procedures are neither complicated nor expensive, it can be used with reasonable economy in field programmes for the control of snail vectors of disease.

The effective dose of endod varies somewhat for the different species of snail tested; in exposures for 6 hours 100% mortality was recorded at 25 ppm in both species of *Biomphalaria* but only half that concentration was needed to kill all *B.* (*P.*) *nasutus*. Responses to the extract by the three species of snail were approximately the same after 24-hour exposures.

These differences in toxicity will result in different field application costs, depending on the species of snail to be controlled. In the laboratory, low concentrations of the extract apparently irritated snails of the species *B.* (*P.*) *nasutus* inducing them to crawl out of the container. Under field conditions, therefore, care will be needed to choose a suitable dosage level since a sublethal dose may induce the snails to avoid contact with the treated water.

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Studies on the prophylactic and radical curative activity of RC-12 against *Plasmodium cynomolgi* in *Macaca mulatta* *

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Abstract

The compound 4-(2-bromo-4,5-dimethoxyphenyl)-1,1,7,7-tetraethyldiethylenetriamine (RC-12) has been shown to be active against the exoerythrocytic (EE) stages of the malaria parasite. Experiments on Plasmodium cynomolgi *in rhesus monkeys showed that single weekly doses of 25 mg per kg of body weight would prevent the development and/or maturation of EE stages. The usefulness of RC-12 for effecting radical cures is, however, still open to doubt.*

The compound 4-(2-bromo-4,5-dimethoxyphenyl)-1,1,7,7-tetraethyldiethylenetriamine (RC-12), developed in the course of studies by Schulemann & Kropp (1930), has been shown to be active against the exoerythrocytic (EE) stages of the malaria parasite. In a report on the antimalarial activity of RC-12, Schmidt et al. (1966) briefly outlined the chemistry of the compound and summarized the results of some studies made with it. They demonstrated that RC-12 was very effective in preventing patent infections of *Plasmodium cynomolgi* in rhesus monkeys *(Macaca mulatta)* when it was administered for a total of 9 days—on the day before the monkeys were challenged with intravenous inoculations of approximately 500 000 sporozoites of *P. cynomolgi*, on the day of challenge, and for a further 7 days after challenge. It was found that 9 of 10 monkeys treated prophylactically with individual doses of 6.25 mg of RC-12 per kg of body weight were protected, and that all the monkeys in a group of 15 treated with individual doses of 25 mg per kg were protected. Schmidt et al. (*op. cit.*) found that RC-12

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had effective tissue schizonticidal activity but only subcurative blood schizonticidal activity.

This report describes experiments on rhesus monkeys infected with *P. cynomolgi* made to investigate the effectiveness of RC-12 as a causal prophylactic drug, particularly when administered on some basis other than in daily doses, and as a radical curative drug.

Material and methods

Initially, 6 monkeys (3 pairs) received oral RC-12 at a dosage rate of 25 mg per kg of body weight for a total of 6 weeks. One pair received the drug once a week, the second pair twice a week, and the third pair three times a week. Each monkey received treatment for 3 weeks; at the end of this time, along with nontreated control animals, they were exposed to interrupted feedings of *Anopheles maculatus* mosquitos infected with *P. cynomolgi*. The first monkey in each pair was exposed on a Monday, a treatment day; the second animal was exposed on a Tuesday, i.e., the day after treatment with RC-12. After exposure to malaria, the monkeys continued to receive RC-12 for 3 weeks. The first pair, therefore, received a total of 6 doses, the second pair 12 doses, and the third pair 18 doses of RC-12. During the treatment period the animals were examined for malaria by means of twice-weekly examinations of thick blood films stained with Giemsa stain. At the end of the 6-week treatment period, the monkeys were observed for a further 8 weeks; they were then splenectomized (Sodeman et al., 1970) and followed up for an additional 3 weeks as a final test of protection. The total observation time for each monkey after exposure to malaria was 14 weeks. The absence of parasites from blood films throughout this period was taken to indicate prevention of infection.

