

Investigation of suspected resistance of *P. falciparum* to Chloroquine in Zambia

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INTRODUCTION

In 1967 Himpoo and MacCallum (1967) reported three cases of possible chloroquine resistance of *P. falciparum* in Broken Hill (Kabwe), Zambia. Although a number of such claims of chloroquine resistance in Africa have been published, a report by a World Health Organization Scientific Group (1967) on the Chemotherapy of Malaria stated that such reports are, as yet, unsubstantiated. So far, therefore, there has been no confirmed incidence of a chloroquine resistant strain of *P. falciparum* in Africa. For this reason, it was decided to investigate the suspected chloroquine resistance reported in Kabwe. This investigation has not yet been completed and it will be continued during the main malaria season 1968—1969. The aim of this paper is to present the results of this investigation during the malaria season 1967—68.

PATIENTS AND METHODS

The investigation was conducted in accordance with the recommendations of the report of a World Health Organization Scientific Group (1967) on the Chemotherapy of Malaria, as detailed below.

Drug regimen

Chloroquine phosphate, in the form of "Avloclor" tablets, was administered in accordance with the following schedule of chloroquine base per kg. body weight:—

Day 0: first dose	—	10 mg/kg
Day 1: second dose	—	10 mg/kg
Day 2: third dose	—	5 mg/kg

Because many of the patients were children and the chloroquine was only available in tablets of 150 mg base of chloroquine, the exact schedule had to be varied slightly to suit the dose of half a tablet, (75 mg base of chloroquine), so as to be as near as possible to the weight of the child. Over the 3 days of treatment, these necessary minor adjustments in the daily dose were calculated so that the total dose over 3 days provided for the administration of 25mg base of chloroquine per kg. All drug doses were given personally by one of the authors.

Blood investigations

Throughout the investigation, thick and thin blood films were used. The initial diagnosis was made by staining a blood film with Field's stain, as this provided a rapid method of diagnosis. All patients so diagnosed had a further slide taken prior to treatment. This slide, and seven subsequent daily slides, were stained with Giemsa stain. Patients in the trial, therefore, had a blood slide taken prior to treatment on the first day of treatment, and a blood slide was taken on each of the seven days following the first day of treatment, i.e. eight slides per patient over eight days. All these slides were read by one of the authors. A count of trophozoites per mm³ was carried out on all of these slides. The trophozoite count was estimated by comparing the number of trophozoites with the number of leucocytes in the blood smear and based on the assumption of 8,000 leucocytes per mm³ of blood.

Chloroquine absorption

To ensure that the oral chloroquine was being absorbed, the urine was tested before treatment and again on the second and seventh day after the commencement of treatment. The urine was tested for the excretion of chloroquine by Wilson and Edeson's method as described in the report of a World Health Organization Scientific Group (1965) on the resistance of malaria parasites to drugs. Details of this test are given at the end of this paper, and this is reproduced from the above-quoted World Health Organization publication.

Selection of patients

Such an investigation requires the daily treatment of patients for three days and for taking daily blood slides for eight days, i.e. the day that treatment commences and the seven subsequent days. It was considered that it would be difficult to carry out such a regimen outside a hospital. In giving treatment, it is necessary to ensure that the patient does not subsequently vomit. In obtaining blood slides it is necessary to obtain a daily blood slide for eight days. If patients are not in hospital, it would be difficult to attain these requirements, and the "failure to complete trial" rate would probably be very high. It was, therefore, decided to admit all patients in the trial to the Kabwe Hospital for treatment and subsequent follow-up investigations.

Patients were, therefore, selected from those attending the hospital who had microscopically diagnosed malaria. Only those with pure *P. falciparum* infections were included in the trial and "mixed infections" were excluded. Patients who vomited following drug administration were also excluded. Infants and children too young to swallow a tablet were not selected for the trial because it is difficult in practice to ensure an exact dose of chloroquine if it is administered as a syrup.

The table gives an analysis of the 47 patients who completed the trial, by age groups and by pre-treatment trophozoite counts per mm³ in logarithmic groups.

Period of trial

In the area of Kabwe, which is at an altitude of about 4,000ft. (1,200m), malaria transmission probably does not occur, or is at least at a low level, during the winter months from June to September. In October, with the onset of the warm weather, malaria transmission commences. Until the onset of the rains in about December, the breeding of malaria vectors is at a low ebb. With the onset of the rains, the breeding of the vectors increases, and serious malaria transmission commences. Therefore, from about January onwards, the incidence of new malaria infections builds up until it reaches its usual peak in April or May. With the onset of the dry and comparatively cold weather in about June, transmission ceases, or is reduced to a very low level. The present investigation was, therefore, commenced on 2nd December, 1967 and, due to other commitments of one of the authors, the investigation ceased on 22nd March, 1968. Further investigations will be resumed during the next malaria season in 1968—69.

RESULTS

During the period of this preliminary investigation, 61 patients commenced treatment, and 47 completed the treatment and also the necessary follow-up investigations prescribed by the regimen of the trial. Of the 14 patients who failed to complete the trial or were excluded from the trial, one died of cerebral malaria soon after admission, one was subsequently diagnosed as a mixed infection of *P. falciparum* and *P. ovale*, one vomited after drug administration, one received a blood transfusion during the trial, and ten absconded from hospital before they had completed the trial.

