



a 66 10 2

THE PROPHYLACTIC AND RADICAL CURATIVE ACTIVITY
OF RC-12 AGAINST PLASMODIUM CYNOMOLGI IN MACACA MULATTA

INDEXED

by

Thomas M. Sodeman,¹ Peter G. Contacos, William E. Collins,
Clinton S. Smith and John R. Jumper
Section on Primate Malaria
Laboratory of Parasitic Diseases
National Institute of Allergy and Infectious Diseases
P. O. Box 80190, Chamblee, Georgia 30341
United States of America



1. INTRODUCTION

RC-12 (1, 2-dimethoxy-4-(bis-diethylaminoethyl) -amino-5-bromo-benzene), developed during the work of Schulemann & Kropp (1930) on aminobenzene derivatives bearing N-dialkylaminoalkyl substituents, was demonstrated to have activity against the exoerythrocytic stages (EE body) of malaria parasites. Schmidt and his co-workers (1966) outlined the chemistry and history of studies with this compound in their report on its activity against Plasmodium cynomolgi, a relapsing tertian malaria of monkeys. They demonstrated that RC-12 was highly effective in preventing infections when administered for a total of nine days: viz: the day prior to spirozoite inoculation, the day of inoculation and for seven days thereafter. Of 10 rhesus monkeys (Macaca mulatta) treated with 6.25 mg per kg body weight, nine were protected and of 15 treated with 25 mg per kg body weight, all were protected. They found RC-12 to have effective tissue schizonticidal activity but only subcurative blood schizonticidal activity.

This report, using P. cynomolgi infected rhesus monkeys, deals with studies to gain further insight into the effectiveness of RC-12 as: (1) a causal prophylactic drug, particularly when given on an other than daily basis; and (2) a radical curative drug.

2. MATERIALS AND METHODS

In the first experiment on causal prophylactic action of the drug six monkeys (three pairs) received oral RC-12 at a dose of 25 mg per kg body weight for a total of six weeks. One pair received drugs once weekly; the second pair, twice weekly; the third pair, three times weekly. Each monkey received the drug for three weeks, at the end of which time, along with untreated control animals, they were exposed to Anopheles maculatus mosquitos infected with P. cynomolgi using the interrupted feedings method. One monkey of each pair was exposed on Monday, a day of drug administration, and the other on Tuesday, the day following the administration of RC-12. After challenge through bites of infective mosquitos, they were continued on RC-12 for three weeks. The first pair therefore, received a total of six doses, the second pair, 12 doses and the final pair, 18 doses of RC-12. During this treatment period following exposure to infection from Giemsa stained blood films the animals were examined twice weekly

¹ Present address: Department of Pathology, University of Michigan, Ann Arbor, Michigan 48104, United States of America.

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.

Ce document ne constitue pas une publication. Il ne doit faire l'objet d'aucun compte rendu ou résumé ni d'aucune citation sans l'autorisation de l'Organisation Mondiale de la Santé. Les opinions exprimées dans les articles signés n'engagent que leurs auteurs.

for malaria parasites. After completion of the six-week treatment period, they were observed for a further eight weeks. They were then splenectomized (Sodeman et al., 1970) and followed for an additional three weeks as a final test of protection. The total observation time for each monkey after challenge through infective mosquitos was 14 weeks. The absence of parasites in blood films throughout this period was taken to indicate prevention of infection.

The results of the first study suggested that further evaluation of the prophylactic effect of the drug administered once a week was needed. To do this, seven other rhesus monkeys were given 25 mg per kg of RC-12 once a week each on a different day of the week. Three weeks after beginning therapy each monkey, along with untreated control monkeys, was subjected to interrupted feedings of A. maculatus mosquitos infected with P. cynomolgi. Initially all animals were exposed on the same day. This resulted in one monkey receiving its fourth dose on the day it was exposed to malaria while the remaining six monkeys received their fourth dose from one to six days after exposure. After the first challenge, RC-12 was continued at the same weekly dose for 14 to 16 weeks. Seventy-one days after the initial dose, the monkeys were again challenged with mosquitos infected with P. cynomolgi. As indicated in Table I, following the first two challenges the pattern of exposure varied from two to five further challenges over the next 33-day period with control animals exposed at the same time. Taking the first listed monkey (T-602) in the table as an example, he received drug once a week and was initially exposed on the day he received RC-12. He was exposed to bites of infective mosquito only on the days he received RC-12. Monkey T-613 was exposed three times on days he received RC-12 (the third, fifth and seventh challenges), once one day, twice five days and once six days after receiving RC-12. This exposure pattern resulted in monkeys being challenged on all days of the week irrespective of the day drug was administered. In several cases exposure occurred on consecutive days. Three weeks after discontinuation of drug administration each monkey was splenectomized. Blood films were examined twice weekly from the day of first exposure until six weeks after splenectomy.