The results of the first study suggested that a further evaluation of RC-12 administered on a once-weekly basis was needed; 7 rhesus monkeys were therefore given 25 mg of RC-12 per kg of body weight once a week, each on a different day of the week. Three weeks after the start of treatment each monkey, along with nontreated monkeys, was exposed to interrupted feedings of *A. maculatus* mosquitos infected with *P. cynomolgi*. After the first challenge, RC-12 treatment was continued for 14–16 weeks. Initially, all animals were exposed on the same day. Thus, one monkey received the 4th dose on the day of exposure to malaria while the remaining 6 monkeys received the 4th dose 1–6 days after exposure. Finally, 71 days after the initial dose of RC-12, the monkeys were rechallenged with mosquitos infected with *P. cynomolgi*. Table 1 shows the pattern of exposure, which varied from 4 to 7 challenges over a period of 33 days, control animals being exposed at the same time. Taking the first monkey (T-602) listed in the table as an example, this animal was treated with RC-12 once a week and was initially exposed to *P. cynomolgi* on the day of treatment. Monkey T-602 was subsequently challenged (exposed to sporozoites) only on days of treatment. Monkey T-613 was exposed 3 times on days when RC-12 was administered, and once 1 day, twice 5 days, and once 6 days after the drug had been administered. This pattern of exposure resulted in monkeys being challenged on all days of the week, irrespective of the day of treatment; in several cases, exposures occurred on consecutive days. Three weeks after the course of treatment was completed each monkey was splenectomized. Blood films were examined twice a week from the day of exposure until 6 weeks after splenectomy.

In the radical curative study, 15 rhesus monkeys along with control animals were exposed to infection by bites of *A. maculatus* or *A. balabacensis* mosquitos infected with *P. cynomolgi*. On the 4th day of patent parasitaemia each animal received either 300 mg of quinine sulfate for 5 days or 50 mg of chloroquine base for 3 days. The monkeys

Table 1. Exposures of rhesus monkeys treated with RC-12 to infection with *P. cynomolgi* (B strain) transmitted by infective *Anopheles* mosquitos

Monkey	Exposures to sporozoites of *P. cynomolgi* [a]						
	1st	2nd	3rd	4th	5th	6th	7th
T-602	0	0	0	0			
T-613	1	5	0	5	0	6	0
T-612	2	0	0	1			
T-611	3	0	0	4	2		
T-610	4	2	3	2	3	3	
T-618	5	1	2	6	3	6	4
T-608	6	5	4	0	6		

[a] No. of days after the weekly administration of RC-12 when the animals were exposed to infection. 0, day of administration.

were divided into 3 groups of 5 animals. Each group was given 5, 6, or 7 consecutive doses of 25 mg of RC-12 per kg of body weight and were then followed up for an average of 9 weeks from the day the RC-12 treatment began. The monkeys were then splenectomized and followed up for 5 more weeks, i.e., for a total observation period of 14 weeks after their first dose of RC-12.

All blood films were prepared and examined by the method of Earle & Perez (1932). Blood films were examined daily for *P. cynomolgi,* beginning 5 days after exposure to sporozoites and continuing until parasites were absent, then twice weekly during the rest of the observation period.

Results

In the first study, 6 rhesus monkeys were treated in pairs with 25 mg of RC-12 per kg of body weight once, twice, and three times a week. None of the animals developed a patent infection, even after splenectomy. At the completion of the study, all 6 monkeys were exposed to blood-induced infections of *P. cynomolgi* and all developed patent infections.

In the second study, 7 rhesus monkeys treated with RC-12 once a week were repeatedly exposed to infective mosquitos. All treated animals were protected but the nontreated control animals developed patent infections. At the end of the study period the monkeys were again exposed to sporozoite-induced infections of *P. cynomolgi* and all developed patent infections.

