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PRINCIPAL INVESTIGATOR: DR. ARBA L. AGER, JR.

PI ADDRESS: UNIVERSITY OF MIAMI - School of Medicine -
CENTER FOR TROPICAL PARASITIC DISEASES
12500 S.W. 152nd.St.
Miami, Florida 33177

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)
Malaria chemoterapeutic studies included a primary antimalarial blood schizontocidal test system (MM test), where 1,500 compounds were evaluated against Plasmodium berghei with 327 exhibiting activity, and a secondary antimalarial program consisting of in depth evaluation of compounds against drug-sensitive and drug-resistant lines. In a 3-dose modified MM test artelinic acid exhibited better activity than 5 analogs of artemisinin and only 2 of the 4 different 5-fluoropyrimidine analogs were active. Neither verapamil, diltiazem, nor prochloroperazine reversed chloroquine resistance in a highly chloroquine-resistant line even when mice were pretreated with phenylhydrazine. The antimalarial activity of primaquine, dapsone, and menocotone was hindered when mice received phenylhydrazine. Alloxan did not influence the antimalarial activity of several compounds. $FeSO_4$ but not $CuSO_4$ enhanced the activity of WR181023. Peroxidized cod-liver oil and menhaden oil had antimalarial activity. In a special diet study the suppressive and curative antimalarial activity of qinghaosu was enhanced in mice maintained on a standard mouse diet containing 5% corn oil and 0 vitamin E. A similar diet deficient in selenium did not (OVER)

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Trypanosoma rhodesiense, Drug-resistant trypanosomes, Pentamidine-resistant trypanosomes, Suramin-resistant trypanosomes, Melarsoprol-resistant trypanosomes, RAI.

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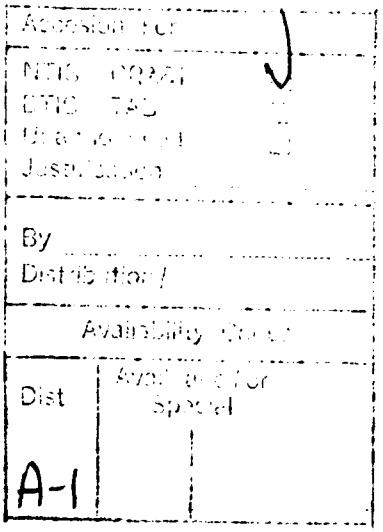
enhance the activity of qinghaosu. When 5% cod-liver oil was used as the fat source the diet alone without qinghaosu exhibited marked antimalarial activity.

A total of 1004 three-level tests with 666 different compounds were performed against drug-sensitive Trypanosoma rhodesiense. Activity was noted in 132 of these compounds. Twenty active compounds were tested against lines resistant to either melarsoprol, pentamidine, or suramin. No cross resistance was noted with suramin however, marked cross resistance with melarsoprol and pentamidine was observed with all compounds except one.



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Carla L Cizer 3/7/90

PI - Signature Date

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INTRODUCTION

Malarial infections are increasing in most endemic areas of the world today. Two major reasons for this increase are; **1)** the occurrence of ***Plasmodium falciparum*** parasites exhibiting resistance to many and often all currently available drugs (multiple drug resistance), **2)** no new anti-relapse agent to replace the toxic primaquine compound for ***Plasmodium vivax*** infections. Other reasons for this increase are inadequate vector control, and the lack of an effective vaccine. It has been estimated for the year of 1986 there were over 400 million cases of malaria resulting in over 2 million deaths worldwide.

The existing repertoire of antimalarial drugs to combat the multiple drug-resistant ***P. falciparum*** parasites are ineffective in many areas of the world. Mefloquine, the only new compound released since pyrimethamine was introduced in the 1950's, has been used in several areas of the world with disappointing results. Parasites of ***P. falciparum*** resistant to mefloquine have emerged. Coupling mefloquine with pyrimethamine and sulfadoxine did not avoid toxicity problems previously encountered with sulfadoxine type compounds.

There is an urgent need for new antimalarial drugs to stop the increasing spread of multiple drug-resistant ***P. falciparum*** parasites and to block relapses of ***P. vivax***. To find new compounds we are currently testing 1,500 compounds per year against drug-sensitive asexual blood stage induced malarial infections in the standard primary antimalarial test system (MM test).

Selected active compounds emerging from this MM test are tested further in a secondary test system (AG test). The various testing involves checking for cross resistance with existing antimalarial compounds, multiple administration of active compounds, detection of synergy between compounds, attempts to reverse chloroquine resistance, testing whether or not pro-oxidant compounds would increase the activity of antimalarial agents, and altering the antioxidant status of mice by dietary manipulation to

increase the activity of qinghaosu.

The control of African sleeping sickness caused by **Trypanosoma rhodesiense** or **Trypanosoma gambiense** in humans has not been effective. Currently available drugs are ineffective due to a combination of drug-resistant parasites and drug toxicity in man. New compounds are needed to combat this disease so we tested 1,000 compounds. Selected active compounds were tested against drug-resistant trypanosome lines.

SCREENING PROCEDURE FOR ASSESSING THE BLOOD SCHIZONTICIDAL ANTIMALARIAL ACTIVITY OF CANDIDATE COMPOUNDS IN PLASMODIUM BERGHEI INFECTED MICE

This mouse malaria (MM) test system was designed to identify new compounds active against asexual blood stages of malaria. Using mice from our breeding colony and a standard inoculum of **P. berghei** it has been possible to produce a consistent disease fatal to 100% of the untreated animals within 6 to 7 days. Active compounds extend the survival time or cure infected mice.

An established disease is less responsive to treatment than a disease in the early stages of development, therefore treatment was deliberately withheld until a moderately high degree of parasitemia was evident. Test compounds were administered subcutaneously in a single dose on the third day postinfection, at which time a 10-15% parasitemia had developed. A similar procedure was followed for the oral administration of selected active compounds.

A compound was classified as "active" if it suppressed the disease and produced an unquestionably significant increase, 100% or more, in the life span of the treated animals over that of the untreated infected controls. A compound was considered to be "curative" if the treated animals remained alive for 60 days after infection with **P. berghei**. Compounds not meeting one of the above requirements were considered inactive.

The severity of the challenge set up in our test system enhances the reliability of our evaluation and the antimalarial potential of the compounds selected for intensive preclinical studies.

METHODS

ANIMAL HOSTS

The total supply of animals needed to screen candidate compounds was obtained from our breeding colony of CD-1 Swiss mice (**Mus musculus**). Test animals weighed 18-20 grams. Weight variations in any given experimental or control group were carefully limited to within 2 to 3 grams. In any given test all animals were approximately the same age.

Animals on test were housed in metal-topped plastic cages, fed a standard laboratory diet and given water **ad libitum**. Once the infected mice had been administered the drug, they were placed in a room maintained at 28.8°C(±2°C), with a relative humidity of approximately 66%.

TEST PROCEDURE

Test animals received an intraperitoneal injection of approximately $6X10^5$ parasitized erythrocytes drawn from donor mice infected 4 days earlier with **P. berghei**. The donor strain was maintained by passage every 4 days in separate groups of mice inoculated with 0.2 cc of a 1:435 dilution of heparinized heart blood.

