

EVALUATION OF NEW ANTIPARASITIC DRUGS AND VACCINES IN THE TROPICS

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1. The Treatment of *P. falciparum* Malaria with a Combination of Quinine and Tetracycline

PROBLEM : With increasing resistance to quinine treatment of *P. falciparum* malaria in Thailand, previously effective courses of seven to ten days of oral quinine therapy are failing and new combination drug regimens are in need of efficacy testing. The Department of Medicine at AFRIMS conducted a study in Phrabuddhabat, Central Thailand from September 1980 - January 1982, which showed that a seven day course of quinine had a cure rate of 60 percent in 15 patients and that a ten day course cured 69 percent of 13 patients (1).

At Mahidol University, School of Tropical Medicine, Dr. Tranakchit Harinasuta reported only a 52 percent cure rate in a 40 patient series originating from the Kampuchean border and treated with three days of quinine in combination with seven days of tetracycline (2). These patients were hospitalized in Bangkok for their entire 28 day follow-up thus excluding the possibility of reinfection.

In 1981 the malaria division of the Thai Ministry of Public Health treated 28 patients with three days of quinine and seven days of tetracycline and achieved a 100 percent cure rate in Kalasin province northeast Thailand (3).

The vanguard of resistant strains of falciparum malaria continues to emerge from the Kampuchean border. This is a pressing problem for the Thai military due to the significant effect of malaria morbidity on troop strength invested to guard that border.

PROGRESS : One hundred patients have been treated from November 1981 to October 15, 1982 at Sa Kaeo Malaria Research Center. Nine different quinine and tetracycline treatment regimens have been compared against mefloquine. These groups have ranged in various combinations from seven and seven to three and six of quinine and tetracycline respectively.

The two treatment regimens which are of current interest are six days of quinine and six days of tetracycline (Q_6T_6) and the three days of quinine and six days of tetracycline (Q_3T_6). The first treatment group (Q_6T_6) had a 100 percent cure rate in 21 patients completing a 28 day follow-up period. The second treatment group (Q_3T_6) had a 72 percent cure rate in 18 patients followed to 28 days. As a control group mefloquine had a 100 percent cure rate in nine patients to date.

In the Q_3T_6 group the mean parasite clearance time (PCT) for the 14 sensitive patients was 91 hours, whereas the mean PCT for the five recrudescing patients was 110.9 hours.

Also in the Q_3T_6 group the mean fever clearance time (FCT) for the 14 sensitive patients was 73 hours and the five recrudescing patients was 108 hours. There was no vomiting in the recrudescing group, which might explain the poor drug response. However, two of the five recrudescing patients returned to camp at day 14, so had been exposed to reinfection for seven days prior to their 21 day parasite count.

Comparing the 23 patients to date in the Q_6T_6 treatment group to the Q_3T_6 group, the mean parasite clearance time of the longer regimen was 86.6 hours and the mean fever clearance time for Q_6T_6 was 63.4 hours. Parasite clearance time was not significantly different but fever clearance time was 10 hours shorter in the longer treatment regimen.

Mefloquine as the standard for malaria treatment in Thailand had a mean PCT in nine patients of 62.9 hours and a mean FCT of 33.7 hours in that same patient group.

The mean initial parasite counts in the mefloquine, Q_3T_6 , and Q_6T_6 treatments groups were 12,516, 22,316, and 19,780 respectively.

FUTURE OBJECTIVES : Future plans include phasing-out the Q_3T_6 treatment group and evaluating only three groups Q_3T_7 , Q_6T_6 and mefloquine in the next year.

2. Treatment of an Acute Case of *Plasmodium malariae* Malaria with Mefloquine

PROBLEM : Mefloquine hydrochloride has been shown to be an effective drug in the therapy of infection due to *P. falciparum* (4-7) and *P. vivax* (8,9). *P. malariae* occurs in Thailand, but is much less common than the other two species. There are no recorded cases of *P. malariae* malaria treated with mefloquine.

