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REPORT NUMBER 3

Phase I Clinical Testing Antimalarial Drugs

Annual Report

LEVEL III

A046648

Richard C. Reba, M.D.

March 1978

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD 17-75-C-5036

BIO-MED, Inc.
110 Irving Street, N.W.
Washington, D. C. 20010

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

From February 1977 through January 1978, the following projects were undertaken under Contract DAMD 17-75-C-5036:

Mefloquine 500 mg weekly for 52 weeks was administered to 50 subjects to evaluate its safety and tolerance. Serial blood samples were obtained from each subject for the performance of drug assay at the Walter Reed Army Institute of Research. Two subjects had telogen effluvium (temporary scalp hair loss). No other

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20. Abstract (Continued)

changes in physical examination or abnormalities in laboratory values were noted that could be attributed to drug ingestion.

A comprehensive study of the pharmacokinetics of mefloquine hydrochloride in 20 subjects was completed. Samples for drug assay were obtained during an 84-day period. The data obtained will provide important information to guide the prophylactic and therapeutic use of this valuable antimalarial drug.

Mefloquine methanesulfonate had been shown in pre-clinical studies to be well tolerated and effective as an intravenou preparation. We initiated a study to determine the safety, tolerance, and pharmacokinetics of this drug but discontinued the study when 6 of the first 8 subjects developed periphlebitis and phlebitis about the infusion site; the complication ensued during or immediately after the infusio of the drug. This intravenous formulation should not be used in subsequent clinical tests.

WR 184,806·H_3PO_4 is a substituted pyridine methanol which was shown to be well tolerated in a short term safety and tolerance study performed during the first year of this contract. A pharmacokinetic study performed using a single oral dose administration was completed on 20 subject Samples for drug assay were collected serially during a 15-day period.

WR 30090·HCl had been shown in previous studies to be inadequately absorbed following oral administration thereby limiting its potential clinical application; a new formulation WR 30090 (oleic acid) showed an eight fold increase in absorption in animal studies. A clinical cross-over study was undertaken to compare the absorption kinetics of the 2 formulations in man but was suspended because of difficulties encountered with the drug assay.

The Walter Reed mefloquine hydrochloride preparation has undergone extensive phase I and II clinical testing including the short and long term safety and tolerance studies mentioned earlier. Detailed pharmacokinetic studies have also been done. We have just completed a clinical study to compare the bioavailability of the Walter Reed and Hoffmann-LaRoche formulations in collaboration with Walter Reed Army Institute of Research and the World Health Organization.

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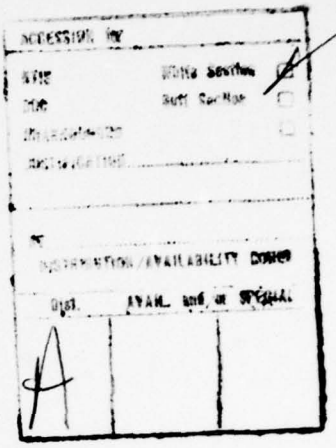

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110 Irving Street, N.W.
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SUMMARY

BIO-MED, Inc. continued Phase I Clinical Testing of antimalarial drugs during this third contract year. The drugs were developed by the antimalarial drug program of the U. S. Army Medical Research and Development Command. Testing was performed at the Washington Hospital Center, Washington, D. C. on subjects recruited from the Washington, D. C. metropolitan area, under contract DAMD 17-75-C-5036.

The safety and tolerance of mefloquine 500 mg weekly over 52 weeks was determined in a study involving 50 subjects. A comprehensive study of the pharmacokinetics of mefloquine was also undertaken. Mefloquine methanesulfonate was shown to be unsafe when administered parenterally because of local tissue irritation. A concomitant decrease in serum haptoglobin was noted with infusion of this drug which is at present unexplained. A pharmacokinetics study of WR 184,806·H_3PO_4 (involving 20 subjects) was completed. A study to evaluate the gastro-intestinal absorption kinetics of WR 30090 (oleic acid) was started but suspended pending the resolution of difficulties with the drug assay. A study to compare the bioavailability of the Walter Reed and Hoffmann-LaRoche formulations of mefloquine hydrochloride has just been completed. This study led to close collaboration amongst Walter Reed Army Institute of Research, the World Health Organization and BIO-MED, Inc.

FOREWORD

Under terms of the contract, Phase I Clinical Testing of antimalarial drugs was performed at the Washington Hospital Center. All protocols were processed by the contractor's Organizational Review and Human Subject (Human Use) Committees prior to submission to the Washington Hospital Center Research Committee.

All protocols were processed and approved by the Washington Hospital Center prior to implementation. The Washington Hospital Center is approved for performance of clinical research by the Department of Health, Education, and Welfare. (DHEW Assurance No. GO 180)

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OBJECTIVES

General: To continue Phase I Clinical Testing of antimalarial drugs at the Washington Hospital Center using methodology which provides maximum protection for the health and welfare of the participating subjects.

Specific:

1. To determine the safety, tolerance, and pharmacokinetics of intravenously administered mefloquine methanesulfonate.

2. To determine the pharmacokinetics of orally administered WR 184,806·H_3PO_4.

3. To determine the pharmacokinetics and compare the bioavailability of WR 30090·HCl and WR 30090 (oleic acid).

4. To determine the pharmacokinetics of orally administered mefloquine.

5. To compare the bioavailability and pharmacokinetics of WR 142,490·HCl and mefloquine hydrochloride·HLR.

6. a) To determine the safety and tolerance of mefloquine hydrochloride administered as weekly oral doses for 52 weeks.
   b) To determine the pharmacokinetics of chronic oral administration of mefloquine hydrochloride.

METHODS AND RESULTS

1. Intravenous Mefloquine Methanesulfonate:
Eight subjects were divided into 2 groups of 4. One group received 0.715 mg/Kg BW and the other 1.73 mg/Kg BW of mefloquine methanesulfonate. The drug was infused intravenously over 60 minutes as a 2 mg/ml solution in 5% dextrose. The study was terminated because of local periphlebitis and phlebitis occurring in 6 of the first 8 subjects. We also noted an unexplained decrease in serum haptoglobin levels after the infusion. The occurrence of local irritation with the drug infusions precludes further clinical tests with this formulation.

2. Pharmacokinetics WR 184,806·H_3PO_4:
The pharmacokinetics of this substituted quinoline methanol antimalarial drug was studied in 4 groups of 5 subjects each. Tablet formulations at 250, 500, 1000 mg dose levels and a cherry syrup formulation containing 250 mg of drug were used. The drug was administered orally to each subject and venous blood obtained before and serially after supervised drug ingestion. Sequential urine samples for drug assay were collected. Transient non-incapacitating light-headedness was reported by 2 of 5 subjects that received 1000 mg of WR 184,806·H_3PO_4. This symptom may have been related to the drug ingestion. At lower dose levels 2 subjects reported a mild headache which was most likely unrelated to the drug ingestion. The mean drug half-life was 25.3 hours, the mean absorption half-time 0.89 hours and the mean t-max was 4.29 hours.

3. Comparative Bioavailability of WR 30090·HCL and WR 30090 (oleic acid):
The clinical usefulness of the HCL derivative of this drug has been limited by its poor gastro-intestinal absorption. A new formulation WR 30090 (oleic acid) showed an eight fold increase in absorption from the gastro-intestinal tract in animal studies. A clinical study to test this preparation WR 30090 (oleic acid) in humans was initiated. This study is now suspended until difficulties with the drug assay are resolved.

4. Mefloquine Pharmacokinetics Following Oral Administration:
Data on human clearance of mefloquine is sparse and suggests a prolonged half-life of 15-25 days. A clinical study was therefore undertaken to investigate the pharmacokinetics of this drug after oral ingestion. Four groups of 4 subjects each received orally either 250, 500, 1000, or 1500 mg mefloquine. Another group of 4 subjects received orally 500 mg mefloquine as an aqueous suspension. The results of this study have been submitted as a report which has not yet been approved by the Clinical Monitor.

5. Comparative Bioavailability of WR 142,490·HCl and Mefloquine Hydrochloride·HLR:
HLR is a commerical pharmaceutical firm with a mefloquine preparation designated mefloquine hydrochloride·HLR. Having completed the clinical study (report in preparation), we sought to compare the bio-availability and pharmacokinetics of WR 142,490·HCl and mefloquine hydrochloride·HLR. We used the classical two-way balanced cross-over design for this comparison.

Three groups of 4 subjects each participated in this study. Blood and urine were collected for drug assay for 21 days following the administration of each drug. A 4-week "wash out" interval was allowed between drug administrations.