To check factors such as changes in the infectivity of our **P. berghei** strain or in the susceptibility of the host, 1 group of mice, which served as the negative control, was infected but not treated. To determine the effect that a drug exerted upon a malarial infection, 2 parameters were measured; the first was an increase in survival time, the second concerned curative action. For comparative purposes, 1 standard compound, pyrimethamine, was administered at 1 level (120 mg/kg) to a group of 15 mice. Pyrimethamine served as a positive control, producing a definite increase in survival time and curative effects. Another function of the positive control involved monitoring 3 procedures; the drug weighing, the preparation of drug solutions and suspensions, and the administration of drugs.

DRUG ADMINISTRATION

Test compounds were dissolved or suspended in peanut oil before they were administered subcutaneously. Compounds to be administered orally were mixed in an aqueous solution of 0.5 % hydroxyethylcellulose-0.1% Tween-80 (HEC).

Treatment consisted of a single dose given subcutaneously or orally 3 days postinfection. At the time of treatment a 10-15% parasitemia had developed. Although the disease was well established, it had not yet caused sufficient debility to affect an evaluation of the test compound's toxicity.

Deaths that occurred before the sixth day, when untreated infected controls began to die, were regarded as the result of a compound's toxic effect and not as the result of action by the infecting parasite.

Each compound was initially administered in 3 graded doses, diluted 4-fold, to groups of 5 mice per dose level. The top dose was 640, 320 or 160 mg/kg of body weight depending upon the amount of compound available for testing. Active compounds were subsequently tested at 6 or 9 dose levels, diluted 2-fold from the highest dose. Successive 6-level tests were performed at respectively lower doses until the lower limit of activity was reached, thus establishing a complete dose-response picture for that compound in a rodent system.

A drug that was toxic for the host at each of the 3 levels initially tested was retested at 6 dose levels diluted 2-fold from the lowest toxic dose.

DRUG ACTIVITY

Acceptance of a drug being sufficiently active for detailed studies was predicated on the margin between the maximum tolerated dose (MTD) and the minimum effective dose (MED) producing a significant effect. An MTD is defined as the highest dose up to 640 mg/kg causing no more than 1 of 5 animals to die from drug toxicity. The MED is defined as the minimum dose increasing the

life span of treated animals by 100% over the life span of untreated controls.

Clearly inactive compounds were rejected after one test, borderline compounds were characterized by a dose-response curve which established the spread the MTD and the lower limit of activity by a determination of drug activity in the dose-level dilution test.

RESULTS

During this year 1,500 compounds were tested for activity against asexual blood stages of **P. berghei**. There were 327 of these compounds which exhibited antimalarial activity.

A total of 297,088 3-dose-level tests were performed from December 1, 1961 through January 31, 1988 (**Table I**). The number of active compounds during part of this time period are summarized in **Table II**.

The specific test data of all compounds tested, (in malaria and trypanosome-drug screens) many of which are commercially or proprietorially discreet, is transmitted to the WRAIR Drug Development Program Chemical/Biological database.

TABLE I

PLASMODIUM BERGHEI MALARIA IN MICE

Compounds Tested

December 1, 1961 - January 31, 1988

TIME PERIOD	NUMBER OF COMPOUNDS TESTED
February 1, 1987 - January 31, 1988	1,500
February 1, 1986 - January 31, 1987	1,507
February 1, 1985 - January 31, 1986	1,500
October 1, 1983 - January 31, 1985	3,390
October 1, 1982 - September 30, 1983	3,026
October 1, 1981 - September 30, 1982	3,020
October 1, 1980 - September 30, 1981	2,998
October 1, 1979 - September 30, 1980	4,826
October 1, 1978 - September 30, 1979	6,175
October 1, 1977 - September 30, 1978	5,375
June, 1976 - September, 1977	7,114
June, 1975 - May, 1976	9,916
June, 1974 - May, 1975	10,604
June, 1973 - May, 1974	11,035
June, 1972 - May, 1973	14,276
June, 1971 - May, 1972	14,874
June, 1970 - May, 1971	18,108
June, 1969 - May, 1970	22,376
June, 1968 - May, 1969	38,150
June, 1967 - May, 1968	40,465
June, 1966 - May, 1967	34,093
June, 1965 - May, 1966	22,731
June, 1964 - May, 1965	13,114
December, 1961 - May, 1964	6,915
TOTAL	**297,088**

TABLE II

PLASMODIUM BERGHEI MALARIA IN MICE

Summary of Active Compounds

June 1, 1970 - January 31, 1988

TIME PERIOD	NUMBER OF COMPOUNDS TESTED	NUMBER OF ACTIVE COMPOUNDS
February 1, 1987 - January 31, 1988	1,500	327
February 1, 1986 - January 31, 1987	1,507	158
February 1, 1985 - January 31, 1986	1,500	74
October 1, 1983 - January 31, 1985	3,390	205
October 1, 1982 - September 30, 1983	3,026	335
October 1, 1981 - September 30, 1982	3,020	574
October 1, 1980 - September 30, 1981	2,998	359
October 1, 1979 - September 30, 1980	4,826	581
October 1, 1978 - September 30, 1979	6,175	969
October 1, 1977 - September 30, 1978	5,375	1,261
June 1, 1976 - September 30, 1977	7,114	1,124
June 1, 1975 - May 31, 1976	9,916	351
June 1, 1974 - May 31, 1975	10,604	616
June 1, 1973 - May 31, 1974	11,035	394
June 1, 1972 - May 31, 1973	14,276	771
June 1, 1971 - May 31, 1972	14,874	593
June 1, 1970 - May 31, 1971	18,108	805
TOTAL	**119,244**	**9,497**

SECONDARY ANTIMALARIAL SCREENING SYSTEM

INTRODUCTION:

DRUG RESISTANCE

Many **P. falciparum** parasites in various geographic areas of the world do not respond to certain standard antimalarial agents while some of these parasites do not respond to any antimalarial agent (multiple drug resistance). The different categories of drug resistance found in **P. falciparum** are summarized below;

1) Resistance to 4-aminoquinolines
   chloroquine

2) Resistance to arylaminoalcohols
   mefloquine (a quinolinemethanol)
   halofantrine (a phenanthrenemethanol)

3) Resistance to cinchona alkaloids
   quinine

4) Resistance to antifol drugs
   pyrimethamine
   proguanil
   Fansidar®
   Fansimef®

5) Resistance to acridines
   atebrine

6) Multiple drug resistance (resistance to two or more of the above compounds).

DRUG ACTIVITY

Toxic reactions in humans can occur with many antimalarials. The following compounds have been shown to cause severe toxic reactions in some patients.

Amodiaquine
Fansidar®
Fansimef®

Collectively, the several types of resistance impair the effectiveness of all the major available antimalarials. Hence, a tremendous need exists for alternate drugs active against the various types of drug-resistant parasites.

