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Enhancement of the Curative Activity of Primaquine by Concomitant Administration of Mirincamycin†

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Mirincamycin, a lincomycin derivative with unequivocal but limited activity against the pre-erythrocytic and persisting exoerythrocytic stages of *Plasmodium cynomolgi*, has been evaluated for capacity to enhance the radical curative potential of the conventional primaquine-chloroquine combination. Established infections with sporozoites of the above plasmodium in rhesus monkeys served this evaluation. The results showed that the dose of primaquine required for cure of 50% of active infections was reduced by one-half to two-thirds by coadministration with 2.5 mg of mirincamycin per kg, 1/16 the 50% curative dose of this lincomycin derivative when used in a mono-drug regimen. The dimensions of the enhancement of the curative activity of primaquine were inversely related to the size of the sporozoite inoculum. The smallest dose of mirincamycin productive of enhancement was 2.5 mg/kg; whether doses larger than 2.5 mg/kg would have been more effective was not determined. There is much to be done before it is known whether a mirincamycin-primaquine combination is useful for suppressive cure or radical cure of the human malarias. Irrespective of that result, the current study serves to focus attention on a somewhat novel approach to the development of more effective and better-tolerated regimens for radical cure, an alternative to the empirical chemical synthesis and screening approach that has dominated searches heretofore.

Primaquine and chloroquine suppressive curative and radical curative regimens were used widely for malaria control purposes during U.S. military operations in Vietnam. The effectiveness of these regimens in preventing overt infections with *Plasmodium vivax* was substantially less than had been anticipated (1–3, 6, 12, 23). This result led to the search for new tissue schizonticides more effective and better tolerated than primaquine, an endeavor supported by synthesis of new agents and their evaluation for curative or prophylactic activity in rhesus monkeys inoculated with sporozoites of *Plasmodium cynomolgi* (15). With respect to uncovering new chemical series with curative activity, this search was not productive (15). It did result in identification of 15 novel 8-aminoquinolines that were two to four times more active than primaquine (16). These compounds are currently awaiting evaluations for tolerability and curative activity in human volunteers.

This report deals with another approach to circumventing the limitations of primaquine uncovered during operations in Vietnam; specifically, enhancing the potential of this 8-aminoquinoline for prophylaxis and radical cure by administering it in combination with well-tolerated doses of a chemically unrelated agent endowed with unequivocal, albeit limited, activity against both early and late tissue stages of the relapsing malarias. Via such enhancement, the doses of primaquine required for both prophylaxis and radical cure might be reduced to levels tolerated by all recipients. The studies recorded here have dealt only with the radical curative aspect of this approach. They have utilized infections with sporozoites of *P. cynomolgi* in rhesus monkeys as the test object and mirincamycin (1′-demethyl-4′-depropyl-4′(*R*)- and -(*S*)-*n*-pentylclindamycin hydrochloride [U-24,729A]) (9–11) as the companion agent. Selection of this lincomycin derivative rested on results of an earlier study which showed that its administration throughout the incubation period at daily doses of 40.0 mg/kg produced striking reductions in numbers of developing pre-erythrocytic parasites in the hepatic parenchyma and provided either full protection against infection or marked delays in onset of parasitemia (21). This study also showed that such doses effected either cure of established infections or substantial prolongation of the relapse interval, indicating that mirincamycin had activity against both secondary and primary tissue stages (21).

This report sums up the results of relatively limited assessments of the capacities of various primaquine-mirincamycin combinations to eradicate established infections with *P. cynomolgi* when these agents were administered in fractions of the doses that in mono-drug regimens were required for cure. The accomplishments of these combinations, even though not all that could be desired in every setting, should encourage broader investigations of the conceptual approach set forth above.

MATERIALS AND METHODS

To simplify evaluations of the several experiments included in this report, design features will be set forth along with the results of the studies that they served. Only procedures common to all experiments will be referred to here.

Monkeys. Sixty-four subadult feral rhesus monkeys (*Macaca mulatta*), 26 females and 38 males, were used in the various studies. Their weights at time of assignment to experiment ranged from 3.5 to 5.1 kg. All were imported directly from India. The procedures used for transporting these subjects from New Delhi, India, to Birmingham, Ala.,

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and conditioning them for experimental studies, the routine colony husbandry practices (including dietary management), and the handling techniques that made the animals available for experimental manipulations at all times without resort to sedatives, tranquilizers, anesthetics, or squeeze cages were identical with those described previously (17).

