malayan Camp-CH/Q and chloroquine-resistant Vietnam Oak Knoll strains of *P. falciparum*. The results of the experiments summarized in the preceding sections were reported in early 1970 to the staff of the Walter Reed Army Institute of Research responsible for direction and management of the Department of the Army Malaria Chemotherapy Program. The question was raised promptly as to whether other 4-aminoquinolines might be as effective or more effective than amodiaquin and amopyroquin against infections with chloroquine-resistant strains. The stock of World War II antimalarials, referred to earlier, containing 42 4-aminoquinolines, made it possible to undertake a pilot test of this question without embarking on a new program of chemical synthesis. To keep evaluations in owl monkeys at a manageable level, it was decided to carry out assessments of the activities of type compounds in vitro, limiting in vivo appraisals to derivatives that exhibited the same or relatively similar in vitro activity against parasites of chloroquine-susceptible and chloroquine-resistant strains. Responsibility for the in vitro assessments was assumed by Karl Rieckmann, Army Medical Research Project, University of Chicago, who had adapted a relatively uncomplicated procedure for identifying infections with chloroquine-resistant plasmodia in the field (34) to measurement of drug activities in the laboratory (33).

Samples of 17 derivatives with distinctive chemical characteristics were drawn from the above stock and together with amodiaquin,

TABLE 3. *Capacities of various 4-aminoquinolines to cure established infections with diverse chloroquine-susceptible and chloroquine-resistant strains of P. falciparum*

Strain		Response to treatment				
Designation	Response to chloroquine[a]	Daily dose[b] (mg of base/kg of body wt)	No. of infections cured[c]/no. treated			
			Chloroquine	Amodiaquin	Amopyroquin	Dichlorquinazine
Uganda Palo Alto	S	1.25	0/22			
		2.5	7/21	0/3	0/2	1/3
		5.0	53/71	2/4	2/4	3/5
		10.0	16/17	6/6	4/4	3/3
Malayan Camp-CH/Q	S	1.25	0/3			
		2.5	0/3	0/3	4/8	0/3
		5.0	9/15	3/6	11/12	0/4
		10.0	11/12	4/4	6/6	4/5
Vietnam Monterey	R	2.5	0/6	0/5		
		5.0	0/15	0/9	0/2	4/9
		10.0	0/4	2/12	4/9	
		20.0	2/35	21/24	8/12	10/10
		40.0	21/30		8/8	
Vietnam Oak Knoll	R	1.25	0/6			
		5.0	0/6	0/3	3/7	2/5
		10.0		2/3	13/15	5/6
		20.0	4/19	8/9	18/18	6/6
Vietnam Smith	R	5.0	0/4			
		20.0	0/11		22/22	
Malayan IV	R	1.25	0/18			
		5.0	1/28		0/2	
		10.0	0/2		0/5	
		20.0	44/66		6/6	

[a] S, Susceptible to chloroquine; R, resistant to chloroquine.

[b] Doses of 1.25 to 20.0 mg/kg were administered once daily between 8:00 and 9:00 a.m. for 7 consecutive days; doses of 40.0 mg/kg were administered in two equal fractions at 8:00 a.m. and 8:00 p.m. over the same time period.

[c] Infections were categorized as cured when parasite clearance was achieved during or immediately after drug treatment and thick blood films were uniformly parasite negative for at least 12 to 13 consecutive weeks after completion of therapy.

amopyroquin, and dichlorquinazine were forwarded as unknowns to Rieckmann. Each of these agents was evaluated for the capacity to inhibit maturation of the ring forms of the Uganda I and Vietnam Marks strains of *P. falciparum*. The former strain differs from the Uganda Palo Alto strain in being susceptible to pyrimethamine as well as to chloroquine and quinine (33). According to studies in human volunteers (6, 33), the Vietnam Marks strain has the same spectrum of drug resistance as the Vietnam Smith strain used previously in our studies.

The first assessments reported by Rieckmann divided the test compounds clearly into two groups: (i) six agents that inhibited maturation of parasites of the Vietnam Marks strain at concentrations of not more than 2.5 times those required for inhibition of the Uganda I strain and (ii) 14 agents that, like the control chloroquine, inhibited the development of parasites of the Marks strain at concentrations of 10 or more times higher than those required for the inhibition of the Uganda I strain. Breaking of the code showed that amodiaquin, amopyroquin, dichlorquinazine, SN-8137, SN-9584, and SN-10274 comprised the first group referred to above (see Fig. 1 for structures of the latter three agents). The initial appraisals were confirmed subsequently for all 20 agents, except SN-8137, which on repeat testing exhibited the same activity differential as chloroquine against the test strains. Table 5 summarizes the observations on the five derivatives with a high order of activity against parasites of the Marks strain, plus observations on SN-8137.