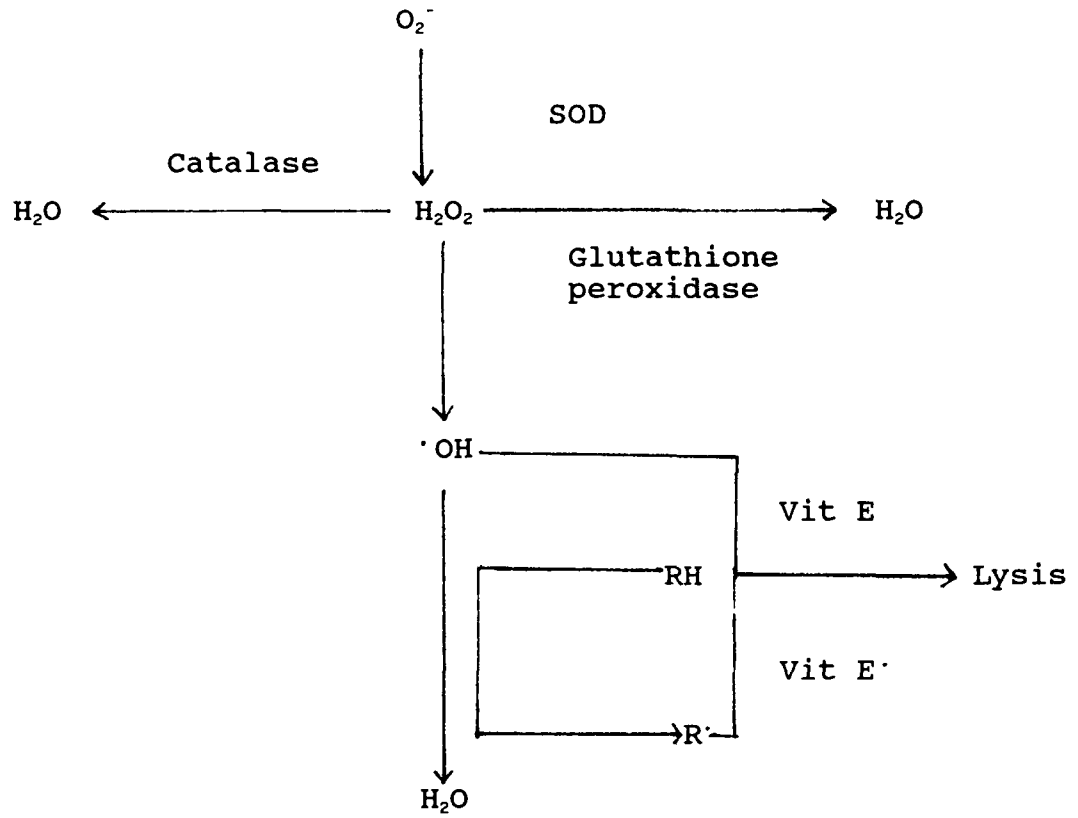
Another approach to antimalarial chemotherapy is by using combinations of synergistically active compounds such as Fansidar ® (pyrimethamine plus sulfadoxine), or the triple combination of mefloquine, pyrimethamine, and sulfadoxine (Fansimef ®)

Unfortunately both of these combinatorial drug regimens share toxicity problems due to the sulfadoxine component. New synergistically active drug combinations are needed.

The ***in vitro*** discovery that verapamil could reverse resistance to chloroquine in ***P. falciparum*** prompted us to verify this in a mouse malaria model. Previous experiments reported last year by us showed no reversal of chloroquine resistance was obtained with verapamil, nifedipine, or diltiazem using the highly chloroquine-resistant line of ***P. berghei***. We have recently developed a line of ***Plasmodium yoelii*** to be moderately resistant to chloroquine, hoping this would be a good ***in vivo*** model to study reversal of chloroquine resistance.

The antioxidant status of red blood cells harboring malarial parasites is an important component often determining the degree of parasitemia and the eventual pathology caused by malarial organisms. The antioxidants are important in inactivating toxic oxygen radicals such as superoxide anions (O_2^-), H_2O_2, OH, and secondary radicals (R). Antioxidants involved in detoxification of the toxic oxygen radicals are superoxide dismutase (SOD), catalase, glutathione peroxidase, and vitamins C and E.

These detoxification reactions of reactive oxygen are depicted in the following diagram.



The reactive oxygen radicals are both inside and outside infected red blood cells.

We did several experiments attempting to study these antioxidant-oxidant reactions during a malarial infection and found some promising leads.

Enhancing a drugs activity by altering the diet of the host is another approach we have taken. Qinghaosu is a compound bearing an endoperoxide grouping which is thought to act against the malarial organism by generating free radicals. These free radicals can damage membranes of the parasite and infected red blood cells. When the vitamin E (an important antioxidant) level of the host is lowered through dietary means the free radicals normally neutralized by vitamin E are free to do membrane damage. Thus this approach of lowering the antioxidant level of vitamin E then treating with a endoperoxide type compound has proven to be a novel new approach to chemotherapy. When we switched the fat source in our vitamin E depleted diets from corn oil to cod-liver oil we found strong antimalarial activity by diet alone. Several dietary experiments are described using this approach.

METHODS

PARASITES

Drug-sensitive lines

Plasmodium berghei KBG-173 (P-line)

Plasmodium yoelii 17 X (X-line)

Drug-resistant lines

P. berghei KBG-173

mefloquine resistant	A-line
chloroquine resistant	C-line
pyrimethamine resistant	M-line
quinine resistant	Q-line
dapsone resistant	S-line
cycloguanil resistant	T-line

P. yoelii 17X

qinghaosu resistant	U-line

ANIMAL HOST

The testing was done in both female and male CD-1 Swiss mice (***Mus musculus***) obtained from our own breeding colony. Four week-

old mice were used for most experiments except 3 week-old weanling male mice were used in the special diet experiments.

TEST DESIGNS

6-DAY SUPPRESSIVE TEST

In this basic 6-day suppressive test,mice were divided into groups of 7 and inoculated with $5X10^4$ parasites intraperitoneally (i.p.). For several drug-resistant lines (A,C, Q, S, and U lines) $15X10^6$ parasitized red blood cells are administered i.p. Drugs were administered twice a day, in a volume of 10 ml/kg on the third, fourth and fifth days after inoculation of parasites. All drugs were mixed in HEC. One group of infected mice received the vehicle alone and served as a negative control group. Blood films were made on the sixth day after inoculation of parasites. Microscopic examination of Giemsa-stained blood smears was made to determine the percentage of cells parasitized. The percent suppression of parasitemia, and significance values for the suppression of parasitemias were then determined. Significance values for the percent suppression of parasitemia were determined by comparing the parasitemia of each treated mouse with the mean parasitemia of the negative control animals. Drug tolerance was reflected by the percent weight change and the proportion of mice that survived treatment.

SYNERGISTIC TEST

Mice were infected i.p. with $5X10^4$ parasitized erythrocytes of the X-line. The drugs were mixed separately then administered either alone or as a mixture orally twice a day on days 3, 4, and 5 after the mice had been infected. The effects were read from blood smears made 1 day after completion of treatment. The dose suppressing 90% of the parasites (SD_{90}) for 1 drug alone and of the mixture were estimated by plotting parasitemia suppressions on probit-log scale graphs. The analyses for synergism were based upon partitioning of the SD_{90} value of each combination in terms of its components. These components were then compared with the respective

SD_{90} values of the corresponding drug alone. If the joint effects were simply additive, each component of a mixture SD_{90} would be expected to be 0.5. If all values were lower than 0.5 the data would indicated synergism. Conversely, if all values were greater than 0.5 the data would indicate antagonism.

3-DOSE MODIFIED MM TEST

A series of artemisinin analogs were administered on days 3, 4 and 5 after inoculation with a regular MM parasite inoculum of $6X10^5$ erythrocytes parasitized with **P. berghei**. Blood films were taken weekly starting on the sixth day after infection and continued for a 60 day period. Mice surviving the 60 day challenge were considered cured.

