Resistance of *Plasmodium falciparum* to antimalarial drugs is a serious impediment to controlling malaria.\(^1\) *P. falciparum* resistance to chloroquine was first reported in Africa in 1979,\(^2\) while clinical evidence of chloroquine resistance emerged in South Africa during the mid-1980s.\(^3\) This prompted a standardised chloroquine *in vivo* therapeutic efficacy study in Mpumalanga, based on the World Health Organization (WHO) protocol,\(^4\) which demonstrated a parasitological failure rate of 48.4% (unpublished Department of Health Report, 1998), necessitating chloroquine’s replacement with sulfadoxine-pyrimethamine (SP) as first-line treatment of uncomplicated *P. falciparum* malaria in Mpumalanga in 1997.\(^5\) The *in vivo* SP cure rate at introduction in Mpumalanga in 1998 was 94.5%, with a combined RI and RII resistance of only 5.5%.\(^5\) A subsequent study conducted 3 years after SP introduction demonstrated continued efficacy of SP in the province. In other African settings, SP monotherapy has not remained effective as first-line treatment of malaria for long before parasitological resistance has developed.\(^7\) Extensive research now supports the implementation of artemisinin-based combination therapies to improve cure rates, decrease malaria transmission and delay resistance.\(^8,9\) To determine whether current levels of SP resistance remain adequate to support its use in combination with artesunate, we conducted an *in vivo* SP therapeutic efficacy study 5 years after implementation in Mpumalanga.
Method and materials

Patients

The study was conducted in Tonga health district, Mpumulanga, between January and June 2002. All patients with clinical features of malaria presenting at the two 24-hour primary health care clinics in Mangweni and Naas, were sequentially tested for *P. falciparum* infection using the rapid *P. falciparum* histidine-rich protein antigen diagnostic test (Core Malaria PI, Core Diagnostics, Birmingham, UK). *P. falciparum*-positive patients were then recruited according to modified WHO guidelines, with inclusion criteria being age 2 years or above, symptomatic uncomplicated *P. falciparum* infection with an asexual parasite density above 1 000 parasites/µl blood, proximity of patient’s home for follow-up, informed consent, and history of fever or axillary temperature above 37.5°C. Exclusion criteria included severe malaria, intolerance of oral therapy, and pregnancy. Criteria for withdrawal included patient’s request and clinical deterioration necessitating hospital referral. Baseline data including age, gender, weight and place of residence were obtained from all study subjects by trained health staff on enrolment at the two study sites.

Treatment

Patients were treated according to the guidelines of the Mpumulanga Department of Health (internal publication), with a single oral administration of SP (Fansidar, Roche, Gauteng, South Africa) at a dose as close as possible (using whole tablets) to 25 mg/kg of sulfadoxine and 1.25 mg/kg of pyrimethamine but not exceeding the maximum dose of 1 500 mg sulfadoxine/75 mg pyrimethamine. After drug administration, patients were observed for 1 hour. If vomiting occurred within 30 minutes of drug administration, the full dose was repeated. If vomiting occurred 30 - 60 minutes after drug administration, an additional half dose was administered. Patients with clinical or parasitological treatment failure were referred to hospital for therapy with quinine.

Laboratory assessment and outcome measures

Clinical and parasitological assessments were conducted routinely on days 1, 2, 3, 7, 14, 21, 28 and 42 post-treatment. At each follow-up visit a thick blood smear was taken, body temperature was recorded and an assessment for adverse events was completed. Fever was defined as an axillary temperature exceeding 37.5°C. Parasite density was measured by counting the number of parasites against 300 leukocytes on a Giemsa-stained, finger-prick thick blood film, assuming a standard leukocyte count of 7 500/µl blood. Parasite clearance time was the period from recruitment to the first of two successive thick smears with no asexual parasites. Fever duration was the time that elapsed between recruitment and axillary temperature being recorded below 37.5°C without a subsequent recorded increase in temperature.

