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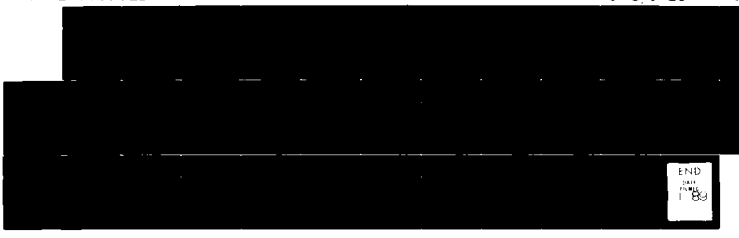

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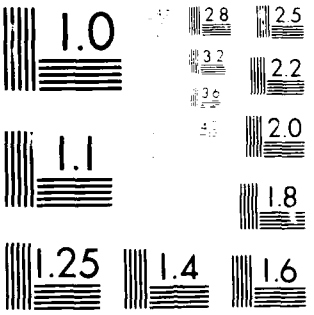
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CHEMOTHERAPY OF RODENT MALARIA

ANNUAL REPORT

PART ONE

WALLACE PETERS MD DSc

SEPTEMBER 1986

Supported by

US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

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Department of Medical Protozoology

London School of Hygiene and Tropical Medicine

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06	15		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Blood schizontocidal action of 2 WRAIR compounds and 3 compounds from other sources is summarised in this report. WR 254594 has slight antimalarial activity and BL 09686 is moderately active. Invermectin shows no evidence of antimalarial activity, whilst doxycycline is shown to be 10 times more active than tetracycline. The quinolone ester, ICI 56780, is highly active when administered subcutaneously but far less so when given orally.

Causal prophylactic activity data are included for 4 WRAIR compounds, ICI 56780 and 5 primaquine metabolites. The most active WRAIR compound is WR 254419. The ICI compound and 4 of the 5 primaquine metabolites also showed tissue schizontocidal activity.

3 WRAIR compounds and ICI 56780 were tested for gametocytocidal activity, none were as active as primaquine.

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19. Continued

Halofantrine and 5 other WRAIR compounds were tested for activity against the sporogonic stages of P.y.nigeriensis, the most active of these was BL 09686. 7 primaquine derivatives tested in this system showed little or no activity.
A method for in vitro testing of drugs against the exoerythrocytic stages of P.yoelii is described and test data showing the direct action of primaquine and ICI 56780 on tissue schizonts are given.
Details of the development of 2 amodiaquine-resistant strains of rodent malaria and cross-resistance studies against a range of drug-resistant strains to 16 different known antimalarials are included.

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

TABLE OF CONTENTS

1. INTRODUCTION

During the first year of Contract DAMD-17-85-C-5172 , two new compounds have been submitted by WRAIR for examination. In addition to investigations on these , studies have continued on a number of compounds submitted previously together with a few miscellaneous drugs of interest in this programme. These investigations have involved testing for activity against blood, tissue and mosquito stages of Plasmodium berghei and P.yoelii.

Concurrently with these investigations we have also been examining cross-resistance patterns of a wide range of resistant lines . Two new amodiaquine-resistant lines have been developed and are now available for inclusion in our blood schizontocidal activity test.

Preliminary studies on the isoenzyme characteristics of some our strains have been carried out and this screening is to be extended to all of the strains of rodent malaria held in our cryobank.

A new technique which has recently been added to our test programme permits the in vitro examination of activity against the exo-erythrocytic schizonts of P.yoelii. To date only one compound, ICI 56780, has been examined in this system and data from this test are included in this report. This compound has also been submitted to WRAIR for evaluation as a hypnozoitocidal agent against P.cynomolgi in Macaca mulatta.

The Annual Report this year has been divided into two volumes. The first of these contains the text of the report together with tables summarising the results obtained in the various test systems. The second volume contains the detailed result sheets relating the tests.

2. ADMINISTRATIVE EVENTS

Staff employed on US Army funds are as follows:

Senior Technologist/ Research Assistant	- Mr B L Robinson	100 % time
Technicians	- Ms A West	100 % time
	- Ms J R Cox	100 % time
Secretary	- Mrs T Sargeaunt	25 % time

Ms West and Ms Cox have been employed on US Army funds for several years as Junior Technicians . They have now both completed their technical education and, having passed the examination for Higher National Certificate in Medical Laboratory Technology (Ms West) and Applied Biology (Ms Cox), have qualified for promotion to a higher grade.