Parasitological elements. All infections were induced by intravenous inoculation of sporozoites of the *B* strain of *P. cynomolgi*. The origin of this strain, its maintenance via serial monkey-to-mosquito-to-monkey passages, the characteristics of untreated sporozoite-induced infections, and the responses of such infections to standard blood and tissue schizonticidal drugs have been detailed elsewhere (17, 18). Sporozoites utilized for inoculation purposes were derived from ground thoraces of *Anopheles freeborni* with heavily infected salivary glands. The procedures used to acquire lots of well-infected mosquitoes, monitor development of gut and salivary gland infections, harvest sporozoites, and enumerate numbers inoculated were identical with those described previously (20), as were the methods and schedules for monitoring parasitic events in the inoculated monkeys from onset of patent parasitemia until infections were categorized as cured or the inoculees were assigned to unrelated studies (17, 18).

Therapeutic agents and their administration. The compounds utilized in the various experiments included mirincamycin hydrochloride (lots 8997-RJR and 10891-RBJ; Upjohn Research Laboratories, Kalamazoo, Mich.), primaquine diphosphate (Eli Lilly & Co., Indianapolis, Ind.), chloroquine diphosphate (Sterling-Winthrop Research Institute, Rensselaer, N.Y.), and pyrimethamine base (Wellcome Research Laboratories, Tuckahoe, N.Y.). These agents were administered over the following range of daily doses: mirincamycin, 0.156 to 10.0 mg/kg, the latter equivalent to one-fourth the dose that cured 50% (CD_{50}) of established infections with sporozoites of the *B* strain of *P. cynomolgi* (21); primaquine, 0.0625 to 0.5 mg/kg, the latter the approximate CD_{50} for such infections (14); chloroquine, 2.5 or 5.0 mg/kg, doses used as companions to primaquine in curative drug regimens to ensure elimination of blood schizonts (14); and pyrimethamine, 0.0094 to 0.6 mg/kg, the latter approximating the CD_{90} for infections with trophozoites of the *B* strain of *P. cynomolgi* (18). Although all agents were administered as water-soluble salts, pyrimethamine having been converted to the hydrochloride at the time of preparation of the stock solution, the above doses and those noted hereafter refer to compound base.

A once-a-day, 7-consecutive-day treatment regimen was used in all assessments, the daily dose being administered between 8:00 and 9:00 a.m., immediately after preparation of blood films. Monkeys were weighed routinely on the afternoons of the day before the first dose and on days 3 and 5 of the treatment course. Doses for days 1 to 3, 4 and 5, and 6 and 7 were based on the respective weighings. Just before dosage, the volumes of the stock solutions required for treatment of a monkey were pipetted into a 125-ml Erlenmeyer flask and diluted to 30 ml with chilled (4°C) distilled water. This volume was transferred to a 50-ml beaker, drawn into a 30-ml glass syringe, delivered via stomach tube to the designated recipient, and followed by a 30-ml tap water rinse of flask, beaker, syringe, and stomach tube. Stock solutions of mirincamycin were prepared fresh daily by grinding the needed amount of this agent in cold (4°C) distilled water and diluting the resulting solution to a concentration of 2 mg/ml. Stock solutions of primaquine (1 mg/ml), chloroquine (5 mg/ml), and pyrimethamine (1 mg/ml, with solution effected by addition of the molar equivalent of 0.1 N HCl) were prepared fresh weekly and stored at 4°C.

RESULTS

Pilot assessment. The initial appraisal of the radical curative activities of combinations of mirincamycin and primaquine was carried out on 10 monkeys drawn from a pool of 24 monkeys committed to two small-scale studies on the prophylactic activities of a miscellaneous group of compounds. The inocula for these experiments, essentially identical in size, were 1.2×10^6 and 2.0×10^6 sporozoites. The prophylactic and therapeutic measures to which the 10 monkeys were exposed before pilot assessment and the responses to these measures have been summarized in Table 1. It should be noted that prophylactic regimens of mirincamycin in doses of 10.0 mg/kg alone, or lower doses in combination with 0.375 mg of primaquine per kg, were ineffective (see Table 1, data on Mmu 8159, 8188, 8281, and 8193).