TABLE 4. *Summary: Calculated ED_{90} values of various 4-aminoquinolines required for suppression and clearance of parasitemias or cure of infections with diverse chloroquine-susceptible and chloroquine-resistant strains of P. falciparum*

Strain		Calculated ED_{90}—daily dose (mg of base/kg of body wt)[b]			
Designation	Response to chloroquine[a]	Chloroquine	Amodiaquin	Amopyroquin	Dichlorquinazine
To effect suppression of parasitemia[c]					
Uganda Palo Alto	S	2.8	<2.5	<2.5	3.5
Malayan Camp-CH/Q	S	2.0	<2.5	<2.5	4.5
Vietnam Monterey	R	30.0	3.0	<5.0	<5.0
Vietnam Oak Knoll	R	30.0	<5.0	<5.0	<5.0
Vietnam Smith	R	20.0		<20.0	
Malayan IV	R	13.0		<5.0	
To effect clearance of parasitemia[c]					
Uganda Palo Alto	S	4.0	<2.5	<2.5	4.0
Malayan Camp-CH/Q	S	3.5	3.5	3.5	7.0
Vietnam Monterey	R	40.0	13.0	13.0	<5.0
Vietnam Oak Knoll	R	ca. 50.0	8.0	8.0	7.0
Vietnam Smith	R	ca. 50.0		<20.0	
Malayan IV	R	17.0		9.0	
To cure infection[c]					
Uganda Palo Alto	S	7.5	7.5	7.5	7.0
Malayan Camp-CH/Q	S	8.5	8.0	8.0	11.0
Vietnam Monterey	R	50.0	21.0	25.0	12.0
Vietnam Oak Knoll	R	ca. 60.0	17.0	12.0	12.0
Vietnam Smith	R	>50.0		<20.0	
Malayan IV	R	28.0		15.0	

[a] S, Susceptible to chloroquine; R, resistant to chloroquine.

[b] Dose, administered once daily for 7 consecutive days, required to suppress or clear parasitemias or cure infections in 90% of the treated subjects.

[c] See Tables 1, 2, and 3, respectively, for definitions of suppression, clearance, and cure.

We are deeply indebted to Rieckmann for permission to present this summary of his evaluations.

In the middle of June 1970, shortly after receipt of Rieckmann's initial assessments, our laboratory embarked on a comparison of the activities of SN-8137, SN-9584, SN-10274, chloroquine, amodiaquin, and amopyroquin against infections with the chloroquine-susceptible Malayan Camp-CH/Q strain and the chloroquine-resistant Vietnam Oak Knoll strain. The latter strain was substituted for the Monterey strain, which had been stored in the frozen state in December 1969 in order to conserve the then short supply of owl monkeys. It was believed that this substitution would not prejudice the results of the current study since the Oak Knoll and Monterey strains have essentially the same levels of resistance to chloroquine and quinine and differ only with respect to susceptibility to pyrimethamine.

Thirty owl monkeys were inoculated with the Malayan Camp-CH/Q strain. Three groups of two subjects each served as untreated and chloroquine- or amodiaquin-treated controls. Four groups, each of six monkeys, were assigned to evaluation of the activities of amopyroquin, SN-8137, SN-9584, or SN-10274, with subgroups of two each treated with 2.5, 5.0, or 10.0-mg/kg doses of the respective compounds. One of the assignees to 10.0-mg/kg doses of SN-9584 died of an overwhelming pneumonia the day prior to scheduled treatment. Thirty-two monkeys were inoculated with the Vietnam Oak Knoll strain. One group of two subjects and two groups of three served, respectively, as untreated and chloroquine- or amodiaquin-treated controls. Four groups of six monkeys were allocated to evaluations of the activities of amopyroquin, SN-8137, SN-9584, or SN-10274, with subgroups of three each receiving 5.0 or 20.0-mg/kg doses of the respective compounds.