Other staff associated with the project but paid from London School sources are :

Professor W Peters (Principal Investigator)	20 % time
Dr D C Warhurst (Biologist)	20 % time
Dr D S Ellis (Electron Microscopist)	10 % time

The refrigerated insectaries are functioning well and we are currently rearing approximately 10,000 adult Anopheles stephensi each week.

A new culture room has just been completed for in vitro studies on exo-erythrocytic stages of P.yoelii and we anticipate being able to incorporate this system as a routine to supplement in vivo testing for causal prophylactic studies before the end of 1986.

The collection of cryopreserved strains of rodent malaria has been supplemented by the addition of two amodiaquine resistant lines. Full screening of compounds for blood schizontocidal

activity now involves a battery of fourteen strains, derived from either P.berghei or P.yoelii, which are resistant to standard antimalarials, in addition to drug-sensitive P.berghei.

An official visit was made to the London School of Hygiene and Tropical Medicine by LTC Willis A. Reid, Jr., Chief of the Department of Parasitology at WRAIR in October 1985.

3. CHEMOTHERAPY STUDIES

3.1 Blood schizontocides

Data are summarised in Table 5 and detailed report sheets are appended as Tables 7 through 18 .

3.1.1 WR 254594 (BL 07762)

Owing to the "flat" nature of the dose response curve obtained from the four day test against P.berghei N strain with this compound, it was necessary to estimate the ED_{90} of 600 mg/kg by graphic interpolation.

3.1.2 BL 09686 (WR Number not known)

This compound, which is appreciably less active than most of the standard antimalarials, has an ED_{90} against N strain of 80 mg/kg X 4 sc.

3.1.3 Ivermectin

Ivermectin is inactive against P.berghei at 3 mg/kg X 4 sc. Shortage of compound prevented screening at higher dosage.

3.1.4 Doxycycline

This 6-deoxytetracycline (ED_{90} - 15.0 mg/kg X 4 sc) was more than ten times as active against P.berghei (N strain) as tetracycline which had an ED_{90} of 170 mg/kg.

Tests against strains resistant to chloroquine, mefloquine and quinine showed no evidence of resistance to doxycycline.

3.1.5 ICI 56780

This quinolone ester was examined in some detail by the Principal Investigator's team in Liverpool some years ago and found to be very active both as a suppressive drug in blood induced infections and also as a causal prophylactic (Ryley and Peters, 1970). In the current series of tests we have confirmed the activity of ICI 56780 against the drug-sensitive P.berghei N strain, when given subcutaneously, and also demonstrated the lack of cross-resistance in strains possessing primary resistance to chloroquine, quinine and mefloquine. Indeed, in the case of the mefloquine resistant line there is evidence of a slight hypersensitivity to this compound. Administered orally, this compound is poorly absorbed and appreciably higher ED_{90} values were obtained.

Strain	Resistant to	ED_{90}	I_{90}
N	-	[illegible]	[illegible]
NS*	Chloroquine	[illegible]	1.4
Q	Quinine	[illegible]	1.1
N1100	Mefloquine	[illegible]	2.5
N	-	57.0 po	1.0
NS*	Chloroquine	44.[illegible]	0.8
Q	Quinine	32.5	0.6
N1100	Mefloquine	[illegible]	0.3

* P.yoelii ssp

Table 1. A summary of "four-day test" data obtained with ICI [illegible] against [illegible] P.berghei [illegible].

3.2 Causal prophylaxis

Causal propnylactic test results are summarised in Table 4 and detailed data may be found in Tables 19 through 32.

3.2.1 BK 74491 (WR 252127 AA)

This compound had a Minimum Fully Effective Dose (MFED) of between 30 and 100 mg/kg X 1 sc . There was no evidence of residual activity at 100 mg/kg.

3.2.2 BK 73127 (WR 251977 AB)

The MFED of this compound was 30 - 100 mg/kg X 1 s.c. No residual activity was detected.

3.2.3 WR 254419 (BL 05844)

WR 254419 is somewhat more active than primaquine. MFED was 30 - 60 mg/kg; in this test primaquine had a MFED of 30 - 60 mg/kg X 1 . There was no residual activity at 60 mg/kg dose but at 30 mg/kg some residual effects were observed.

3.2.4 WR 242511 BL 7116

The MFED of WR 242511 was between 30 and 60 mg/kg X 1 with no evidence of any residual activity.

3.2.5 ICI 56780

This quinolone ester had an MFED of 60 - 90 mg/kg X 1 sc in the causal prophylactic test but slight residual activity is apparent even at 10 mg/kg.

