The Therapeutic Efficacy of Sulfadoxine/Pyrimethamine against *Plasmodium falciparum* in Yemen

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Key Words

Drug-resistant malaria • Therapeutic efficacy • Sulfadoxine • Pyrimethamine • *Plasmodium falciparum* • Yemen

Abstract

Objective: The aim of this study was to determine the sensitivities of *Plasmodium falciparum* clinical isolates to sulfadoxine/pyrimethamine (SP) using in vivo and in vitro methods.

Subjects and Methods: In vivo and Mark III in-vitro test techniques according to World Health Organization protocols of antimalarial drug tests were used to determine the SP susceptibility of the *P. falciparum* isolates from 100 malaria patients of both sexes between the ages of 3.5 and 45 years and living in Tihamah, Yemen. The study was conducted between 19 March and 12 May 2005. **Results:** In vivo: no therapeutic failure occurred; the clinical outcome matched the parasitological response and all patients were parasite free by day 3 and remained so on days 7, 14 and 28. In vitro: all the *P. falciparum* isolates developed to schizonts in zero-drug-concentration wells, but were inhibited in 40 nmol/l of SP; the mean effective concentration (EC₉₉) was 67.17 nmol/l.

Conclusion: Our findings showed that the SP combination is still effective for the treatment of uncomplicated *P. falciparum* malaria in Yemen. It is recommended that further

studies be carried out to address the importance of dihydropteroate synthetase/dihydrofolate reductase mutations as predictive markers of sulfadoxine/pyrimethamine resistance in Yemen.

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Introduction

Malaria remains an important public health concern in countries where transmission occurs regularly [1]. Antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control; today, drug resistance has been implicated in the spread of malaria to new areas. Drug resistance has also played a significant role in the occurrence and severity of epidemics in some parts of the world [2, 3].

Resistance to all known antimalarial drugs, with the exception of the artemisinin derivatives, has developed to various degrees in several countries [4–7]. Parasite resistance to sulfadoxine/pyrimethamine has developed very quickly in Southeast Asia [8]. In Africa, sulfadoxine/pyrimethamine (SP) resistance was low until 5–6 years ago [9]. In most malaria-endemic countries in sub-Saharan Africa they have abandoned the use of SP because of high

levels of resistance and are currently using artemisinin combination therapies [8, 9]. However, SP is relatively cheap, safe and relatively available, and therefore might still be useful in countries like Yemen if resistance is low.

The rapid spread of antimalarial drug resistance over the last few decades has increased the need for monitoring in order to ensure proper management of clinical cases, allow for early detection of changing patterns of resistance and suggest where national malaria treatment policies should be revised [10, 11]. Malaria is one of the most serious health problems in Yemen. Approximately 60% of the population live in areas with malaria transmission, and the annual incidence rate is 5,263/100,000, with 35,000 deaths/year; *P. falciparum* accounts for more than 90% of malaria cases. Most of the studies on antimalarial drug efficacy carried out in the past 2 decades were mainly in vivo studies based on the standard WHO 7-day test [12, 13]. In the present study, both in vivo and in vitro studies using Fansitab® tablets (SP, produced by Shaphaco Pharmaceutical Industries, Yemen) were carried out. Fansitab tablets were selected because they are easily administered, cheap and widely available in Yemen.

Subjects and Methods

Patients of both sexes between the ages of 3.5 and 45 years living in Tihamah (Bajil city and surrounding villages, high endemic area of malaria) in the Republic of Yemen suffering from fever and/or other malaria symptoms were screened at field hospitals and Malaria Control Centers. This study was conducted between 19 March and 12 May 2005.

The study was approved by the Ethical Committees of the Faculty of Medicine, Sana'a University, and the Ministry of Health. Informed written consent was obtained from patients or their parents. Fever was defined as a body temperature of 37.5°C or above. Patients were asked for any history of diarrhea and vomiting.