As indicated by the early responses to doses of 5.0 mg/kg (Fig. 3), the activities of SN-8137 and SN-9584 against infections with the chloroquine-susceptible Malayan Camp-CH/Q strain compared favorably with the activities of chloroquine, amodiaquin, and amopyroquin. However, it would be an error to conclude that these five compounds have equal antimalarial activ-

TABLE 5. *Capacities of chloroquine and related 4-aminoquinolines to block in vitro maturation of blood schizonts of the chloroquine-susceptible Uganda I strain and the chloroquine-resistant Vietnam Marks strain of P. falciparum*[a]

Compound[b]	Concn required for >90% inhibition of maturation (μg of salt/liter of blood)		Ratio of activities (Uganda I vs. Vietnam Marks)
	Uganda I strain	Vietnam Marks strain	
Chloroquine	250	2,500	10
Amodiaquin	50	100	2
Amopyroquin	50	100	2
Dichlorquinazine	250	500	2
SN-8137	100 (100)[c]	1,000 (250)[c]	10 (2.5)[c]
SN-9584	250	500	2
SN-10274	250	500	2

[a] We are indebted to Karl Rieckmann and co-workers of the Army Medical Research Project, The University of Chicago, for making these data available and especially to Rieckmann (Malaria Research Program, University of New Mexico, Albuquerque, N.M.) for permission to present them in this report.

[b] See Fig. 1 for structures of the various compounds.

[c] The result of the initial test, not confirmed.

ity. As Fig. 3 and Tables 1 and 3 show, chloroquine, amodiaquin, and amopyroquin regularly effected suppression of parasitemia at doses of 2.5 mg/kg and cured a high proportion of infections at doses of 5.0 mg/kg. Doses of 5.0 mg of SN-8137 or SN-9584 per kg of body weight were required for suppression of parasitemia (Fig. 3), and doses of 10.0 mg/kg were required for cure of either primary or retreatment infections. These comparative data have led to the conclusion that SN-8137 and SN-9584 have approximately one-half the activity of chloroquine, amodiaquin, or amopyroquin against infections with the chloroquine-susceptible Malayan Camp-CH/Q strain. SN-10274 was clearly less active than SN-8137 or SN-9584. Doses of 10.0 mg/kg were required for suppression of parasitemia, and doses of 20.0 mg/kg were required for cure (Fig. 3).

With respect to activity against infections with the chloroquine-resistant Vietnam Oak Knoll strain, Fig. 4 shows that SN-8137 and SN-9584 occupy a position between that of chloroquine on one extreme and those of amodiaquin and amopyroquin on the other. At daily doses of 20.0 mg/kg of body weight, chloroquine had no significant effect on the evolution of parasitemia; SN-8137 and SN-9584 suppressed parasite development; amodiaquin and amopyroquin effected parasite clearance. Infections in two of three recipients of 20.0-mg/kg doses of amodiaquin and in all three recipients of such doses of amopyroquin were cured.

The above assessments of the relative positions of the five 4-aminoquinolines against infections with the Oak Knoll strain are reinforced by the responses of primary treatment failures to retreatment with the same agent ("+" items in Fig. 4). Retreatment with chloroquine at daily doses of 40.0 mg/kg (20.0 mg/kg twice daily) temporarily reduced the parasitemias of two subjects and cured one infection. Infections of five of six primary treatment failures on SN-8137 and six of six failures on SN-9584 were cured by retreatment with the same 40.0-mg/kg regimen. Retreatment with 10.0-mg/kg doses of amopyroquin effected cure of the three infections that had been suppressed by primary treatment with doses of 5.0 mg/kg.

SN-10274 exhibited less activity than any other agent against infections with the Oak Knoll strain. Doses of 5.0 or 20.0 mg/kg had little effect on parasitemia. Retreatment of each of the six primary treatment failures with doses of 40.0 mg/kg led to no more than a slight suppression of parasitemia.

Although the data are limited, they suffice to show that the decrement in therapeutic activity of either SN-8137 or SN-9584, in the face of chloroquine resistance, is less than that for chloroquine itself, but is substantially greater than the reduction in activity for either amodiaquin or amopyroquin. Although from the practical viewpoint the accomplishments of SN-8137 and SN-9584 were disappointing, they certify to the absence of solid cross-resistance among the 4-aminoquinolines and thereby encourage further study of representatives of this class of drug.