REVERSING CHLOROQUINE RESISTANCE

An attempt to reverse chloroquine resistance in the highly chloroquine-resistant C-line was done in mice pretreated with phenylhydrazine for 2 days before infection. The phenylhydrazine was given to cause the mice to produce large numbers of reticulocytes which are preferentially invaded by the chloroquine-resistant parasites. Then on days 3, 4 and 5 after infection either verapamil, ditilizam, or prochlorpromazine were administered 30 minutes after chloroquine. The results were determined by comparing the SD_{90} values of the drug combinations with the SD_{90} values of each component administered singly.

DEVELOPMENT OF A MODERATELY CHLOROQUINE-RESISTANT LINE

A line started from **P. yoelii** was placed under chloroquine drug pressure to develop a line moderately resistant to chloroquine. This was done by increasing chloroquine pressure at weekly intervals. As the line was being developed several standard and new antimalarials were tested for activity against this line.

OXYGEN RADICALS

A series of compounds which generate oxygen radicals were administered alone or combined with antimalarial agents in order to increase the oxidative stress in the infected red blood cell leading to its eventual lysis and the demise of the parasite. Selected pro-oxidants were also tested for antimalarial activity. All the compounds were accessed for suppressive activity in the 6-day suppressive test and curative activity was determined by monitoring survival times for 60 days postinfection.

DIETARY STUDIES

Three special diet experiments were performed to study the effect of corn oil, lard, and cod-liver oil diets deficient in vitamin E on the antimalarial activity of qinghaosu. Weanling male mice were fed their diets for either 9, 4, or 9 weeks for experiments 1, 2, and 3 respectively before infection with **P. yoelii** ($5X10^4$ parasites). A control group at this time was also bled and plasma vitamin E determinations were made. Mice were dosed orally with qinghaosu b.i.d. on days 3, 4 and 5 postinfection. On the sixth day, blood films were made and suppressive antimalarial activity was assessed by determining the percent parasitemia. Survival times were monitored for 60 days postinfection.

RESULTS

6-DAY SUPPRESSIVE TEST

Artelinic acid was more active orally than artemisinin and arteether when the compounds were administered orally (**Table III**).

SYNERGISTIC TESTS

Test results to detect synergistic activity between two compounds are described in various areas of this result section depending on the nature of the agents. These areas are primarily combination testing to reverse chloroquine resistance and oxygen radical generators combined with pro-oxidants to enhance antimalarial activity.

3-DOSE MODIFIED MM TEST

The suppressive and curative antimalarial activity of artemisinin and several of its analogs were tested in a series of 6 special tests. The results are shown in **Table IV.** Artelinic acid appears to be the best of the analogs tested. Four different 5-fluoropyrimidine analogs were tested with the results shown in **Table V.** Two of these analogs (PR-1 and 4) exhibited slight antimalarial activity. The survival time of the mice receiving these two analogs was extended at least 4 days longer than the infected non-treated controls. No curative activity was found with any of the 4 compounds.

REVERSING CHLOROQUINE RESISTANCE

Attempts to reverse chloroquine resistance in the highly chloroquine-resistant C-line were not successful with verapamil, diltiazem, or prochloroperazine when combined with chloroquine (**Table VI**). Since quinine is also not effective against this chloroquine-resistant line the same three reputed chloroquine reversing agents used above did not allow quinine to suppress the C-line parasites. Phenytoin did not reverse chloroquine resistance in a line of **P. yoelii** which has a low number of chloroquine-resistant parasites.

DEVELOPMENT OF A MODERATELY CHLOROQUINE-RESISTANT LINE

A new line of **P. yoelii** was placed under chloroquine drug pressure and several antimalarials were tested against it for activity. The antimalarials tested were mefloquine, quinine, WR 171669, and WR 180409. These compounds were still as active against this line as they were against the drug-sensitive line of **P. yoelii**. This line exhibited only a 2-fold increase resistance to chloroquine.

OXYGEN RADICALS

The results of all the oxygen radical work is shown in **Table VII**. The administration of phenylhydrazine appeared to hinder the

antimalarial activity of primaquine, dapsone, and menoctone. Alloxan did not alter the antimalarial activity of chloroquine, trifluperazine, t-butylhydroperoxide or mefloquine. Iron dextran did not enhance the activity of primaquine, however iron sulfate did enhance the activity of WR 181023 but not artelinic acid. Peroxidized cod-liver oil and menhaden oil did exhibit antimalarial activity. Copper sulfate did not increase the antimalarial activity of primaquine. Malonaldehyde administered subcutaneously (s.c.) and t-butyldroperoxide i.p. and p.o. exhibited antimalarial activity while H_2O_2 did not.

DIETARY STUDIES

A diet containing 5% corn oil without vitamin E prefed to mice 9 weeks before infection with **P. yoelii** potentiated the antimalarial activity of qinghaosu. A similar diet deficient in selenium but supplemented with vitamin E did not enhance the activity of qinghaosu (**Table VIII**). In a second experiment two vitamin E deficient diets, one with corn oil and the other with cod-liver oil, were tested for their ability to enhance the activity of qinghaosu. These diets were prefed to mice for 4 weeks before infection with **P. yoelii**. The mice fed the vitamin E deficient cod-liver oil diet exhibit suppressed parasitemias and enhanced curative activity regardless of the level of qinghaosu (**Table IX**).

A third experiment was performed to compare the activity of qinghaosu in mice fed two vitamin E-deficient diets containing either 5% lard or 5% corn oil.

The parasites were suppressed to similar levels in mice receiving both vitamin E-deficient diets (**Table X**). There was an increased curative effect in the lard group receiving 8 mg/kg qinghaosu.

TABLE VI

SUPPRESSIVE AND CURATIVE ANTIMALARIAL ACTIVITY OF COMPOUNDS ATTEMPTING TO REVERSE CHLOROQUINE RESISTANCE

EXPERIMENT NUMBER	COMPOUND NAME	PARASITE LINE	ANTIMALARIAL ACTIVITY SUPPRESSIVE	CURATIVE
496*	Chloroquine + Verapamil	C	Inactive	None
	Chloroquine + diltiazem	C	Inactive	None
	Chloroquine + prochlorperazine	C	Inactive	None
	Quinine + verapamil	C	Inactive	None
	Quinine + diltiazem	C	Inactive	None
	Quinine + prochlorperazine	C	Inactive	None
505	Chloroquine + phenytoin	X	Inactive	None

* Mice were pretreated with phenylhydrazine on days -2 and -1 before infection.

TABLE VII

SUPPRESSIVE AND CURATIVE ANTIMALARIAL ACTIVITY OF OXYGEN RADICAL GENERATORS AND PRO-OXIDANTS

EXPERIMENT NUMBER	COMPOUND NAME OR WR NO.	PARASITE LINE	ANTIMALARIAL ACTIVITY SUPPRESSIVE	ANTIMALARIAL ACTIVITY CURATIVE
495	Phenylhydrazine (P)	X	-	-
496	P + chloroquine	C	+	-
	P + quinine	C	+	-
497	P + chloroquine	X	+	-
	P + primaquine	X	+	+
	P + dapsone	X	+	+
	P + menoctone	X	+	+
522	P + menhaden oil	X	+	-
523	P + menhaden oil	X	+	-
524	P + menhaden oil	X	+	-
499	Alloxan (A)	X	-	-
	A + chloroquine	X	+	+
	A + trifluperazine	X	+	+
	A + primaquine	X	+	+
500	A + chloroquine	X	+	+
	A + t-butylhydroperoxide	X	+	+
	A + mefloquine	X	+	+
501	A (orally)	X	-	-
	A (intraperitoneally)	X	-	-
513	A + t-butylhydroperoxide	X	+	-
	A + WR 181023	X	+	-

TABLE VII (Cont.)

