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# DRUG EVALUATION IN THE PLASMODIUM FALCIPARUM - AOTUS MODEL

ANNUAL/FINAL REPORT

Richard N. Rossan

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The objective of these studies was to evaluate experimental antimalarial drugs in the Panamanian night monkey (Aotus l. lemurinus) experimentally infected with Plasmodium falciparum. Four folic acid inhibitors were tested against infections of the pyrimethamine-resistant Vietnam Smith strain. Of the four drugs evaluated, pyrimethamine, proguanil, cycloguanil, and a proguanil analog (WR 250417), only the proguanil analog had antimalarial activity. A total 3.0 mg/kg dose cured infections in 3 of 7 treatments, a 30.0 mg/kg total dose cured 6 infections out of 8 treatments, and a total dose of 150.0 mg/kg cured infections in 4 of 4 treatments.

Six calcium channel blockers were administered with chloroquine in trials to reverse chloroquine-resistance in vivo of Vietnam Smith/RE infections. Only desipramine cleared primary parasitemias but did not cure infections.

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19. Trials with other drugs, ketotifen, cyproheptadine, combretestatin, and an aryl-amine, showed no effect uppon primary parasitemias. Two neuroleptic phenothiazines, chlorpromazine and prochlorperazine, administered with chloroquine, cleared parasitemias and cured infections. This is the first demonstration of in vivo reversal of chloroquine-resistance.

Four derivatives of artemisinin, the active antimalarial principal of the Chinese herb qinghaosu, were selected for evaluation against Vietnam Smith/RE infections. Arteether and artemether (both oil soluble), were administered at doses ranging from 0.25 to 64.0 mg/kg (i.m., q.12hx3). Primary and repeat treatments with arteether cured 36 of 58 (62%) infections. Artemether, in primary and repeat treatments, cured 35 of 49 (71%) infections. The water soluble derivatives, artesunate and artelinate, were each administered in doses of 16.0, 32.0 and 64.0 mg/kg (i.m., q.12hx3). Artesunate cleared parasitemias in 9 of 9 Aotus and artelinate cleared parasitemias in 6 of 10 monkeys. Neither drug cured infections. The oil-soluble derivatives cure P. falciparum infections, in contrast to the water-soluble derivatives, which do not cure infections, at the doses administered.

## SUMMARY

The purpose of the studies under this contract was to infect Aotus lemurinus lemurinus (Panamanian night monkey) with strain(s) of Plasmodium falciparum of known drug sensitivity/resistance and to use the model for evaluation of experimental antimalarial drugs. The Vietnam Smith and Vietnam Smith/RE strains were used, both resistant to maximally tolerated doses of chloroquine, quinine, and pyrimethamine. Monkeys were infected by inoculation of P. falciparum blood stages from suitable monkey donors.

Three areas of drug evaluation were pursued during this contract period:

1. Assessment of folic acid inhibitors.

2. In vivo reversal of chloroquine resistance.

3. Evaluation of artemisinin derivatives.

Four folic acid inhibitors were tested against infections of the pyrimethamine-resistant Vietnam Smith strain. The anticipated cross-resistance was demonstrated with pyrimethamine (WR 002978), proguanil (WR 003019), and cycloguanil (WR 005473); these drugs had no effect upon parasitemias. In contrast, a proguanil analog (WR 250417) had significant antimalarial activity. A total dose of 3.0 mg/kg cured three infections of a total of seven treatments, a total dose of 30.0 mg/kg cured six infections of a total of eight treatments, and a total dose of 150.0 mg/kg cured infections in 4 of 4 monkeys.

In vivo trials to reverse chloroquine resistance of P. falciparum parasites were predicated upon the successful in vitro reversal with verapamil (a calcium channel blocker) plus chloroquine. The mechanism of chloroquine resistance reversal is putatively due to the prevention, by the channel blocker, of the active efflux of chloroquine from the parasitized erythrocyte, resulting in parasitocidal levels of chloroquine. The desideratum for the demonstration of in vivo reversal of chloroquine resistance is the administration of a calcium channel blocker (or similar acting drug) plus chloroquine in a defined regimen during the ascending phase of the primary parasitemia with subsequent clearance of parasitemia and infection cure.