DISCUSSION

The results of the current studies have shown that the 4-aminoquinoline derivatives, amodiaquin, amopyroquin, and dichlorquinazine, are essentially as active as chloroquine against established infections with chloroquine-susceptible strains of *P. falciparum* in owl monkeys, but that each of the aforementioned compounds is less active against infections with chloroquine-resistant strains than against infections with chloroquine-susceptible strains. This finding provides general support for the concept that chloroquine-resistant strains of *P. falciparum* are cross-resistant to other 4-aminoquinolines. More importantly, however, these investigations have shown that infections with such resistant strains, not curable when treated with the maximally tolerated doses of chloroquine, can be cured regularly by treatment with well-tolerated doses of amodiaquin, amopyroquin, and dichlorquinazine. This finding has led to the conclusion that, at least with the strains of *P. falciparum* examined in this study, the dimensions of cross-resistance are limited,

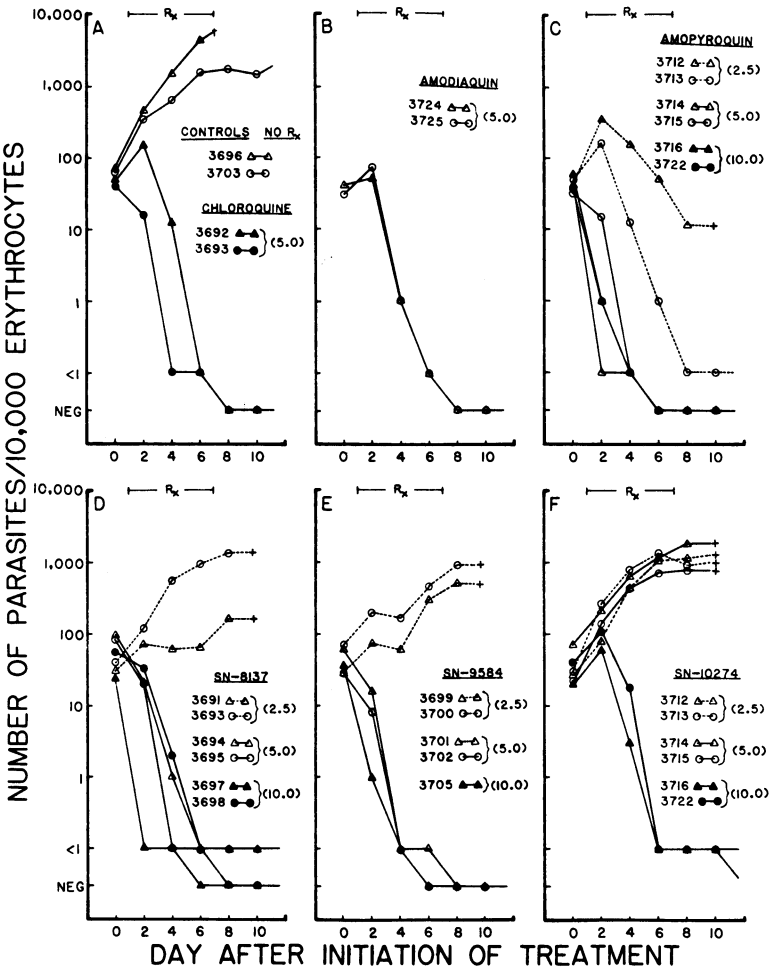

FIG. 3. *Comparative activities of chloroquine, amodiaquin, amopyroquin, SN-8137, SN-9584, and SN-10274 in owl monkeys infected with the chloroquine-susceptible Malayan Camp-CH/Q strain of P. falciparum. +, Point of retreatment with increased dose of same drug; †, death from malaria.*

sufficiently so that some 4-aminoquinolines, including the three derivatives mentioned above, retain substantial levels of therapeutic utility in the face of chloroquine treatment failures. This implies discrete, but nonetheless important, differences in the actions of various 4-aminoquinolines on the malaria parasite.