As a result of mefloquine testing done under this contract and the phase II testing of the drug done elsewhere, we now are in collaboration with the World Health Organization and Walter Reed Army Institute of Research (WRAIR) to compare the bioavailability of a mefloquine preparation manufactured by a pharmaceutical firm to a Walter Reed formulation. The increased need for pharmacokinetic data has led to close collaboration with the Department of Pharmacology, WRAIR, where the drug assays are performed.

Although these studies require prolonged subject compliance and frequent venipuncture for blood samples with their attendant rising costs, the information gained will permit the design of more reasonable and productive clinical study protocols. In addition to providing maximum information these protocols will also minimize the risks to the subjects.

6. Chronic Safety and Tolerance, WR 142,490

Mefloquine 500 mg weekly was administered over 52 weeks to 50 subjects to evaluate its safety and tolerance. Serial blood samples were obtained from each subject for the performance of drug assay at the Walter Reed Army Institute of Research. Two subjects had telogen effluvium (temporary scalp hair loss). No other changes in physical examination or abnormalities in laboratory values were noted that could be attributed to drug ingestion.

CONCLUSIONS

The third contract year has been characterized by an increasing number of detailed pharmacokinetic studies in addition to continuation of initial safety and tolerance testing of new antimalarial drugs.

The emphasis on mefloquine testing performed under this contract in addition to the phase II testing of the drug elsewhere, has culminated in a collaborative project with the World Health Organization and Walter Reed Army Institute of Research to compare the bioavailability of a preparation manufactured by a pharmaceutical firm with the Walter Reed formulation. It is anticipated that this drug will be manufactured by the pharmaceutical firm for additional clinical testing.

The increased emphasis on pharmacokinetics studies has required close collaboration between the Department of Pharmacology, Walter Reed Army Institute of Research where the drug assays are performed and by BIO-MED, Inc. Although these studies required prolonged subject compliance and frequent blood collection to obtain samples for drug assay with attendant increased costs, the information derived permits more logical and productive protocol design for other clinical studies. The net result is to limit clinical testing and exposure of subjects to risk to protocols which will provide maximum information.

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EXPERIMENT NO. 5: WR 142,490·CH_3SO_3H: MEFLOQUINE METHANESULFONATE SAFETY, TOLERANCE, AND PHARMACOKINETICS OF INTRAVENOUS ADMINISTRATION

STUDY SUMMARY

INTRODUCTION:

Patients who are seriously ill with P. falciparum malaria require immediate treatment with an effective blood schizonticide. For many years parenterally administered chloroquine or quinine has been used for this purpose. However, the development of drug resistance has precluded the use of chloroquine for this purpose in certain areas of the world. Intravenously administered quinine has retained its ability to suppress parasitemia, though some resistance to this drug has also been documented. In addition, intravenous quinine is not tolerated in some individuals because of serious toxicity. For these reasons an alternative parenteral drug is being sought.

Mefloquine hydrochloride administered orally is a highly effective drug against multidrug-resistant strains of P. falciparum. Therefore an intravenous preparation was formulated. Mefloquine methanesulfonate (WR 142,490·CH_3SO_3H) is a considerably more soluble salt form than mefloquine hydrochloride. The drug is an oderless, slightly bitter white powder, formulated in 50 ml vials, containing 250 mg of the salt, as a freeze-dried preparation. The sources and preparation of drug components, manufacturing methods, controls and manufacturing facilities are detailed in the IND.

Pre-clinical studies suggested mefloquine methanesulfonate would be a safe and effective intravenous preparation for the treatment of malaria in human subjects.

This study, designed to test the safety, tolerance, and pharmacokinetics following intravenous administration, was terminated when symptoms and signs of local irritation occurred in six of the first eight subjects participating in the study.

METHODS AND MATERIALS:

Subject Selection:

Eight subjects were recruited from the Washington, D.C. metropolitan area. Candidates were hired as temporary employees for study purposes.

Candidates for employment underwent qualifying examinations to obtain the subjects for study. The medical evaluation included a comprehensive history and physical examination, chest x-ray, electrocardiogram, urinalysis, white blood cell and differential count, red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet count, glucose, BUN, creatinine, Na^+, K^+, Cl^-, CO_2, uric acid, total protein, albumin, globulin, calcium, phosphate, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, LDH, total bilirubin, G6PD.

Qualified candidates were presented with a complete written explanation of the background and procedures to be used in the study and all details of the protocol as it involved the individual subjects. Candidates were interviewed in a group and individually in the presence of an investigator and member of the Human Use Committee. Each participant was given the opportunity to ask questions. Following this, the Consent Form (page 10) was read and signed in the presence of a witness, investigator, and member of the Human Use Committee.

The subjects were housed in a controlled environment on Nursing Unit 5-W at the Washington Hospital Center for 24 hours before drug administration and for 48 hours afterward.

Drug Administration:

1. The drug was supplied in vacuum-stoppered vials as a sterile freeze-dried powder. Each vial contained mefloquine methanesulfonate, 250 mg. Thirty milliliters of sterile water for injection, U.S.P. was injected rapidly into the vial to avoid caking and speed dissolution. The resulting solution was then added to 5% Dextrose Injection U.S.P. q.s. to 125 ml for each 250 mg (vial) drug added. This final solution contained 2 mg/ml of mefloquine methanesulfonate in 5% Dextrose for intravenous administration. The volume of infusion for each subject was calculated by multiplying his body weight in kilograms by the mg/kg dose scheduled and dividing by 2.

2. The study schedule: There was an interval of one week between study periods.

Study Period	No. of Subjects	Incremental Increase	Total Dose (mg/70kg)	Dose (mg/kg)	Rate* (mg/kg/min)
1	4		50	0.715	0.012
2	4	2.5	120	1.73	0.029

*The mefloquine methanesulfonate was given at a constant rate during a 60 minute interval under the direct supervision of a physician investigator. Electrocardiographic monitoring with a cathode ray oscilliscope was used for five hours from the start of the infusion.

At 6 a.m. on the day of infusion the subjects drank 360 ml of Sustacal (Mead Johnson product) containing a total of 360 calories. At approximately 9 a.m. the infusion was started by a member of the investigating team.

Measured amounts of water were taken ad lib until 5 p.m. at which time the subjects returned to the regular diet they selected from the hospital menu.

The schedule for clinical and laboratory evaluations of the subjects is outlined below:

SCHEMATIC STUDY PLAN

INTRAVENOUS ADMINISTRATION - WR 142,490·CH_3SO_3H

DAY	0*	1*	2*	3	7	9	11	15
Dose		X						
Physical Exam	X		X		X			
Interview	X	X	X	X	X			
Complete Vital Signs	X	X	X	X	X			
ECG (Rhythm Strip LII)	X	X	X		X			
Laboratory Tests+	X		X	X	X			
PA Chest X-Ray	X	X			X			
Drug Assay (Selected Subjects)		X	X	X	X	X	X	X

*Controlled Environment

+Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, CO_2, Uric Acid, T. Protein, Albumin, Globulin, Calcium, Phosphate, Cholesterol, Triglyceride, Alka. Phos., SGOT, SGPT, LDH, T. Bilirubin, CBC (differential and indices), Platelets, Urinalysis, Serum Haptoglobin. Chest X-Ray day 1 end of infusion; six foot portable.

For drug assay, 10 ml venous blood was obtained immediately before drug administration and every one-half hour for 4 hours, hourly for 4 hours, every 2 hours for 4 hours, daily for 14 days, and on days 21, 28, and 35 after drug administration. Approximately 310 ml of blood was required from each subject.

The schematic presents the schedule for hematologic and biochemical determinations. A staff nurse obtained 20 ml venous blood while the subject was fasting before breakfast. Five milliliters of whole blood was anticoagulated for the hematologic determinations.

RESULTS:

Symptoms and Physical Findings:

Eight subjects participated in the study, in two groups of four; individuals in group I received 0.715 milligrams per kilogram, and those in group II received 1.73 milligrams per kilogram over approximately one hour.

Two of the four subjects in group I complained of "burning" or "cramping" above the site of drug infusion in the forearm extending to the antecubital fossa soon after the beginning of drug infusion (i.e. within 15 minutes). Erythema of the forearm extending to the antecubital fossa which was noted during the infusion resolved spontaneously in both subjects within 30-45 minutes. One subject developed a palpable non-tender cord extending along the vein from the infusion site in the forearm to the antecubital fossa (nine centimeters); this subject also developed a palpable axillary node (right axilla), soft and non-tender, which was noted on day 35 and resolved spontaneously. The venous cord in this subject was barely palpable by 10 weeks after infusion. The other subjec from this group who complained of discomfort had no associate physical findings.