3.2.6 Primaquine metabolites

The recent arrival of some primaquine metabolites from Professor Strother has enabled us to continue some of the investigations which were interrupted by shortage of material. [illegible]

are :

i. 5-hydroxyprimaquine (5HPQ)

Fully active (MFED = 10 - 30 mg/kg X 1 sc). Some residual activity at 30 mg/kg.

ii. 5-hydroxy-6-desmethyl primaquine (DHPQ)

The MFED of this metabolite was 30 - 60 mg/kg with no evidence of residual activity. At 60 mg/kg , however, this compound is toxic with seven out of ten treated mice dying.

iii. 5,6-dihydroxy-8-aminoquinoline (AQD)

AQD was found [illegible] active but, [illegible] fully active dose was not realised. The MFED [illegible] of 60 mg/kg.

iv. 6-methoxy-8-aminoquinoline (MAQ)

The MFED of MAQ [illegible]. There was [illegible] evidence [illegible] residual activity [illegible] at this dose.

v. [illegible] primaquine

[illegible]

[illegible] Gametocytocidal activity

Data from tests for gametocytocidal activity are summarized in Table [illegible] and detailed data sheets are appended as Tables [illegible].

[illegible] WR [illegible] (BL[illegible])

WR [illegible] appears to have some [illegible] gametocytocidal activity with approximately [illegible] of infection occurring at a dose of [illegible] mg/kg X 1 sc.

3.3.2 WR 254594 (BL 07762)

This compound shows activity of the same order as WR 254419. The maximum tested dose (100 mg/kg) produced 41 per cent suppression of oocyst development in A.stephensi fed on treated mice.

3.3.3 BL 09686 (WR number not known)

Only slight suppression (38 per cent) of gametocyte infectivity resulted from a single dose of 100 mg/kg of this compound.

3.3.4 ICI 56780

This compound showed a fairly high level of almost [illegible] activity against P.y.nigeriensis in this test. The ID_{50}, however, is 185 mg/kg as compared with 32 mg/kg for primaquine.

3.4 Sporontocidal activity

Summarised sporontocidal activity test results are presented in Table [illegible] and detailed data are presented in Table [illegible].

3.4.1 WR 171669 - BK 84507 (Halofantrine)

The standard screening concentration of 0.3 per cent in sucrose solution produced almost 100 per cent suppression of development of oocysts. Halofantrine is to be examined in an extended dose range and the results of the full test will be reported at a later date.

3.4.2 BK 74471 (WR 252127 AA)

This compound was completely inactive in the screening test.

3.4.3 BK 73127 (WR 51977 AB)

The screening test of this compound caused more than a [illegible]

cent inhibition of oocyst development and further tests are being undertaken to assess the effects of higher doseage.

3.4.4 WR 254419 (BL 05848)

WR 254419 was toxic to A.stephensi at the screening dose and only one mosquito survived from the 25 originally infected. Dissection of this sole survivor, however, showed no sign of any suppression of oocyst numbers and there is probably no activity at the maximum tolerated dose.

3.4.5 WR 254594 (BL 07762)

This compound was also toxic to mosquitoes, although slightly less so than WR 254419. At the screening dose four mosquitoes survived and dissection showed that there was approximately 40 per cent inhibition of oocyst development. It is questionable whether this is due to true sporontocidal activity or is a manifestation of generalised cytotoxicity.

3.4.6 BL 07665 - WR number not known

This compound showed a good level of sporontocidal activity, giving almost 90 per cent inhibition of infection at the screening dose. Further tests are to be performed to confirm these data and to evaluate the compound in an extended dose range.

3.4.7 Primaquine metabolites

The following seven metabolites of primaquine have been examined for sporontocidal activity :

- 5-methoxy-8-aminoquinoline (MAQ) = WR 15081

  Inactive at 0.05 %

- 5,6-dihydroxy-8-aminoquinoline (AQ) = WR 6865

  Inactive at 0.05 %

3. 6-hydroxy-8-aminoquinoline (AQL) = WR 6890

   Slightly active at 0.05 %

4. 5,6-dimethoxy-8-aminoquinoline (DM8AQ)

   Slightly active at 0.05 %

5. 5-hydroxy-primaquine (5HPQ)

   Inactive at 0.05 %

6. 5-hydroxy-6-desmethyl-primaquine (DHPQ)

   Inactive at 0.05 %

7. carboxymetabolite of primaquine

   Slightly active at 0.05 %

3.5 In vitro studies against exo-erythrocytic stages of *P. yoelii*

3.5.1 Materials and methods

In vitro studies on the activity of compounds against pre-erythrocytic schizonts of *P. yoelii* 17X are carried out using a modification by Millet *et al* (1988) of the technique described by Lambiotte *et al* (1984).