In the pilot assessment, combinations of primaquine and mirincamycin were tested for capacity to cure five primary attacks, four first relapses, and one second relapse (Table 1). All 10 of these infections were cured, 8 by dosage with 0.188 mg of primaquine per kg daily, plus doses of 2.5 mg of mirincamycin per kg for 7 and 10.0 mg of mirincamycin per kg for the 8th. The two remaining infections were cured by daily doses of 0.375 mg of primaquine per kg, with mirincamycin at doses of 2.5 mg/kg in one case and 10.0 mg/kg in the second. Comparison of the performance of the primaquine (0.188 mg)-mirincamycin (2.5 mg) combination with that of the conventional primaquine-chloroquine combination against infections with the same strain of *P. cynomolgi* (14) indicated that the curative activity of this 8-aminoquinoline had been enhanced approximately threefold by concomitant administration with 1/16 the CD_{50} of mirincamycin when this lincomycin derivative was utilized in a mono-drug regimen (21).

Primary assessments. The first of these appraisals had a triple purpose: first, to ascertain whether the capacity of mirincamycin to enhance the radical curative activity of primaquine, as exhibited in the pilot study, could be replicated in infections not previously treated during either the incubation period or primary attack; second, to assess the dimensions of this enhancement in terms of effective doses of both agents; and third, to determine whether the enhancement of the curative activity of primaquine by mirincamycin could be duplicated by another agent with limited tissue schizonticidal activity—a compound, for example, like pyrimethamine (5). These purposes were served by an experiment with a group of 24 monkeys, each inoculated with 1.6×10^6 sporozoites. Parasitemias of all monkeys were patent 8 days after inoculation. Three days after the onset of patency, when parasitemias (primary attacks) ranged from 10 to 26 parasites per 10^4 erythrocytes, these monkeys were assigned to diverse drug regimens (Table 2). Two were treated with mirincamycin alone (2.5 or 10.0 mg/kg daily), eight with a combination of mirincamycin (0.156 to 2.5 mg/kg) plus primaquine (0.0625 to 0.5 mg/kg), two with pyrimethamine alone (0.15 or 0.6 mg/kg), eight with a combination of pyrimethamine (0.0094 to 0.15 mg/kg) plus primaquine (0.0625 to 0.5 mg/kg), two with primaquine alone (0.25 or 0.5 mg/kg), and one with chloroquine alone (5.0 mg/kg). The 24th monkey, whose parasitemia persisted throughout the entire study, served as an untreated control. To ensure elimination of blood schizonts, a result not regularly attainable with mirincamycin at doses of 2.5 mg/kg

TABLE 1. Pilot study of the effects of concomitant administration of mirincamycin on radical curative activity of primaquine

Test object		Agents administered			Response to:	
		Special or companion				
Mmu no.	Type of evaluation[a]	Designation	Daily dose (mg/kg)	Primaquine daily dose (mg/kg)	Prophylactic regimen[b]	Radical curative regimen[c]
8159	Prophylactic	Mirincamycin	10.0	0	− (22)	
	Radical cure (P)	Mirincamycin	2.5	0.188		+ (152)
8188	Prophylactic	Mirincamycin	10.0	0	− (28)	
	Radical cure (P)	Mirincamycin	2.5	0.375		+ (93)
8189	Prophylactic			0.375	− (16)	
	Radical cure (P)	Mirincamycin	2.5	0.188		+ (159)
8158	Prophylactic			0.375	− (10)	
	Radical cure (P)	Chloroquine	2.5	0.375		− (35)
	Radical cure (R_1)	Mirincamycin	10.0	0.188		+ (174)
8281	Prophylactic	Mirincamycin	0.156	0.375	− (13)	
	Radical cure (P)	Mirincamycin	2.5	0.188		+ (160)
8193	Prophylactic	Mirincamycin	0.625	0.375	− (42)	
	Radical cure (P)	Mirincamycin	2.5	0.188		+ (128)
8182	Prophylactic	WR-158, 122[d]	10.0	0	− (11)	
	Radical cure (P)	WR-158, 122	10.0	0.375		− (87)
	Radical cure (R_1)	Mirincamycin	2.5	0.188		+ (140)
8232	Prophylactic	Chlorguanide	20.0	0.375	− (22)	
	Radical cure (P)	Chlorguanide	20.0	0.375		− (43)
	Radical cure (R_1)	Mirincamycin	2.5	0.188		+ (141)
8237	Prophylactic	WR-49, 808[e]	5.0	0	− (0)	
	Radical cure (P)	Pyrimethamine	0.6	0		− (22)
	Radical cure (R_1)	Pyrimethamine	0.6	0.375		− (36)
	Radical cure (R_2)	Mirincamycin	2.5	0.188		+ (136)
8222	Prophylactic	Oxytetracycline	80.0	0.375	− (20)	
	Radical cure (P)	Oxytetracycline	80.0	0.375		− (12)
	Radical cure (R_1)	Mirincamycin	10.0	0.375		+ (189)