All four subjects in group II developed sensations of "cramping", "contraction", "pain", and "burning", starting at the site of infusion and generally extending up the forearm to the antecubital fossa, and beginning soon after the initiatio of drug infusion. One subject complained of a "flushed feeling" in the skin over his upper chest without other complaint or other physical findings. Three of the four subjects devel oped a palpable venous cord, with the same three subjects showing erythema over the course of the vein which resolved in minutes to hours. A vascular consultant described the fi ings in the forearm and the antecubital space of all four sub jects as consistent with acute superficial venous thrombophl bitis.

Laboratory Values:

In all subjects there were various randomly distributed laboratory abnormalities, which were not thought to be drug induced or related. None of these abnormalities were considered to be pathologic in view of the associated history and clinical examination. One subject developed an elevated SGOT, SGPT, and LDH serum levels, detected on day 35 with spontaneous resolution, the etiology of which was not determined.

A decrease in serum haptoglobin levels 24 hours after the infusion compared with baseline values was noted in all eight subjects, although in general the haptoglobin levels remained within 95% confidence limits. Hemolysis was not otherwise suggested by changes in hematocrit, hemoglobin, or LDH levels, and the exact significance of the changes in haptoglobin is uncertain. It may be related to the thrombosis with associated local inflammation and low grade hemolysis.

Electrocardiograms:

No changes occurred in electrocardiographic rhythm strips examined after drug administration in any subject.

DISCUSSION:

The drug was not tolerated in six of the eight subjects infused. The occurrence of symptoms and signs in two of four subjects at the lower rate of infusion and uniform intolerance when the rate was doubled for group II suggests a relationship to the rate of infusion (i.e. amount over time). A lower drug concentration and slower delivery rate might be tolerated, but may not be clinically feasible.

The haptoglobin changes are of interest but were not associated with evidence of significant hemolysis, and are difficult to explain.

CONCLUSIONS AND RECOMMENDATIONS:

In this study six of eight subjects (all received mefloquine methanesulfonate intravenously in a concentration of 2 milligrams per milliliter) developed findings consistent with vein wall inflammation.

At this concentration and rate of infusion the drug appeared to cause significant venous wall irritation, inflammation, and thrombosis.

Additional findings of changes in the serum haptoglobin may be significant, though the exact pathophysiologic explanation has not been determined.

The current formulation of mefloquine for intravenous use is not considered practical for further investigation at the current concentration.

Development of a better tolerated formulation is recommended.

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EXPERIMENT NO. 6: WR 184,806·H_3PO_4

PHARMACOKINETICS FOLLOWING

ORAL ADMINISTRATION

STUDY SUMMARY

INTRODUCTION:

WR 184,806·H_3PO_4 is the code name for a substituted quinoline methanol proposed for antimalarial clinical trials. It is a white homogenous powder which is relatively insoluble in water. It is expected to be better absorbed and its biological half-life to be shorter than mefloquine and therefore potential problems of drug accumulation during multiple dosing should be minimized.

Thirty-eight young healthy adult male subjects have been administered single and multiple doses of WR 184,806·H_3PO_4 by the oral route in acute safety and tolerance studies at the Washington Hospital Center, Washington, D.C. The drug was well tolerated up to 1000 mg in single doses and to 400 mg when administered every 8 hours for 72 hours in multiple dose studies. Symptoms of intolerance included primarily light-headedness with associated difficulties concentrating and focusing, insomnia, unusual dreams, and less commonly, mild gastrointestinal symptoms. In all cases the symptoms were mild and of less than 24 hours duration.

This study was performed to determine sequential blood concentrations and urinary excretion of WR 184,806·H_3PO_4 following oral administration of single doses.

METHODS AND MATERIALS:

Subject Selection:

Twenty subjects were recruited from the Washington, D.C. metropolitan area. Candidates were hired as temporary employees for study purposes.

Candidates for employment underwent qualifying examinations to obtain the subjects for study. The medical evaluation included a comprehensive history and physical examination, chest x-ray, electrocardiogram, urinalysis, white blood cell and differential count, red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet count, glucose, BUN, creatinine, Na^+, K^+, Cl^-, CO_2, uric acid, total protein, albumin, globulin, calcium, phosphate, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, LDH, total bilirubin, G6PD.

Qualified candidates were presented with a complete written explanation of the background and procedures to be used in the study and all details of the protocol as it involved the individual subjects (pages 7 through 9). Candidates were interviewed in a group and individually in the presence of an investigator and a member of the Human Use Committee. Each participant was given the opportunity to ask questions. Following this, the Consent Form (page 10) was read and signed in the presence of a witness, investigator, and member of the Human Use Committee.

The subjects were housed in a controlled environment on Nursing Unit 5-W at the Washington Hospital Center for 24 hours before drug administration and for 48 hours thereafter.

Drug Administration:

Four groups of 5 subjects each, designated Groups 25, 26, 27, and 28 were studied. The subjects in Group 25 were administered a single 250 mg oral dose of WR 184,806·H_3PO_4 and those in Groups 26 and 28 were administered a single 500 mg and 1000 mg oral dose of WR 184,806·H_3PO_4 respectively. Group 27 subjects received 250 mg WR 184,806·H_3PO_4 in aqueous solution.

At 6:00 a.m. on the day of dosing the subject drank 360 ml of Sustacal (Mead-Johnson product) containing a total of 360 calories. At approximately 8:00 a.m. the drug was swallowed in the presence of a member of the investigating team.

Measured amounts of water were taken ad lib until 5:00 p.m. at which time the subjects were permitted their regular diet.

The lot of formulated WR 184,806·H_3PO_4 used was Lot B-514.

Specimen Collection:

Specimen collection for WR 184,806·H_3PO_4 Drug Assay was as follows: 10 ml venous blood were obtained prior to drug administration and following dosing at 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, and 360 minutes; at 8, 12, 24, 36, 48, 72, and 96 hours; and on days 7, 9, 11, and 15. The specimens were stored at minus 20°C in teflon capped glass tubes pending transport to Department of Pharmacology, WRAIR, for drug assay. Twenty-four hour urines were collected for drug assays on days 0, 1, and 2 from 0801 hours to 0800 hours. Storage and transport as directed by WRAIR. Blood specimens were stored at minus 20°C. in teflon capped glass tubes and transported in dry ice.

Clinical and Laboratory Evaluation:

The following schedule was used:

SCHEMATIC STUDY PLAN

WR 184,806·H_3PO_4 - PHARMACOKINETICS - ORAL DOSE

DAY	0*	1*	2*	3	4	7	9	11	15
Dose		X							
Physical Exam	X		X			X			
Interview	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X		X			
ECG (Rhythm Strip)	X		X						
Laboratory Tests+	X		X	X		X			
Blood for Drug Assay		X	X	X	X	X	X	X	X

*Controlled Environment

+Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, CO_2, Uric Acid, T. Protein, Albumin, Globulin, Calcium, Phosphate, Cholesterol, Triglyceride, Alka. Phos., SGOT, SGPT, LDH, T. Bilirubin, CBC (differential and indices), Platelets, Urinalysis. Additional studies were done as clinically indicated.

RESULTS:

Symptoms:

Two of the 5 subjects administered 1000 mg WR 184,806·H_3PO_4 developed symptoms: subject Code No. 116 felt "woozy" for 1 hour starting 30 minutes after drug ingestion. Subject Code No. 119 was light-headed for 1 hour starting 8 hours after drug administration. Two of the remaining 15 subjects receiving WR 184,806·H_3PO_4 experienced mild headache of 4 hours duration: Subject Code No. 104 receiving 250 mg had onset seven hours after dosing and Subject Code No. 106 had onset 6 hours after ingesting 500 mg WR 184,806·H_3PO_4. No other potentially drug related symptoms were observed.

Physical Findings:

No physical examination changes occurred potentially attributable to drug ingestion.

Laboratory Values:

The laboratory values of the sample population for Antimalarial Drug Project studies (n>100) excluding post drug dosing values were used to establish the normal range ($\bar{x}$ + 2 SD) as presented (BMI-ME4) on page 11. Each subject had multiple abnormal laboratory values reported. The abnormalities were marginal and inconsistent and no pattern was detected suggestive of drug association. Values beyond the normal range are tabulated on pages 12 through 15.

Trace proteinuria was detected on one occasion each prior to drug administration for Subject Code Nos. 108 and 118. Similarly Subject Code No. 119 had 8 wbc/hpf on one examination prior to drug ingestion. Subject Code No. 107 had abacterial urethritis manifested by 5-15 wbc/hpf and penile abrasions associated with vigorous sexual activity. Follow-up urinalysis was normal.

Electrocardiograms:

No change in electrocardiographic rhythm strips occurred following drug administration.