Response to treatment was assessed according to the 2003 WHO classification for low to moderate transmission areas.10 Adequate clinical and parasitological response (ACPR) was defined as absence of parasitaemia on day 42 irrespective of axillary temperature without previously meeting any of the criteria for early treatment failure or late clinical or parasitological failure. Early treatment failure was defined as the development of danger signs or severe malaria on day 1, 2 or 3, in the presence of parasitaemia; parasitaemia on day 3 with axillary temperature ≥ 37.5°C; parasitaemia on day 2 higher than day 0 count; or parasitaemia on day 3 a 25% of count on day 0. Late clinical failure was defined as the development of danger signs or severe malaria after day 3 in the presence of parasitaemia or the presence of parasitaemia and axillary temperature ≥ 37.5°C on any day from day 4 to day 42, without previously meeting any of the criteria for early treatment failure. Late parasitological failure refers to the presence of parasitaemia on any day from day 7 to day 42 and axillary temperature < 37.5°C, without previously meeting any of the criteria for early treatment failure or late clinical failure.

Parasitological success was defined as conversion from a positive smear at recruitment to a negative smear by day 7, remaining negative until the end of the 42-day follow-up period. Levels of resistance were defined as follows: (i) RI resistance (recrudescence) — a negative blood film before day 7 and reappearance of parasites during the remaining follow-up period; (ii) RII resistance — axillary temperature ≥ 37.5°C on day 2 and parasitaemia ≥ 25% of day 0, or axillary temperature ≥ 37.5°C on day 3 and any level of parasitaemia; (iii) RIII resistance — less than 75% reduction in parasite density by 72 hours.7

Polymerase chain reaction (PCR) amplification of the polymorphic genetic markers MSP1, MSP2 and GLURP was used to differentiate between true recrudescence and new infections.11

Ethical considerations

Approval for the study protocol was obtained from the Mpumulanga Department of Health Ethics Committee and the University of Cape Town Research and Ethics Committee. Informed consent was obtained before enrolment from each patient or the guardians of minors. The recruited subjects were informed that they were free to withdraw their consent at any time during the study. Treatment was provided free of charge, as is the norm for public-sector malaria treatment in Mpumulanga.
Results

Baseline information

One hundred and fifty-two patients were recruited between January and June 2002. Three patients (2%) moved from the study area on day 2 ($N=2$) or day 7 ($N=1$), and were therefore unable to complete follow-up. Baseline characteristics are summarised in Table I. The mean dose of pyrimethamine administered was 1.59 mg/kg with 32/152 (21%) receiving less than the recommended 1.25 mg/kg dose of pyrimethamine.

Clinical and parasitological responses

Median fever clearance time was 69 hours and median parasite clearance time was 72 hours. Of the 149 patients (98%) who completed follow-up to day 42, 134 (90%) had an adequate clinical and parasitological response. Fifteen patients (10%) failed treatment — 2 (1.3%) experienced early treatment failure, and PCR confirmed recrudescent infection in all 13 patients (8.7%) who had either late parasitological ($N=11$) or late clinical ($N=2$) failure. These recrudescent infections occurred on day 21 ($N=5$), day 28 ($N=3$) and day 42 ($N=4$).

Gametocyte carriage was prevalent throughout follow-up, and peaked on day 14 when 67/146 study subjects (45.9%) carried gametocytes (Fig. 1). Intention-to-treat analysis of the prevalence of patients carrying gametocytes at any time following treatment has increased significantly from 40/132 (30.3%) in 1998 to 52/119 (43.7%) in 2000 (odds ratio (OR) 1.79 (1.03 - 3.10), and to 83/152 (54.6%) in 2002 (OR 2.77 (1.65 - 4.66)). This trend is highly significant ($p = 0.00004$, chi square test for trend).

Clinical adverse event monitoring was conducted throughout the study and no adverse events were observed.