Table 2 is a summary of the results obtained in the radical curative study. All animals had negative blood films 2–6 days after their initial treatment with quinine or chloroquinine. Altogether, 11 of the 15 monkeys were cured of their infection and had no relapse in the 14 weeks of observation. Patent infections developed in monkeys that received RC-12 for 5 days (monkey T-532), 6 days (T-566 and 576, and 7 days (T-542). The animals treated for 5 and 7 days developed patent infections 40 days after exposure. The monkeys treated for 5 and 6 days that developed infections were splenectomized, treated with 50 mg of chloroquine

Table 2. Radical curative activity of RC-12 against *P. cynomolgi* (B strain) in rhesus monkeys

Monkey	Doses of RC-12 [a]	Cured	No. of days after RC-12 to positive blood film	No. of days after RC-12 to splenectomy	No. of days after RC-12 to final blood film
T-484	5	yes	—	62	91
T-414	5	yes	—	69	99
T-530	5	yes	—	64	111
T-531	5	yes	—	64	111
T-532	5	no	38	39	—
T-486	6	yes	—	55	84
T-416	6	yes	—	62	92
T-565	6	yes	—	58	94
T-566	6	no	40	41	93
T-576	6	no	40	101	130
T-492	7	yes	—	56	85
T-483	7	yes	—	59	101
T-529	7	yes	—	56	85
T-542	7	no	38	—	—
T-569	7	yes	—	57	86

[a] 25 mg of RC-12 per kg of body weight once a day.

for 4 days, and followed up for a further period. The monkey treated for 5 days relapsed 26 days later. Both monkeys treated for 6 days showed no further relapses. All nontreated control animals exposed along with the treated monkeys developed patent infections.

Discussion

The experiments on the prophylactic and radical curative effects of RC-12 suggest a high degree of activity against the EE stages of the malaria parasite. The results of the first study indicated that a single dosage regimen of 25 mg per kg once a week would prevent the development and/or maturation of EE stages. However, the animals were exposed on the day of treatment or 1 day after treatment. To be effective, a single weekly dose of RC-12 would have to prevent the maturation of EE stages in the face of multiple challenges on all days of the week, irrespective of the day of treatment. The second study was established to test such a premise, and the data obtained confirmed that, despite repeated exposures to malaria, a single weekly dose of RC-12 prevented infection. The results of these two studies suggest that when RC-12 is administered once a week sufficient is retained in the body to prevent the development of EE stages. These results appear more favourable than the statement of Schmidt et al. (1966) that " doses of RC-12 spaced seven days apart are not more than 50 % protective even when one of these doses is administered immediately after sporozoite challenge ". The difference may be accounted for, however, by differences in the dosage rates since Schmidt et al. (*op. cit.*) did not define the dosage they employed. If it was lower than the dosage we used, one dose a week may not have prevented infection. Studies made in this laboratory by Held et al. (unpublished data) on the effects of RC-12 on the morphology of EE stages suggested that treatment must be given within 4 days of exposure to prevent the development of patent parasitaemia. In our study, the maximum possible period between exposure to *P. cynomolgi* and administration of RC-12 was 3 days. Further study is necessary to determine if the loading period of 3 weeks used in these studies can be shortened, and to find an effective method to measure plasma RC-12 so that the actual effective level can be determined and the half-life of the drug in circulation studied.

The study of the radical curative activity, although promising, was not uniformly successful; 25 mg of RC-12 per kg failed to destroy all the EE stages in the liver when given to all the monkeys for 5, 6, or 7 consecutive days. Chloroquine was subsequently administered to the animals treated for 5 and 6 days that developed parasitaemia. Both monkeys treated for 6 days failed to show a second relapse during the observation period and following splenectomy, suggesting that RC-12 destroyed all but a few well-developed EE stages. The monkey treated for 5 days relapsed even after chloroquine therapy, indicating that not all the EE stages had been destroyed. In view of these failures, the usefulness of RC-12 for radical cures at the dosage levels used in this study is questionable.

The results of the prophylactic studies are most encouraging in the search for a causal prophylactic drug, particularly since RC-12 affords protection when administered only once a week. Such a causal prophylactic drug would offer a means to tackle the problem of resistant malaria by preventing erythrocytic infection.

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