Of the six calcium channel blockers evaluated, WR 255694 (verapamil), WR 255695 (nifedipine), WR 256287 (verapamil analog), WR 255693 (diltiazem), WR 256975 (Bepridil), and WR 149244 (desipramine), only desipramine partially fulfilled the

desideratum. A three-day treatment course of desipramine plus chloroquine cleared parasitemias, but the infection was not cured. It was recognized at the onset of these trials that the high concentrations of drugs to achieve in vitro reversal of chloroquine resistance might be incompatible with host viability.

One drug tested during the initial phase of these studies was WR 256410, a chlorpromazine analog. When administered with chloroquine, during the primary ascending phase of parasitemia, this drug combination had no effect upon parasitemia. Since in vitro reversal of chloroquine resistance was achieved with chlorpromazine and chloroquine, an in vivo pilot evaluation of this drug combination was initiated. Chlorpromazine (WR 2173) or prochlorperazine (WR 6379), both neuroleptic phenothiazines, administered with chloroquine during the ascending phase of primary parasitemias of Vietnam Smith/RE infections cleared parasitemias and cured infections. This is the first demonstration of in vivo reversal of chloroquine resistance with a well-tolerated drug regimen.

Recent studies of in vivo reversal of chloroquine-resistance have used the following drugs: WR 267634, ketotifen; WR 35917, cyproheptadine; WR 268766, combretestatin; and 87/209, an aryl-amine. None of these drugs, administered with chloroquine, had antimalarial activity against primary parasitemias of the Vietnam Smith/RE strain. Promethazine, WR 2158, plus chloroquine cleared primary parasitemias, but did cure infection. Additional studies with promethazine are planned.

Four derivatives of artemisinin, the active antimalarial principal of the Chinese herb qinghao, were selected for evaluation in the P. falciparum - Aotus model. Two derivatives, WR 255131 (arteether) and WR 254986 (artemether), are oil soluble; WR 255663 (artelinate) and WR 256283 (artesunate) are water soluble. Limited toxicity evaluation of the two oil soluble derivatives showed that a dose of 64.0 mg/kg administered intramuscularly q.12x3 doses evoked no overt toxic reaction in Aotus. Drug intolerance was associated with intravenous administration of the water soluble derivative. When administered intramuscularly, neither artesunate nor artelinate provoked overt toxicity.

For assessment of antimalarial activity, both arteether and artemether were administered at doses ranging from 0.25 to 64.0 mg/kg, intramuscularly, q.12hx3 doses. Primary treatment with arteether cleared parasitemias in 25 of 29 monkeys and cured infection in 15 of 28 animals. Repeat treatment with arteether cured infection in 21 of 30 monkeys. Overall, arteether cured 36 of 58 (62%) infections. Primary treatment with artemether cleared 24 of 33 parasitemias and cured 19 of

33 infections. Repeat treatments with artemether cured 16 of 20 infections. Overall, artemether cured 35 of 49 (71%) infections.

The antimalarial activity of the water soluble derivatives, artesunate and artelinate, was evaluated by intramuscular administration, q.12hx3 doses, of doses of 16.0, 32.0, and 64.0 mg/kg. Artesunate cleared parasitemias in 9 of 9 Aotus and artelinate cleared parasitemias in 6 of 10 monkeys. Neither drug cured infections. All treatment failures were re-treated with either arteether or artemether.

In the Aotus - P. falciparum model, neither water soluble artemisinin derivative cured infections. Both of the oil-soluble derivatives cured infections; and artemether was more effective than arteether, at the doses administered.

Further evaluation was initiated of the proguanil analog alone (WR 250417), at doses of 0.1, 1.0, and 10.0 mg/kg(x3), and in combination with sulfadiazine (WR 75557), at a dose ratio of 1:20. Results to date indicate that the antimalarial activity of the proguanil analog is not enhanced by the concommitant administration of sulfadiazine.

# FOREWORD

In conducting the research described in this report, the investigator 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 Commission of Life Sciences, National Research Council (NIH Publication No. 86-23, Revised 1985).