EXPERIMENT NUMBER	COMPOUND NAME OR WR NO.	PARASITE LINE	ANTIMALARIAL ACTIVITY SUPPRESSIVE	CURATIVE
522	A + menhaden oil	X	+	-
504	Fe dextran (Fd)	X	+	-
	Fd + menhaden oil	X	-	-
505	Fd + primaquine	X	+	+
507	Fd + primaquine	X	-	-
508	$FeSO_4$ (Fs) p.o.	X	-	-
	Fs s.c.	X	-	-
	Fs i.p.	X	-	-
512	Fs + WR 181023	X	+	+
	Fs + artelenic acid	X	+	+
514	Fs (PO) + menhaden oil	X	+	+
519	Fs + menhaden oil	X	+	+
	Fs + SuperMaxEPA	X	-	-
	Fs + Norwegian CLO	X	+	+
506	$CuSO_4$ $5H_2O$ (Cu)	X	+	+
507	Cu + primaquine	X	+	+
521	Cu + Omega-3 oil	X	-	-
	Cu + SuperMaxEPA	X	-	-
	Cu + Norwegian CLO	X	+	+
	Cu + menhaden oil	X	+	+
504	Menhaden oil (MO)	X	+	-
	MO + primaquine	X	+	+

TABLE VII (Cont.)

EXPERIMENT NUMBER	COMPOUND NAME OR WR NO.	PARASITE LINE	ANTIMALARIAL ACTIVITY SUPPRESSIVE	CURATIVE
506	MO + primaquine	X	+	+
507	MO + primaquine	X	+	+
	MO + primaquine + Fd	X	-	-
514	MO + Fs i.p.	X	-	-
	MO + Fs p.o.	X	+	+
518	MO		+	+
519	MO + Fs	X	+	+
521	MO + Cu	X	+	+
523	MO + P	X	-	-
524	MO + P	X	-	-
518	Cod-liver oil (Peroxidized)	X	+	+
519	Menhaden oil (Peroxidized)	X	+	+
521	Menhaden oil (Peroxidized)	X	+	+
522	Menhaden oil (Peroxidozed)	X	+	+
518	Omega-3 oil	X	-	-
519	Omega-3 oil	X	+	+
521	Omega-3 oil + Cu	X	-	-
518	SuperMaxEPA	X	+	+

TABLE VII (Cont.)

EXPERIMENT NUMBER	COMPOUND NAME OR WR NO.	PARASITE LINE	ANTIMALARIAL ACTIVITY SUPPRESSIVE	CURATIVE
519	SuperMaxEPA + Fs	X	-	-
521	SuperMaxEPA + Cu	X	-	-
518	Norwegian CLO	X	-	-
519	Norwegian CLO	X	-	-
	Norwegian CLO + Fs	X	+	+
521	Norwegian + Cu	X	+	+
495	Primaquine	X	+	+
	WR 181023	X	+	+
501	t-butylhydroperoxide p.o.	X	+	+
	t-butylhydroperoxide i.p.	X	+	+
510	Malonaldehyde p.o.	X	-	-
	Malonaldehyde s.c.	X	+	+
	Malonaldehyde i.p.	X	-	-
511	H_2O_2 s.c.	X	-	-
	H_2H_2 i.p.	X	-	-

TABLE III

SUPPRESSIVE AND CURATIVE ANTIMALARIAL ACTIVITY OF ARTEMISININ, ARTELINIC ACID, AND ARTEETHER vs. P. YOELII IN A 6-DAY SUPPRESSIVE TEST

EXPERIMENT NUMBER	COMPOUND NAME	MG/KG/DAY ORAL	PARASITEMIA D+6	NO. MICE ALIVE D+60/ TOTAL
515	Artemisinin	16	21	1/7
		8	36	0/7
		4	41	0/7
		2	43	0/7
	Artelinic Acid	16	3	4/7
		8	4	2/7
		4	40	0/7
		2	43	0/7
	Arteether	16	2	4/7
		8	3	2/7
		4	10	0/7
		2	35	0/7
517	Artemisinin	32	5	2/7
		16	31	0/7
		8	48	0/7
		4	41	0/7
	Artelinic Acid	32	0.4	3/7
		16	2	4/7
		8	4	5/7
		4	37	0/7
	Arteether	32	0	1/7
		16	2	3/7
		8	4	2/7
		4	32	2/7

TABLE IV

SUPPRESSIVE AND CURATIVE ANTIMALARIAL ACTIVITY OF ARTEMISININ, AND ARTELINIC ACID vs *PLASMODIUM BERGHEI* IN A 3 DOSE MODIFIED MM TEST

SPECIAL TEST NO.	COMPOUND NAME OR BN	MG/KG/DAY	VEHICLE	ROUTE	NO. MICE ALIVE D+60 TOTAL
1	Artemisinin	640	P*	SC	5/5
		160	P	SC	5/5
		40	P	SC	2/5
2	Artemisinin	80	P	SC	2/5
		40	P	SC	3/5
		20	P	SC	0/5
		10	P	SC	0/5
		5	P	SC	0/5
		2.5	P	SC	0/5
3	Artelinic Acid	640	5%Na_2HCO_3	SC	5/5
		160	"	SC	5/5
		40	"	SC	5/5
	Na Artesunate	640	"	SC	5/5
		160	"	SC	0/5
		40	"	SC	0/5
4	Artelinic Acid	160	"	SC	5/5
		80	"	SC	0/5
		40	"	SC	0/5
		20	"	SC	0/5
		10	"	SC	0/5
		5	"	SC	0/5
		2.5	"	SC	0/5
		1.25	"	SC	0/5
		0.675	"	SC	0/5

TABLE IV (Cont.)

SPECIAL TEST NO.	COMPOUND NAME OR BN	MG/KG/DAY	VEHICLE	ROUTE	NO. MICE ALIVE D+60 TOTAL
4	Na Artesunate	160	5%Na_2HCO_3	SC	1/5
		80	"	SC	0/5
		40	"	SC	0/5
		20	"	SC	0/5
		10	"	SC	0/5
		5	"	SC	0/5
		2.5	"	SC	0/5
		1.25	"	SC	0/5
		0.625	"	SC	0/5
5	BL 49135	320	P	SC	5/5
		80	P	SC	5/5
		20	P	SC	1/5
	BL 49144	320	P	SC	3/5
		80	P	SC	0/5
		20	P	SC	0/5
	BL 49153	320	P	SC	5/5
		80	P	SC	1/5
		20	P	SC	0/5
6	Artemisinin	320	HEC@	O+	0/5
		80	"	"	0/5
		20	"	"	0/5
	Artelinic Acid	320	"	"	5/5
		80	"	"	0/5
		20	"	"	0/5
	Arteether	320	"	"	2/5
		80	"	"	0/5
		20	"	"	0/5

