RESEARCH NOTE

PYRIMETHAMINE-SULFADOXINE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN LAO PDR

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Abstract. A 28-day *in vivo* treatment trial to evaluate the efficacy of pyrimethamine/sulfadoxine (Fansidar©, PS) was conducted in 21 Lao patients with uncomplicated *Plasmodium falciparum* malaria. Sixteen patients (76%) were completely cured with PS without any reappearance of asexual stage parasitemia during the follow-up examination. On the other hand, 5 patients (24%) failed to respond to this trial medication, resulting in recrudescence of asexual stage *P. falciparum* malaria. PS resistance resulted in higher prevalence of post-treatment gametocytemia, 25% gametocyte carriers among PS sensitive cases versus 75% of the resistant cases. These findings suggest that although the level of PS resistance is still valid for treatment of malaria in the study area of Lao PDR, post-treatment induction of gametocytemia among resistant cases may result an increase in transmission rate of PS resistant falciparum malaria.

INTRODUCTION

Lao PDR is a developing country in Southeast Asia bounded by borders with Myanmar, Cambodia, China, Thailand, and Vietnam. Malaria is the most serious public health problem in Lao PDR, accounting for an estimated 1,561 deaths from 1998 to 2002, as recorded by Center of Malariology, Parasitology and Entomology (CMPE), Vientiane. Although the limited use of impregnated bed nets and other preventive measures, such as health education, have been playing a big role to gradually reduce the morbidity and mortality due to malaria in recent years, there has been growing concern due to increasing frequency of treatment failure over the period of past decade. Because an alarmingly increasing emergence of resistant falciparum malaria to chloroquine, the recommended first-line antimalarial agent for uncomplicated malaria in the country, has become evident in a series of *in vivo* and *in vitro* studies in Lao PDR (Tawil, 1977; Giboda *et al*, 1992; Pillai *et al*, 2001; Mayxay *et al*, 2003), has been suggested that a revised national policy for treatment of malaria is needed. Indeed, local doctors are now facing enormous troubles in giving treatment to malaria patients with chloroquine, as treatment failure due to resistance not only complicates the disease but also increases the transmission rate of resistant malaria.

Under these circumstances, a combination of pyrimethamine and sulfadoxine (PS), the recommended second-line antimalarial agent in Lao PDR, has become the drug of choice for treat-

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ment of malarial patients in the country. Although there are very few reports to support or refute the current use of PS, local and sporadic observations suggest that PS-resistant falciparum malaria is also developing. In view of the deteriorating multidrug-resistant malaria in the countries bordering Lao PDR (Gomes *et al*, 1978; White, 1992; Smithuis *et al*, 1997), the importation of PS-resistant malaria from the neighboring countries is a possibility, especially because of recent population movements across the porous borders.

With this view in mind, the present study was undertaken to assess the efficacy and appropriateness of PS so as to justify whether it has potential for prevention and treatment of malaria in a selected region of Lao PDR. The study also focused on the influence of PS treatment on gametocytemia. Although this study is confined to *in vivo* treatment trial only, it should deserve attention as an essential step for further studies on the efficacy of PS, including genotype analysis to understand the mechanism of resistance.

MATERIALS AND METHODS

With approval from Ministry of Health, Lao PDR, the present study was conducted from February to August 2003 in close co-operation with senior CMPE officials, who helped in the selection of study sites as well as in field settings, language interpretation and technical expertise. Based on a preliminary small-scale survey, this study was conducted during the rainy season in the field settings of rural areas of Lao Ngam and Khongsedon districts of Saravan Province, a malaria endemic area with moderate to low transmission, located about 800 km south of Vientiane. The residents of the study areas belong to two ethnic groups, namely, Lao Luom (lowland) and Lao Theung (upland), who live by cultivation and farming.

RESULTS

A total of 1,192 people were examined for active case detection of *Plasmodium falciparum* by microscopy using thick and thin smears of finger prick blood. Twenty-nine samples were positive for *P. falciparum*, one of which was also identified as mixed-infection with *P. vivax*. Eight cases were positive for *P. vivax* by microscopic examination of Giemsa's stained blood film. Overall prevalence encompassing *P. falciparum* (2.4%), *P. vivax* (0.7%) and a mixed infection (0.1%) was 3.1%.