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## EXPERIMENTAL PROCEDURES

The monkey-adapted Plasmodium falciparum strain, Vietnam Smith/RE (resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine) was used to induce experimental malaria infections in Aotus lemurinus lemurinus for the evaluation of the antimalarial efficacy of candidate drugs. Infected blood, with sodium citrate (2.5%) as the anticoagulant, from untreated Aotus was diluted appropriately with chilled saline (0.85%), such that each milliliter contained 5,000,000 parasites, and this amount was injected into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Early-Perez method and reported as the number of parasites per cmm.

Blood films from untreated Aotus, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If a recrudescence occurred, blood films were obtained again on a daily basis.

The schema depicted in Figure 1 represents the designs of a typical drug evaluation study. Parasitemias were evaluated daily during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two times per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescences occurred during a 100 day examination period, the infection was considered to have been cured.

Drug doses were calculated as mg base per kg of body weight. Stock solutions of water soluble compounds, at appropriate concentrations, were prepared with distilled water and stored at 8°C for the treatment period. If a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

Oral administration of drugs was effected by gastric intubation with a 14 French catheter. The total amount of fluid administered, drug solution or suspension, and rinse was 14 ml.

As indicated in the appropriate sections, some water soluble drugs were administered intramuscularly; other water insoluble drugs were diluted in sesame oil and administered intramuscularly.

# INTRODUCTION

This is the Final Report for Contract DAMD17-87-C-7163, "Drug Evaluation in the Plasmodium falciparum - Aotus Model." The funded portion of the contract was from 15 May 1987 to 14 May 1990. Annual Reports for these three years have been submitted and the Final Report will summarize the results.

The primary purpose of the study supported by the contract was to infect Aotus lemurinus lemurinus (Panamanian night monkey) with a strain(s) of Plasmodium falciparum of known drug susceptibility/resistance and to use this system to evaluate the activity of candidate antimalarial drugs or combinations thereof. The presence of chloroquine-resistant P. falciparum strains in densely populated geographical areas, and the rapidly acquired resistance to newly-introduced antimalarial drugs makes it imperative to identify new drugs and/or seek novel combinations effective against these malaria parasites. The P. falciparum - Aotus model serves a pre-clinical test system for new drugs.

# ANTIMALARIAL ASSESSMENT OF FOUR FOLIC ACID INHIBITORS

The emergence of resistance to dihydrofolate reductase inhibitors, in both P. falciparum and P. vivax, has impacted adversely on the treatment of such infections. Cross resistance among this class of drugs offers a challenge to develop new and effective agents. Three standard folic acid inhibitors and new analog of proguanil were evaluated against infections of the pyrimethamine-resistant Vietnam Smith strain of P. falciparum.

A. WR 005473AM (BN:BK 40388)

Cycloguanil administered at doses of 1.0, 10.0, and 50.0 mg/kg(x3) had either no effect or a suppressive effect upon parasitemias.

B. WR 003019AM (BN:BL 18309)

Proguanil, administered at doses of 1.0, 10.0, and 50.0 mg/kg(x3) had no effect upon parasitemias.

C. WR 002978AK (BN:BK 39401)

Pyrimethamine, administered at the maximum tolerated dose of 2.5 mg/kg(x3), was ineffective against this pyrimethamine-resistant strain.

D. WR 250417AA (BN:BK 47734)

This proguanil analog was evaluated against primary parasitemias and treatment failures with cycloguanil and proguanil. A dose of 1.0 mg/kg(x3) cured 3 of 7 (43%) infections, 10.0 mg/kg(x3) cured 6 of 8 (75%) infections, and a dose of 50.0 mg/kg(x3) cured 4 of 4 infections. There was no overt toxicity associated with this analog.

Additional evaluation of WR 250417AD (BM 03125) included the concomitant administration of sulfadiazine, WR 7557AW (BG 59677). The rationale of administering such a drug combination was to ascertain if sulfadiazine would enhance the effectiveness of the proguanil analog at doses lower than 10.0 mg/kg(x3). The drug combination was administered at doses of a 1:20 ratio, proguanil analog: sulfadiazine. Administration of proguanil alone at doses of 0.1 and 1.0 mg/kg(x3) did not cure infections. The antimalarial activity of these doses plus sulfadiazine was not enhanced when administered with sulfadiazine.

There was the expected cross resistance of infections of this pyrimethamine-resistant strain to cycloguanil and proguanil. The proguanil analog was effective, at tolerated doses, in curing infections, and may eventually be used to treat infections in humans.