Of the 47 patients who completed the trial, 46 were free of parasites on the 7th day after commencement of treatment. The remaining case, who was free of parasites on the 4th to 6th day, had one parasite in the blood smear on the 7th day. Of the 46 patients, 3 were cleared of parasites on the 2nd day after treatment, a further 23 on the 3rd day, a further 18 on the 4th day, and the remaining 2 on the 5th day. One of these cases, recorded as being cleared of parasites on the 2nd and 3rd day after treatment did, in fact, show one parasite in the blood smear taken on the 4th day, but the slides on the 5th to the 7th days were free of parasites.

Of the 14 patients who failed to complete the trial, 9 remained under observation for sufficient time to assess the effect of chloroquine on the parasitaemia for

several days. In all these patients, the parasitaemia was showing a satisfactory response to chloroquine therapy.

Of the 47 patients completing the trial, 32 had pyrexia on admission. In this context, the term "pyrexia" indicates a patient with an oral temperature above 99°F. (37.2°C.). 7 patients still had pyrexia on the day after treatment. 2 patients had pyrexia on the second or third day after treatment but both of these patients had been afebrile for at least one day between admission and this subsequent pyrexia. No patient showed any pyrexia after the third day following the commencement of treatment.

The urinary excretion of chloroquine was found to be positive on the 2nd day after the commencement of treatment in all patients.

DISCUSSION

When the resistance of *P. falciparum* to chloroquine is reported this is difficult to disprove. If such a resistant strain is rare, it would be necessary to investigate a very large number of infections to detect the resistant strain. It is, therefore, necessary to investigate many such infections which are sensitive to chloroquine before it is possible to say that the presence of a resistant strain is unlikely.

In the present investigations, 47 patients completed a satisfactory investigation. Of these, 45 patients were cleared of their parasitaemia in a manner indicating that the parasite was sensitive to chloroquine. Of the other two patients, one was clear of parasites from the 2nd day onwards except for one parasite on the 4th day, and the other was clear of parasites on the 4th day onwards, except for one parasite on the 7th day. It is possible to argue that these two patients showed an early recrudescence of the parasite. It is, however, considered that the presence of one single parasite on a slide in these circumstances in these two patients was more likely to be due to a technical cause during staining, such as the transfer of the parasite from another slide or a failure to clean the slide properly before its re-use. However, the reaction of the parasite to chloroquine in these two patients indicated in every other way that the parasite was sensitive to the drug.

So far in this investigation, 61 patients suffering from malaria were treated with chloroquine in Kabwe and the reaction of *P. falciparum* to chloroquine therapy was studied in detail. Chloroquine does not usually cause a clearance of parasites on the first day after treatment commences and sometimes the trophozoite count may even increase on this first day. From the 2nd day after treatment a reduced trophozoite count should be observed but complete clearance of the trophozoites is not usually achieved until the 3rd or 4th day and this may even be delayed until the 5th day after treatment commences. The presence of gametocytes on any day is unimportant as chloroquine has no effect on the gametocytes of *P. falciparum*.

It is, therefore, considered that the parasites in this trial did not show any indication of resistance of *P. falciparum* to chloroquine. The number of patients

in this investigation is, however, insufficient to claim that it is probable that a chloroquine resistant strain of *P. falciparum* does not exist in the area of Kabwe. It is, therefore, planned that this investigation will continue in the next main malaria transmission season from December 1968 to May 1969. In the meantime, this preliminary investigation has failed to substantiate the report of suspected chloroquine resistance of *P. falciparum* in the area of Kabwe.

SUMMARY

Treatment with chloroquine of 61 patients with a *P. falciparum* infection in the Kabwe (Broken Hill) hospital in Zambia has failed to substantiate the report of Himpoo and MacCallum (1967) of a suspected chloroquine resistant strain of *P. falciparum* in the area of Kabwe. In view of the small number of investigations carried out so far in this trial, it is intended to continue this investigation during the main malaria transmission season from December 1968 to May 1969.

ACKNOWLEDGEMENTS

This investigation was conducted in agreement between the Government of the Republic of Zambia and the World Health Organization. We are grateful to the Permanent Secretary, Zambian Ministry of Health, for permission to publish this paper.

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TEST FOR THE PRESENCE OF CHLOROQUINE IN URINE

The following qualitative tests for detection of the presence of chloroquine in urine can be easily applied in the field:—

1. Wilson & Edeson's test (1954)

Reagent:

Mercuric chloride (HgCl ₂)	6.8g)	Mayer-
Potassium iodide (KI)	24.9g)	Tanret's
Distilled Water	500ml)	reagent

Method: A few drops of Mayer-Tanret's reagent are added to a few millilitres of fresh clear urine. A white turbidity appears which disappears on heating and reappears as the urine cools down. If albumin is present the turbidity increases on heating. The test can be carried out, however, if the albumin is removed by boiling and subsequent filtration. The test is more sensitive if the urine is cold (half an hour in the refrigerator) (Pille and Lambourg, 1958). The sensitivity of the test is about 0.4—1.0mg per 100ml. This test becomes positive within 12 hours after the administration of a single dose of 600mg of chloroquine base and remains positive in most subjects for 5—6 days.