[a] P, Primary attack; R_1, first relapse; R_2, second relapse. In the prophylactic regimen, test agent(s) was administered once daily, the day before sporozoite challenge, the day of challenge, and for 7 days thereafter. In the radical curative regimen, test agents were administered once daily for seven consecutive days.

[b] −, Failure to provide complete protection; numbers in parentheses are days of delay in onset of patency relative to onset in untreated controls, which occurred on day 8 after sporozoite challenge.

[c] +, No evidence of relapse; −, relapse in observation period of (number of days) after last dose of test agent.

[d] WR-158, 122, 2,4-Diamino-6-(2-naphthyl)-sulfonylquinazoline.

[e] WR-49, 808, 2-Hydroxy-3-(8-cyclohexyloctyl)-1,4-naphthoquinone.

or less (21) or pyrimethamine at doses of 0.15 mg/kg or less (18), all recipients of mirincamycin and pyrimethamine, with or without primaquine, as well as the two recipients of primaquine alone, were treated simultaneously with chloroquine at a dose of 5.0 mg/kg daily. Relapses (those of the original recipient of chloroquine alone excepted) were treated according to the following pattern. The first relapse was always treated with a different drug or drug combination than that administered during the primary attack, whereas the second was treated with the same drug or combination employed in the primary attack. Rotation of these regimens was continued until infections were cured or the purposes of the experiment were served. Successive relapses of the monkey originally treated with 5.0 mg of chloroquine per kg were treated with 2.5-mg/kg doses of this agent, an amount that regularly effects clearance of parasitemia (18).

Of the 87 treatment courses delivered in this experiment, only 14 (16%) effected radical cure (Table 2). Primaquine administered at a daily dose of 0.5 mg/kg in combination with chloroquine accounted for two of these cures, a result compatible with earlier experience (14). The combination of pyrimethamine with primaquine and chloroquine also effected two cures. One of these, which included primaquine at a usually curative dose, 0.5 mg/kg, was to be expected (14). The other, attained with doses of 0.375 mg of primaquine, 0.6 mg of pyrimethamine, and 5.0 mg of chloroquine, although not anticipated, was acceptable, for the same dose of primaquine in combination with 2.5 mg of chloroquine per kg effected cure of 7 of 25 infections in an earlier study (14). The remaining 10 cures were effected by 31 treatment courses with combinations of mirincamycin, primaquine, and chloroquine. All were attained with a mirincamycin dose of 2.5 mg/kg. There was one cure among eight recipients of primaquine at a dose of 0.188 mg/kg, four among five recipients of 0.25 mg of primaquine per kg, and four among six recipients of 0.375 mg of primaquine per kg. The 10th cure, at a dose of 0.5 mg of primaquine per kg, was to be anticipated (14). As judged by length of relapse

TABLE 2. Effects of concomitant administration of mirincamycin versus those of pyrimethamine on radical curative activity of primaquine