# IN VIVO TRIALS TO REVERSE CHLOROQUINE RESISTANCE

## A. Introduction

The identification, (1,2) of chloroquine-resistance in P. falciparum and the world-wide spread of such resistant strains, has challenged malariologists to develop new and effective drugs. The introduction of new drugs into the armamentarium generally has resulted in rapid resistance to them. Recent reports (3,4) indicated the in vitro reversal of chloroquine resistance by a calcium channel blocker - verapamil. It was proposed that chloroquine-resistant falciparum parasites prevent the accumulation of chloroquine in the infected erythrocyte and thus escape the drug's cytocidal action. A calcium channel blocker, or other similar acting drug, stops the active efflux of chloroquine which then accumulates to a parasite-toxic level. Based upon these in vitro studies, trials were initiated to determine if in vivo reversal of chloroquine-resistance is feasible in the Aotus model.

The desideratum of in vivo reversal of chloroquine resistance is the administration of one course of treatment with the test drug plus chloroquine during the ascending phase of the primary parasitemia, followed by clearance of parasitemia and infection cure, as indicated by no recrudescence during the 100-day post-treatment period.

B. Response of Vietnam Smith strain infections of P. falciparum to chloroquine (WR 1544BM; BN:AR 20613) and isolation of the Vietnam Smith/RE strain.

The Vietnam Smith strain, isolated in 1971(5), was identified as having RIII resistance to chloroquine. Parasites have been passaged by infected blood, with occasional cryopreservation and thawing of parasites. The strain is putatively resistant to maximum tolerated daily doses of 20.0 mg/kg of chloroquine in Aotus.

Primary treatment of Smith strain infections with chloroquine (20.0 mg/kgx3) or (40.0, 20.0, and 20.0 mg/kg) did not clear parasitemias, but retreatment with these doses cleared parasitemias, but did not cure infections. Recrudescent parasites were subinoculated into malaria naive Aotus and these infections treated with chloroquine. Suppression only of parasitemia was noted and this strain, termed Smith/RE, was used for in vivo reversal experiments.

C. WR 255694AB (BN:BL 22009), verapamil

The first calcium channel blocker to be evaluated for its effectiveness in reversing chloroquine resistance was verapamil. Verapamil alone and verapamil plus chloroquine were administered at diverse doses and regimens against Smith and Smith/RE strain infections of P. falciparum. In a total of 15 primary treatments, the infection was cured in one Aotus with verapamil (25.0 mg/kgx3) plus chloroquine (20.0 mg/kgx3). Drug toxicity was accountable for 5 deaths.

Results of these initial trials, while disappointing, were not unexpected due to the high concentrations of verapamil required for in vitro reversal of chloroquine-resistance. These high concentrations would be difficult to sustain in vivo and retain host viability.

D. WR 255695AC (BN:BL 21995), nifedipine

This calcium channel blocker plus chloroquine was administered in a total of 24 treatments, of diverse doses and regimens. Five of these infections were cured, but only after retreatment. Consequently, cure could not be attributed to reversal of chloroquine resistance.

E. WR 256287AA (BN:BL 28636)
WR 256287AB (BN:BL 51153)

This Hoffman LaRoche drug is a structural analog of verapamil and is putatively more active as a calcium channel blocker in humans. Also, it is 4x more effective than verapamil in reversing chloroquine resistance in vitro. Of 17 total treatments with WR 256287 plus chloroquine, two infections were cured, but only after retreatment.

F. WR 2564100AA (BN:BL 30887)

This drug is a chlorpromazine analog prepared by Smith Kline French. When administered with chloroquine, no infections were cured in a total of five treatments.

G. WR 255693AC (BN:BL 48567), Diltiazem

One infection of a total of 15 treatments with diltiazem plus chloroquine was cured. However, cure was obtained after retreatment.

H. WR 256975AA (BN:BL 39755), Bepridil

Bepridil, a calcium channel blocker, plus chloroquine did not cure any infections in a total of 10 treatments.