The foregoing conclusion provokes numerous questions, including the following. (i) Are the demonstrated activities of amodiaquin, amopyroquin, and dichlorquinazine against infections with chloroquine-resistant strains of *P. falciparum* in owl monkeys compatible with published assessments of the activities of these agents against infections with this plasmodium in humans or infections with other plasmodia in experimental animals? (ii) Is there a theoretical basis, or any rational explanation, for the superior activities of amodiaquin, amopyroquin and dichlorquinazine against infections with chloroquine-resistant parasites? (iii) Should attention be directed to both currently available and newly synthesized 4-aminoquinolines in an effort to uncover a derivative or derivatives with greater activity than amodiaquin, amopyroquin, or dichlorquinazine against infections with chloroquine-resistant strains of *P. falciparum*? If so, are there reliable, comparatively simple, and economical approaches to this end? In approaching these questions, it is necessary to rest heavily on experiences with amodiaquin since comparatively few rounded clinical or experimental investigations have been pursued with either amopyroquin or dichlorquinazine.

Before turning to the first question, attention should be directed to the similarity of the doses of amodiaquin (also chloroquine) required to

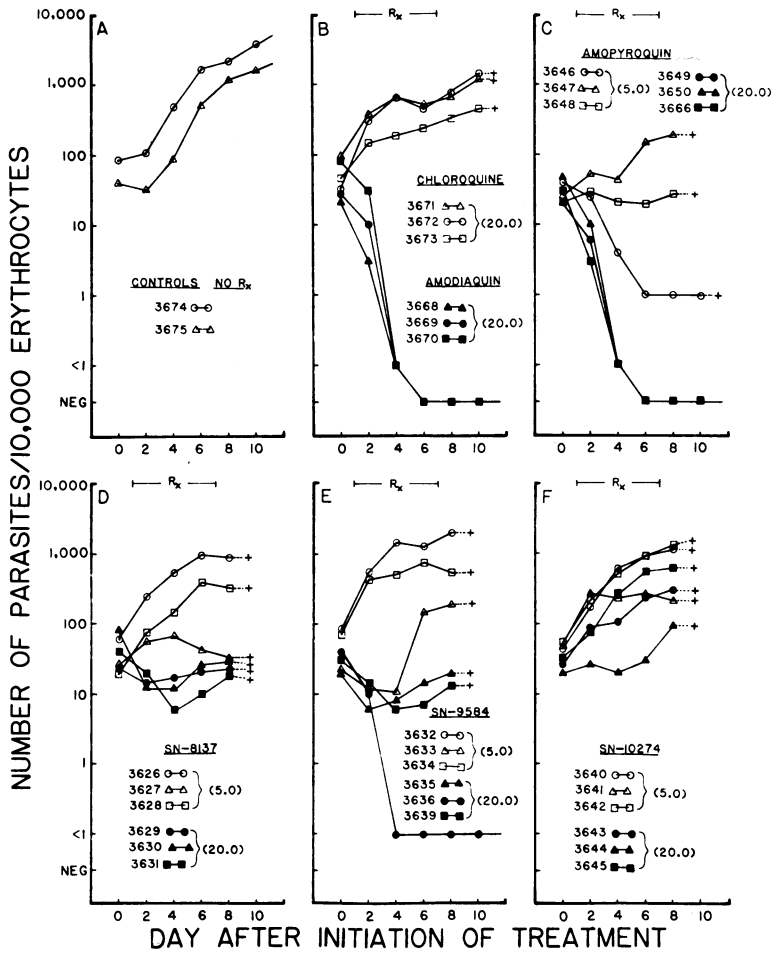

FIG. 4. *Comparative activities of chloroquine, amodiaquin, amopyroquin, SN-8137, SN-9584, and SN-10274 in owl monkeys infected with the chloroquine-resistant Vietnam Oak Knoll strain of P. falciparum. +, Point of retreatment with increased dose of same drug.*

cure infections with chloroquine-susceptible strains of *P. falciparum* in humans and owl monkeys. The total doses for such achievements approximate 20.0 mg/kg of body weight (1.4 g to a 70-kg patient) for humans, and 52.5 mg/kg for owl monkeys (Table 4). These quantities equate (13) to 740 and 630 mg/m^2. The essential identity of the doses required to achieve a comparable end point in these two dissimilar hosts should engender confidence in use of the owl monkey-human malaria model for resolving some of the problems of chloroquine resistance in the human malarias.