P* = Peanut oil - HEC@ = 0.5% Hydroxyethylcellulose 0.1% Tween 80 - O+ = Oral

TABLE V

ANTIMALARIAL ACTIVITY OF 5-FLUOROPYRIMIDINE ANALOGS ADMINISTERED S.C. vs I.P. IN *PLASMODIUM BERGHEI* INFECTED MICE

COMPOUND CODE	MG/KG/DAY FOR 3 DAYS	ROUTE	VEHICLE	AVERAGE NO. DAYS MICE SURVIVED
	0	i.p.	S*	6.5
PR-1	0.1	i.p.	"	11.0
PR-2	0.1	i.p.	"	6.6
PR-3	7	i.p.	"	6.3
PR-4	7	i.p.	"	11.8
	0	s.c.	P+	6.3
PR-1	0.1	s.c.	"	10.4
PR-2	0.1	s.c.	"	6.4
PR-3	7	s.c.	"	6.1
PR-4	7	s.c.	"	12.0

S* = Saline

P+ = Peanut oil

TABLE VIII

EFFECT OF SELENIUM AND VITAMIN E DEFICIENCY ON THE SUPPRESSIVE AND CURATIVE ANTIMALARIAL ACTIVITIES OF QINGHAOSU

DIETARY SUPPLEMENT [+]	DOSE OF QINGHAOSU (MG/KG) 0	8	32	128
Suppressive assay (%) Parasitemia)				
Vit E + Se[*]	55	39	2	<1
Se	43	<1	1	<1
Vit E	57	39	1	<1
Curative assay (60-d survival)				
Vit E + Se	0 of 8	1 of 7	7 of 7	5 of 7
Se	5 of 8	7 of 7	7 of 7	6 of 7
Vit E	0 of 8	2 of 7	7 of 7	7 of 7

[+] Fat source 5% corn oil

[*] SE = Selenium

TABLE IX

EFFECT OF VITAMIN E-DEFICIENT DIETS CONTAINING EITHER CORN OIL OR COD-LIVER OIL ON THE ANTIMALARIAL ACTIVITY OF QUINGHAOSU

DIETS	DOSE OF QHS (MG/KG)			
	0	4	8	16
Suppressive assay (% Parasitemia)				
CLO -VE	2	1	2	0.5
CO -VE	39	44	26	22
CLO +VE	46	48	43	27
CO +VE	52	53	56	25
Active assay (60-d survival)				
CLO -VE	6 of 7	7 of 7	7 of 7	7 of 7
CO - VE	0 of 7	0 of 7	1 of 7	0 of 7
CLO +VE	0 of 7	1 of 7	0 of 7	0 of 7
CO +VE	0 of 7	0 of 7	0 of 7	0 of 7

CLO = Cod-liver oil

CO = Corn oil

VE = Vitamin E

TABLE X

EFFECT OF VITAMIN E-DEFICIENT DIETS CONTAINING EITHER CORN OIL OR LARD ON THE ANTIMALARIAL ACTIVITY OF QINGHAOSU

	DOSE OF QHS (MG/KG)			
	0	8	32	128
Suppressive assay (% parasitemia)				
Chow + VE	52	40	2	0.01
Lard + VE	57	57	0.5	0
CO + VE	63	55	0.7	0
CLO + VE	62	55	1.4	0
Lard -VE	40	47	1.2	0
CO -VE	56	44	0.4	0
CLO -VE	0	ND	ND	ND
Curative assay (60-d survival)				
Chow -VE	1 of 5	2 of 7	7 of 7	6 of 7
Lard +VE	1 of 7	0 of 6	7 of 7	4 of 7
CO +VE	0 of 7	1 of 6	7 of 7	5 of 7
CLO +VE	0 of 6	1 of 7	6 of 7	6 of 7
Lard -VE	3 of 7	4 of 7	6 of 7	4 of 6
CO -VE	0 of 6	0 of 7	7 of 7	4 of 7
CLO -VE	5 of 7	ND	ND	ND

ND = Not determined

A SCREENING PROCEDURE FOR THE EVALUATION OF TRYPANOCIDAL ACTIVITY OF CANDIDATE COMPOUNDS IN TRYPANOSOMA RHODESIENCE INFECTED MICE

INTRODUCTION

The World Health Organization estimates that there were about 20,000 new cases of African trypanosomiasis last year with 45 million people living in endemic areas. The various species of the vector (**Glossina**) are found over 4.5 million square miles in Africa. African trypanosomiasis has a very high mortality rate and has considerable importance as a public health problem, especially in this age of increasing foreign travel. The few drugs available for use today are toxic and parasite resistance to these agents is commonly found.

No new trypanocidal drugs have been introduced since the synthesis of pentamidine in 1939. Four drugs are currently available for the treatment of human trypanosomiasis caused by **Trypanosoma rhodesiense** or **Trypanosoma gambiense**. Two of these drugs, suramin and pentamidine are used in the treatment of the blood parasite (trypomastigote), but lack of efficacy in the treatment of central nervous system infections with trypomastigotes. Melarsoprol and nitrofurazone are used for the treatment of trypomastigotes in the central nervous system.

All of these drugs have severe side effects resulting in poor therapeutic indices. The use of suramin may lead to nausea, vomiting, shock and loss of consciousness. It can also cause exfoliate dermatitis, albuminuria, hematuria and ultimately renal failure. Pentamidine use may lead to fatal hypertension, hypoglycemia, diabetes and renal dysfunction. Administration of melarsoprol may lead to lethal encephalopathy and in 10 to 15 percent degeneration of the seminiferous tubules. This drug is also

associated with causing hemolytic anemia in glucose 6-phosphate dehydrogenase deficient patients.

Compounding the problem of low therapeutic indices is the problem of trypanosomal drug resistance. Human trypanosome strains are commonly resistant to at least 1 chemotherapeutic agent. With some patients, their infection is resistant to 2 or more antitrypanosomal drugs.

Therefore, there is a definite need to develop and test compounds that are potentially active against resistant strains of **T. rhodesiense** and that are less toxic than the existing drugs. Further testing also needs to be done using different routes of administration and combinations of 2 or more drugs.

The test system described herein was developed specifically to evaluate the trypanosomal activity of large numbers of candidate compounds. Based on blood induced **T. rhodesiense** infections in mice, it acts as a primary screen or as a secondary screen and/or confirmatory test. This test gives a precise quantitative evaluation of chemical compounds that demonstrate potentially useful therapeutic and/or prophylactic activity in **T. rhodesiense** infections. Consequently, it is also a helpful guideline in the synthesis of new related active agents.

All candidate compounds are obtained from the chemical inventory of the Division of Experimental Therapeutics at the Walter Reed Institute of Research.

METHODS

ANIMAL HOSTS

CD-1 Swiss mice (**Mus musculus**) used in this screening procedure weighed 25 to 28 grams with weight variation in any given experimental or control group carefully limited to 3 grams. Male and female mice approximately the same age were used.

Animals were housed in metal-topped plastic cages, fed a standard laboratory diet and given water **ad libitum**. After drug

treatment, mice were kept in a room maintained at a temperature of 28.8°C (±2°C) with a relative humidity of 66% (±2%).

INOCULATION OF PARASITES

Test animals received an intraperitoneal injection of 0.2 cc of a $1.5X10^4$ dilution of heparinized heart blood drawn from a donor mouse infected 3 days earlier (approximately $1.3X10^4$-$1.7X10^4$ trypomastigotes).

The donor line was maintained by 3-day blood passes; each animal received 0.1 cc of a $1:1.5X10^4$ dilution of heparinized heart blood drawn from a mouse harboring a 3-day infection.