COMMENT:

It is possible that the mild and transient symptoms of lightheadedness in 2 of the 5 subjects receiving 1000 mg WR 184,806·H_3PO_4 were drug related. A drug association is possible but not probable for the mild headache occurring at lower dose levels (250 and 500 mg) in 2 of the twenty subjects.

No other observations of possible drug effect were made in the course of the study.

CONCLUSIONS:

WR 184,806·H_3PO_4 is a well-tolerated drug. Subject compliance was superior in reporting for specimen collection. Conclusions await report of drug assay values and their analysis.

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EXPERIMENT NO. 8: COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF WR 30090·HCl AND WR 30090·(OLEIC ACID)

STUDY SUMMARY

INTRODUCTION:

WR 30090·HCl has been shown to be an effective antimalarial in humans against chloroquine resistant strains of P. falciparum. However, poor absorption following oral administration has limited the usefulness of WR 30090·HCl as an antimalarial. Previous Phase I studies have demonstrated that the drug is well tolerated in volunteers given 1.4 gm/day orally for 10 consecutive days. A regimen of 230 mg three times daily for 6 days was about 90% curative against P. falciparum infections in U.S. soldiers in Southeast Asia. WR 30090·(Oleic Acid) was formulated to improve drug absorption following oral administration. Compared to the WR 30090·HCl formulation, WR 30090·(Oleic Acid) increased oral absorption by approximately 800% in beagle dogs. Both preparations of WR 30090 administered in single oral doses of 1,2,3, and 4 gm per kilogram to mice and rats were without apparent toxic effects.

A method for measuring the concentration of WR 30090 in blood by high pressure liquid chromatography was developed at the Walter Reed Army Institute of Research. Therefore, it appeared bioavailability and relevant pharmacokinetic studies were feasible. However, non-predictable difficulties with the assay methodology developed and the study was suspended after the initial dose levels.

This report includes results of the initial dose levels of a crossover study comparing the two formulations.

METHODS AND MATERIALS:

Subject Selection:

Four subjects were recruited from the Washington, D.C. metropolitan area. Candidates were hired as temporary employees for study purposes.

Candidates for employment underwent qualifying examinations to obtain the subjects for study. The medical evaluation included a comprehensive history and physical examination, chest x-ray, electrocardiogram, urinalysis, white blood cell and differential count, red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet count, glucose, BUN, creatinine, Na^+, K^+, Cl^-, CO_2, uric acid, total protein, albumin, globulin, calcium, phosphate, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, LDH, total bilirubin, G6PD.

Qualified candidates were presented with a complete written explanation of the background and procedures to be used in the study and all details of the protocol as it involved the individual subjects. Candidates were interviewed in a group and individually in the presence of an investigator and member of the Human Use Committee. Each participant was given the opportunity to ask questions. Following this, the Consent Form (page 10) was read and signed in the presence of a witness, investigator, and member of the Human Use Committee.

The subjects were housed in a controlled environment on Nursing Unit 5-W at the Washington Hospital Center for 24 hours before drug administration and for 48 hours thereafter.

DRUG ADMINISTRATION:

A cross over method using a two week interval between dosing was used as follows:

			STUDY WEEK			
			1	2	3	4
Group	Dose (mg)	Subj.				
A	*250 +35	1 2 3 4	HCl* HCl OA+ OA		OA OA HCl HCl	

*WR 30090·HCl
+WR 30090·(free base Oleic Acid)

The capsules were ingested at 8:00 a.m. in the presence of a research nurse. The following lots were used:

WR 30090·HCl - lot number E-310
WR 30090·(Oleic Acid) - lot number A-503

The schedule used for clinical and laboratory evaluation for each subject is presented in schematic form:

SCHEMATIC STUDY PLAN

WR 30090 - CLINICAL EVALUATION OF TWO FORMULATIONS:

FREE BASE AND HYDROCHLORIDE SALT

DAY	0*	1*	2*	3	4	7	9	11	14**
Dose		X							
Physical Exam	X		X			X			
Interview	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X		X			
ECG (Rhythm Strip LII)	X	X(after dosing)							
Laboratory Tests+	X		X						
Blood for Drug Assay++	X	X	X	X	X	X	X	X	X

*Controlled environment

+Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, CO_2 Uric Acid, T. Protein, Albumin, Globulin, Calcium, Phosphate, Cholesterol, Triglycerides, Alka. Phos., SGOT, SGPT, LDH, T. Bilirubin, CBC (differential and indices), Platelets, Urinalysis. Additional studies will be done as clinically indicated.

**Day 0 of 2nd study interval.

++Drug Assay: Each subject immediately prior to drug administration and after dosing at 30,60,90 minutes and at 2,3,4,5, 6,8,12,24,36,48,72 hours; and again on study days 7,9,11,& 14.

RESULTS:

Symptoms and Physical Findings:

None of the four subjects had symptoms or findings attributed to drug administration.

Only one subject had any symptoms. This subject (code no. 210) had constipation for two days following study entry for

administration of 30090·HCl and epigastric discomfort with belching upon study entry for administration of the oleic acid formulation. In both instances, the symptoms were mild and attributed to entry into a controlled environment.

Each subject had one or more physical findings not attributed to drug ingestion: Subject code no. 209 had bilateral paronychia unchanged during study. Subject code no. 210 had sunburn on study entry and recurrence of a periodontal infection on the last study day. Subject code no. 211 had pectus excavatum and finally subject code no. 212 had a mild left otitis externa unchanged during the study interval.

Laboratory Values:

The laboratory values of the sample population (n>100) excluding post drug administration values were used to establish the normal range ($x \pm 2$ SD) page 11. All values outside the normal range are included in the composite table on page 12. All subjects had multiple values beyond the range. However, the abnormalities were minimal and inconsistent and not considered to be significant or drug related.

Electrocardiograms:

No electrocardiographic rhythm strip changes occurred after drug administration in any subject.

COMMENT:

The tolerance of both formulations at the low dose levels used was expected. The 30090·HCl formulation has been tolerated at dose levels of 1.4 grams daily for 10 days in previous clinical testing and 1,2,3, and 4 grams per kilogram of each formulation has been administered to mice and rats without apparent toxic effect.

CONCLUSIONS AND RECOMMENDATIONS:

No adverse effect was attributable to the administration of 35 mg 30090·(Oleic Acid) or 250 mg 30090·HCl. The study was suspended because of inadequacy of the drug assay methodology. It is recommended that the study be renewed when drug assay techniques are perfected.

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FINAL REPORT

EXPERIMENT NO. 9:

MEFLOQUINE (WR 142,490·HCl): PHARMACOKINETICS FOLLOWING ORAL ADMINISTRATION

INTRODUCTION:

Mefloquine (WR 142,490·HCl), a substituted quinoline methanol, has been shown to be an effective single dose agent in the treatment of chloroquine resistant falciparum malaria. Its prophylactic effectiveness against chloroquine resistant falciparum malaria inoculated by infected mosquitoes has also been demonstrated. Four volunteers were fully protected following a single dose of 1000 mg of mefloquine when challenged two weeks later. The therapeutic effectiveness of this drug when administered as a single dose and the duration of prophylactic effect suggest that the kinetics of its distribution and elimination from the body are prolonged.

Early Phase I testing with mefloquine in humans established that the drug was well tolerated in single doses as high as 2000mg when administered orally in a slowly dissolving tablet form (250 mg each, Lafayette Lot #E443). Other studies with the drug administered as an aqueous suspension (in orange juice) or in a rapidly dissolving tablet form (250 mg each, INTERx Lot #B512) resulted in complaints of transient light-headedness and gastrointestinal intolerance (diarrhea, abdominal cramps, and occasional nausea and vomiting) following single doses of 1250 mg, 1500 mg, and 1750 mg.

The results of the present pharmacokinetic study will be used in conjunction with previous results to develop a new formulation designed to improve gastrointestinal tolerance and optimize bioavailability. The single doses used in this study were 250 mg, 500 mg, 1000 mg, and 1500 mg. It was anticipated that at the highest dose level, the subjects might have temporary non-incapacitating symptoms. However, the broad spectrum of doses was required in the experiment to establish whether or not the drug behaves according to first order pharmacokinetics. There are no other known immediate or long-term effects expected following the single doses used in this study.

An accurate analytic method for mefloquine in blood and urine specimens is now available in the laboratories of the Department

of Pharmacology at the Walter Reed Army Institute of Research. This provided an opportunity to develop detailed pharmacokinetic information on the parent compound in man. The sparse information previously available indicated that the drug does have a prolonged biologic half-life in man (15-25 days) which probably accounts for its efficacy as a single dose antimalarial. Following a single dose of 500 mg in one individual, a peak level of 0.710 ug/ml of whole blood was obtained 6 hours after dosing with an elimination half-life of 26 days. In a second individual administered a single 1500 mg dose of mefloquine, a peak level of 2.528 ug/ml of whole blood was obtained at 7 hours with an elimination half-life of 16 days. In both cases the pattern of blood concentration behaved according to a first order kinetic system consisting of a two compartment open model. Blood levels observed in several individuals taking 500 mg of the drug weekly for 52 weeks were at or below the level that would be predicted at plateau with an expected elimination half-life of 20 days (i.e. < 2.5 ug/ml).