In the radical curative study, 15 rhesus monkeys were exposed to infection along with control animals by bites of A. maculatus or A. balabacensis mosquitos infected with P. cynomolgi. On the fourth day of patent parasitaemia, each animal received either five daily doses each of 300 mg quinine sulfate or three daily doses of 50 mg chloroquine base. The monkeys were divided into three groups of five animals. Each group was administered either five, six or seven consecutive daily doses of RC-12 (25 mg per kg body weight). From the first day they received RC-12 they were followed for an average of nine weeks; they were then splenectomized and followed for five more weeks or for a total observation period of 14 weeks after their first dose of RC-12. Blood films were stained with Giemsa and examined for P. cynomolgi daily, beginning five days after exposure to the infective mosquitos until parasites were absent and then twice weekly throughout the remainder of the observation period. All blood films were made and examined using the Earle-Perez method (1932).

3. RESULTS

In the first study on causal prophylactic action, six rhesus monkeys were treated in pairs with 25 mg/kg of RC-12 once, twice and three times a week. None of the animals developed patent infections even after splenectomy. At the completion of the study, all six monkeys were exposed to blood-induced infections of P. cynomolgi and all developed patent infections.

In the second study on prophylactic action, seven rhesus monkeys, receiving RC-12 at 25 mg/kg once a week, were repeatedly exposed to challenges through bites of malaria infected mosquitos. All were protected while the untreated control animals developed patent infections. At the completion of the drug study period, the monkeys were again exposed to sporozoite induced infections of P. cynomolgi. All developed patent infections.

Table 2 gives a summary of the results obtained in the radical curative study. In all animals blood films were negative two to six days after the start of their initial treatment with quinine or chloroquine. Following their regimen of RC-12, 11 of the 15 monkeys were considered cured of their infection as no relapses had occurred during the 14-week observation period. Patent relapse infections developed in one of the five monkeys which had received RC-12 for five days (monkey T-532), in two of the five treated for six days (T-566 and T-576) and in one of the group treated for seven days (T-542). The five and seven day treated animals developed a patent infection 38 days after exposure and both six day treated animals did so 40 days after exposure. The five and six day treated monkeys that developed relapses were splenectomized, treated with 50 mg chloroquine daily for four days, and followed for an additional period. The five day treated monkey relapsed again 26 days later. Both six day treated monkeys showed no further relapses. All untreated control animals exposed to malaria infection along with the treated monkeys developed patent parasitaemia.

4. DISCUSSION

The experiments on the prophylactic and radical curative effects of RC-12 suggest a high degree of activity against the EE forms of *P. cynomolgi*. The results of the first study of the prophylactic effects indicated that a single dose regimen of 25 mg/kg once a week would prevent the development and/or maturation of exoerythrocytic parasites. However, the animals were exposed to infection on the day of treatment or one day after treatment. If a single weekly dose was to be effective, it would have to prevent maturation of the EE body in the face of multiple challenges on all days of the week, irrespective of the day of drug administration. The second study on the prophylactic effects was established to test such a premise. The data obtained confirmed that despite repeated exposures to bites of malaria infective mosquitos, a once a week dose of 25 mg/kg RC-12 prevented infection. The results of these two studies suggest that RC-12 is retained in the body at sufficient levels when administered once a week as to prevent EE body development. These results do not support and appear more favourable than the statement of Schmidt et al. (*loc. cit.*) that "doses of RC-12 spaced seven days apart are not more than 50 per cent. protective even when one of these doses is administered immediately after sporozoite challenge". The authors failed to publish the dose of RC-12 they used. If it was lower than in our study one dose a week may have not¹ prevented infection. Unpublished data of studies conducted in this laboratory by Held et al.¹ on the effects of RC-12 on the morphology of the EE body suggested that the drug must be administered within four days of exposure to prevent patent parasitaemia. In our study the maximum interval between exposure to malaria and administration of drug was three days. Further study is necessary to determine if the loading period of three weeks used in these studies can be shortened and to find an effective method to measure plasma levels of RC-12, so that the actual effective level can be determined and the half life of the drug in circulation studied.

The study on the radical curative activity, though promising was not uniformly successful. Twenty-five mg per kg of RC-12 failed to destroy all the EE bodies in the liver when given for five, six or seven consecutive days in all of the monkeys. Chloroquine was subsequently administered to the five and six day treated animals that redeveloped parasitaemia. Both six day treated animals failed to show a second relapse through the observation period and following splenectomy, suggesting that RC-12 destroyed all but a few well developed EE bodies that resulted in the initial infection. The relapsing of the five day treated animal even after chloroquine therapy indicated that not all EE bodies had been destroyed. In the face of these failures, the usefulness of RC-12 for radical cures at the dosage and schedules used in this study is questionable.