As for the first question, we have been unable to identify any serious incompatibilities between published appraisals of the activity of amodiaquin against human infections with chloroquine-resistant strains of *P. falciparum* and assessments in owl monkeys infected with such strains. The great majority of investigators (3-5, 8, 14, 19, 24, 29, 30, 31, 35, 46-48), all but one of whom (48) have worked with very small groups of subjects infected with a variety of chloroquine-resistant strains, have found that such infections cannot be cured by treatment with 1.4-g doses of amodiaquin, the dosage recommended for cure of infections with sensitive strains. This finding is compatible with observations in owl monkeys that show that doses that cure infections with susceptible strains do not cure infections with resistant strains. However, three other groups of investigators (15, 33, 43), who have pursued the issue of cross-resistance in much greater depth, have reported that amodiaquin, although not regularly curative, is significantly more active than chloroquine in volunteers or patients infected with resistant strains. One of these studies (43)

involved work with 30 volunteers of comparable immune status, infected with the highly chloroquine-resistant Vietnam Marks strain. Chloroquine, administered to 10 of these volunteers in total doses ranging from 1.5 to 3.0 g, produced temporary clearance of parasitemia in 3 subjects and suppression of parasite multiplication in 1 and was without effect on the course of disease in 6. In contrast, amodiaquin, administered to 20 volunteers in total doses of 1.5 to 3.6 g, cured 4 infections, produced temporary clearance of parasitemia in 15 subjects, and was without effect in only 1. A second study (15), pursued in Thailand, compared the activities of chloroquine and amodiaquin in 45 patients with naturally acquired infections with chloroquine-resistant strains. Eleven patients were treated with 1.5 g of chloroquine. Temporary clearance of parasitemia was achieved in a single patient; parasitemias persisted in 10 subjects. Thirty-four patients received amodiaquin in doses of 1.5 and 2.0 g. Cure was achieved in 13 subjects, and temporary clearance of parasitemia was achieved in 17. In four subjects, parasitemias persisted. The superiority of amodiaquin in these studies was demonstrable at doses that were relatively less than those found necessary to cure infections with chloroquine-resistant strains in owl monkeys. It would be interesting to know whether the accomplishments of amodiaquin in humans could be improved by administration of doses three- to fourfold greater than those commonly employed, assuming that doses of such magnitude can be delivered without evoking untoward reactions.

The current observations on the activity of amodiaquin, amopyroquin, and dichlorquinazine against infections with chlorquine-resistant strains of *P. falciparum* are not in harmony with published observations (17, 27, 39) on the activities of these 4-aminoquinolines in mice infected with chloroquine-resistant strains of *P. berghei*. The latter are solidly cross-resistant to amodiaquin and amopyroquin (17, 27, 39) and under comparable conditions to dichlorquinazine (42). This dichotomy between the two sets of observations is not surprising, considering that they were based on different plasmodial species, one with a quotidian and the other with a tertian cycle of development, and a variety of other distinguishing features. It is important to emphasize that, apart from their response to the relevant 4-aminoquinolines, chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum* are biologically indistinguishable. They are identical in virulence, exhibit the same morphology, and have the same age of erythrocyte preference and predilection for sequestration in deep vascular loci. Chloroquine resistance is a stable characteristic, persisting unaltered through years of passage through the untreated host (personal observation, manuscript in preparation). On the other hand, the biological features of chloroquine-resistant and chloroquine-susceptible *P. berghei* are quite different. Chloroquine-resistant strains are less virulent than susceptible strains (39), and, unlike the latter strains, produce little pigment (26), multiply selectively in reticulocytes (28), and preferentially localize in deep vascular sites (18). Furthermore, in most strains of *P. berghei*, resistance is an unstable characteristic, with reversal to the susceptible state occurring rapidly upon passage through normal untreated mice (16, 39).

The above broad biological differences between chloroquine-resistant *P. berghei* and *P. falciparum*, although doubtless important, may be less significant than the differences in levels of chloroquine resistance. Resistant strains of *P. berghei* are developed via extended serial transfer of the parasite through mice treated with increasing doses of chloroquine, up to the maximally tolerated level. The resulting strains have a very high level of resistance. The infections that they produce cannot be controlled by treatment with 30 to 200 times the dose of chloroquine required to control infections with the parent susceptible strains (16, 17, 27, 32, 39). Whereas, because of toxicity limitations, the dimensions of chloroquine resistance in *P. falciparum* cannot be fully assessed, our studies in owl monkeys, as well as those of McKelvey and co-workers (20) in patients, suggest that the difference between the susceptibilities of sensitive and resistant strains are at most not more than eightfold and in some strains may be considerably less. Thus, the challenges posed to amodiaquin, amopyroquin, and dichlorquinazine in infections with chloroquine-resistant *P. berghei* are of a different magnitude than those posed by infections with chloroquine-resistant *P. falciparum*. Until studies with the two parasites can be carried out at comparable resistance levels, it would be advisable to postpone concerns with incompatibilities. (Subsequent to preparation of this manuscript, it was learned that a strain of *P. berghei*, designated N-67, is now available which may make such comparison possible. According to a personal communication from F. Benazet, Rhone-Poulenc, Vitry, France, infections with this strain respond to 10 to 20 times the dose of chloroquine required for control of infections with the parent susceptible strain. More importantly, infections with the