With the foregoing information, this definitive protocol for study of the oral pharmacokinetics of mefloquine was designed. The sampling times for blood collection were selected to minimize the variance estimate of the slope of each of the 3 kinetic phases anticipated by the model. A total of 32 blood specimens (192 ml) were obtained for drug level analysis over a period of 12 weeks. The long duration of the study was required to provide specimens for analysis at 3-4 times the elimination half-life of the drug. This study was completed by all subjects admitted. The assay results are not available at the time of reporting.

The previous IND with Supplements and Clinical Summary were available at all processing committee meetings and were available at all times in the Office of the Clinical Director of BIO-MED, Inc.

METHODS AND MATERIALS:

Subject Selection:

Twenty healthy male subjects aged 21 to 45 years, weighing 55-90 kg and within 10% of their ideal body weight were employed for the study. They were recruited from the Washington, D.C. metropolitan area. Candidates were hired by BIO-MED, Inc., as temporary employees for study purposes.

Candidates for employment were screened to obtain the subjects for study. The medical evaluation included a comprehensive history, physical examination, chest X-ray, electrocardiogram, urinalysis, white blood cell and differential count, red blood

cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet count, glucose, BUN, creatinine, sodium, potassium, chloride, carbon dioxide, uric acid, total protein, albumin, globulin, calcium, phosphate, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, LDH, total bilirubin, and G6PD determinations.

Qualified candidates were presented with a complete explanation of the background and procedures to be used in the study and all details of the protocol as it involved the individual subjects. They were interviewed in a group and individually in the presence of an investigator and a member of the Human Subject Committee. Each participant was given the opportunity to ask questions. Following this, the "Explanation for Potential Subjects" was read and initialed by those participating. The formal consent form was signed in the presence of a witness, investigator, and member of the Human Subject Committee.

During the first five days of the study the subjects were housed in a controlled environment on Nursing Unit 5-W at the Washington Hospital Center. Thereafter they reported according to the protocol schedule (page 6).

Subject Assignment - Drug Administration:

Five groups, designated Groups I-V, were studied. Four subjects each in Group I, II, III, and IV were administered a single 250 mg, 500 mg, 1000 mg, or 1500 mg oral dose of WR 142,490·HCl (Lot #E555). The participants in Groups I-IV and the investigators did not know in advance the dose level at which the individual was being tested. Each individual received a total of 6 tablets, with placebo tablets added to the number of active drug tablets required at each dose level. In this way the assignment of subjects to dose levels was randomized. The individual drug containers were provided by the Department of Pharmacology at WRAIR and labelled Group I A-D, Group II A-D, Group III A-D, and Group IV A-D. Each subject received some active compound, but the subject and investigators did not know what dose he received. There were four individuals at each of the four different dose levels. A fifth group of four individuals (Group V) were given a single oral dose of 500 mg as an aqueous suspension. The drug was administered in the presence of a member of the investigating team.

At 6:00 AM on the day of dosing (Day 1) the subject drank 360 ml of Sustacal (Mead Johnson product) containing a total of 360 calories. The drug was administered at 8:00 AM. Water was taken

ad lib until 5:00 PM at which time the subjects had the regular diet selected from the hospital menu. Lunch was omitted on day 1 only.

Clinical and Laboratory Monitoring:

The clinical and laboratory evaluation of the subjects is outlined below:

SCHEMATIC STUDY PLAN

WR 142,490·HCl -- PHARMACOKINETICS -- ORAL DOSE

STUDY DAY	0*	1*	2*	3*	4*	7	84
Dose		X					
Physical Exam	X		X			X	X
Interview	X	X	X	X	X	X	X
Vital Signs	X	X	X	X		X	X
ECG (Rhythm Strip)	X		X				X
Laboratory Tests+	X		X	X		X	X

*Controlled Environment

+Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, Carbon Dioxide, Uric Acid, Total Protein, Albumin, Globulin, Calcium, Phosphate, Cholesterol, Triglyceride, Alkaline Phosphatase, SGOT, SGPT, LDH, Total Bilirubin, CBC (differential and indices), Platelets, and Urinalysis. Additional studies were done as clinically indicated.

The schedule for hematologic and biochemical blood tests is indicated. For these tests 20 ml venous blood were obtained by a staff nurse while the subject was fasting before breakfast. Five ml blood were taken for determination of white blood cell and differential count, red blood cell count, hematocrit, hemoglobin, MCV, MCH, MCHC, and platelet count. Fifteen ml of the venous blood specimen were centrifuged and the serum separated. The serum was divided into two samples. One sample was stored in the refrigerator as a "back-up" until the biochemical lab report was received: thereafter it was stored in the freezer until released by the investigator. The other sample was used on

the day obtained to determine the values for serum glucose, BUN, creatinine, sodium, potassium, chloride, carbon dioxide, uric acid, total protein, albumin, globulin, calcium, phosphate, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, LDH, and total bilirubin.

Drug Assay Specimen Collection:

Specimen collection for WR 142,490·HCl drug assay was as follows: six ml venous blood were obtained by a staff nurse prior to drug administration and following dosing at the times specified in the table on the next page. The specimens were collected in heparin rinsed syringes and stored at -20°C in teflon capped glass tubes pending transport to Department of Pharmacology, WRAIR, for drug assay. Twenty-four hour urines were collected for drug assays on Days 0, 1, 2, and 3, from 0801 to 0800 hours.

BLOOD SPECIMEN COLLECTION FOR DRUG ASSAY

DAY OF THE STUDY	SPECIMEN NUMBER	SPECIMEN TIME		BLOOD VOLUME ml
Day 1	1	0	Prior to Dosing	6
	2	1 hr	9:00 AM	6
	3	2 hr	10:00 AM	6
	4	4 hr	NOON	6
	5	6 hr	2:00 PM	6
	6	8 hr	4:00 PM	6
	7	10 hr	6:00 PM	6
	8	11 hr	7:00 PM	6
	9	12 hr	8:00 PM	6
	10	13 hr	9:00 PM	6
	11	15 hr	11:00 PM	6
Day 2	12	20 hr	4:00 AM	6
	13	24 hr	8:00 AM	6
	14	28 hr	NOON	6
	15	34 hr	6:00 PM	6
Day 3	16	48 hr	8:00 AM	6
	17	56 hr	4:00 PM	6
	18	63 hr	11:00 PM	6
Day 4	19	72 hr	8:00 AM	6
	20	76 hr	NOON	6
	21	80 hr	4:00 PM	6
HOME				
Day 5	22	96-98 hr	8:00-10:00 AM	6
Day 7	23	144-146 hr	8:00-10:00 AM	6
Day 8	24	168-170 hr	8:00-10:00 AM	6
Day 14	25	312-314 hr	8:00-10:00 AM	6
Day 21	26	480-482 hr	8:00-10:00 AM	6
Day 28	27	648-650 hr	8:00-10:00 AM	6
Day 35	28	816-818 hr	8:00-10:00 AM	6
Day 49	29	1152-1154 hr	8:00-10:00 AM	6
Day 63	30	1488-1490 hr	8:00-10:00 AM	6
Day 77	31	1824-1826 hr	8:00-10:00 AM	6
Day 84	32	1992-1994 hr	8:00-10:00 AM	6
				TOTAL 192 ml

RESULTS:

Symptoms and Physical Findings:

Symptoms are tabulated as Table I, page 16. Symptoms compatible with drug intolerance occurred at all dose levels. Symptoms were less frequent and less typical in subjects administered 250 and 500 mg mefloquine as compared with symptoms occurring at the 1000 and 1500 mg dose levels. Symptoms of light-headedness occurred in 3 of the 4 subjects receiving 1000 mg and may have been drug related in 2 subjects. The symptoms were mild and not incapacitating. The four subjects administered 1500 mg mefloquine all had symptoms attributed to drug ingestion manifested by combinations of malaise, fatigue, insomnia, headache, anorexia, nausea, light-headedness with difficulty focusing, abdominal cramps, and watery bowel movement. One subject (Code No. 186-B) had rotational vertigo of 2 hours duration starting 2 hours after drug ingestion. Light-headedness of a non-incapacitating nature persisted in this subject for six days and in another subject (Code No. 176-D) for five days.

No changes in physical findings attributed to drug ingestion occurred in any subject.