Primary cultures of rat liver parenchymal cells (Wistar strain) from albino laboratory rat are prepared by perfusing the liver with collagenase and harvesting dissociated hepatocytes. After washing with HEPES buffer, the cells are resuspended in MEM with added foetal calf serum (FCS) and penicillin/streptomycin. The cell suspension is adjusted to 2 x 10⁵ hepatocytes/ml.

25 µl drops are dispensed into petri dishes (two separate drops into each dish) and the cultures are incubated at 37°C for 24 hours.

Sterile dissections of salivary glands are made from 150

female A.stephensi which have been infected with P.yoelii 17X strain fourteen days previously. The glands are homogenised in MEM (with FCS and antibiotics) and, after counting the sporozoites, the volume is adjusted to give a concentration of approximately 100,000 sporozoites / ul.

The medium is removed from the hepatocyte cultures, replaced with 0.25 ul of sporozoite suspension and the cultures are incubated at 37°C to allow the sporozoites to enter the hepatocytes. After two hours incubation, 1 ml of MEM with FCS, antibiotics and cortisone is added to each of the untreated control cultures and solutions of drug dissolved in the same medium to the test plates. The medium and drug are renewed after 24 hours incubation and the cultures are fixed with methanol after 48 hours incubation prior to staining with Giemsa stain.

The total number of schizonts present in each culture are counted and the mean count at each of the drug concentrations is expressed as a percentage of the mean count [illegible].

Where it is necessary to utilise a solvent [illegible] for preparation of the drug, additional control cultures containing the same concentration of solvent as is present in the treated cultures must be included to check for any possible interference arising from the solvent.

References:

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2. Lambiotte,M., Landau,I., Thierry,N. and Miltgen,F. (1981) C.R.Acad.Sc. Paris, 293, 431 - 433.

3.5.2 Results

When examined in the P.y.nigeriensis causal prophylaxis test, the quinolone ester ICI 56780 was found to be fully active but the presence of residual activity at the lower point of the MFED range made it unclear whether that activity was truly causal prophylactic. In the in vitro test, however, activity against the exo-erythrocytic schizont may be directly observed and a compound which relies entirely on residual activity against emergent blood stages can be identified by its lack of action against the tissue stage.

Compound	mg/l	48 hour schizont counts 1	2	3	4	Mean	% Control
Medium control	-	32	25	32	37	31.50	100
Ethanol control	-	29	30	33	38	32.50	100
Primaquine	0.1	24	27	26	21	24.25	77.0
	1.0	7	4		3		
ICI 56780	0.1	10	5	8	8	7.75	24.5
	1.0	3					

Table 2. Results of an in vitro test of ICI 56780 and primaquine diphosphate against exo-erythrocytic stages of P.yoelii 17X in primary hepatocyte culture.

In this test an ethanolic solution of ICI 56780 was added to the medium to give concentrations of 0.1 and 1.0 mg/l. An ethanol control was included and primaquine was used as a positive drug control. From the data obtained (Table 2) it was shown that the lower of these concentrations caused approximately [illegible] per cent suppression of infection, while the higher dose [illegible]

blocked infection completely. This compares favourably with primaquine which whilst fully effective at 1.0 mg/l caused only 23 per cent suppression at 0.1 mg/l. Two Petri dishes, i.e. four cultures, were used for each group.

No evidence of toxicity was found in either primaquine or ICI 56780 treated preparations stained supravitally with 0.25 per cent Trypan blue in HEPES buffer.

From the results of this test it can be concluded that ICI 56780 has a direct action on the pre-erythrocytic schizont of P.yoelii and that, in this strain at least, the level of activity is greater than that of primaquine.

3.6 Development of drug resistance

Two lines of rodent malaria resistant to amodiaquine have been developed by the two per cent relapse technique. The first of these (designated NAM) was produced by exposing successive passages of the drug sensitive P.berghei N strain to 50 mg/kg X 1 dose of amodiaquine at the time of passage. This was the maximum dose which had been found to permit recrudescence in a preliminary experiment. Development of this line was fairly slow, with 20 passages elapsing over a period of almost six months before a high level of resistance was reached. The course of acquisition of resistance in the NAM line is graphically illustrated in Figure 1. The stability of this resistance has not yet been tested by withdrawal of drug pressure, but cryo-preservation has no discernable effect.