PROGRESS : A 30 year old Thai male presented with a history of fever, chills, headache, backache and dizziness for 12 days. In his work, he travelled throughout Thailand and had been frequently exposed to malaria vectors for the previous month. He was free of symptoms, except for slight dizziness, at the time of admission and his temperature at that time was 36.7°C . He weighed 124 lbs. He denied taking any antimalarials since the onset of symptoms and his serum contained no detectable levels of quinine or sulfa. His initial parasite count was 5220/cu. mm. A diagnosis of vivax malaria was made and the patient

was enter into the vivax study and treated with mefloquine 1500 mg.p.o.

Examination of additional slides established the correct diagnosis of *P. malariae*. This was confirmed in all slides, including those obtained on admission, by the presence of numerous band forms of trophozoites, lack of enlargement of parasitized red cells, absence of Schuffner's stippling, the presence of coarse pigment typical of *P. malariae* and the low numbers of merozoites. The patient remained relatively free of symptoms, his parasite clearance time was 166 hours and his fever clearance time was 93 hours. This is much higher than comparable figures for vivax malaria treated with mefloquine in the same study (mean PCT = 59 hours and mean FCT = 28 hours).

FUTURE OBJECTIVES : Study is complete.

3. The Comparative Bioavailability and Renal Clearance of the Combination of Quinine and Tetracycline Given Simultaneously or Sequentially

PROBLEM : Over the last ten years in Southeast Asia, with the growing resistance of *Plasmodium falciparum* to chloroquine and fansidar, the drug combination of quinine and tetracycline has been widely utilized (10). Published recommendations advise simultaneous use of quinine and tetracycline (11, 12, 13). Although quinine provides a rapid clearance of parasites, the tetracycline portion of the drug combination produces a radical cure (14). Therefore any factors which would hamper absorption of tetracycline should lead to treatment failures.

It has been postulated that since quinine is a base, it might decrease the absorption of tetracycline which is known to have poor bioavailability in the presence of alkalis (15,16) and food, both of which result in an elevated gastric pH. This pharmacokinetic study was designed to analyze the amount of both tetracycline and quinine available in the blood after two methods of administration.

PROGRESS : From 15 June to 15 October 1982, fifteen patients have been treated under this protocol. Six subjects have received sequential treatment with quinine sulfate 650 mg q hr p.o. for three days followed by tetracycline HCl 250-250 mg-500 mg given q 8 hr p.o. for a seven day course. Nine patients have received quinine sulfate 650 q 8 hr p.o. x three days administered simultaneously with tetracycline HCl 250 mg-250 mg and 500 mg q 8 hr p.o. for a total of seven days.

Five of the total patients in both treatment groups fell into the high parasite count group, $> 20,000/\text{mm}^3$ (mean parasite count 55,265), and ten of the remaining fifteen patients were in the low parasite count group, $< 20,000/\text{mm}^3$ (mean parasite count 5201). The goal is to have equal numbers of high and low initial parasite counts in each treatment group. Twenty-eight day follow-ups have not been obtained on these patients since it was initially designed as a pharmacokinetic study. There were no RII or RIII drug resistance patterns encountered in this study.

One patient returned with recurrent *P. falciparum* malaria on day 42 having only been in Saraburi and Hua Hin since his hospital stay. He denied any excursions to malaria transmission areas. He could be classified as an RI treatment failure.

Only one patient had any persistently abnormal lab values in the first seven days of his treatment course. He was QTK 10, a 46 year old male, who had a slightly elevated SGOT between 59-66 on day 0, 3 and 7 of his hospital course. His other liver function studies returned to normal as his disease resolved.

Mean parasite clearance time in the sequential treatment group was 88 hours and in the simultaneous treatment group was 93.4 hours. Mean fever clearance time in the sequential treatment group was 64 hours and in the simultaneous treatment group was > 75 hours. This project is targeted to have 25 patients at completion and we have treated 60 percent of the patients to date.

The serum and urine collected on each patient at timed intervals throughout the 7-10 day hospital course have not yet been analyzed for quinine and tetracycline levels. The WRAIR collaborator on this protocol is Dr. Lawrence Fleckinstein of the Division of Experimental Therapeutics.

FUTURE OBJECTIVES : In the remaining ten patients in the study we plan to have them return weekly for parasite counts for a 28 day follow-up period.

We are also examining each patient's serum protein electrophoresis and serum glycoprotein electrophoresis for changes in the normal pattern with acute malaria as previously reported in experimentally induced malaria in a non-immune population (17, 18). If there is a significant shift in globulins one might expect greater drug binding and decreased availability of the active drug.