Treatment regimen						Responses to treatment regimens			
Daily dose (mg/kg)[a]				Recipients		Relapses		Cures	
Mirincamycin	Pyrimethamine	Chloroquine	Primaquine	No.	Attack[b]	No.	Days after last dose[c]	No.	No. of days of follow-up after last dose[c,d]
2.5	0	5.0	0	2	P, R_1	2	10, 12	0	
10.0	0	5.0	0	2	P, R_1	2	10, 15	0	
0.156	0	5.0	0.25	2	P, R_1	2	7, 97	0	
0.312	0	5.0	0.25	2	P, R_1	2	10, 29	0	
0.625	0	5.0	0.25	2	P, R_1	2	13, 19	0	
1.25	0	5.0	0.25	2	P, R_1	2	38, 25	0	
2.5	0	5.0	0.0625	1	P	1	10	0	
2.5	0	5.0	0.125	2	P, R_1	2	17, 19	0	
2.5	0	5.0	0.188	8	2 R_1, 6 R_2	7	19, 20, 21, 27, 30, 65, 69	1	103_{R_1}
2.5	0	5.0	0.25	5	P, 2 R_1, 2 R_2	1	13_P	4	190, 130, 113, 190
2.5	0	5.0	0.375	6	R_3	2	56, 104	4	111, 113, 130, 140
2.5	0	5.0	0.5	1	P	0		1	124
0	0.15	5.0	0	2	P, R_1	2	6,12	0	
0	0.6	5.0	0	2	P, R_1	2	13, 12	0	
0	0.0094	5.0	0.25	2	P, R_1	2	17, 17	0	
0	0.0188	5.0	0.25	2	P, R_1	2	10, 33	0	
0	0.0375	5.0	0.25	2	P, R_1	2	10, 33	0	
0	0.075	5.0	0.25	1	P	1	13	0	
0	0.15	5.0	0.0625	1	P	1	6	0	
0	0.15	5.0	0.125	2	P, R_1	2	13, 14	0	
0	0.15	5.0	0.25	2	P, R_1	2	17, 68	0	
0	0.15	5.0	0.5	2	P, R_1	1	73	1	106_{R_1}
0	0.6	5.0	0.188	7	2 R_1, 5 R_2	7	20, 24, 26, 30, 31, 36, 40	0	
0	0.6	5.0	0.375	4	R_3	3	44, 47, 81	1	142
0	0	5.0	0.25	2	P, R_3	2	11, 16	0	
0	0	5.0	0.5	2	P, R_1	0		2	103, 106
0	0	5.0, 2.5[e]	0	19[f]	P, R_1–R_{18}	19	10, 8, 7, 6, 6, 7, 7, 6, 7, 6, 8, 14, 12, 10, 10, 12, 14, 12, 13	0	

[a] Agents administered concomitantly, once daily for 7 consecutive days.
[b] P, Primary attack; R_1, first relapse, etc. Numerical prefix refers to number of attacks in a category.
[c] Days listed in order of attack, with primary first, etc.
[d] Inferior suffix refers to attack treated.
[e] Dose was 5.0 mg/kg for primary attack, 2.5 mg/kg for relapses 1 through 18.
[f] Data on this regimen were derived from a single monkey treated during the primary attack and 18 relapses.

intervals (60 days and greater), there were four "near cures" among recipients of regimens containing mirincamycin, primaquine, and chloroquine, one with doses of 0.156 mg of mirincamycin plus 0.25 mg of primaquine (interval, 97 days), two with doses of 2.5 mg of mirincamycin plus 0.188 mg of primaquine (intervals, 65 and 69 days), and one with doses of 2.5 mg of mirincamycin plus 0.375 mg of primaquine (interval, 104 days).

With respect to the missions of the above experiment, these results indicated that pyrimethamine, a compound with limited tissue schizonticidal activity (5, 18), does not share with mirincamycin the capacity of this lincomycin derivative to enhance the curative activity of primaquine against infections with the *B* strain of *P. cynomolgi*, a conclusion compatible with the results of a 1952 study on infections with the *M* strain (19). The results also suggested that daily doses of 2.5 mg of mirincamycin per kg are the smallest that will enhance the activity of primaquine. Lastly, they showed that the enhancement achieved in established infections in which there was no drug intervention during the incubation period and no effective intervention during the primary attack was substantially less than that attained in the pilot study, in which the monkeys had been exposed to a variety of prophylactic and therapeutic measures, some of which were close to being effective. Since exposure to these subeffective measures could have reduced the numbers of persisting tissue stages, the forms responsible for relapse, it seemed reasonable to question to what extent the capacity of mirincamycin to enhance the curative activity of primaquine was related to the dimensions of this tissue stage burden.