The SAM line, which was developed from P.yoelii ssp NS strain, was put under a higher level of drug pressure as the

parent strain is inherently less sensitive to amodiaquine than P.berghei N strain. The dose selected from the preliminary test was 100 mg/kg X 1 sc. Resistance developed very rapidly, with an appreciable level being apparent after only four passages (Figure 2). Withdrawing drug pressure after 55 passages has had no effect on the level of resistance to date (15 passages).

Cross-resistance studies on these two strains are scheduled and the results of these will be included in a subsequent report.

3.7 Cross-resistance studies

Over a period of years, initially in Liverpool and latterly in London, the Principal Investigator and his team have developed strains of rodent malaria which are resistant to a wide range of antimalarials. Whilst isolated experiments have been carried out to determine the levels of primary resistance and, in a few cases, cross-resistance patterns to some of the range of standard antimalarials, no comprehensive studies have been made. We are currently attempting to rectify this omission by testing all of our existing resistant lines against a battery of sixteen different antimalarials and the results obtained so far are presented in Tables 52 through 222 (summarised in Tables 7 and 8).

3.8 Isoenzyme studies

Preliminary experiments to establish experimental techniques have now been completed for seven isoenzymes and marker strains have been selected. Clones of these strains are being made in order to prepare pure standard lysates and data from the electrophoretic studies on these and other strains will be presented in a subsequent report. The isoenzymes which we are currently stu-

to study are listed below, together with a list of marker strains, and techniques for other enzymes are being investigated.

3.8.1 Isoenzymes and marker strains

Techniques are available for the following isoenzymes:

1. Glucose phosphate isomerase (GPI)
2. Glutamate dehydrogenase (GDH)
3. Glucose-6-phosphate dehydrogenase (G6PD)
4. Hexokinase (HK)
5. Lactate dehydrogenase (LDH)
6. Malate dehydrogenase (MDH)
7. 6-phosphogluconate dehydrogenase (6PGD)

The following parasites will be employed as markers:

1. *P. berghei* N796 (= Keyberg 173)
2. *P. bergnei* NK65
3. *P. yoelii* 17X
4. *P. y. nigeriensis*
5. *P. chabaudi*
6. *P. vinckei*

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5. APPENDICES

SUMMARY TABLES

SUMMARY OF FIELD SCREENING (ORAL 4 DAY TEST) DATA

		N		NS		N 1100		Q		RC							
		ED 50	ED 90	ED 50	I 90	ED 50	I 90	ED 50	I 90	ED 90	I 90	ED 90	I 90	ED 90	I 90	ED 90	I 90
WR 254594 BL 07762 LON 2024	sc	10.0	600														
WR BL 09686 LON 2046	sc	31.0	80.0														
Ivermectin LON 2025	sc	-	NA 3.0														
Doxycycline LON 2023	sc	2.3	15.0	14.6	1.0	15.2	1.0	26.0	1.7	10.5	0.7						
ICI 56780	sc	0.5	1.3	1.8	1.4	0.7	0.5	1.4	1.1								
LIV 1001	po	7.6	57.0	44.0	0.8	18.5	0.3	32.5	0.6								

TABLE 4

SUMMARY OF CAUSAL PROPHYLACTIC TESTS

LON		MFED mg/kgx1	RESIDUAL ACTIVITY at D+2	COMMENT
	PRIMAQUINE	30-60	NIL	
1956	WR BK 74491	30-100	NIL	
1957	WR BK 73127	30-100	NIL	
2010	WR 254419 BL 05848	3.0-10.0	NIL	
2024	WR 254594 BL 07762	>100	NIL AT 100	
1001	ICI 56780	30-60	PRESENT AT 10	
2062	5-hydroxyprimaquine	10-30	PRESENT AT 10	TOXIC AT 30
2063	5-hydroxy-6-desmethyl primaquine	30-60	NIL AT 60	TOXIC AT 60
2059	5,6-dihydroxy-8AQ	>60	NIL AT 60	
2058	6-methoxy-8-AQ	>60	NIL AT 60	
2064	carboxymetabolite of PQ	-	NIL AT 60	INACTIVE AT 60

PRINCIPAL INVESTIGATOR: PROFESSOR W. PETERS
DEPARTMENT OF MEDICAL PROTOZOOLOGY
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TABLE 5

SUMMARY OF GAMETOCYTOCIDAL ACTIVITY

WR	BN	LON	GD_{50}	GD_{90}	PI_{90}
PRIMAQUINE DIPHOSPHATE		1711	1.3	33.0	1.0
254419	BL 05848	2010	> 100	-	-
254594	BL 07762	2024	> 100	-	-
?	BL 09686	2046	> 100	-	-
ICI 56780		1001	22.5	185.0	0.18