4. The Pharmacokinetics of Intravenous Quinine in Patients with Naturally Acquired Falciparum Malaria

PROBLEM : Quinine remains the most useful drug for treating severely ill malaria patients or those with parasites resistant to other drugs. The success of quinine treatment depends in large measure on achieving adequate drug concentrations in the blood, and there are known to be large inter-individual variations in plasma quinine levels following oral or intravenous therapy (19,20). Previous bioavailability and pharmacokinetics studies have not used analytical methods specific for quinine (19,20,21). This investigation will use the highly sensitive and specific high performance liquid chromatography to measure pharmacokinetics parameters such as quinine clearance, volume of distribution and protein binding with the goal of identifying major factors accounting for variability in quinine disposition in patients undergoing therapy.

PROGRESS : Since the study started in January 1982, two female and 12 male patients have been admitted. Plasma from the first 10 patients was sent to WRAIR in May 1982 and plasma from the second four patient series was sent in August 1982 for determination of quinine levels. The age distribution of the patients to date is 10/14 have been 20-30 years of age, 2/14 have been between 30-40 years of age and 2/14 patients have been in the 40-45 years of age group.

The mean initial parasite count in patients to date is 29,149. The mean parasite clearance time currently is 100 hours, and mean fever clearance time is 90 hours. One patient failed to clear and was classified as an RII resistant case.

FUTURE OBJECTIVES : This study is the first of other anticipated future investigations of quinine pharmacokinetics and the factors contributing to the variability of steady state quinine levels. The ultimate goal of these studies is to improve antimalarial drug therapy with quinine by identifying factors affecting quinine disposition and to improve the predictability of drug levels. We shall also test the pharmacokinetics of quinine and tetracycline (this combination is the only effective, commercially available therapy for drug resistant malaria in Thailand) when given simultaneously or sequentially.

5. The Treatment of *Plasmodium falciparum* Malaria with Halofantrine a Phenanthrenemethanol

PROBLEM : Due to rapidly emerging drug resistant strains of *Plasmodium falciparum* malaria in Thailand, the development and clinical testing of new antimalarial classes of drugs is of utmost importance. For effective outpatient treatment of malaria, single dose or short term therapy with a cure rate approaching 100 percent is the goal of the Army Drug Development Program. Halofantrine WR 171,669, a 9-phenanthrenemethanol completed Phase II drug trials with experimentally induced malaria in healthy volunteers in 1981. It was tested in 27 non-immune subjects infected with Vietnam Smith strain of *P. falciparum* and three other non-immune subjects, one infected with Cambodian Buchanan strain of *P. falciparum* and two infected with Chesson strain of *P. vivax* (22). At that time, all patients were cured except two of four patients on 1500 mg single dose and one of three patients at 1000 mg single dose. Three of three patients were cured at 250 mg q 6 hr x four and at 500 mg q 12 hr x two. Eight of eight patients were cured at 1000 mg followed six hours later by 500 mg. Therefore to be conservative, in the Army's first field trial of the drug, the higher 1500 mg split dose over one day was chosen as the drug regimen to be compared in a double blind efficacy trial against mefloquine as a 1500 mg single dose.

PROGRESS : From June 28 to October 15, 1982, at Ft. Taksin, Chantaburi, in a population of Royal Thai Marines, 19/38 patients were treated with halofantrine and 19/38 patients were treated with mefloquine for *P. falciparum* malaria. Of those patients receiving halofantrine in a dose of 1000 mg followed six hours later by 500 mg. 13/19 were sensitive, 1/19 experienced an RII pattern of resistance and 5/19 experienced an RI pattern of resistance (23). In the second group of patients receiving mefloquine as a 1500 mg single dose 18/19 were sensitive and 1/19 exhibited an RII pattern of drug resistance.

The double-blind design of the study was interrupted after 30 patients were treated and six recrudescences had occurred. It was vital to know if both drugs were failing or if the majority of treatment failures were in the halofantrine drug group.

Mean parasite clearance time for halofantrine was 79 hours and for mefloquine 90 hours. Mean fever clearance time for halofantrine was 56.7 hours and for mefloquine 62 hours.