Discussion

$P. falciparum$ asexual parasites remain sensitive to SP in Mpumalanga, with a 90% cure rate at 42 days, and only 2 (1.3%) early treatment failures after 5 years of programme use as first-line treatment for uncomplicated malaria. There was no statistically significant difference in SP cure rates between introduction and 3 and 5 years later (chi-square for trend $p = 0.14$). The baseline data at SP introduction in 1998 demonstrated the sensitivity of $P. falciparum$ to SP, with a cure rate of 94.7% (125/132) and a combined RI and RII resistance of 5.5%.

This efficacy was maintained in 2000 when the ACPR rate was 93.3% (111/119). No RIII failures were detected in either of these studies. The sustained clinical and parasitological efficacy of SP for 5 years in Mpumalanga is in marked contrast to findings in Malawi, where the SP parasitological failure rate in patients followed up for 28 days was 51%, 5 years after implementation of SP as first-line malaria treatment.

Despite sustained SP efficacy, the prevalence of gametocytes in 2002 remained high throughout the follow-up period, with 55% of patients carrying gametocytes on at least 1 follow-up visit. This significant increase in gametocyte carriage since SP introduction, despite sustained high asexual parasitological cure rates, is cause for concern. Increasing gametocyte carriage may represent an early indication of impending SP therapeutic failure. Gametocytes are responsible for the ongoing transmission of malaria, as they are the stage in the parasite’s lifecycle responsible for re-infection of $Anopheles$ mosquitoes from infected humans. There are convincing indications that antimalarial resistance is facilitated by the relatively higher gametocyte carriage rates in resistant compared with sensitive infections.12 Treatment for malaria should eliminate asexual parasites and ideally also reduce gametocyte stages. The combination with an artemisinin derivative offers the
advantage of decreasing gametocyte carriage. The recently published meta-analysis of 5,948 individual patients' data from 16 randomised trials studying the effect of artesunate addition to standard treatment of P. falciparum malaria demonstrated a significant impact of combination therapy against gametocytæmia, with a summary OR of 0.11 (95% confidence interval (CI): 0.09 - 0.15) for the presence of gametocytes at day 7. This meta-analysis demonstrated a mean decrease in log gametocyte count equivalent to a decrease of 46% (95% CI: 41 - 50%) in the geometric mean gametocyte count curve.

These findings indicate that combining SP with an artemisinin-derivative should be highly effective in the primary treatment of uncomplicated malaria in Mpumalanga. In areas where SP remains highly effective, this artemisinin-based combination therapy (ACT) has a number of advantages over artether-lumefantrine including greater effectiveness if patients are only partially adherent (as SP is administered as a single dose); artesunate plus SP, unlike artether-lumefantrine (Coartem), is not dependent on fat for absorption; there are no weight limitations for AS or SP; and drug costs are currently lower.

Experience from other southern African areas suggests that ongoing surveillance remains essential. Data from KwaZulu-Natal12 years after introduction of monotherapy with SP demonstrated an SP parasitological failure rate of 55% on day 14 and 81% at 28 days' follow-up. Similar findings have recently been published from Malawi,7 where 10 years after the implementation of SP the parasitological failure rate was 38% at day 14 and 73% after 28 days' follow-up. Although artemisinin-based combination antimalarial therapy appears to provide protection against the development of resistance and there are established therapeutic precedents from the treatment of the other micro-organisms, regular standardised in vivo evaluations are essential to confirm ongoing efficacy.

The findings from this study in Mpumalanga support rapid implementation of the artemisinin-based combination of artesunate plus SP as first-line treatment of uncomplicated falciparum malaria in Mpumalanga, coupled with regular standardised monitoring of treatment efficacy.

This study was nested within the South-east African Combination Antimalarial Therapy (SEACAT) evaluation which received core financial support from the United Nations Development Programme World Bank WHO Special Programmes in Tropical Diseases Research (WHO TDR).

References


Accepted 8 November 2004.