N-67 strain respond to treatment with amodiaquin and a relative of dichlorquinazine, designated 12,494-R.P., whereas infections with conventional chloroquine-resistant strains are totally refractory to treatment with these compounds.)

With respect to the second question, there is as yet no adequately supported concept of the mode of action of chloroquine or other 4-aminoquinoline on any plasmodium, although there are numerous provocative hypotheses. Despite this deficit, there are a series of observations that appear to provide a reasonable explanation for the activity of amodiaquin in the face of chloroquine resistance. These observations had their origin in the work of Macomber and coworkers (22), who pursued a complicated, but well-executed, study in groups of mice infected with either chloroquine-susceptible or chloroquine-resistant strains of *P. berghei* and treated with various doses of [^{14}C]quinoline ring-labeled chloroquine at an appropriate time in the infection. At various times after treatment, animals were sacrificed and critical comparisons were made of the concentrations of the label in erythrocytes of noninfected mice and in the parasitized and nonparasitized erythrocytes of mice infected with susceptible and resistant strains. The results showed that the concentrations of the label in erythrocytes (parasitized and nonparasitized) obtained from mice infected with the chloroquine-susceptible strain were 25-fold greater than the concentrations in erythrocytes drawn from noninfected mice. More importantly, under identical dosage and harvesting conditions, the concentrations of the label in erythrocytes derived from mice infected with the chloroquine-resistant strain were only one-tenth of the concentrations in erythrocytes obtained from mice infected with the susceptible strain. Macomber et al. (22) concluded that "The results . . . suggest that chloroquine is selectively toxic because it attains higher concentrations in parasitized cells than in normal tissue cells and that plasmodial resistance to chloroquine is based on an impairment of the mechanism by which such drug levels are accumulated."

Fitch (10) pursued the above suggestion with a simple, but very precise, in vitro procedure based on measuring the uptake of labeled chloroquine by parasitized erythrocytes from medium to which this compound had been added in concentrations well within the range of those found in plasma of patients treated with this drug. He confirmed the essential correctness of Macomber's observations on the difference in chloroquine binding by erythrocytes parasitized with chloroquine-susceptible and chloroquine-resistant strains of *P. berghei* and went on to demonstrate that, although of lesser magnitude, there was also a marked difference in binding of labeled chloroquine by parasitized erythrocytes derived from owl monkeys infected with the chloroquine-susceptible Malayan Camp-CH/Q strain versus those infected with the chloroquine-resistant Vietnam Monterey and Oak Knoll strains (11, 12). On the basis of critical physicochemical measurements, he concluded that the parasites of the chloroquine-resistant strains of *P. falciparum* were deficient in high-affinity binding sites for chloroquine (11). The observations most relevant to the current findings came from a comparison of the uptakes of ^{14}C-labeled chloroquine and ^{14}C-labeled amodiaquin by parasites of the Malayan Camp-CH/Q and Vietnam Oak Knoll strains (12). This comparison showed that although the association constants for binding of the two compounds were identical, the chloroquine-susceptible Malayan Camp-CH/Q parasites bound two to three times as much amodiaquin as chloroquine. More importantly, binding of amodiaquin by parasites of the Malayan Camp-CH/Q and Vietnam Oak Knoll strains was essentially identical, whereas binding of chloroquine by the latter resistant strain amounted to only 30 to 40% of the binding by the susceptible Malayan Camp-CH/Q strain. The resulting differences in localization of chloroquine and amodiaquin in erythrocytes parasitized with susceptible and resistant strains of *P. falciparum* seem to go a long way toward explaining why amodiaquin has activity in the face of chloroquine resistance.