The second of the primary appraisals was focused on the above question. It involved an experiment with 30 monkeys, 15 inoculated with 8.5×10^3 sporozoites and 15 with 8.5×10^5 sporozoites, respectively at least 10^3 and 10^5 infective doses (17). All 30 had patent parasitemias 8 days after inoculation. Four days later, the recipients of the larger

TABLE 3. Effects of concomitant administration of mirincamycin on radical curative activity of primaquine as influenced by size of sporozoite inoculum

No. of sporozoites per inoculum	Treatment regimen					Responses to treatment			
	Daily dose (mg/kg)[a]			Recipients		Relapses		Cures	
	Mirincamycin	Chloroquine	Primaquine	No.	Attack[b]	No.	Days after last dose[c]	No.	No. of days of follow-up after last dose[c,d]
8.5×10^3	2.5	2.5	0	1	P	1	30	0	
	10.0	2.5	0	1	R_1	1	36	0	
	2.5	2.5	0.094	2	R_2	2	37, 56	0	
	2.5	2.5	0.188	4	P	0		4	109[e]
	2.5	2.5	0.375	4	P	0		4	109[e]
	0	2.5	0.188	2	P	2	32, 50	0	
	0	2.5	0.375	4	2 P, 2 R_1	3	13, 50, 76	1	102_{R_1}
	0	2.5	0.5	2	R_1	0		2	105, 108
	0	2.5	0	7[f]	P, R_1–R_6	7	10, 10, 15, 22 25, 32, 44	0	
8.5×10^5	2.5	2.5	0	2	P, R_1	2	8, 10	0	
	10.0	2.5	0	1	R_2	1	40	0	
	2.5	2.5	0.188	4	P	4	8, 18, 18, 24	0	
	2.5	2.5	0.375	8	4 P, 4 R_1	4	53, 54, 56, 56	4	104, 107, 115, 115
	10.0	2.5	0.188	4	3 R_1, R_2	3	27, 37, 86	1	106_{R_1}
	0	2.5	0.188	2	P	2	11, 14	0	
	0	2.5	0.375	4	2 P, 2 R_1	3	11, 17, 26	1	106_{R_1}
	0	2.5	0.5	2	R_1	0		2	106, 108
	0	2.5	0	11[g]	P, R_1–R_{10}	11	4, 8, 7, 8, 11, 11, 10, 5, 10 13, 18	0	

[a] Agents administered concomitantly, once daily for 7 consecutive days.
[b] P, Primary attack; R_1, first relapse, etc. Numerical prefix refers to number of attacks in a category.
[c] Days listed in order of attack, with primary first, etc.
[d] Inferior suffix refers to attack treated.
[e] Same number of days follow-up for all monkeys in this group.
[f] Data on this regimen were derived from a single monkey treated during the primary attack and six relapses.
[g] Data on this regimen were derived from a single monkey treated during the primary attack and 10 relapses.

inoculum had parasitemias ranging from 11 to 91 parasites per 10^4 erythrocytes. Six days after onset of patency, the recipients of the smaller inoculum had parasitemias ranging from 12 to 56 parasites per 10^4 erythrocytes. At these target times, 14 of the 15 monkeys in each inoculum group were assigned to dosage regimens as follows: one to 2.5 mg of mirincamycin plus 2.5 mg of chloroquine per kg, four to 2.5 mg of mirincamycin plus 0.188 mg of primaquine plus 2.5 mg of chloroquine per kg, four to 2.5 mg of mirincamycin plus 0.375 mg of primaquine plus 2.5 mg of chloroquine per kg, two to 0.188 mg of primaquine plus 2.5 mg of chloroquine per kg, two to 0.375 mg of primaquine plus 2.5 mg of chloroquine per kg, and one to 2.5 mg of chloroquine per kg. The dose of chloroquine used in these regimens, one-half that used in the preceding study, was wholly adequate for elimination of parasitemia (14). Relapses in recipients of the above regimens were usually retreated with the drug or drug combination utilized in treatment of the primary attack, but at higher doses of primaquine or, occasionally, mirincamycin. The exceptions to this were the recipients of 2.5 mg of mirincamycin plus 0.094 mg of primaquine plus 2.5 mg of chloroquine per kg. One of these monkeys had received 10.0 mg of mirincamycin plus 2.5 mg of chloroquine per kg, the second 0.375 mg of primaquine plus 2.5 mg of chloroquine per kg. Relapses in the recipients of chloroquine alone were treated with this same drug at a dose of 2.5 mg/kg. The 15th monkey in each inoculum group served as an untreated control. The parasitemias of these subjects were patent throughout the 302-day observation period.