TABLE 6

SUMMARY OF SPORONTOCIDAL ACTIVITY

WR	BN	LON	ACTIVITY	% SUPPRESSION
CYCLOGUANIL			ACTIVE	95.2
PYRIMETHAMINE			ACTIVE	84.8
HALOFANTRINE		1955	ACTIVE	47.6
252127	BK 74491	1956	INACTIVE	0
251977	BK 73127	1957	ACTIVE	54.3
254419	BL 05848	2010	INACTIVE *	0
254594	BL 07762	2024	SLIGHTLY ACTIVE*	40.7
?	BL 09686	2046	ACTIVE	79.3
MAQ (Strother) = WR 15081			INACTIVE	0
AQD (Strother) = WR 6865			INACTIVE	0
AQL (Strother) = WR 6890			SLIGHTLY ACTIVE	33.1
DM8AQ (Strother)			SLIGHTLY ACTIVE	32.4
5HPQ (Strother)			INACTIVE	0
DHPQ (Strother)			INACTIVE *	0
Carboxymetabolite of PQ (Strother)			SLIGHTLY ACTIVE	32.4

* TOXIC TO MOSQUITOES

All compounds tested at the screening concentration of 0.05 per cent in 5 per cent sucrose solution.

TABLE 7(A & B). A summary of ED_{90} values obtained with a series of strains of P.berghei and P.yoelii against a range of antimalarials.

Strain	Primary Resistance
1.P.berghei	
N	Drug sensitive
RC	Chloroquine
Q	Quinine
N 1100	Mefloquine
NH	Halofantrine
P	Primaquine
B	Cycloguanil
PYR	Pyrimethamine
ORA	Sulfonamides
MEN	Menoctone
NPN	Pyronaridine
N 1708	WR 228258
2. P.yoelii	
NS	Chloroquine
NS 1100	Mefloquine
SH	Halofantrine
SPN	Pyronaridine
NS 1708	WR 228258
P.y.nigeriensis	
NIG	No induced resistance

COMPOUND	N	RC	Q	N/1100	NH	P	B	PYR	ORA	MEN	NPN	N1708
CHLOROQUINE	3·1	230	»60	4·5	7·0	2·3	4·8	3·5	3·6	3·0	25·0	10·2
AMODIAQUINE	2·6	420	»30	20·0	5·4	2·0	2·1	3·3	2·6	4·5	32·0	5·2
PRIMAQUINE	4·8	13·0	18·5	9·0	10·5	74·0	6·4	24·0	2·6	3·3	8·4	7·0
QUININE HCl	118	2500	»600	1700	210	140	170	130		40·0	900	175
CINCHONINE HCl	125	3250	»600	400	290	85·0	50·0	91·0		60·0	550	90·0
MEFLOQUINE	4.6	14.3	»60	540	9.0	13.5	6·0	5·6	4.4	2·5	6·8	5·3
HALOFANTRINE	1·1		»100	135	3·6	1·5	4·2	2·3		0·7	3·5	1·5
ARTEMISININ	4·2	630	»30	17·0	10·5	12·0	8·2	4·8	2·7	6·2	90·0	5·9
PYRIMETHAMINE	0·12	0·05	0·03	0·04	0·26	0·17			0·5	0·4	0·21	0·01
SULFADOXINE	4·4	0·62	0·13	0·04	2·7	0·39	0·71	1·9	29·0	0·34	0·1	1·2
PYR:SULF (1:3)	0·32	0·06	0·01	0·08	0·16	1·1		3·0	0·48	0·07	0·03	0·1
CYCLOGUANIL	3·3	3·6	3·4	2·5	6·4				44·0	5·2	10·0	3·7
MENOCTONE	1·4	11·0	1·8	1·2	1·6	2·1	9·0	7·2	2·7	»30	1·8	2·3
FLOXACRINE	1·0	0·27	0·5	0·3	0·8	0·39	0·4	0·38	0·4	1·0	0·3	0·6
CLINDAMYCIN	36·0	56·0	9·7	2·9	57·0	6·4	27·0	6·0	8·8	7·5	9·0	27·0
PYRONARIDINE	0·71	10·0	>100	1·6	0·75	1·0	1·4	1·1	1·5	0·73	13·5	0·67