It should be noted that during the 84 day study interval, some subjects had symptoms and findings obviously not related to drug ingestion, primarily of a nose-throat-respiratory nature. These symptoms and signs are not tabulated, but are included in the Individual Final Summaries of the Appendix.

Laboratory Values:

The laboratory values of the sample population ($n > 100$) excluding post drug administration were used to establish the normal range ($x \pm 2$ SD) presented on page 19. All values outside the normal range are included in Table IIB, pages 20 through 23. All values in two subjects were within the normal range. Six subjects had consistent or recurrent values outside the normal range considered to be individual variants in the absence of other abnormalities suggesting an active disease process. One subject (Code No. 178-B) had a moderate elevation of SGPT day 0 through 7 with inconsistent elevation of SGOT. On day 84, SGOT and SGPT values were reported as 122 and 328 units respectively. Follow-up values were normal. The values outside the normal range for the remaining eleven subjects were marginal and inconsistent.

Electrocardiograms:

No changes occurred in electrocardiographic rhythm strips after drug administration in any subject.

DISCUSSION:

The formulation of mefloquine used for this study was not well tolerated by any subject at the 1500 mg dose level. Intolerance was manifested by combinations of dizziness with associated difficulty focusing, gastrointestinal symptoms, insomnia, and malaise. Although of variable duration, symptoms were most frequent and severe within the first eight hours after drug administration and not incapacitating except in one subject (Code No. 186-B). This subject was incapacitated by rotational vertigo for 2 hours starting 2 hours after drug administration. Physical examination performed during the episode of rotational vertigo was unrevealing. Similarly, physical examination in the other subjects during the interval of symptomatology remained unchanged.

Symptoms compatible with intolerance to mefloquine occurred in some subjects at all dose levels. This may be attributed to anticipation engendered by detailed presentation of intolerance manifestations to the subjects both written and verbal at the time of study entry. However, at the 1000 mg mefloquine dose level, two subjects (Code Nos. 177-A and 188-D) developed recurrent, fleeting episodes of light-headedness during the first 24 hours after drug administration which were possibly drug related. In any event, the symptoms were so fleeting and minimal that the 1000 mg dose mefloquine is well tolerated.

There were no objective clinical nor laboratory observations suggesting mefloquine effect.

CONCLUSIONS AND RECOMMENDATIONS:

The drug assay values are not available at the time of reporting. It is recommended they be included as a supplement to this report. Mefloquine was not well tolerated by any subject at the 1500 mg dose level. Poor tolerance was manifested by combinations of dysphoria, light-headedness with associated difficulty focusing, mild gastrointestinal symptoms, insomnia, and in one subject rotational vertigo of 2 hours duration.

Therefore, it is recommended 1500 mg mefloquine be considered a poorly tolerated dose for the formulation used.

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ANTIMALARIAL DRUG PROJECT

EXPERIMENT NUMBER 10

TITLE:	COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) AND MEFLOQUINE HYDROCHLORIDE·HLR
PRINCIPAL INVESTIGATOR:	RICHARD C. REBA, M.D.
CLINICAL DIRECTOR:	KEVIN G. BARRY, M.D.
ASSISTANT TO THE DIRECTOR:	PAMELA G. GUHA, M.D.
CONSULTING AND ORGANIZATIONAL REVIEW COMMITTEE MEMBERS:	JAMES A. CURTIN, M.D. STUART H. DANOVITCH, M.D.

Richard C. Reba

RICHARD C. REBA
PRINCIPAL INVESTIGATOR

TITLE:

Comparative Bioavailability and Pharmacokinetics of WR 142,490·HC
(Mefloquine Hydrochloride) and Mefloquine Hydrochloride·HLR*.

PURPOSE:

To compare specific bioavailability parameters of two preparations of Mefloquine·HCl following single oral dose administration to healthy human male subjects, i.e.: peak blood levels, time to peak level, blood level-time patterns and area under concentration-time curve.

RATIONALE:

Malaria remains a leading cause of death among the world population. Therefore, development of effective prophylaxis and therapy maintains a high priority. Chloroquine resistance has emerged as a major problem in the prevention and treatment of P. falciparum malaria except in Africa.

Mefloquine hydrochloride, a substituted quinoline methanol, has been shown to be an effective single dose agent in the treatment of chloroquine resistant P. falciparum malaria. Its prophylactic effectiveness against chloroquine resistant P. falciparum malaria inoculated by infected mosquitoes has also been demonstrated. Clinical studies in humans showed that 10 subjects who received 250 mg of Mefloquine·HCl (WR 142,490) weekly for 8 weeks were protected when exposed to mosquitoes heavily infected with multi-drug resistant P. falciparum. The drug administered to human subjects in single doses up to 1000 mg was well tolerated. Intolerance at higher doses was manifested by temporary light-headedness, diarrhea, abdominal cramps, occasional nausea and/or vomiting. Symptoms were dose related and mild in all cases.

In addition, 12 healthy young males have completed a year long study during which each subject received a single weekly dose of 500 mg of mefloquine (WR 142,490·HCl) without significant adverse clinical or laboratory effects. It appears that this antimalarial is well tolerated and deserving of additional clinical investigations in man.

A variety of formulations have been used in previous tolerance and therapeutic studies. Clinical results in infected volunteers and blood level determinations in a limited number of pharmacokinetic studies indicate considerable variation in the bioavailability of these formulations. The use of a new formulation in field studies (such as the HLR formulation) must therefore be supported by prior demonstration of adequate bioavailability.

*F. Hoffmann-La Roche, & Co.

Comparative bioavailability of the HLR formulation and an established effective formulation by measurement of blood levels of parent compound will be studied.

PLAN:

Twelve healthy male subjects aged 21 to 45 years, weighing 60-85 kg and within 10% of their ideal body weight will be employed for the study. They will be recruited from the Washington, D.C. metropolitan area. Candidates will be hired by BIO-MED, Inc., as temporary employees for study purposes.

Candidates for employment will be screened to obtain the subjects for study. The medical evaluation will include a comprehensive history and physical examination, chest X-ray, electrocardiogram, urinalysis, white blood cell and differential count, red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet count, glucose, BUN, creatinine, Na^+, K^+, Cl^-, CO_2, uric acid, total protein, albumin, globulin, calcium, phosphate, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, LDH, total bilirubin, and G6-PD.

Qualified candidates will be presented with a complete explanation of the background and procedures to be used in the study and all details of the protocol as it involves the individual subjects. They will be interviewed in a group and individually in the presence of an investigator and a member of the Human Use Committee. Each participant will be given the opportunity to ask questions. Following this, the consent form will be read and those wishing to participate will sign it in the presence of a witness, an investigator and a member of the Human Use Committee.

During the first five days of the study the subjects will be housed in a controlled environment on Nursing Unit 5-W at the Washington Hospital Center. Thereafter, they will report according to the schedule in Table II.

The IND with Supplements and Clinical Summary will be available at all processing committee meetings and are available at all times in the Office of the Clinical Director of BIO-MED, Inc.

METHODS:

1. A classical two way balanced cross-over design will be used. Each subject will be randomly assigned to one of two possible sequences of drug administration, within the limitations of the design.
2. Three groups of 4 subjects will be admitted to the study sequentially. Within each group the two formulations will be administered at a dose of 4 tablets* to two subjects each, by random assignment. Following a "wash out" period of four weeks, each subject will be given 4 tablets* of the alternate formulation. Follow-up and sampling times following each administration are listed in Tables I and II.

   *The four tablets for the Walter Reed formulation will constitute a single dose of 1000 mg of Mefloquine·HCl, and for the F. Hoffman-La Roche, & Co. preparation a single oral dose of 1100 mg Mefloquine·HCl.

3. On the day of drug administration, subjects will be permitted to drink water ad lib until 1 hr prior to and after 2 hours after drug administration. Breakfast will be withheld. The drug will be administered in the presence of a member of the investigating team at 8:00 a.m. Subjects will return to a regular diet at 2:00 p.m.

4. The clinical and laboratory evaluations of subjects is outlined in Table 1.

TABLE I: SCHEMATIC STUDY PLAN FOR EACH DOSING

DAY OF STUDY	0*	1*	2*	3*	4*	5	7	8	14	21
Dose		X								
Physical Exam	X		X				X			
Interview	X	X	X	X	X		X			
Vital Signs	X	X	X	X	X	X	X			
ECG	X	X	X							
Laboratory Tests+	X		X	X			X			
Drug Assay++		X	X	X	X	X	X	X	X	X

*Controlled Environment

+Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, CO_2, Uric Acid, T.Protein, Albumin, Globulin, Calcium, Phosphate, Cholesterol, Triglyceride, Alk.Phos., SGOT, SGPT, LDH, Total Bilirubin, CBC (differential and indices), Platelets, Urinalysis. Additional studies will be done as clinically indicated.