One group of infected, untreated mice was included as a negative control to check both the infectivity of the **T.rhodesiense** (CT-Wellcome strain) and the susceptibility of the murine host. In order to determine the effect a drug exerted on a trypanosome infection, 2 parameters were measured; **1)** the increase in mouse survival time and **2)** drug curative action. For comparative purposes, 2 standard antitrypanosomal compounds, stilbamidine isethionate and 2-hydroxystilbamidine isethionate, were administered subcutaneously at one dose (26.5 mg/kg) to separate groups of 10 mice each. The same positive controls were administered orally at 53 mg/kg when compounds were tested orally. These 2 diamidines served as positive controls, producing definite increase in survival time and curative effects.

DRUG ADMINISTRATION

Test compounds were dissolved or suspended in peanut oil before they were administered subcutaneously. Compounds to be administered orally were mixed in an aqueous solution of HEC.

Treatment consisted of a single dose, given subcutaneously or orally, 2 to 3 hours after the injection of parasites. Deaths that occurred before the 4th day, when untreated infected controls begin to die, were regarded as a result of toxic action by the drug, not the lethal effects of the parasites.

Each compound was initially administered in 3 graded doses diluted 4-fold to groups of 5 mice per dose level. The top dose was either 424, 212, or 106 mg/kg, depending on the amount of compound available for testing. Active compounds were subsequently tested at 6 dose levels, diluted 2-fold from the highest dose. If necessary, successive 6-level tests were performed at respectively lower doses until the lower limit of activity was reached.

A drug that was toxic for the host at each 3 levels initially tested was retested at 6 dose levels diluted 2-fold from the lowest toxic dose.

DRUG ACTIVITY

Acceptance of a drug as being sufficiently active for detailed studies was predicated on the margin between the maximum tolerated dose (MTD) and the minimum effective dose (MED) producing a significant effect. A MTD is defined as the highest dose up to 424 mg/kg causing no more than 1 of 5 animals to die from drug activity. The MFD is defined as the minimum dose increasing the life span of treated animals by 100% over the live span of untreated infected controls.

Clearly inactive compounds were rejected after 1 test and border-line compounds after 2 tests. Active compounds are characterized by dose-response curves, which establish the spread between the MTD and the lower limit of activity by a determination of drug activity in the dose-level dilution tests. Treated animals alive at the end of 30-days were considered cured.

RESULTS

CONTROLS

Mice inoculated with trypomastigotes but receiving no drug (negative control group) all routinely died within 4 to 5 days. Mice serving as positive controls, receiving 26.5 mg/kg of stilbamidine, or 26.5 mg/kg of 2-hydroxystilbamidine, usually survived for the duration of the experiment (30 days).

COMPOUNDS TESTED

Data for all the new compounds is summarized in **Table XI.** There were 132 active compounds out of 666 tested.

TABLE XI

SUMMARY OF TRYPANOSOMA RHODESIENSE DRUG-SCREEN TEST

Report Period	Number of three-level tests	Number of compounds tested	Total number of active compounds	Number of compounds active by route of administration			
				oral only	S.C.[1] only	Oral and S.C.	I.P.[2] only
2/1/87 - 1/31/88	1,004	666	132	0	132	0	0
2/1/86 - 1/31/87	1,006	794	89	8	31	50	0
2/1/85 - 1/31/86	1,060	816	67	21	31	15	0
10/1/83 - 1/31/85	2,372	2,056	142	1	141	0	0
10/1/82 - 9/30/83	2,069	1,788	82	0	75	7	0
10/1/81 - 9/30/82	1,994	1,960	67	3	60	2	2
10/1/80 - 9/30/81	2,043	1,222	62	6	50	6	0
10/1/79 - 9/30/80	4,780	3,462	88	3	78	7	0
10/1/78 - 9/30/79	3,158	2,783	125	7	116	2	0
10/1/77 - 9/30/78	4,025	3,032	77	9	54	14	0
6/1/76 - 9/30/77	4,235	4,235	396	17	270	109	0
6/1/75 - 5/31/76	1,653	1,653	257	59	198		
6/1/74 - 5/31/75	1,826	1,826	298	73	225		
6/1/73 - 5/31/74	1,581	1,581	185	93	92		
8/1/72 - 5/31/73	3,030	3,030	68				
TOTAL	**35,836**	**30,904**	**2,135**	**300**	**1,553**	**212**	**2**

[1] S.C. = Subcutaneous

[2] I.P. = Intraperitoneal

DRUG-RESISTANT TRYPANOSOME TEST

INTRODUCTION

Drug-resistant parasites of **T. rhodesiense** and **T. gambiense** in humans severely complicate the chemotherapy approach to this disease. Pentamidine, the best available drug to treat the blood stream trypomastigotes stages of both species of African trypanosomes, is no longer effective in many areas of Africa because resistant parasites are commonly found. Suramin is the other drug used against the trypomastigotes of **T. rhodesiense**. It must be administered intravenously often with numerous toxic side effects. This leaves no safe drug available for treatment of trypomastigote stages in the blood of humans. Once trypomastigote stages cross the blood brain barrier the only compounds used are melarsoprol and nitrofurazone. Parasites resistant to melarsoprol are frequently found and toxicity is a severe problem. Nitrofurazone has numerous toxic side effects leaving no reliable therapeutic treatment for human cerebral African trypanosomiasis.

Many new compounds are cross resistant with parasites resistant to both pentamidine and melarsoprol. Therefore new compounds must be tested for degree for cross resistance patterns to established antitrypanosomal compounds.

The resistance of **T. rhodesience** to selected antitrypanosomal compounds can be induced by repeated drug pressure in an **in vivo** test system. This was achieved by infecting mice with a standard inoculum of parasites, administering the test compound at a dose just below the curative level, and passing parasites from these animals to a new set of mice when the parasitemia rose to a desirable level. Passes were made every 3 to 4 days with drug doses being increased as resistance develops at each dose level.

This type of study can establish the rate at which **T. rhodesiense** acquires resistance in mice to selected compounds. Degrees of cross resistance to trypanosomicidal compounds found to

be active against the drug-sensitive line in primary screening tests may also be determined.

Lines of trypanosomes have been developed which are completely resistant to the following compounds.

RESISTANT LINES	HIGHEST DOSE RESISTANCE ACHIEVED
Pentamidine	212.0 mg/kg
Melarsoprol (Mel-B)	424.0 mg/kg
Suramin	543.0 mg/kg

METHODS

ANIMALS HOSTS

CD-1 Swiss mice (**Mus musculus**) used in this screening procedure weighed 20 to 24 grams with weight variations in any given experimental or control group carefully limited to 3 grams. Both male and female mice were used and were approximately the same age.

Animals were housed in metal-topped plastic cages, fed a standard laboratory diet and given water **ad libitum**. After drug treatment, mice were kept in a room maintained at a temperature of 28.8° (±2°C) with a relative humidity of 66% (±2%).

INOCULATION OF PARASITES

Giemsa-stained blood smears from donor mice infected 3 days earlier with **T. rhodesiense** trypomastigotes were microscopically examined to determine parasitemias (i.e., number of trypomastigotes in a field of 100 erythrocytes). One set of test animals was infected with the drug-sensitive line of parasites and received an intraperitoneal injection of 0.2 cc of a $1:1.5X10^4$ dilution of heparinized heart blood drawn from a donor mouse harboring a parasitemia of 30-35% (approx. $1.3X10^4$ - $1.7X10^4$ trypomastigotes). Other sets of mice were similarly infected with each drug-resistant line to be tested. Blood dilutions were made such that all mice infected with the resistant lines received approximately the same

number of trypomastigotes as mice infected with the drug-sensitive line.