Treatment courses delivered to the 14 monkeys inoculated with 8.5×10^3 sporozoites totaled 27, of which 11 effected cures (Table 3). These cures were attained in all four recipients of the regimen of 2.5 mg of mirincamycin plus 0.188 mg of primaquine plus 2.5 mg of chloroquine, all four recipients of the regimen of 2.5 mg of mirincamycin plus 0.375 mg of primaquine plus 2.5 mg of chloroquine, one of four recipients of the regimen of 0.375 mg of primaquine plus 2.5 mg of chloroquine, and both recipients of the regimen of 0.5 mg of primaquine plus 2.5 mg of chloroquine. Near cure, as reflected by a relapse interval in excess of 60 days, was attained in only one subject, a recipient of the regimen of 0.375 mg of primaquine plus 2.5 mg of chloroquine.

Treatment courses delivered to the 14 monkeys inoculated with 8.5×10^5 sporozoites totaled 38, of which 8 effected cures (Table 3). These cures were attained in four of eight recipients of the regimen of 2.5 mg of mirincamycin plus 0.375 mg of primaquine plus 2.5 mg of chloroquine, one of four recipients of the regimen of 10.0 mg of mirincamycin

plus 0.188 mg of primaquine plus 2.5 mg of chloroquine, one of four recipients of the regimen of 0.375 mg of primaquine plus 2.5 mg of chloroquine, and both recipients of the regimen of 0.5 mg of primaquine plus 2.5 mg of chloroquine. Near cure, again judged by the length of the relapse interval, was achieved in a single monkey, a recipient of the regimen of 10.0 mg of mirincamycin plus 0.188 mg of primaquine plus 2.5 mg of chloroquine. It is worth noting that uncommonly long relapse intervals of 53 to 56 days were encountered in four other subjects, all recipients of the regimen of 2.5 mg of mirincamycin plus 0.375 mg of primaquine plus 2.5 mg of chloroquine.

Although the results of this experiment are in basic agreement with the results of earlier studies which indicated that mirincamycin had the capacity to enhance the curative activity of primaquine, they do suggest that the dimension of this enhancement is inversely related to the size of the sporozoite inoculum. An attempt has been made to quantify this relationship in recipients of variable doses of primaquine, 2.5 mg of mirincamycin, and either 2.5 or 5.0 mg of chloroquine per kg by calculating (8) the CD_{50}s of the 8-aminoquinoline for infections induced by 8.5×10^3 sporozoites (Table 3) and the combined infections induced by 8.5×10^5 sporozoites (Table 3) and 1.6×10^6 sporozoites (Table 2). The CD_{50}s for the respective groups, covering 10 and 33 treatment courses, were 0.14 and 0.26 mg of primaquine per kg. These CD_{50}s compared with those of 0.40 and 0.42 mg of primaquine per kg when this agent was delivered with chloroquine only against 8 infections induced with the smaller inoculum and 12 induced with the larger inoculum. This assessment gains credence from the near identity of these CD_{50}s for primaquine administered in combination with chloroquine only with the 0.45-mg/kg value obtained previously in a study on 124 infections with the *B* strain (14).

DISCUSSION

The results of the experiments summarized in this report showed that (i) mirincamycin, administered in a dose of 2.5 mg/kg concomitantly with primaquine, effected a significant reduction in the dose of this 8-aminoquinoline required for cure of established infections with sporozoites of *P. cynomolgi*, and (ii) the dimensions of this reduction were inversely related to the size of the sporozoite inoculum. The aforementioned dose of mirincamycin was the least that enhanced the curative activity of primaquine. Whether the dimensions of enhancement observed in this study can be augmented by doses of mirincamycin larger than 2.5 mg/kg is an important issue that remains to be determined.