++Drug Assay - Each subject immediately prior to drug administration and after dosing at 1,2,4,6,8,10,11,12,13,15,20,24,28,34, 48,56,63,72,76,80 hours and on days 5,7,8,14, and 21.

The schedule for hematologic and biochemical blood tests is indicated. For these tests 27 ml venous blood will be obtained while the subject is fasting before breakfast. Seven ml blood will be used for determination of white blood cell and differential count, red blood cell count, hematocrit, hemoglobin, MCH, MCHC, MCV and platelet count. Twenty ml of the venous blood specimen will be centrifuged and the serum separated. The serum will be divided into two samples. One sample will be stored in the refrigerator as a "back-up" until the biochemical lab report is received: Thereafter it will be stored in the freezer until released by the investigator. The other sample will be used on the day obtained to determine the values for serum glucose, BUN, creatinine, Na^+, K^+, Cl^-, CO_2, uric acid, total protein, albumin, globulin, calcium, phosphate, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, LDH, total bilirubin.

5. Specimen collection for Mefloquine·HCl drug assay will be as follows: 6 ml venous blood will be obtained in a heparin rinsed syringe. It will be placed in 16 x 125 mm glass test tubes with screw-on teflon lined caps. Specimens will be obtained prior to drug administration and following dosing at the times specified in Table II. The specimens will be stored at -20°C pending transport to Department of Pharmacology, WRAIR, for drug assay. A Specimen Collection Worksheet (BMI-WS-1) will be completed by the staff nurse on each subject participating in the study.

   Twenty-four hour urine specimens will be collected for drug assay on Days 0, 1, and 2 from 8:01 AM to 8:00 AM (See Table II).

6. On the last study day for each subject final physical and laboratory evaluation will be done. All abnormal findings will cause follow-up until normalcy, stabilization or proper medical disposition is secured.

7. Emergencies: Immediate action will be taken to first care for the subject in emergent need, including consultation and hospitalization if warranted, and second to notify the WHO and Army Monitors and the Washington Hospital Center authorities immediately by telephone and by written communication within 24 hours.

LABORATORY DATA:

All laboratory data will be recorded daily for each subject. Any deviation from normal will be brought to the attention of the investigator.

DATA ANALYSIS:

From each subject, individual blood level versus time data will by analyzed separately for each formulation. The data will be fit by a digital computer program by least squares criterion to a nonlinear function (sum of exponentials). The estimated parameter of interest for comparison will be the area under the curve determined by integration from 0 to infinity. In addition, the peak blood level, time to peak blood level, and mean blood levels will be compared. This will be done by an analysis of variance with subjects, periods, treatments, and "experimental error" as sources of variance. In addition, 95% C.I. for the area under the curve for each formulation will be computed using standard techniques.

REPORTS:

Progress and final reports will be submitted to the World Health Organization.

SUBJECT ACCEPTABILITY CRITERIA:

Subject acceptability criteria are based upon the precept that the risks of participation should be slight, and comparable for all subjects. Following this guideline, certain subjects are rejected routinely: For example, subjects with organic heart murmurs, splenomegaly or active lesions on chest X-ray. The presence of conditions which do not increase risk or potentially compromise the validity of the study as illustrated by epidermophytosis, "shotty lymphadenopathy", or scarred tympanic membranes are not routinely cause for rejection. Deviations of laboratory values of 3 standard deviations from the mean are cause for rejection. Deviations between 2 and 3 standard deviations from the mean are generally cause for rejection dependent upon the particular test and associated clinical and laboratory observations. For example, a serum sodium 153 mEq/L of itself would not, whereas a serum calcium of 11.2 mg/dl would cause rejection. Subjects shall be healthy men, age 21-45 years, weighing 60-85 kg, and within 10% of ideal body weight.

When doubt exists concerning entry acceptance of a subject for any reason, a decision is made following consultation with fellow M.D. investigators and other specialists, as appropriate. In this manner questionable candidates are given full consideration and the integrity and ethics of the Research Team protected.

EXPERIMENT NO. 10: COMPARATIVE BIOAVAILABILITY and PHARMACOKINETICS of WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) and MEFLOQUINE HYDROCHLORIDE·HLR

TABLE II: DRUG ASSAY COLLECTION

DAY OF THE STUDY	SPECIMEN NUMBER	BLOOD SPECIMEN TIME		BLOOD VOLUME (ml)
1	1	0	Prior to dosing	6
	2	1 hr	9:00 AM	6
	3	2 hr	10:00 AM	6
	4	4 hr	NOON	6
	5	6 hr	2:00 PM	6
	6	8 hr	4:00 PM	6
	7	10 hr	6:00 PM	6
	8	11 hr	7:00 PM	6
	9	13 hr	8:00 PM	6
	10	14 hr	9:00 PM	6
	11	15 hr	11:00 PM	6
2	12	20 hr	4:00 AM	6
	13	24 hr	8:00 AM	6
	14	28 hr	NOON	6
	15	34 hr	6:00 PM	6
3	16	48 hr	8:00 AM	6
	17	56 hr	4:00 PM	6
	18	63 hr	11:00 PM	6
4	19	72 hr	8:00 AM	6
	20	76 hr	NOON	6
	21	80 hr	4:00 PM	6
HOME				
5	22	96-98 hr	8:00-10:00 AM	6
7	23	144-146 hr	8:00-10:00 AM	6
8	24	168-170 hr	8:00-10:00 AM	6
14	25	312-314 hr	8:00-10:00 AM	6
21	26	480-482 hr	8:00-10:00 AM	6
			TOTAL	156 (ml)

	URINE SPECIMENS	
0	1	8:01 AM to 8:00 AM
1	2	8:01 AM to 8:00 AM
2	3	8:01 AM to 8:00 AM

TABLE III: TOTAL AMOUNT OF BLOOD WITHDRAWN FOR EACH STUDY SUBJECT FOLLOWING EACH DRUG ADMINISTRATION

	HEMATOLOGY	CHEMISTRY	BLOOD ASSAY	TOTAL (ml)
DAY 0	7	20		27
1			66	66
2	7	20	24	51
3	7	20	18	45
4			18	18
5			6	6
7	7	20	6	33
8			6	6
14			6	6
21			6	6
			TOTAL	264 (ml)

DRUG ASSAY
Specimen Collection Worksheet

Experiment #10: COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) AND MEFLOQUINE HYDROCHLORIDE·HLR

Name:_____ Code:____ Age:____ Ht:____cm. Wt:____kg.

First Dosing

Drug Formulation:_____
Total Dose:_____mg Dose(mg/kg):_____
Date & Time Dosed:_____

Second Dosing

Drug Formulation:_____
Total Dose:_____mg Dose(mg/kg):_____
Date & Time Dosed:_____

Whole Blood Collection

Spec. No.	P.D.*	Sched	Actual
1	-15 min.		
2	1 hr		
3	2 hr		
4	4 hr		
5	6 hr		
6	8 hr		
7	10 hr		
8	11 hr		
9	12 hr		
10	13 hr		
11	15 hr		
12	20 hr		
13	24 hr		
14	28 hr		
15	34 hr		
16	48 hr		
17	56 hr		
18	63 hr		
19	72 hr		
20	76 hr		
21	80 hr		
22	96-98 hr		
23	144-146 hr		
24	168-170 hr		
25	312-314 hr		
26	480-482 hr		

Whole Blood Collection

Spec. No.	P.D.*	Sched	Actual
1	-15 min.		
2	1 hr		
3	2 hr		
4	4 hr		
5	6 hr		
6	8 hr		
7	10 hr		
8	11 hr		
9	12 hr		
10	13 hr		
11	15 hr		
12	20 hr		
13	24 hr		
14	28 hr		
15	34 hr		
16	48 hr		
17	56 hr		
18	63 hr		
19	72 hr		
20	76 hr		
21	80 hr		
22	96-98 hr		
23	144-146 hr		
24	168-170 hr		
25	312-314 hr		
26	480-482 hr		

24 Hr. Urine Collections

STUDY DAY	TIME Begin	TIME End	TOTAL Hrs.	TOTAL Min.	Vol (ml)
0		0800			
1	0801	0800			
2	0301	0800			

24 Hr. Urine Collections

STUDY DAY	TIME Begin	TIME End	TOTAL Hrs.	TOTAL Min.	Vol (ml)
0		0800			
1	0801	0800			
2	0801	0800			

* Post Dose

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EXPLANATION FOR POTENTIAL SUBJECTS
ANTIMALARIAL DRUG PROJECT
EXPERIMENT NUMBER 10
Comparative Bioavailability and Pharmacokinetics
of WR 142,490·HCl (Mefloquine Hydrochloride)
and Mefloquine Hydrochloride·HLR

GENTLEMEN:

This document explains the nature of the study, its purpose, procedures, risks and benefits. You will be given the opportunity after reading it to ask additional questions. If you then choose to participate as a research subject, you will be asked to initial the last page signifying that you have read and understand its contents prior to obtaining your formal written consent to participate in the study. The subject will participate for a total of 50 days.

