

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 105: Chloroquin Levels and Excretion Rates in Thai and American Volunteers

Project No. 3A 025601 A 811: Military Medical Research Program
S. E. Asia

Task 01: Military Medical Research Program
S. E. Asia

Subtask 01 Military Medical Research Program
SEASIA (Thailand)

Reporting Installation: U.S. Army-SEATO Medical Research
Laboratory, APO 146, San Francisco,
California

Division of Special Projects

Department of Biochemistry-Malaria

Period Covered by Report: 1 April 1963 to 31 March 1964

Principal Investigator: Katchrinnee Pinswasdi, M.D.

Associate Investigators: Udaya Sandhinand, M.D.
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Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

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ABSTRACT

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The purpose of this study was to determine the effectiveness of chloroquin as a therapeutic and prophylactic agent against malaria infections in Thailand. Serum was obtained from healthy Thai and American personnel taking various doses of the drug at therapeutic or prophylactic levels. The serum chloroquin levels of these individuals were determined at intervals to

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determine the rate of absorption and excretion of the drug. In addition, sera were obtained from patients with proven active cases of falciparum malaria, and serial counts of the parasite levels in the blood were compared with serum levels of chloroquin. A significant number of these patients were found with malignant tertian infections which appeared to be resistant to chloroquin. Morphological differences were detected between P. falciparum parasites from resistant and susceptible infections. There also appeared to be differences in the rates of cerebral infections seen from various parts of Cholburi Province. Detailed studies were made on a member of the Laboratory staff who contracted malaria despite what should have been more than adequate chloroquin prophylaxis. His infection proved to be highly resistant to chloroquin and pyrimethamine, but susceptible to quinine. Similar observations were made on a Thai national. Chloroquin resistance appears to be an important problem in some parts of Southeastern Thailand, and the currently recommended prophylactic schedule of the U.S. Armed Forces will not protect all individuals from P. falciparum infections.

BODY OF REPORT

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Objective: To study the effect of chloroquin and other synthetic drugs on the course of malaria parasitemia in Thailand. To determine the effectiveness of these drugs as malarial therapeutic and prophylactic agents.

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Description: Biochemical methods (spectrophotofluorometry) were used to determine the levels of chloroquin attained in the serum of humans after ingestion of the drug, and the duration of these levels. Baseline studies were conducted on normal (non-malarious) individuals, and on persons naturally infected with malaria. During the study an opportunity also arose for investigation of an individual naturally infected with a strain of P. falciparum which proved to be resistant to chloroquin. Initially an extraction procedure utilising ethylene dichloride as a solvent was used to prepare the serum samples for the spectrophotofluorometer. The recovery obtained was not satisfactory, and the procedure of McChesney, Banks and McAuliff (Antibiotics and Chemotherapy, 1962) was substituted. The method presently used is a further modification of that technique. Serum samples were obtained from several groups of participating Thais and Americans, according to schedules which will be outlined below. Fresh serum was extracted when possible, but some samples were frozen before extraction. The parasite counts referred to were made by counting the number of parasites in a stained (Giemsa) thick smear, in relation to a standard count of 500 leucocytes.

Progress: The serum obtained for analysis fell into a number of categories during the year, and for purposes of clarity these will be discussed separately below.

Chloroquin Serum Levels in Normal Individuals

Before attempting to correlate serum levels of chloroquin with malaria infections, information was sought on the serum levels of the drug which would be reached in healthy Thais and Americans. This was designed to obtain baseline data where the problem of treatment and severe illness did not interfere with the drug administration and blood sampling. Several treatment schedules were used, and the three phases of the study of normal individuals were as follows:

Phase I: Fifteen Thai adults were given a full therapeutic dose of 1500 mg. of chloroquin base. The drug was administered orally as a phosphate salt in two doses of 450 mg. (four hours apart) on the first day, and 300 mg. on each of the two following days. The first day dosage schedule is slightly different from the World Health Organization recommendation of 600 mg. followed by 300 mg. after an interval of six hours. It had been observed by a number of workers that Thais showed slight side effects from the initial 600 mg. dose. These side effects (headache, dizziness) were minimized by the schedule used. Blood was drawn just before the first ingestion of drug and at 2, 4, 12, 24, 48, 72, 96, 216 and 336 hours thereafter. Figure 1 illustrates the mean serum level of chloroquin for this group, at the times indicated. The highest level of chloroquin in the serum was

reached at hour 12. At that time 900mg. of the drug had been ingested, and 8 hours had elapsed since the last dose. The subsequent administration of two 300 mg. doses established a maintenance level of chloroquin in excess of 20 μ g/L. This level persisted for 14 days. A serum chloroquin concentration of 10-20 μ g/L is adequate for suppression of malaria parasites according to Berliner et al. (Journal of Clinical Investigation, 1948).

Phase II: Fifteen U.S. Military personnel were placed on a therapeutic schedule of 1500 mg. chloroquin base. The initial dose was 600 mg., followed by 300 mg. six hours later. An additional 300 mg. dose was administered on each of the succeeding two days. The participants were observed for any possible side effects, but these were minimal, and all participants carried on their normal military duties. Blood was drawn on the same time schedule as in Phase I. The full series was completed on two participants, but technical difficulties were encountered in obtaining readings at the required fluorescent wavelengths of 390-400 m μ during the processing of the remaining thirteen samples. Attempts to obtain reading on these sera after repair of the instrument were hampered by hemolysis of the sera which had been frozen in the interim. This phase of the study will be repeated using a large enough number of participants to assure significant results.

Phase III: Thirteen Thai adults were administered a single tablet containing 300 mg. of chloroquin base and 45 mg. of primaquin base. A blood sample was obtained just before administration of the drug and additional samples were obtained at 24 hour intervals until chloroquin serum levels had decreased below 10 μ g/L. This is the standard U.S. Army prophylactic drug, designed to be taken once a week. The average chloroquin serum level in these individuals was 15 μ g/L at 72 hours and 0 μ g/L at 168 hours. Thus, no protective chloroquin was present in the blood on the seventh day, on which the next prophylactic dose should have been taken.

Most of the observations on normal individuals will be repeated to obtain additional data on therapeutic and prophylactic effects. However, certain facts are already apparent from the data at hand. The different dosages and time schedules of chloroquin in the therapeutic doses established different serum levels and maintenance periods. Some modification of the present recommendations for treatment may be indicated after further study. The essential absence of chloroquin from the serum of participants in the trial of chloroquin-primaquin prophylactic drug combination before the end of one week may indicate the necessity for changes in the regime.

Chloroquin Serum Levels and Malaria Parasite Density

Clinical data, blood specimens for chloroquin determination and finger tip blood smears for parasite counts were obtained from a group of patients

admitted to Cholburi Provincial Hospital with a confirmed diagnosis of malaria. The study was conducted with the cooperation of the Director of the Hospital. The hospital is located southeast of Bangkok on the Gulf of Thailand. It serves a region which contains a number of highly malarious areas. One of these, Khao Mai Kaeo, is the site of the epidemiological and entomological investigations reported in Studies Number 51 and 52. During the first part of the study (1 October - 16 November) forty one patients were studied, while during the second part (19 February - 1 April) nineteen were examined. These patients were from many parts of the Province.

Almost all of the patients were charity cases, and many of them left the hospital before the entire series of bleeding and other observations could be completed. There was considerable resistance to the number of bleedings required, especially among those patients who showed a rapid response to chloroquin treatment. Under the circumstances, a number of elements in the original design could not be completed. These included: collection of urine samples, observations on relapse following apparently successful treatment, and blood samples on all patients 48 hours after completion of the full course of 1500 mg chloroquin base. Many of the patients left the hospital before the end of the 48 hour period. Originally, blood was obtained at the time of institution of treatment and at 12, 24, 48, 72 and 96 hours thereafter. Later, the bleeding schedule was changed to 0, 72, 96 and 120 hours, in the hope that the reduced number of bleedings would encourage more patients to complete the series. The serum was separated at the hospital and frozen until it could be delivered to the main laboratory in Bangkok for chloroquin determination. At each bleeding peripheral blood smears (thick and thin films) were made for parasite counting and identification. Many of the sera were stored for a considerable period of time due to the malfunction of the spectrophotofluorometer referred to above. Determination of chloroquin level were made on all the sera possible, but full series were not completed on all of the patients in the study. The following observations were made:

a. Plasmodium falciparum is the predominant parasite species in the Province and all of the selected cases were parasitized by this species. Apparently, the less serious cases caused by P. vivax do not appear for hospitalization in very great numbers. Of the cases examined, 47% were parasitized by presumptively chloroquin resistant strains of P. falciparum, based on the persistence of asexual parasites in the peripheral blood 48 hours after the ingestion of a full therapeutic dose of 1500 mg. of the drug. The serum chloroquin levels at this time were much higher than the reported therapeutic level of 10-20 µg/L

b. In the October-November period 26% of the patients had circulating gametocytes on admission; in the February-April period the gametocyte rate was 37%. The number of patients with heavy infections (1000-4500 asexual parasites/500 WBC) was also higher in the February-April period.

c. Over half of the patients had received some treatment before admission to the hospital. Some of them had serum chloroquin levels as high as 280 µg/L on admission.

d. About 15% of the patients showed an increase in parasite density after administration of the drug; the rest showed the expected decrease.

e. Parasites from patients with chloroquin resistant strains appeared to differ in morphology from susceptible strains.

Further modification of the study plan will be made in order to keep patients with chloroquin resistant strains of malaria in the hospital for longer periods for additional drug studies. In addition, an effort will be made to follow the course of parasitemia in those patients who show gametocytes in the blood following clinical cure by chloroquin. A much larger group of patients will be examined to determine the validity of the preliminary observation on the morphological differences among strains.

An additional clinical observation was made during the Cholburi Hospital study which has important implications, and deserves further study. There appeared to be a correlation between the locality in the Province where malaria was contracted and the organ affected in pernicious attacks. In some parts of the Province the malignant tertian attacks took a cerebral form, but in other areas severe parasite attacks seemed to affect the liver, with a corresponding decrease in cerebral attacks. Plans have been made for a more detailed study of this apparent difference.

Observations on Chloroquin Resistant Malaria

As noted in the report of the clinical study at Cholburi Provincial Hospital, a number of cases were seen which were classed as presumptive chloroquin resistant malaria, based on finding parasites in the blood 48 hours after completion of therapy. Additional cases were seen among the U.S. and Thai personnel of this Laboratory engaged in field studies of malaria further south in the Province (see Study 52). Blood specimens for isolation of the suspected chloroquin resistant malaria strains were obtained from five cases during the year, frozen, and forwarded to WRAIR for further study. Chloroquin levels were not run on these cases since quinine had been administered to most of them prior to bleeding. Collection of additional samples is still in progress at the Cholburi Hospital. A report was

received from WRAIR that the blood of one of the U.S. team members who contracted malaria at Khao Mai Kaeo had been successfully transferred to prisoner volunteers at Stateville Prison, Illinois. In the volunteer the P. falciparum strain was resistant to chloroquin, but responded readily to pyrimethamine. The donor (a visiting scientist from WRAIR) had contracted his infection at Khao Mai Kaeo despite chloroquin prophylaxis. Later in the year another member of the U.S. staff contracted malaria despite chloroquin and combined chloroquin-primaquin prophylaxis. In this case it was possible to make biochemical as well as parasitological observations, and these are presented below:

Case Report - Drug Resistant Malaria

The patient (JMN) is a caucasian, 24 years of age, an officer of the Medical Service Corps, U.S. Army. Two days before entering the Khao Mai Kaeo study area he ingested two standard issue chloroquin phosphate tablets. On the first day in the area he took an additional combined chloroquin-primaquin tablet. Two nights were spent observing mosquito collections in the area, after which he returned to Bangkok, a non-malarious area. Fourteen days after entering the study area he experienced prodromal symptoms of headache and slight fever. He was admitted to the hospital six days after onset of symptoms, and blood for isolation attempts was obtained on the second and third day after admission. He was treated with camoquin, atabrine and intramuscular quinine and on leaving the hospital received a fourteen day treatment of 15 mg. of primaquin daily. He remained symptomless and aparasitemic for a period of three weeks. The first relapse occurred two days after completion of the primaquin treatment. Chloroquin treatment was instituted (1800 mg. orally in two days) in conjunction with parasite counts and bleeding for chloroquin serum determinations. Parasites decreased sharply as the chloroquin serum level rose, but parasites persisted for six days, at which time the counts began to rise. The patient was then given oral pyrimethamine (200 mg. in a four day period). The initial response to pyrimethamine was a sharp rise in the number of parasites to levels higher than in the first relapse. The number of parasites slowly decreased, but large numbers were present four days after completion of the pyrimethamine treatment. As noted above, a strain isolated from another U.S. member of the team had been reported as being sensitive to pyrimethamine. To confirm the lack of effectiveness in the case of JMN, another course of pyrimethamine was given. Again, the parasite count increased sharply, followed by a drop in numbers. When it became obvious that neither drug would affect a radical cure the patient was given quinine. Quinine dihydrochloride was administered intravenously (800 mg. in 1000 ml of 5% glucose-normal saline), followed by a course of six oral tablets (2000 mg.) daily for ten days. The parasite count dropped sharply, but a few parasites were present four days later, so the intravenous course was repeated. His blood was free of asexual parasites after completion of the quinine treatment. Observation on the case is still in progress and he remains parasite free at present (some 50 days post-treatment).

A similar history was noted by a recognized Thai scientist in a Thai adult inoculated with blood from a chloroquin resistant P. falciparum infection. Daily blood films became positive on the third day after inoculation of blood from a febrile patient who had failed to respond to chloroquin. The patient became quite ill on the sixth day post-inoculation and a total dose of 3000 mg. of chloroquin base was administered orally. Blood from this case was made available to this Laboratory for biochemical study. The chloroquin level was higher in this patient than in JMN and it persisted at higher levels for a longer period. The parasitemia was suppressed temporarily, for 13 days. A relapse occurred on the fourteenth day, and the number of parasites and rate of increase were much higher than in the initial attack. A course of pyrimethamine was started, but the high level of parasitemia required interruption of this treatment by quinine. A total dose of 21.6 g. of quinine was administered in a combined intravenous and oral regime in a ten day period. Response to quinine was satisfactory, and comparable to that seen in JMN. The parasites from JMN, from the Thai patient and from the patients suspected to have chloroquin resistant strains of P. falciparum at Cholburi Hospital were similar in morphology.

Summary: Biochemical procedures were used to study the level of chloroquin in the sera of Thai and U.S. personnel who were taking various dosages of the drug. In normal individuals therapeutic serum levels of 20 µg/L persisted for as long as 14 days after ingestion of 1500 mg. of the drug. When used as a prophylactic drug (300 mg. chloroquin base plus 45 mg. primaquin base) there was some evidence that protective levels in the serum may disappear before the end of the weekly interval between doses. Further observations will be required. When chloroquin levels were compared with numbers of parasites in patients at the Cholburi Provincial Hospital a large number of patients were found whose infections seemed to be resistant to the drug. There appeared to be a difference in the morphology of P. falciparum parasites of chloroquin resistant and susceptible strains. There also appeared to be a geographical separation of clinical manifestation of pernicious attacks, with cerebral symptoms predominating in some areas, and apparent liver damage predominating in other parts of the Province.

Infection of a U.S. member of the staff while working in the field permitted detailed observation on the course of a proven case of chloroquin resistant P. falciparum. The parasite strain did not respond to chloroquin or primaquin, but apparently did respond to quinine. Chloroquin levels and parasite counts were determined during the course of the patients illness. Blood specimens were also obtained from a Thai adult with an induced

infection of a chloroquin resistant strain of P. falciparum, and the response to treatment was the same as that observed in the U.S. individual.

Conclusion: Differences were detected in serum chloroquin levels reached in individuals taking various dosages of the drug. These observations will be extended to permit recommendations on therapeutic dosage and prophylactic dosage. A significant number of patients were found with P. falciparum infections which resisted treatment with chloroquin. Observations on members of the staff who contracted malaria during field studies confirmed the fact that neither chloroquin nor chloroquin-primaquin tablets taken at or above the recommended dosages protected all individuals from infection with P. falciparum.