TABLE 7A

COMPOUND	NS	NS/1100	SH	SPN	NS1708	NIG						
CHLOROQUINE	56·0	27·0	80·0	220	21·5	6·7						
AMODIAQUINE	18·0	4·8	»100	420	31·0	6·3						
PRIMAQUINE	8·4	18·4	9·2	13·7	9·0	19·5						
QUININE HCl	290	600	190	920	200							
CINCHONINE HCl	220	70·0	»600	1600	155							
MEFLOQUINE	7·2	640	»100	20·0	7·5							
HALOFANTRINE	1·0	22·5	375	3·4	0·9							
ARTEMISININ	10·0		»30	20·5	7·8							
PYRIMETHAMINE	0·11		0·11	0·07	0·1	0·16						
SULFADOXINE	0·26		0·21	0·08	0·14	0·18						
PYR : SULF (1:3)	0·1		0·19	0·08	0·1	0·04						
CYCLOGUANIL	6·9		6·8	11·5	5·0	12·3						
MENOCTONE	4·5		3·8	4·3	3·5							
FLOXACRINE	0·56		0·46	0·52	0·58	0·44						
CLINDAMYCIN	55·0	18·5	14·0	24·0	24·0							
PYRONARIDINE	1·2	1·4	>100	33·5	1·4	0·7						

TABLE 7B

TABLE 8(A & B) Blood schizontocidal test results expressed as Resistance Factors (I_{90}). ED_{90} values of each strain are compared with that of P.berghei N strain (I_{90} of N strain = 1.0)

COMPOUND	N	RC	Q	N 1100	NH	P	B	PYR	ORA	MEN	NPN	N1708
CHLOROQUINE	1·0	74·2	»19·4	1·5	2·3	0·7	1·5	1·1	1·2	1·0	8·1	3·3
AMODIAQUINE	1·0	161·5	»11·5	7·7	2·1	0·8	0·8	1·3	1·0	1·3	12·3	2·0
PRIMAQUINE	1·0	2·7	3·9	1·9	2·2	15·4	1·3	5·0	0·5	0·7	1·8	1·5
QUININE HCL	1·0	21·2	»5·1	14·4	1·8	1·2	1·4	1·1	1·6	0·3	7·6	1·5
CINCHONINE HCl	1·0	26·0	37·6	3·2	2·3	0·7	0·4	0·7	0·5	0·5	4·4	0·7
MEFLOQUINE	1·0	59·8	»13·0	117·4	2·0	2·9	1·3	1·2	0·9	0·5	1·5	1·2
HALOFANTRINE	1·0	>27·3	»90·9	122·7	3·3	1·4	3·8	2·1	1·7	0·6	3·2	1·4
ARTEMISININ	1·0	150	»7·1	4·0	2·5	2·9	2·0	1·1	1·8	1·5	21·4	1·4
PYRIMETHAMINE	1·0	1·3	0·3	0·3	2·2	1·4			4·2	3·3	1·8	0·08
SULFADOXINE	1·0		0·03	0·01	0·6	0·09	0·16	0·4		0·08	0·02	0·27
PYR : SULF (1:3)	1·0		0·03	0·25	0·5	3·4		9·4		0·22	0·09	0·3
CYCLOGUANIL	1·0	0·9	1·0	0·8	1·9					1·6	3·0	1·1
MENOCTONE	1·0	7·9	1·3	0·9	1·1	1·5	6·4	5·1	1·9	»21·4	1·3	1·6
FLOXACRINE	1·0		0·5	0·3	0·8	0·39	0·4	0·38		1·0	0·3	0·6
CLINDAMYCIN	1·0	1·6	0·3	0·08	1·6	0·2	0·8	0·2	0·2	0·2	0·25	0·75
PYRONARIDINE	1·0		>140·8	2·3	1·1	1·4	2·0	1·5		1·0	19·0	0·9