## I. WR 149244AD (BN:BL 54261), desipramine

Desipramine (Norpramin), is a tricyclic psychotropic drug. Some drugs in this class are weak antimalarials and are calcium antagonists. In vitro reversal of chloroquine resistance by desipramine in P. falciparum was reported (5) at concentrations similar to those in patients treated for depression. In a total of 20 treatments with desipramine plus chloroquine two infections were cured after retreatment. However, primary treatment with desipramine plus chloroquine, cleared the parasitemia in 2 of 2 Aotus, but did not cure the infections. This result indicated the potential of this drug combination to reverse chloroquine resistance in vivo. Desipramine plus chloroquine was toxic to Aotus and further evaluation discontinued.

J. WR 2173AL (BN:BK 20886), chlorpromazine

Chlorpromazine, a neuroleptic phenothiazine, is another drug used for in vivo trials of chloroquine resistance reversal. When administered with quinine against Vietnam/Smith/RE infections, suppression of parasitemia occurred. Retreatment of the infection with chlorpromazine plus chloroquine cured the infection in 2 of 5 Aotus.

Chlorpromazine (10.0 mg/kgx7) plus chloroquine (20.0 mg/kgx7), administered as a primary treatment, cured infection in 1 of 4 monkeys. Primary treatment with chlorpromazine (20.0 mg/kgx7) combined with chloroquine cured the infection in 3 of 6 Aotus. These drug regimens did not provoke overt toxicity in Aotus.

## K. WR 6379AF (BN:BM 1907), prochlorperazine

A second neuroleptic phenothiazine, prochlorperazine, was administered with chloroquine (20.0 mg/kgx7) against primary parasitemias of the Smith/RE strain. Prochlorperazine (10.0 mg/kgx7) plus chloroquine cleared parasitemia in 2 of 2 monkeys, but without cure. Retreatment with prochlorperazine (20.0 mg/kgx7) plus chloroquine cleared parasitemias. A recrudescence occurred in one monkey, and the infection was cured in the other animal.

Prochlorperazine (20.0 mg/kg) plus chloroquine cured the infection in 2 of 2 Aotus.

L. Toxicity evaluation of
WR 267634AC (BN:BM 01916), ketotifen
WR 235917AB (BN:BL 08170), cyproheptadine

Two tricyclic antihistamines, ketotifen and cyproheptadine, are to be evaluated for their capacity to reverse chloroquine-resistance in vivo. Prior to the initiation of this experiment, the toxicity of each of these drugs plus chloroquine (20.0 mg/kgx7) was evaluated in Aotus (cured of malaria infection). A 10.0 and 20.0 mg/kg(7) dose of ketotifen and cyproheptadine, each with chloroquine, did not evoke overt toxicity and no animal lost a significant amount of body weight.

M. WR 267634AC(BN:BM 01916), ketotifen

This tricyclic antihistamine when administered at doses of 10.0 and 20.0 mg/kg(x7) had no activity against Vietnam Smith/RE parasitemias. A dose of 10.0 mg/kg plus chloroquine (20.0 mg/kgx7) had no effect upon the primary parasitemia in one Aotus and suppressed the parasitemia in one Aotus.

Ketotifen administered at 20.0 mg/kg plus chloroquine (20.0 mg/kg) suppressed the parasitemia in 2 of 2 monkeys.

Retreatment with 20.0 mg/kg of ketotifen plus chloroquine cleared parasitemia, without cure, in 2 of 2 Aotus. When a dose of 40.0 mg/kg of ketotifen plus chloroquine (20.0 mg/kg), was used for retreatment, the infections were cured in 3 of 3 monkeys.

N. WR 035917 (BN:BL 08170), cyproheptadine

Cyproheptadine, a tricyclic antihistamine, when administered alone (10.0 and 20.0 mg/kg) and, at these doses, in combination with chloroquine (20.0 mg/kg), had no effect upon primary parasitemias of the Vietnam Smith/RE strain of P. falciparum.

Retreatment with a dose of 20.0 mg/kg of cyproheptadine plus chloroquine cleared the parasitemias, without cure, in 4 of 4 Aotus. Retreatment with cyproheptadine (40.0 mg/kg) plus chloroquine cured the infection in 2 of 2 monkeys.

O. WR 268766AA(BN:BM 05245), combretestatin

This compound at a dose of 10.0 and 20.0 mg/kg, plus chloroquine, had a suppressive effect only upon parasitemias of the Vietnam Smith/RE strain.