As the recorded observations showed, concomitant delivery of mirincamycin in doses of 2.5 mg/kg reduced the dose of primaquine required for cure by one-half in monkeys inoculated with 10^5 infective doses and by two-thirds in subjects inoculated with 10^3 infective doses, respectively the equivalents of large- and moderate-sized sporozoite challenges. Assuming that these are the greatest reductions that can be effected by any dose of mirincamycin and that reductions of these dimensions will carry over to human infections with *P. vivax*, one must inquire whether they are large enough to improve clinical acceptance of primaquine. If concomitant dosage with mirincamycin does not enhance the toxicity of primaquine, that question can probably be answered in the affirmative. With respect to evoking toxic reactions, including hemolysis related to glucose-6-phosphatedehydrogenase (G-6-PD) deficiency, primaquine has a very steep dose-response curve (7). Since the doses employed in the currently recommended weekly suppressive curative and daily radical curative regimens evoke reactions in only 3% of recipients (7), one would anticipate that essentially all would tolerate one-half these doses. Even individuals with unusual sensitivity to hemolytic agents might well tolerate dosage with primaquine at this level.

If judged only by performance in rhesus monkeys, there would be little basis for concern with the toxicity of mirincamycin. In our earlier therapeutic studies (21), doses of 40.0 mg of mirincamycin per kg were administered daily for 7 to 9 days to 25 monkeys, with no evidence of untoward reactions. An evaluation of toxicity carried out in the Upjohn Laboratories showed that course doses of 80.0 and 160.0 mg/kg daily for 28 days were each tolerated by a group of four monkeys (privileged communication, Upjohn Technical Report, 10 July 1972; J. E. Gray, R. N. Weaver, and G. D. Stowman, U-24,729A, 4-pentyl-7-chloro-7 deoxylincomycin hydrochloride. Subacute oral toxicity in the rhesus monkey). Since the dose of mirincamycin that enhanced the curative activity of primaquine was only 1/64 of the 160.0-mg/kg dose, the margin of safety of mirincamycin for rhesus monkeys would be very substantial.

The real issue, however, is the tolerability of mirincamycin in humans. This has to be a matter of substantial concern, for mirincamycin is closely related structurally to clindamycin, application of which has been associated with severe episodes of pseudomembranous colitis and a significant number of fatalities (24). These reactions were evoked by daily doses of 8.0 to 36.0 mg of clindamycin per kg, or 296 to 1,332 mg/m^2. The 2.5-mg/kg dose of mirincamycin that enhanced the curative activity of primaquine in the rhesus monkey translates to a dose of 37.5 mg/m^2, approximately one-eighth the dose of clindamycin that produced pseudomembranous colitis in treated patients. The relevance of this calculation to the present situation depends on whether the toxicity of mirincamycin for humans is no greater than that of clindamycin. If so, the differential in dose just might give mirincamycin the margin of safety required of an agent or regimen that is applied without close medical supervision, as is the case in field use of antimalarial agents. Carefully monitored studies, of substantial size, of the tolerability of combinations of mirincamycin with primaquine in humans will be required before embarking on such field trials.

Whether the capacity of mirincamycin to enhance the curative activity of primaquine is great enough to be useful clinically and whether its special chemical structure presents obstacles, real or hypothetical, to exploiting the benefits of a combination mirincamycin-primaquine regimen, are clearly important issues that merit carefully controlled investigation. However these studies turn out, the observations summarized in this report are of some importance. They serve to focus attention on an approach to circumventing the liabilities of primaquine that has received scant attention heretofore. Specifically, they provide conceptual support for examining agents structurally unrelated to the 8-aminoquinolines and with certifiable, though limited, prophylactic or radical curative properties for capacities to enhance the curative activity of primaquine. There are at least two available agents in addition to mirincamycin that merit such evaluation. They are (i) RC-12 [1,2-dimethoxy-4-(bis-diethylaminoethyl)-amino-5-bromobenzene], which has highly reproducible prophylactic and radical curative activity against infections with *P. cynomolgi* in rhesus monkeys (22; personal observations to be published) and has been evaluated only superficially against infections with *P. vivax* in humans (4), and (ii) floxacrine [7-chloro-10-hydroxy-3-(4-

trifluoromethylphenyl)-3,4-dihydroacridine-1,9-(2*H*,10*H*)-dione], which has a substantial capacity to prevent *P. cynomolgi* infections, although limited capacities for cure (13).

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