The incidence of post dosing symptoms in the first twenty four hours after halofantrine was 2/19 with headache, 4/19 with nausea, 5/19 with diarrhea, 4/19 with vomiting, 3/19 with abdominal pain and 5/19 with no symptoms.

The number of patients with post dosing symptoms in the first 24 hours after mefloquine administration were as follows: 4/19 with vomiting, 5/19 with nausea, 6/19 with diarrhea, 7/19 with abdominal pain and 3/19 patients with no symptoms.

The cure rate for mefloquine was 95 percent in this patient population, while cure rate for halofantrine was 74 percent.

Due to previous treatment success in the Phase II human trials with halofantrine (22, 23, 24), good predicted efficacy by the aotus model (25); and low *in vitro* inhibitory doses required with halofantrine in the multidrug resistant Smith strain (26) and in three strains from various areas of Thailand currently (27), we are attributing this poor cure rate to poor drug absorption.

FUTURE OBJECTIVES : In an effort to improve the cure rate with halofantrine, we plan to amend our current treatment regimen and to give halofantrine in three or four 500 mg doses spaced six hours apart.

A bioassay of the drug in the patient's plasma against parasitized red cells in the hypoxanthine microtiter plate *in vitro* model will be used to document absorption of the drug.

This method will not give biochemical levels of the drug but it will give the additive growth inhibition to the parasite of both drug and its active metabolite at various sampling times in the patient's course.

6. *In vitro* Antimalarial Drug Sensitivity Testing

PROBLEM : In Thailand, *Plasmodium falciparum* is now resistant to conventional antimalarial drugs. This resistance varies from almost complete in the case of chloroquine and other 4-aminoquinolines and pyrimethamine/sulfadoxine to moderate but increasing for quinine. Quinine at high therapeutic dose continues to be effective when combined with tetracycline. Two new antimalarial drugs, mefloquine and halofantrine, have been introduced into Thailand and are now in various stages at field evaluation. *In vitro* antimalarial drug sensitivity testing provides an objective means of quantifying dose-response characteristics for individual drugs and thus the identification of resistance patterns in Thailand.

A radioisotope microdilution technique has been adopted to test antimalarial activity *in vitro* under field conditions. The technique was

standardized in the central Bangkok laboratory. The technique is based on incorporation of (³H) hyposanthine by parasitized PBC in microculture. Inhibition of uptake of (³H) hyposanthine by the parasites serves as an indicator of antimalarial activity. This technique has proven more sensitive and precise than traditional microscopic methods. It also permits large scale testing with fewer personnel. At present four drugs are being tested *in vitro* as part of an antimalarial drug efficacy study at Chantaburi in cooperation with the Royal Thai Navy and Marine Corps. The drugs used for sensitivity testing are mefloquine, halofantrine, quinine and chloroquine. At Chantaburi about 50 cases will be studied. Data from this study will be compared to data from other geographical areas of Thailand. The project will accomplish the following (1) provide *in vitro/in vivo* correlation of antimalarial drug response; (2) establish base-line quantitative data (ID 50); (3) permit identification and collection of drug resistant malaria strains; and (4) allow comparative testing of malaria strains from treatment failures when they occur. Comparative studies are also being done to determine whether cryopreserved strains can be directly used for testing by the radioisotope technique. A case of RII mefloquine resistance has been observed at Chantaburi in which sensitivity testing confirmed a decreased susceptibility to mefloquine.

FUTURE OBJECTIVES : Antimalarial drug resistance is an on-going problem. It is essential that antimalarial drug sensitivity testing be done on a continuing basis. The question of whether cryopreserved malaria strains can be used directly in the radioisotope technique needs to be answered since this will permit use of a centralized testing facility. Studies will also be initiated to permit the addition of other antimalarial drugs to the testing scheme.

7. Effect of Antimalarial Drugs on Human Lymphocyte Response to Mitogenic Lectins

PROBLEM : Since immunosuppression is a characteristic of malaria infection the possibility that an antimalarial agent may itself compromise immune responsiveness becomes an important clinical consideration. A drug induced decrease in host immune capacity during malaria infection could result in a prolonged parasite clearance time and subsequent delayed recovery from the disease. Similarly, the compromise to the patient may result in increased susceptibility to intercurrent illness. There is also the concern for malaria endemic populations where suboptimal chemoprophylaxis may combine with the disease itself so as to compromise vaccine employment - especially a prospective malaria vaccine.