Among 29 *P. falciparum* positive cases, 8 cases were excluded from *in vivo* treatment trial according to the exclusion criteria, including pregnancy and positive cases diagnosed by the presence of gametocytes only. The remaining twenty-one cases (10 males and 11 females with age of 2-45 years, 2 adults and 19 children) met the inclusion criteria for the *in vivo* treatment study with signs and symptoms of acute uncomplicated *P. falciparum* malaria (fever less than 39.5ºC on enrolment or a history of fever within the previous 24 hours), single infection with *P. falciparum*, and initial parasitemia (asexual stage parasites) of more than 1,000 per microliter blood. The cases were, with informed consent, administered PS (1.25 mg pyrimethamine/kg body weight) under strict supervision (day 0) and were followed up on days 2, 3, 7, 14, 21 and 28 for axillary temperature measurements, thick and thin blood smears and blood spots on filter paper. Giemsa's stained blood smears were read by microscopists of CMPE and filter paper blood was used for analysis of MSP-1 and MSP-2 genes of *P. falciparum* to distinguish recrudescence from re-infection. The efficacy of treatment was determined by blood examination in which no asexual parasitemia was detected after the treatment.

Table 1 summarizes the results of the 28-day *in vivo* treatment trial with PS. Sixteen (76%) of 21 *P. falciparum* patients recruited in the *in vivo* trial were completely cured with PS without any reappearance of asexual parasitemia during the follow-up examination. Five (24%) patients failed PS treatment, suggesting the possibility of infection due to recrudescence. For 3 of the 5 resistant cases, the parasitologic failure to respond to PS was graded as R III resistance, as manifested by the recurrent parasitemia within the first week of the follow-up examination. The other resistant cases had recurrence during days 14-28, which was consistent

Table 1
Results of a 28-day *in vivo* therapeutic trial with pyrimethamine/sulfadoxine against *Plasmodium falciparum* malaria in Saravan Province of Lao PDR.

Features	Result
Number of patients	21
Mean age (Range)	11 (2-45) years
Percent male	47.6
Parasitologic response	
Sensitive	16/21 (76%)
Resistance (total)	5/21 (24%)
RI/RII resistance	2/21 (10%)
RIII resistance	3/21 (14%)

Treatment failure was defined by the presence of asexual stage parasites in the blood during the follow-up period. RI/RII resistance indicates late treatment failure while RIII indicates early treatment failure.

with RI/ RII resistance, as identified by the analysis of MSP-1 and MSP-2 genes of *P. falciparum* using isolates from filter paper samples (data not shown).

DISCUSSION

Because of the emergence of high degree of chloroquine-resistant falciparum malaria in Lao PDR, PS seems to be a good candidate for replacement therapy. Data on the efficacy of PS, however, are not available to support this view. Only recently Schwöbel *et al* (2003) demonstrated that PS resulted in 17.9% treatment failure in a 14-day *in vivo* trial in Attapu Province, Lao PDR. This finding is not so inconsistent with our 28-day trial with PS which resulted in 24% treatment failure. Even if we had conducted our study for 14 day, it would have produced 14% (3 out of 21) treatment failure. Although we cannot extrapolate these findings to other areas of Lao PDR, knowledge from unpublished sources (CMPE record) indicates that PS resistance, even with its developing stage, exists within a safe range.

On the other hand, the question is that PS resistance is associated with post-treatment induction of gametocytemia. In the present study, we observed 80% (4 out of 5) gametocyte carriers among patients having therapeutic failure to PS, as compared with that of only 25% (4 out of 16) gametocyte carriers among patients having an adequate therapeutic response. In an epidemiological study in Gambia, von Seidlein *et al* (2001) also observed the association of PS treatment with high prevalence of post-treatment gametocytemia. However, the study failed to make clear whether resistance is the prime (essential) contributor to post-treatment gametocytemia. Further studies are necessary to establish the association of PS resistance with post-treatment induction of gametocytemia, since gametocytes do not contribute to disease pathology but it has impact on the spread of malaria. Induction of gametocytemia by treatment failure may worsen the situation by spreading resistant falciparum malaria. The high gametocyte prevalence among PS resistant patients suggests that the continuous use of PS is in contrast to conventional treatment policy. Because of the poor socio-economic infrastructure of Lao PDR, it is quite difficult for the policy makers to go beyond PS (or chloroquine), which is easily available and affordable. von Seidlein *et al* (2001) have shown that PS combined with artesunate at a single dose cleared both sexual and asexual parasitemia in Gambian patients, and this can be chosen as an affordable cost-effective antimalarial, provided that the therapy is effective in Lao PDR.

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