Chloroquine-resistant *Plasmodium falciparum* malaria in the Kavango region of Namibia

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Summary

The sensitivity to chloroquine of *Plasmodium falciparum* from the Kavango region of Namibia was determined by a 24-hour test *in vitro*. Twenty-six isolates were successfully tested, of which 11 were resistant to a low degree, schizogony being inhibited at 8 pmol/well. The results of the Dill-Glazko test for the presence of 4-aminoquinolines in urine indicate that chloroquine is not widely used in the area.

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Chloroquine-resistant *Plasmodium falciparum* malaria was first reported from Africa in 1979 and has since spread throughout most of southern Africa,[1] its occurrence having serious implications for the treatment and control of the disease. The mechanisms for the development of chloroquine-resistant malaria have been shown to include adaptation and mutation of genes followed by selection under drug pressure.[2] The level of resistance is considered to be influenced by dosage and duration of use of the drug. Continued use of chloroquine in areas where resistance occurs may lead to a higher degree of stable resistance. It is therefore essential that background levels of drug sensitivity be closely monitored in order to control the disease adequately.

In vitro chloroquine resistance was first detected in the Ovambo region of Namibia in 1984.[3] Unconfirmed reports of non-response to treatment with chloroquine have also been received from the Kavango region of Namibia. However, no drug sensitivity studies have been carried out in this area of southern Africa. In May and June 1988, an investigation of the *in vitro* sensitivity to chloroquine of *P. falciparum* in the Kavango region was carried out. The results of this study are reported here.

Materials and methods

Venous blood was collected in lithium/heparin tubes from suspected malaria patients reporting to Andara Mission Hospital and several clinics in the Kavango area between 23 May and 1 June 1988. A urine sample was collected from each patient for testing by the Dill-Glazko method[4] for the presence of 4-aminoquinolines. The blood and urine specimens were transported on ice to a field laboratory where they were processed within 12 hours of collection. The laboratory consisted of a thatch-roofed hut, with reed walls and cement floor. A pair of portable 220 V generators supplied power to a microscope, a centrifuge, an incubator and an automatic pipette. To minimise contamination of the isolates to be cultured, all plastic and glassware, as well as the melamine table top that served as a working surface, were swabbed down with 70% enthanol, and all pipettes, tubes and bottles were flamed before use. In addition, surgical gloves were worn during the entire procedure to protect both the operator and the isolates.

Thick and thin blood smears were prepared from each blood sample, stained with Giemsa's solution and examined microscopically for the presence of *P. falciparum*. Isolates were considered to be unsuitable for the *in vitro* test if they contained less than 500 parasites/μl and were diluted with fresh O-positive red blood cells if the parasitaemia exceeded 100 000 parasites/μl. Isolates were further excluded if the patient was known to have taken antimalarial drugs in the recent past and/or if the Dill-Glazko test was positive.

Chloroquine sensitivity *in vitro* was determined by a modification of the Rieckmann microtechnique, as described by Freese *et al.*[5] Briefly, infected erythrocytes were washed twice in RPMI 1640 culture medium and suspended to 5% in medium to which 10% non-immune human AB serum had been added. Aliquots (50 μl) of the suspensions were added to the wells of microtitre plates predosed with 1, 2, 4, 5, 7, 8, 16 and 32 pmol chloroquine. Control wells contained no drug. For each isolate tested, 3 wells per chloroquine concentration were used. The plates were agitated for a few seconds to dissolve the drug and then incubated at 38°C in an atmosphere of 3% oxygen, 4% carbon dioxide and 93% nitrogen for 24 - 28 hours. After incubation, a smear was prepared from each well, allowed to dry and stored in slide boxes. These were transported to the Institute's laboratory in Durban where they were examined microscopically for the presence of schizonts (i.e. parasites with more than 2 nuclei). Tests were considered successful when at least 5% of trophozoites in the control wells had developed to schizonts. Isolates were classified as resistant when schizont development occurred at a concentration of $\geqslant$ 5,7 pmol chloroquine per well.

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Results

Blood specimens were collected from 118 patients and 55 of these were found to contain *P. falciparum* trophozoites. Forty of these malaria-positive patients were under 20 years of age. Six isolates were excluded from the test as no urine samples were available and 3 were excluded because the urine samples were positive for 4-aminoquinolines.

Of the 46 isolates tested, 8 were removed from the test before schizont development could take place and 12 did not mature at all. Fifteen of the 26 successfully tested isolates were susceptible with total inhibition occurring at 4 pmol chloroquine per well. The remaining 11 isolates were resistant to chloroquine, all being inhibited at 8 pmol per well.

The Dill-Glazko test showed 18 of the 101 urine samples tested to be positive for 4-aminoquinolines.

Discussion

The results of this investigation confirm the presence of chloroquine-resistant *P. falciparum* malaria in the Kavango region of Namibia. Forty-two per cent of the isolates successfully tested were inhibited at 8 pmol chloroquine, which is indicative of RI resistance.[6] Similar results were obtained by Isaacson *et al.*,[3] who found, in a study of 7 isolates from the Ovambo region of Namibia, that 5 isolates were sensitive to chloroquine, being inhibited at $\leqslant$ 6 pmol per well, and 2 were resistant, being inhibited at 12 pmol per well. These *in vitro* chloroquine sensitivities are considerably lower than those found in northern KwaZulu, where the majority of isolates tested were found to be inhibited at 32 pmol per well.[5]

The low level of chloroquine resistance in the Kavango area may be related to the degree of drug pressure. The small number of urine specimens positive for 4-aminoquinolines in the Dill-Glazko test indicates that chloroquine is not widely used in the area. It is possible that under this relatively low drug pressure, parasites from this area have tended not to develop higher levels of resistance. However, future monitoring of drug sensitivity in this area is considered essential in order to assess whether or not the situation remains stable.

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REFERENCES

1. Spracklen FHN, Whittaker RG. Malaria 1984. Part II. Drug resistant malaria. *S Afr Med J* 1984; **66:** 211-216.
2. Beale GH. The genetics of drug resistance in malaria parasites. *Bull WHO* 1980; **58:** 799-804.
3. Isaacson M, Cox GA, Sieling WL. *In vitro* confirmation of chloroquine-resistant *Plasmodium falciparum* in southern Africa. *S Afr Med J* 1984; **66:** 209-210.
4. Lelijveld J, Kortmann H. The eosin colour test of Dill and Glazko: a simple field test to detect chloroquine in urine. *Bull WHO* 1970; **42:** 477-479.
5. Freese JA, Sharp BL, Ngxongo SM, Markus MB. *In vitro* confirmation of chloroquine-resistant *Plasmodium falciparum* malaria in KwaZulu. *S Afr Med J* 1988; **74:** 576-578.
6. Report of a WHO Scientific Group. Advances in malaria chemotherapy. *WHO Tech Rep Ser* 1984; No. 711.