PROGRESS : Mitogenic lectin induced lymphocyte blast transformation provides an established assay for evaluation of cellular immune responsiveness. We have standardized an *in vitro* mitogenic lectin assay to assess whether selected antimalarial drugs suppress cellular immune responsiveness in human lymphocytes. Preliminary studies show that the new antimalarial drugs, mefloquine and halofantrine, suppress normal lymphocyte response to phytohemagglutinin (PHA), Concanavalin A (Con A) and pokeweed mitogen (PWM).

FUTURE OBJECTIVES : These preliminary observations need to be confirmed in a large population sample. These studies are currently underway. Studies are also being done to assess whether these antimalarial drugs have an effect in lymphocyte culture (MLC).

8. Hypoxanthine Metabolism by Human Malaria Infected Erythrocytes : Focus for the Design of New Antimalarial Drugs

PROBLEM : The development of resistance to almost all conventional antimalarial drugs by *Plasmodium falciparum* makes critical the need to discover new chemotherapeutic agents. Truly rational approaches to antimalarial chemotherapy have been hampered by a lack of basic biochemical understanding of host-parasite relationships in human malaria infection. We have used malaria culture techniques along with novel chromatographic procedures (28) to study purine metabolism during the intraerythrocytic (IE) growth cycle of *P. falciparum*. Identification of differences in host-parasite purine metabolism could present appropriate targets for design of new antimalarial drugs.

PROGRESS : We have identified the major metabolic pathways used by IE *P. falciparum* to synthesize both guanosine and adenosine nucleotides from the precursor base, hypoxanthine (29,30). We next selected inhibitors specific for the purine enzymes associated with these essential nucleotide pathways (31). Hadacidin, but not alanosine, blocked synthesis of adenosine nucleotides from hypoxanthine via IMP. Hadacidin and alanosine are known to inhibit adenylosuccinate synthetase. The lack of inhibition by alanosine may be due to an inability to form the active metabolite, L-alanosyl-AICOR, which requires an active *de novo* purine pathway. We have shown that *P. falciparum* does not synthesize purines *de novo* (32). Bredinin and mycophenolic acid interfered with synthesis of guanosine nucleotides from hypoxanthine via IMP. These agents are inhibitors of IMP dehydrogenase. These studies confirm the importance of hypoxanthine as a precursor for synthesis of purine nucleotides and nucleic acids by malaria infected erythrocytes.

FUTURE OBJECTIVES : These studies identify biochemical targets associated with the human malaria parasites' metabolism of hypoxanthine which are essential for synthesis focus on these unique features of parasite purine metabolism and the classes of inhibitors effective against them could lead to the design of new antimalarial drugs. We plan to continue assessment of specific purine enzyme inhibitors with special emphasis on the salvage enzyme, hypoxanthine phosphoribosyl-transferase.

9. Evaluation of Thai Medicinal Plant Preparations for Antimalarial Activity against Drug-Resistant Strains of *Plasmodium falciparum*

PROBLEM : Antimalarial drug resistance in Thailand is a major health problem that continues to intensify. There is an urgent need to identify new compounds effective against malaria resistant to chloroquine and to pyrimethamine-sulfonamide combinations. Thai medicinal plants with putative antimalarial activity offer a unique source for biological and chemical study to elucidate an active antimalarial principle for use against drug-resistant *P. falciparum*.

Botanical preparations are a special interest to Thailand because they represent a natural resource with considerable economic potential.

PROGRESS : An intensive chemical analysis was made on *Eurycoma longifolia*, a Thai medicinal plant exhibiting strong *in vitro* schizonticidal activity on natural isolates of *P. falciparum*. Five different crude chloroform extracts obtained were tested and only one fraction (fr.a) was found with strong schizonticidal effect (1.5653 g/ml blood suspension). Further chemical isolation of this major fraction was made and four different compounds were obtained. Different concentrations of each compound isolated were tested for *in vitro* inhibitory effect on *P. falciparum* development. Results revealed that compound "a" was the only component of *Eurycoma longifloria* that exhibited schizonticidal activity (0.3945 g/ml blood suspension).