P. 87/209, aryl-amine

This compound administered for 7 days at a dose of 10.0 mg/kg plus chloroquine (20.0 mg/kg) suppressed parasitemias in 2 of 2 Aotus. A dose of 20.0 mg/kg of 87/209 plus chloroquine cleared the parasitemia in 1 of 2 Aotus, but the infection was not cured.

Q. WR 2158AJ(BN:BL 50610), promethazine

Promethazine (Phenergan) is an antihistamine. When a dose of 10.0 mg/kg plus chloroquine was administered for 7-days, the parasitemia was cleared in 1 of 2 Aotus. A dose of 20.0 mg/kg of promethazine plus chloroquine cleared the parasitemia in 1 of 2 monkeys, but without cure.

R. Conclusions

When the studies to reverse chloroquine resistance in vivo were initiated, there was doubt that the high concentration of calcium channel blockers required for in vivo reversal could be obtained in vivo and still sustain host viability. This proved to be true with most of the channel blockers evaluated. Desipramine met the desideratum of clearing primary parasitemias, but did not cure primary infections.

Other drugs have been evaluated recently in pilot studies to determine their capacity to reverse chloroquine-resistance in vivo. None of these drugs - ketotifen, cyproheptadine, combretestatin, and an aryl-amine - had activity against primary parasitemias of the Vietnam Smith/RE strain. Promethazine plus chloroquine cleared parasitemias, but did not cure infections.

Two neuroleptic phenothiazines, chlorpromazine and prochlorperazine, appear to have fulfilled the desideratum of in vivo chloroquine resistance, viz. administration (with chloroquine) in a defined treatment course against the primary ascending parasitemia followed by parasite clearance and infection cure. Additional studies are planned with these drugs. The potential use of chlorpromazine plus chloroquine to cure drug resistant P. falciparum infections in man is of significance to malaria chemotherapy.

# EVALUATION OF THE ANTIMALARIAL EFFICACY OF FOUR ARTEMISININ DERIVATIVES

## A. Introduction

An herb, qinghao (Artemisia annua L.), has been used in China for more than 400 years against the chills and fever of malaria (6). The active antimalarial principal of the herb has been identified as a 15-carbon sesquiterpene lactone endoperoxide and named artemisin. Studies in China with patients infected wtih P. falciparum or P. vivax showed that artemisin, an oil soluble derivative (artemether), and a water soluble derivative (artesunate) possessed significant antimalarial activity. Synthesis and selection of new artemisinin derivatives yielded an oil soluble ethyl ether derivative, arteether, and a water soluble derivative, sodium artelinate. These two newly synthesized derivatives, and artemether and artesunate were selected for comparison of their antimalarial efficacy against infections of the multi-drug resistant Vietnam Smith/RE strain of P. falciparum in Aotus.

B. Limited toxicity evaluation

As no studies with these four artemisinin derivatives had used Aotus, it was necessary to evaluate the toxicity of at least the highest dose to be administered for antimalarial evaluation. Drug toxicity was monitored by body weight and overt symptoms in monkeys cured of malaria.

1. WR 255131AE (BN:BL 48816), arteether
   WR 254986AB (BN:BL 26767), artemether

   Each of these drugs, dissolved in sesame oil, was administered at a dose of 64.0 mg/kg(i.m.), q.12hx3 doses to one monkey. Neither drug provoked overt toxicity nor was there significant body weight loss attributed to the drugs.

2. WR 255663AG (BN:BL 54038)
   WR 255663AH (BN:BL 55866), artelinate

   Sodium artelinate, a water soluble artemisinin derivative, was administered intravenously in the first toxicity trial. Using a 30 mg/ml stock solution, a dose of 64.0 mg/kg, q.6hx3 doses, was lethal for one monkey. This dose, administered in a 10.0 mg/ml stock solution, was not lethal, although hypotonia occurred after doses 2 and 3. Lower doses did not produce overt toxicity.

3. WR 256283AA (BN:BL 28556), artesunate

   This water soluble derivative, sodium artesunate, was converted to artesunic acid immediately prior to administration by the addition of sodium bicarbonate (5%). Intravenous administration of 64.0 mg/kg, q.6hx3 doses, produced no overt toxic reaction.