This study involves taking by mouth the drug mefloquine hydrochloride. Mefloquine is similar to quinine and has been approved by the Food and Drug Administration for investigational studies. The drug has been administered to more than 100 subjects to determine its safety and tolerance, and ability to prevent and cure malaria.

Previous studies with this drug established that it was well tolerated in single doses up to 1000 mg. Doses of 1250, 1500, and 1750 mg occasionally caused transient light-headedness or gastrointestinal symptoms. At 1250 mg only mild diarrhea lasting for ½ to 3 hours after taking the drug was reported. There was no associated nausea, vomiting or abdominal pain. At 1500 mg two subjects reported a transient sense of light-headedness, two subjects had mild diarrhea without other symptoms, and one subject vomited five minutes after taking the drug. At 1750 mg there was mild to moderate diarrhea, with the number of stools varying from 1 to 6 over a period of 35 minutes to 6 hours after taking the drug. Occasional nausea and mild abdominal cramps were also reported by some subjects.

This study is to compare the bioavailability of two different formulations. It will be conducted using one tablet formulation provided by Walter Reed Army Institute of Research, and the other provided by F. Hoffman-La Roche, & Co. The dose to be administered in both cases is 4 tablets*. You may experience some of the symptoms discussed above. No other symptoms or long term effects are anticipated.

*The four tablets for the Walter Reed formulation will constitute a single dose of 1000 mg of Mefloquine·HCl, and for the F. Hoffman-La Roche, & Co. preparation a single oral dose of 1100 mg Mefloquine·HCl.

We will study these two preparations by using a two way cross-over design. You will be assigned in a random manner to one of 3 groups designated Group I through III, each group containing 4 subjects. You will receive one drug preparation on one occasion and four weeks later the other formulation. You will be required to remain on the research unit for five days at the beginning of each period.

On the day the drug is to be administered, breakfast will be withheld. You will be permitted to drink water as you wish except for 3 hours encompassing the time of drug administration. The drug will be administered in the presence of a member of the investigating team at 8:00 AM. At 2:00 PM you may resume the normal diet you select from the hospital menu until you are discharged from the unit.

The purpose of the present study is to accurately determine the pattern in which the drug appears and disappears from your body. Blood (6 ml) will be drawn at the times specified in Table II after you take the drug to measure the amount of drug present. You will notice that the frequency of specimens is much greater during the early days of the study. In addition, we will collect all of your urine during the first 3 days encompassing the time of drug administration. You will be admitted to the research unit for the first 5 days and seen on brief visits for a period of 3 weeks. On the fourth week you will be readmitted to the research unit for a second 5 day period, after which you will be seen on brief visits thereafter per schedule. The long period of follow-up is required because of the expected slow release of the drug from the body. It is important that the blood be obtained as nearly as possible to the times specified in Table II and that you eat a light breakfast (i.e. cereal, milk, juice, coffee, bread -- no eggs or bacon) on the days you come in for blood drawing. It is also important that you avoid taking any other medication during the entire period and avoid the use of alcohol. Such factors as time of day, meals, alcohol, other drugs, and lack of proper sleep may affect the level of drug in your blood on any given day.

It is expected that the amount of drug remaining in your blood on the last day of the study will be very low. However, the late specimens are just as important as the early specimens in obtaining an overall accurate assessment of the way the drug is handled by the body. Therefore, please do not start the study if you anticipate difficulty in adhering to the schedule.

The amount of blood to be withdrawn for the entire study will be 555 ml, which is obtained over a seven week period and is about 39 ml more than a unit of whole blood that many people donate at American Red Cross Center Blood Banks. Instead of repeated venipunctures we will place a small teflon catheter in an arm vein and obtain the blood samples from it during the first fifteen (15) hours. In this way repeated venipunctures may not be necessary. The specifics for the study are presented in the schematic on the following page.

SCHEMATIC STUDY PLAN FOR EACH DOSING

DAY OF STUDY	0*	1*	2*	3*	4*	5	7	8	14	21
Dose		X								
Physical Exam	X		X				X			
Interview	X	X	X	X	X		X			
Vital Signs	X	X	X	X	X		X			
ECG	X		X				X			
Laboratory Tests[+]	X		X	X			X			
Drug Assay[++]		X	X	X	X	X	X	X	X	X

*Controlled Environment

[+]Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, Carbon Dioxide, Uric Acid, Total Protein, Albumin, Globulin, Calcium, Phosphate, Cholesterol, Triglyceride, Alkaline Phosphatase, SGOT, SGPT, LDH, Total Bilirubin, CBC (differential and indices), Platelets, and Urinalysis. Additional studies will be done as clinically indicated.

[++]Drug Assay - Each subject immediately prior to drug administration and after dosing at 1, 2, 4, 6, 8, 10, 11, 12, 13, 15, 20, 24, 28, 34, 48, 56, 63, 72, 76, 80 hours and on days 5, 7, 8, 14, and 21.

On the days indicated by *, the participants in the study will remain in a controlled environment. The entire group will remain together with a member of the Research Unit Staff and will function according to their direction. Facilities provided while participating in the study include room and board with a study-lounge area. Recreation (tennis and basketball) is also provided if weather conditions permit and supervision is available.

You have already had many of the examinations listed on the schematic as a part of your qualification examination. The laboratory tests require urine collections and venipunctures to obtain blood specimens. This will not affect you except for temporary discomfort associated with obtaining blood from your arm vein.

On the last study day for each subject final physical and laboratory evaluation will be done. All abnormal findings will cause follow-up until normalcy, stabilization or proper medical disposition is secured.

The Human Use Committee members are also looking after your safety. They insure that you are not subjected to undue risk and discomfort. A member of this committee will be available to speak with you at the Washington Hospital Center. After members of the investigating team and Human Use Committee are satisfied that you understand the study and written informed consent form you will be permitted to sign it. No subject may participate without a signed consent. By signing the informed consent you signify that the study has been explained to you with regard to its risks and requirements and you wish to participate.

It should be clear that your participation in this study is of no therapeutic value to you personally. The benefit, rather, is to others who live in parts of the world where malaria is a serious problem, and to Americans, civilian and military, who may travel to these areas. For this reason especially, your participation must be entirely voluntary with full knowledge of the personal risks and general benefits involved. Furthermore, you retain the right to withdraw your consent at any time without prejudice.

If after reading this document, you have any additional questions, please ask them before affixing your initials below and signing the consent form.

_____
Initials of Participant

SUBJECT AGREEMENT

CONSENT TO PARTICIPATE AS A STUDY SUBJECT

I, _____, hereby give my informed consent to participate as a study subject in the study entitled, "Comparative Bioavailability and Pharmacokinetics of WR 142,490·HCl (Mefloquine Hydrochloride) and Mefloquine Hydrochloride·HLR."

The implications of my voluntary participation; the nature, duration and purpose; the methods by which it is to be conducted and the inconveniences and hazards which may reasonable be expected have been explained to me by Dr. _____, and are set forth in the document entitled, "EXPLANATION: ANTIMALARIAL DRUG PROJECT EXPERIMENT NUMBER 10, Comparative Bioavailability and Pharmacokinetics of WR 142,490·HCl (Mefloquine Hydrochloride) and Mefloquine Hydrochloride·HLR," which I have signed.

I understand that with all drug administration and clinical investigation there are associated potential discomforts and risks. The discomforts and potential risks of participation as a subject in this study have been explained to me and I freely and voluntarily accept them. I understand that I will attain no direct therapeutic benefits from participation in the study.

All questions and inquiries I have made regarding the study have been answered to my satisfaction and I understand that I have the right to ask questions concerning the study at any time and have them answered to my satisfaction. Further, I understand I am free to withdraw, without prejudice, my consent and participation from the project at any time; however, I may be requested to undergo further examination, if in the opinion of the attending physician, such examinations are necessary for my health or well-being.

I consent to the taking and publications of any photographs in the course of the study for the purpose of advancing medical science, provided that my identity will remain confidential.

I certify that I have read and understand the above consent and that the explanations therein were made to me and that all inapplicable paragraphs, if any, were stricken before I signed.

_____	_____
Date	Witness - Human Use Comm. Cert.
_____	_____
Signature	Investigator Certification
_____	_____
Address	Witness

REAFFIRMATION OF CONSENT:

_____	_____
Date	Witness
_____	
Signature	

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