Chemical characterization and structural formula elucidation were available on two compounds. The compound "a" with strong *in vitro* schizonticidal activity was a lactone. The other compound was a scopolatin with no activity.

FUTURE OBJECTIVES : An attempt to confirm the anti-malarial activity of *Eurycoma longifloria* lactone on different strains of *P. falciparum* is being made. The evaluation of *in vitro* inhibitory effect by morphological comparison is a tedious and laborious technique as compared to the parasites' uptake of the labelled nucleic acid precursor-hypoxanthine. This technique is being standardized and will make possible large scale studies for *in vitro* comparison of different compounds on various strains of *P. falciparum*.

10. Evaluation of *Plasmodium cynomolgi* Sporozoites Induced Infections of Captive Born *Macaca fascicularis*

PROBLEM : India has ceased exportation of rhesus monkeys which are used in the *Plasmodium cynomolgi* antimalarial compound testing model. A systematic evaluation of captive born *Macaca fascicularis* has not been completed to determine if this species could be used to supplement scarce rhesus monkeys.

Sporozoite infection of 18 cynomolgus monkeys with *Plasmodium cynomolgi* has been completed. Three groups consisting of low dose (< 3 million), high dose (> 3 million); and high dose-splenectomized were infected IV with sporozoites. Preinfection and weekly acute infection period CBCs were performed. Parasitemia curves are being followed for a period of 120 days. When gametocytes of both sexes were present, mosquitoes were fed to determine if sporozoite production and thus a complete monkey-mosquito-monkey cycle could be maintained. The low dose intact monkeys did not develop a substantial or persistent parasitemia. An appreciable percentage of the high inocular intact monkeys did not develop parasitemias of sufficient persistence to make them a useful model. Additionally, only one of eight developed a gametocytemia which resulted in successful sporozoite production. Every monkey in the high dose, splenectomized group produced respectable gametocytemias and all mosquito feedings resulted in oocyst or sporozoite production. Some splenectomized high inocula monkeys developed parasitemias indistinguishable from parasitemia patterns in intact rhesus. The other splenectomized monkeys produced satisfactory initial parasitemias followed by persistent but very low parasite numbers. These late

parasitemias may not be of sufficient magnitude to be useful. An extremely high leukocytosis was observed in the high dose, splenectomized group during high parasitemias.

FUTURE OBJECTIVES : Determine persistence of parasitemias in high inocula splenectomized cynomolgus monkeys following chloroquine administration. Also, primaquine will be given at less than curative doses to evaluate tissue stage persistence. All monkeys will be followed for a minimum of 120 days.

11. Evaluation of the Efficacy of Ribavirin and Triacetyl Ribavirin in Japanese Encephalitis Infections

PROBLEM : Japanese encephalitis virus (JEV) is endemic in Southeast Asia with case fatality rates between 10 and 90 percent. JEV infection is a serious threat to local populations and military forces deployed anywhere in this region. The ability to select within 24 hours a subpopulation of patients at highest risk has made the use of an effective antiviral drug more attractive. Previous studies have shown activity of ribavirin against JEV infections *in vitro* but its *in vivo* efficacy is limited by its inability to pass the blood-brain barrier. Therefore other derivatives such as triacetyl ribavirin as well as intrathecal ribavirin treatment regimens are being evaluated.

PROGRESS : The most significant development in these studies has been the detection of ribavirin in serum and CSF of treated monkeys by high performance liquid chromatography (HPLC). Previously pharmacokinetic data could only be obtained by using radioactive drugs or by performing biological assays requiring expensive logistical support and yielding results of low accuracy. This technique is currently being used to determine the ability of triacetyl ribavirin to pass the blood-brain barrier of treated monkeys. Experimental JEV infections have been performed and LD₅₀ determined.

Toxicity studies have been completed with intrathecal ribavirin in monkeys and a subtoxic dose will be evaluated to determine ribavirin efficacy by this treatment.

FUTURE OBJECTIVES :

1. Promising ribavirin derivatives should be given to monkeys i.v. to determine their ability to pass the blood-brain barrier.
2. The derivative that is most effective at entering the CNS should be tested for efficacy in monkeys with experimental JE.

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