The history of antimalarial drug ingestion within the past 2–4 weeks was taken. If known, the name and quantity of the drug were recorded. Only patients with negative urine tests to 4 aminoquinolines and sulphonamides were enrolled. Exclusion criteria were patients with complicated diseases, such as cerebral malaria, anemia, jaundice and bronchopneumonia; patients whose peripheral blood smear (thick film) contained mixed infections from *P. falciparum*, *P. vivax*, *P. malariae* and/or *P. ovale*. The diagnosis for all patients was confirmed by the presence of asexual forms (trophozoites) of *P. falciparum*. Enrolled patients were registered and given special codes.

In vivo Test

One hundred patients (66 male and 34 female), whose parasite counts were not less than 10,000 but not more than 80,000 asexual parasites per mm³ of blood, were selected based on WHO se-

Table 1. Distribution of 100 patients with *P. falciparum* malarial infections according to their age and sex

	Male, %	Female, %	Total
Age (years)			
<5	13	14	27
5–15	13	7	20
>15	40	13	53
Total	66	34	100

lection protocols [14]. They were registered and given special codes. Treatment was started on day 0. The clinical examination, including axillary temperature, was repeated on days 3, 7, 14 and 28. In addition, the blood slide examination was repeated on the same days. The axillary temperature was recorded to 1 decimal point with an electronic thermometer.

Laboratory Testing of Antimalarial Drugs Susceptibility (in vitro Test)

The WHO in vitro antimalarial drug susceptibility test (Mark III) technique [15] was used to determine the antimalarial drug susceptibility of the *P. falciparum* isolates and the schizont maturation inhibition concentration (the lowest drug concentration in which no such schizonts were observed).

Data Analysis

This was carried out using test record sheets and probit analyzing program (WHO analyzing program version 2) and Epi Info version 6 (Centers for Disease Control, Atlanta, Ga., USA, 2001) to determine the percentage of schizont maturation inhibition, and the effective concentration (EC) values and their 95% confidence intervals.

Results

The distribution of malarial infections based on age and gender is given in table 1. In males, the most infected were >15 years of age, while in females it was those <5 and >15 years that were most infected.

In vivo Test Results

No therapeutic failure was observed, and the clinical response showed that patients had adequate clinical and parasitological responses. Six patients were lost to follow-up. Of these, 5 were lost on day 3 and 1 on day 7. The clinical outcome matched the parasitological response, and the malaria parasite was absent from the blood on day 3 and remained so on days 7, 14, and 28 for all patients (table 2).

Table 2. The percentage of schizont maturation inhibition by a Mark III in vitro test

Drug concentra- tion, Pmol/well	Drug concentra- tion, nmol/l	SMI, %
0.25	5	47.97
0.5	10	68.74
1.0	20	89.55
2.0	40	100.00
4.0	80	100.00
8.0	160	100.00
16.0	320	100.00

Pmol/well = Part mole per wells; SMI = percentage of schizont maturation inhibition.

Table 3. The EC of SP by the Mark III in vitro test

	Mean, nmol/l	95% CI	
		lower	higher
EC ₁	0.4474	0.2041	0.9810
EC ₁₆	1.8781	1.5226	2.3166
EC ₅₀	5.4820	4.4445	6.7618
EC ₈₄	16.0017	12.9731	19.7373
EC ₉₀	21.8007	13.6508	34.8164
EC ₉₅	32.2419	18.2135	57.0753
EC ₉₉	67.1702	30.6356	147.2741

In vitro Test Results

The schizont minimum inhibition percentages and effective concentrations (EC₅₀, EC₈₄, EC₉₀, EC₉₅ and EC₉₉) are shown in tables 2 and 3, where 100% of schizont inhibition occurred in 2 pmol of drug concentration, EC₅₀ = 5.4820 nmol/l (95% CI: 4.4445–6.7618) and EC₉₀ = 21.8007 nmol/l (95% CI: 13.65–34.8164).

Discussion

Resistance to SP combinations (such as Fansitab) is now widespread in many parts of *P. falciparum* foci in the world [1, 16]. The data derived from our study for evaluating the efficacy of SP are essential, not only for maintaining confidence that current treatment recommendations are adequate in relation to malaria patients' needs, but also for generating convincing evidence that current treatment recommendations are in need of change. More-

over, when such evaluations are conducted over time and in selected sites in different malaria foci in Yemen, we should be able to monitor drug efficacy in a way that will suggest changes in treatment recommendations or policies to be made early enough to minimize the impact of a failing treatment regimen.