C. Antimalarial assessment of artemisinin derivatives

1. WR 255131AE, arteether

Arteether was dissolved in sesame oil and administered intramuscularly, q.12hx3 doses, at doses ranging from 0.25 to 64.0 mg/kg. In a total of 29 primary treatments, parasitemias were cleared in 25 monkeys, resulting in infection cure of 15 of 28 (54%) Aotus. Repeat treatments were at doses ranging from 1.0 to 32.0 mg/kg. Parasitemias were cleared in 29 of 30 treatment courses, and infections cured 21 of 30 treatments. Overall, 36 of 58 treatments (62%) resulted in infection cure.

2. WR 254986AB, artemether

This artemisinin derivative, dissolved in sesame oil, was administered intramuscularly, q.12hx3 doses, at doses ranging from 0.25 to 64.0 mg/kg. Primary treatments cleared parasitemias in 24 of 29 monkeys, and cured infection in 19 of 29 (66%) Aotus. Repeat treatments, at doses of 1.0 to 16.0 mg/kg, cleared parasitemia in 21 of 21 animals, and cured infection in 16 of 20 (80%) treatments. Overall, artemether cured 35 infections in 49 treatments (71%).

3. WR 256283AA, artesunate

Pilot antimalarial evaluation of sodium artesunate used a dose of 64.0 mg/kg, in each of five Aotus. When administered intravenously, q.6hx3 doses, the parasitemia was suppressed. Two monkeys received the drug intravenously, q.12hx3 doses - the parasitemia in one animal was cleared (with recrudescence), and one animal died one day post treatment of drug toxicity. Intramuscular administration, q.12hx3 doses, cured the infection in one Aotus, and one monkey died (drug toxicity) on day 4 post-treatment. Artesunate was then administered at doses of 16.0, 32.0, and 64.0 mg/kg, intramuscular, q.12hx3 doses. Primary treatments cleared parasitemia in 9 of 9 Aotus, but no infection was cured. Recrudescences were re-treated with either arteether or artemether.

4. WR 255663AH, artelinate

A dose of 64.0 mg/kg was used for the pilot evaluation of sodium artelinate. When administered

(in primary treatments) intravenously (q.6hx3 doses), two animals died of drug toxicity, the parasitemias in one monkey was suppressed, and parasitemia in one animal was cleared, with recrudescence. Intravenous administration (q.12hx3 doses) cleared parasitemia in 3 of 3 Aotus, but with recrudescence. Intramuscular administration (q.12hx3 doses) cleared parasitemia, with recrudescence, in 2 of 2 Aotus.

Additional antimalarial evaluation used intramuscular administration (q.12hx3 doses) as follows: doses of 16.0 and 32.0 mg/kg each cleared parasitemia in 2 of 3 monkeys, without infection cure; a dose of 64.0 mg/kg cleared parasitemia in 2 of 4 monkeys, but with recrudescences. Recrudescences in all cases were re-treated with either artemether or arteether.

## D. CONCLUSIONS

Both oil-soluble artemisinin derivatives, arteether and artemether, cured infections of the multi-drug resistant Vietnam Smith/RE strain of P. falciparum in Aotus. Infection cures were obtained at drug doses well-tolerated by Aotus. There is an indication that artemether is somewhat more effective than arteether in curing these infections: artemether cured 71%, while arteether cured 62%.

At tolerated doses in the Aotus model, neither water soluble artemisinin derivative, artesunate or artelinate, cured infections of the Smith/RE strain. Parasitemias were cleared, but with recrudescence.

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# LIST OF PERSONNEL WHO RECEIVED SUPPORT UNDER CONTRACT

R. N. Rossan, Ph.D.
A. Escajadillo, DVM
N. Obaldia III, DVM
L. Martinez
F. Durham
G. Cisneros
M. Gormaz
M. Brewer
K. Thompson
T. Gonzalez
R. De Gracia
C. Marin
O. Bostic
B. Zaldivar
O. Guerrero
R. Rojas, Jr.

FIGURE 1

SCHEMA FOR DRUG EVALUATION AGAINST

PLASMODIUM FALCIPARUM

INDUCED INFECTIONS IN AOTUS LEMURINUS LEMURINUS