Groups of 10 mice per group infected with the drug-sensitive line and with each drug-resistant line but receiving no drug served as negative controls.

DRUG ADMINISTRATION

Test compounds were dissolved or suspended in either peanut oil for subcutaneous administration or HEC for oral administration. Compounds were given 1 hour following challenge with trypomastigotes.

Compounds doses were diluted 2 or 4-fold from a level that had been projected to be fully curative. Five mice were used for each dose level.

CROSS RESISTANCE DETERMINATION

Each compound was tested against the drug-sensitive line and the 3 drug-resistant lines. Mice surviving 30 days postinfection were considered cured. The degree of cross resistance (fold resistant) was obtained by the following calculation.

$$\textbf{Cross-resistance} = \frac{CD_{50}\ \text{Drug-resistant line}}{CD_{50}\ \text{Drug-sensitive line}}$$
(Fold resistant)

CD_{50} is the lowest mg/kg level of a compound curing at least 3 of 5 mice.

RESULTS

EXPERIMENTAL DATA

Infected non-treated control mice for all lines of trypanosomes died on either day 4 or 5 postinfection.

Little of any cross resistance to suramin (4-fold or less) was found with the compounds tested. Marked cross resistance (>8-fold)

to both pentamidine and melarsoprol was noted with all compounds except BL 63005.

TABLE XII

ANTITRYPOMASTIGOTE ACTIVITY OF COMPOUNDS ADMINISTERED SUBCUTANEOUSLY AGAINST THE DRUG-SENSITIVE LINE, AND LINES RESISTANT TO EITHER PENTAMIDINE, MELARSOPROL, OR SURAMIN

Bottle Number	Parasite Line	CD 50	Fold-resistant
AJ 78221	DSL	>212	-
	MRL	>212	-
	PRL	>212	-
	SRL	>212	-
AM 78566	DSL	53	-
	MRL	>53	>1
	PRL	>53	>1
	SRL	53	1
AQ 72907	DSL	55	-
	MRL	>106	>2
	PRL	>106	>2
	SRL	106	2
BK 40673	DSL	0.4	-
	MRL	>13	>33
	PRL	>13	>33
	SRL	0.1	1
BK 40726	DSL	6.6	-
	MRL	>106	>16
	PRL	>106	>16
	SRL	6.6	1

TABLE XII (Cont.)

Bottle Number	Parasite Line	CD 50	Fold-resistant
BK 62820	DSL	0.42	-
	MRL	>13	>31
	PRL	>13	>31
	SRL	0.42	1
BL 63005	DSL	3.33	-
	MRL	6.66	2
	PRL	3.33	1
	SRL	3.33	1
BL 22250	DSL	0.14	-
	MRL	>1.66	>11
	PRL	>1.66	>11
	SRL	0.14	1
BL 22303	DSL	0.06	-
	MRL	>3.33	>55
	PRL	>3.33	>55
	SRL	0.21	3
BL 24085	DSL	1.66	-
	MRL	>106	>63
	PRL	>106	>63
	SRL	4.2	1
BL 24147	DSL	0.21	-
	MRL	>3.33	>15
	PRL	>3.33	>15
	SRL	0.21	1

TABLE XII (Cont.)

Bottle Number	Parasite Line	CD 50	Fold-resistant
BL 31053	DSL	1.66	-
	MRL	>13.3	>8
	PRL	>13.3	>8
	SRL	0.21	1
BL 35542	DSL	1.66	-
	MRL	>26.5	>16
	PRL	>26.5	>16
	SRL	0.83	1
BL 00021	DSL	0.21	-
	MRL	>3.3	>15
	PRL	>3.3	>15
	SRL	0.21	1
BL 10054	DSL	6.6	-
	MRL	>106	>16
	PRL	>106	>16
	SRL	13.3	2
BL 12174	DSL	0.8	-
	MRL	>13	>16
	PRL	>13	>16
	SRL	3.3	4
BL 18818	DSL	3.3	-
	MRL	>53	>16
	PRL	>53	>16
	SRL	6.6	2

TABLE XII (Cont.)

Bottle Number	Parasite Line	CD 50	Fold-resistant
BL 19806	DSL	0.8	-
	MRL	>26	>33
	PRL	>26	>33
	SRL	0.8	1
BL 20658	DSL	6.6	-
	MRL	>53	>8
	PRL	>53	>8
	SRL	6.6	1
BL 21584	DSL	0.21	-
	MRL	>6.6	>31
	PRL	>6.6	>31
	SRL	0.21	1

CONCLUSIONS

In the primary antimalarial test system over 300 of 1500 compounds tested showed blood schizonticidal activity. This testing needs to be continued in order to find new chemical classes active against malaria and also to evaluate compounds emerging out of the lead directed synthesis program.

Selected active compounds emerging from this lead directed synthesis program were tested in a 3-day multiple dose system and one of these, artelinic acid, appears to exhibit the best antimalarial activity. In this same system two different 5-fluoropyridine analogs exhibited some suppressive but not curative antimalarial activity.

Attempts to reverse chloroquine resistance in a highly chloroquine-resistant line pretreated with phenylhydrazine were not successful. The compounds used in conjunction with chloroquine were verapamil, diltiazen, and prochlorperazine. This needs to be followed further with a line moderately resistant to chloroquine. We have developed such a line and will continue these studies using this line.

Several oxygen radical generating compounds e.g., phenylhydrazine, and alloxan were tested in combination with three antimalarial compounds. Phenylhydrazine hindered the curative activity of primaquine, dapsone, and menoctone. Alloxan did not influence the curative activity of chloroquine or mefloquine. Iron sulfate did enhance the activity of WR 181023 but not artelinic acid, while copper sulfate did not alter primaquines activity. Peroxidized cod-liver oil and menhaden fish oil both exhibited antimalarial activity.

Dietary factors can greatly influence the growth of malarial parasites alone and they can also enhance the activity of qinghaosu. A diet containing corn oil without vitamin E enhanced the antimalarial activity of qinghaosu while a similar diet depleted of selenium (a component of the antioxidant glutathione

peroxidase) had no influence on qinghaosu. When cod-liver oil was substituted for corn oil in a vitamin E-depleted diet the parasitemia was suppressed even without qinghaosu. When lard was used as the fat source the antimalarial effects were similar to a corn oil based diet. Dietary manipulation of the diet is a fruitful area of enhancing the antimalarial activity of selected compounds.

There were 1004 three-level tests performed with 666 different compounds evaluated for activity against drug-sensitive **Trypanosoma rhodesiense**. A total of 132 of these were active. Twenty of the active compounds were further tested against three different drug-resistant lines. These lines were resistant to either melarsoprol, pentamidine or suramin. Marked cross resistance to both melarsoprol and pentamidine was noted with all compounds except BL 63005. No cross resistance with suramin was found. There will be no further drug testing against **T. rhodesiense** at the request of WRAIR.

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CHEMOTHERAPY ASPECTS

Joaquin Ardavin — Merida Aviles

Rosa Fontela — Richard May

CARE AND MAINTENANCE OF ANIMAL COLONY

Ian Squires — Phillip Roberts

MAINTENANCE OF LABORATORY COMPLEX

Leon Johnson - Fernando Carulo

STAFF ASSOCIATE

Maria Isabel Ager

PRINCIPAL INVESTIGATOR

DR. ARBA L. AGER, JR.

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