The selection of the in vivo test in our study was intended to provide information needed to develop an evidence-based policy for the treatment of malaria, and this is similar to the situations in Africa, South America and Asia, where studies have used the WHO in vivo methodology to provide information on the efficacy of SP as well as initial evaluations of potential alternative treatment regimens [17, 18].

Our in vivo and in vitro findings on the efficacy of SP in the study area are in agreement with results by the WHO in 2 other foci of malaria in Yemen [19]. Our results are also similar to those in the rest of the Arabian peninsula and East Africa [20], where the failure of treatment by SP was not recorded in spite of the high prevalence of the SP-resistant genotypes dihydrofolate reductase/dihydropteroate synthetase (dhfr/dhps), where parasites are generally wildtype in dhps/dhfr, but doubly mutated in dhfr/dhps. In addition, triple mutant alleles of dhfr are now common in Sudan and Saudi Arabia, Tanzania, Kenya and Uganda [21, 22].

The question of primary interest was the possible reappearance of parasites within the observation period indicating treatment failure, since it was difficult to keep patients in a mosquito-free environment to prevent reinfection. In spite of that, the possibility of reinfection was excluded – a fact that was indicated by the absence of parasites in patients' blood during the 28 days of follow-up and observation.

In this study, the selected target age groups of <5, 5–15 years and adults emphasized treatment efficacy in all age groups including children under 5 years of age. The reason for the selection of the age group <5 years was that, even in populations with little acquired immunity (as occurs in areas of low or high seasonal malaria transmission), younger children often have a less favorable therapeutic response to antimalarial drugs than older children and adults, owing to the absence or low level of acquired immunity [23].

The duration of posttreatment follow-up in our study was 28 days. The length of time that is appropriate for assessing response in vivo has been a topic of recent debate. Accumulated experience from a number of studies conducted in a variety of settings has raised important observations and issues that are relevant to this debate. One of

the disadvantages of studies with a shorter follow-up (i.e. <14 days) is that they will underestimate overall treatment failure rates; this is especially true of drugs with longer elimination half-life. Drug clearance is the parameter that defines the length of the follow-up in the therapeutic efficacy test: for our tested drug (SP) the half-life is 100 h (4 days), so clearance would take $6 \times 4 = 24$ days, so follow-up should therefore be for a minimum of 28 days.

In addition, the disadvantage of a long follow-up period is that the longer the duration of posttreatment follow-up, the greater the chance of reappearance by parasites which might be caused by reinfection rather than recrudescence. Inability to distinguish between reinfection and recrudescence can lead either to an overestimated rate of failure (if all reappearances are assumed to be recrudescence) or to an underestimated rate of failure (if reappearances are assumed to be reinfections). This risk increases with increasing transmission intensity [1, 23]. To solve this problem, molecular techniques can help to distinguish recrudescence from reinfection. However, these techniques are not currently available in Yemen, and they require specialized equipment and training that exceed the typical capacity of our University.

EC refers to the concentration of a drug that induces a response between the baseline and maximum. It is commonly used as a measure of drug potency, e.g. the EC_{50} of a graded dose response curve represents the concentration of a compound where 50% of its maximal effect is

observed. The EC values are also related to the inhibition concentration, which is a measure of a compound's inhibition. In our study, using the in vitro drug sensitivity assay, the EC values for the tested isolates demonstrated a relatively high sensitivity to SP ($EC_{50} = 5.4820$ nmol/l, 95% CI: 4.4445–6.7618, $EC_{90} = 21.8007$ nmol/l, 95% CI: 13.65–34.8164; table 3). Our results were similar to those reported in Bangladesh [24]. Therefore, this drug proved to be an interesting option for treating uncomplicated falciparum malaria in Yemen.

Conclusion

Our findings showed that the SP combination is still effective for the treatment of uncomplicated *P. falciparum* malaria in Yemen. It is recommended that further studies be carried out to address the importance of dhps/dhfr mutations as predictive markers of SP resistance in Yemen.

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