The third and final question posed earlier in this section has two parts, one concerned with the merit of pursuing further investigations with currently available and newly synthesized 4-aminoquinolines and a second pertaining to approaches to such studies, assuming that they are indicated. Before addressing these issues, it should be noted that, on the basis of available data, it is doubtful whether the demonstrated capacities of amodiaquin, amopyroquin, or dichlorquinazine to control infections with chloroquine-resistant strains of *P. falciparum* can be exploited in practice. The best results achieved with amodiaquin (15, 43) were far from optimal. In view of the steep dose-response curves of the relevant 4-aminoquinolines, cures might be attained regularly by doubling the largest dose of amodiaquin employed in the above studies. However, such a dosage increase would probably be pushing the limits of tolerability of amodiaquin, an allowable procedure as

a lifesaving measure in a critically ill patient under close observation in a hospital environment, but not acceptable for unsupervised suppressive use in the field or for treatment of developed disease under minimal supervision. The value of the current observations on amodiaquin and related compounds is, therefore, not at the practical therapeutic level, but rather in identifying the limits of cross-resistance and focusing attention on the possibility of developing a 4-aminoquinoline with usable therapeutic activity against infections with chloroquine-resistant plasmodia.

Having arrived at the above conclusion, there would appear to be substantial merit to evaluating the activities of currently available 4-aminoquinolines and to the synthesis and evaluation of new derivatives. To date, only 20 compounds, from what must be a large pool of derivatives remaining from the World War II Malaria Chemotherapy Program, have been examined. The results of this limited exploration, revealing five agents with significant activity against infections with chloroquine-resistant strains, should encourage the release of compounds of this class now being held in diverse "drug repositories." Studies on available agents will doubtlessly have to be supplemented by studies on newly synthesized compounds. Hopefully, these will be prepared on a rational basis. Although not skilled in chemical theory, it seems to us that the investigations of Marquez et al. (23) provide at least one reasonable theoretical approach to the synthesis of amodiaquin congeners.

As for evaluation of various 4-aminoquinolines, it should be emphasized that for reasons set forth earlier, use of infections with chloroquine-resistant strains of *P. berghei* for this purpose is clearly contraindicated. Ideally, the evaluation should be pursued in owl monkeys infected with a chloroquine-resistant strain of *P. falciparum* well established in the laboratory (e.g., the Smith strain), but this may not be possible because of limited availability of these subjects. Even if they were in unlimited supply, it would probably be wise to initiate studies of new derivatives in vitro, starting with culture procedures such as have been developed by Rieckmann et al. (34) and Siddiqui et al. (38). In these systems, groups of test compounds could be compared for activities against parasites of chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum* derived from infected owl monkeys. Agents that exhibited essentially equal activity against both parasites could then be examined for binding to susceptible and resistant parasites, utilizing the basic techniques evolved by Fitch (10), but measuring the concentrations of the compound by fluorometric rather than radiolabel procedures, since preparation of every labeled compound to be studied would not be practical. (This sequence of evaluations would be helpful at all times, but especially so when the supply of a new agent is limited.) Compounds that bind equally well to susceptible and resistant parasites would then be evaluated in infected owl monkeys. The ultimate evaluations for tolerability and therapeutic and suppressive activities would proceed from this point in conventional order.

In concluding this report, it should be emphasized that interest in developing another 4-aminoquinoline as a possible replacement for chloroquine rests on the remarkable worldwide performance of this drug for a period of close to 30 years in suppressing and treating infections with all three human plasmodia. The premier position of chloroquine rests on the fact that it is very well tolerated in recommended doses, that it controls parasitemia and fever more rapidly than any other drug except the related amodiaquin, that it has a steep dose-response curve (making for uniformly reliable activity), and that it is extensively localized in tissues and slowly degraded, even then to a metabolite with essentially the same antimalarial activity as the parent compound (unpublished observation of L. H. Schmidt). Except in those limited regions where chloroquine-resistant strains of *P. falciparum* have appeared, it is still the agent of choice for suppression and treatment of malaria. The pioneering studies (44) on the 4-aminoquinolines showed that most 7-chloro derivatives were endowed with many of the same pharmacological characteristics as chloroquine. It is our opinion that uncovering a compound with the biological properties of chloroquine, but effective in the face of resistance to this agent, would contribute importantly to malaria chemotherapy.

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