TABLE 8A

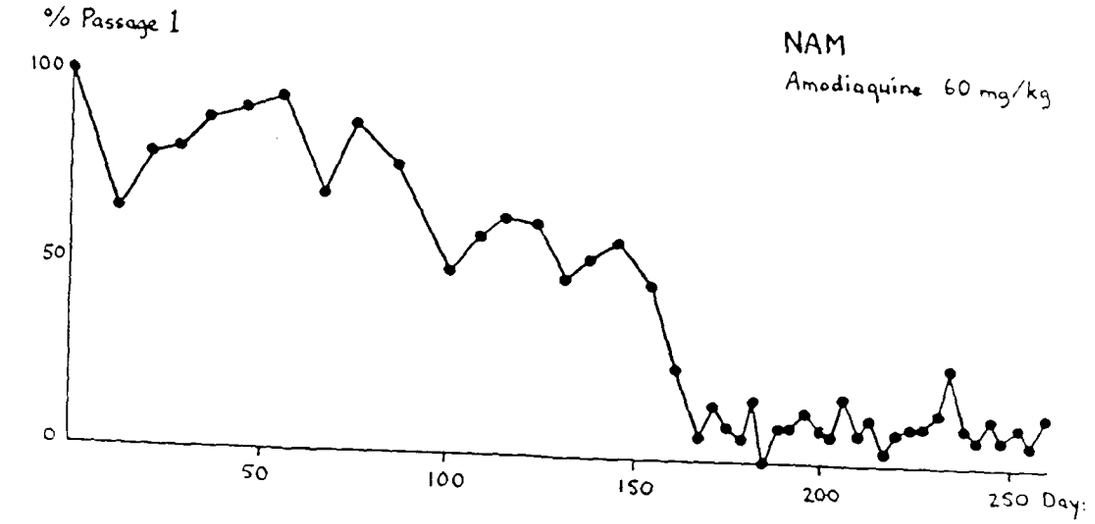
COMPOUND	NS	NS 1100	SH	SPN	NS 1708	NIG						
CHLOROQUINE	18·1	8·7	25·8	71·0	6·9	2·2						
AMODIAQUINE	6·9	1·8	»38.5	161·5	11·9	2·4						
PRIMAQUINE	1·8	3·8	1·9	2·9	1·9	4·1						
QUININE HCl	2·5	5·1	1·6	7·8	1·7							
CINCHONINE HCl	1·8	0·6	»4.8	12·8	1·2							
MEFLOQUINE	1·6	139.1	»21·7	4·3	1·6							
HALOFANTRINE	0·9	20·5	340·1	3·1	0·8							
ARTEMISININ	2·4		»7·1	4·9	1·9							
PYRIMETHAMINE	0·92		0·92	0·58	0·83							
SULFADOXINE	0·06		0·05	0·02	0·03							
PYR : SULF (1:3)	0·31		0·59	0·25	0·31							
CYCLOGUANIL	2·1		2·1	3·5	1·5							
MENOCTONE	3·2		2·7	3·1	2·5							
FLOXACRINE	0·56		0·46	0·52	0·58							
CLINDAMYCIN	1·5	0·5	0·4	0·7	0·7							
PYRONARIDINE	1·7	2·0	>140.8	47·2	2·0							

TABLE 8B

5.2 FIGURES

FIGURE 1. Graphs showing the acquisition of resistance to amodiaquine by the NAM strain, which was developed by the two per cent relapse technique from P.berghei N strain exposed to 60 mg/kg amodiaquine (X 1 sc), and the SAM strain which is derived from P.yoelii ssp. NS strain under amodiaquine pressure of 100 mg/kg X 1 sc.

FIGURE 1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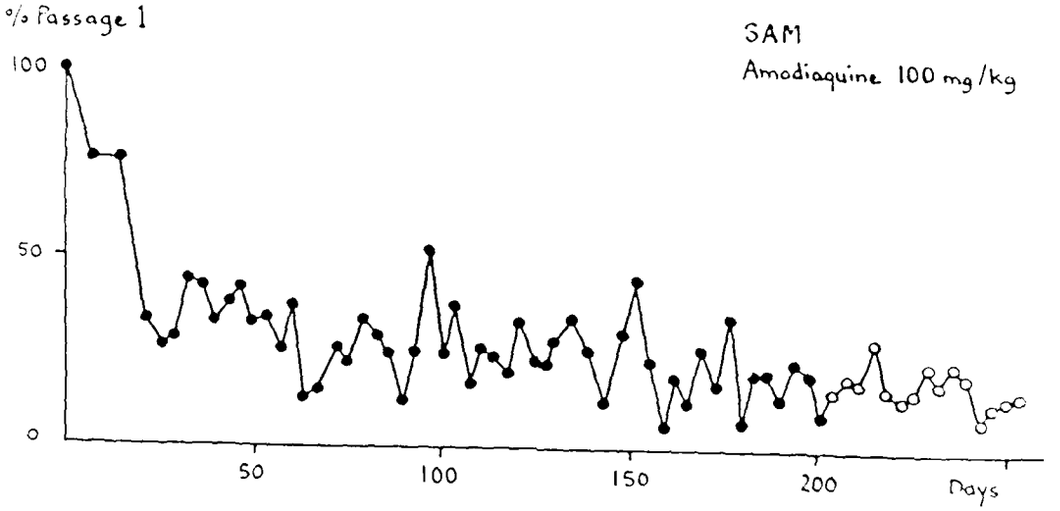
100
50
0
50
100
150
200
250 Days


% Passage 1
SAM
Amodiaquine 100 mg/kg
100
50
0
50
100
150
200
Days

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