¹ Held, J. R., Contacos, P. G., Sodeman, T. M. & Jumper, J. R. (1967).

The results of the prophylactic studies are most encouraging in the search for a causal prophylactic drug, particularly in that RC-12 affords protection against P. cynomolgi when administered only once a week. The need for such a drug with this type of action against human malaria parasites is immediate with the increasing occurrence of resistant malaria. Such a causal prophylactic drug would represent an approach for handling the problem of resistant malaria by preventing erythrocytic infection.

Summary

RC-12 is a pyrocatechol compound shown to have antimalarial activity against the exoerythrocytic stage of Simian malaria. Two studies were conducted to define the prophylactic activity of 25 mg/kg of RC-12 in rhesus monkeys (Macaca mulatta) exposed to Plasmodium cynomolgi, a relapsing tertian malaria through infective mosquito bites. Results indicated that RC-12 once a week, when begun three weeks prior to first exposure, resulted in protection despite repeated challenges during the period of prophylactic treatment.

A third study conducted on the radical curative activity of RC-12, showed promising results though not uniform success. Of 15 rhesus monkeys infected with P. cynomolgi and treated with chloroquine (three days) or quinine (five days) and subsequently five, six or seven days of RC-12, four relapsed.

REFERENCES

- Earle, W. C. & Perez, M. (1932) Enumeration of parasites in the blood of malaria patients, J. Lab. Clin. Med., 17, 1124
- Schmidt, L. H. et al. (1966) Studies on the antimalarial activity of 1, 2-dimethoxy -4-(bis-diethylaminoethyl)-amino-5-bromobenzene, Bull. Wld Hlth Org., 34, 783-788
- Schulemann, W. & Kropp, W. (1930) Verfahren zur Darstellung von N-dialkylaminoalkylderivaten aromatischer: Aminoxy-und Diaminverbindungen, German Patent No. 499826 (United States Pat. 1 757 394 of 6 May 1930)
- Sodeman, T. M. et al. (1970) A technique for splenectomy of non-human primates, J. trop. Med. Hyg., 37, 33-35

RESUME

Le RC-12 est un dérivé du pyrocatechol qui s'est révélé actif contre les stades exo-érythrocytaires des parasites du paludisme simien. Deux études ont été effectuées pour déterminer l'activité prophylactique d'une dose de 25 mg/kg de RC-12 chez les singes rhésus (Macaca mulatta) exposés à Plasmodium cynomolgi (agent d'une fièvre tierce à rechute) par piqûre de moustiques infectants. Il est apparu que le traitement hebdomadaire par le RC-12, entrepris trois semaines avant la première exposition, protégeait les singes contre des inoculations répétées pendant la période d'administration prophylactique.

Une troisième étude portant sur l'effet curatif radical du RC-12 a fourni des résultats prometteurs, bien que le succès n'ait pas été uniforme. Sur quinze singes rhésus infectés par P. cynomolgi et traités par la chloroquine (trois jours) ou la quinine (cinq jours), puis par le RC-12 pendant cinq, six ou sept jours, quatre ont eu des rechutes.

TABLE 1. THE PATTERN OF EXPOSURE TO INFECTION WITH PLASMODIUM CYNOMOLGI (B STRAIN) BY THE BITES OF INFECTED ANOPHELES DURING THE TREATMENT WITH RC-12

Monkey	Exposures to sporozoites of <u>P. cynomolgi</u>						
	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		

* Days after weekly RC-12 that animal was exposed to malaria (example: 0 = day of drug; 1 = 1 day after drug).

TABLE 2. RADICAL CURATIVE ACTIVITY OF RC-12 AGAINST PLASMODIUM CYNOMOLGI (B STRAIN) IN MACACA MULATTA

Monkey	Number of * doses RC-12	Cured	Number of days after RC-12 to positive film	Number of days after RC-12 to splenectomy	Number of days after RC-12 to last 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

* 25 mg/kg body weight once a day.

The purpose of the WHO/MAL series of documents is threefold:

- (a) to acquaint WHO staff, national institutes and individual research or public health workers with the changing trends of malaria research and the progress of malaria eradication by means of summaries of some relevant problems;
- (b) to distribute to the groups mentioned above those field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;
- (c) to make available to interested readers some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

It should be noted that the summaries of unpublished work often represent preliminary reports of investigations and therefore such findings are subject to possible revision at a later date.

The mention of manufacturing companies or of their proprietary products does not imply that they are recommended or endorsed by the World Health Organization.