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Evaluation of Fosmidomycin in Adults with Acute Uncomplicated *Plasmodium falciparum* Malaria

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Abstract

The treatment of malaria is becoming increasingly difficult due to the development of *Plasmodium falciparum* strains resistant to the commonly used antimalarials. There is thus an urgent need for new, effective, and safe, antimalarial drugs. This study determined the efficacy of fosmidomycin among patients with uncomplicated *P. falciparum* infection. Fifteen patients were enrolled to receive fosmidomycin 1,200 mg orally every 8 hours for 7 days on an inpatient basis, followed by scheduled outpatient visits on days 14, 21, and 28 post-treatment. Five patients withdrew from the study: 1 had an equivocal pregnancy test result, 1 developed signs of severe malaria, 1 withdrew consent, and 2 did not receive the full treatment course. Per-protocol analysis showed a 100% cure rate in the first 7 days, dropping to 22% at 28-day follow-up. No serious adverse effects were observed. The result of this study showed that fosmidomycin cleared parasites well in the first 7 days; however, the recrudescence rate was high during the 28 days' follow-up period. The drug should be useful in the treatment of acute uncomplicated *P. falciparum* malaria infection, but combination therapy is needed to reduce recrudescence, thus improving cure rate.

Keywords: *Plasmodium falciparum*, fosmidomycin, isopentenyl diphosphate

Introduction

The treatment of malaria is becoming increasingly difficult, due to the development of *Plasmodium falciparum* strains resistant to the commonly used antimalarials. In addition to the chloroquine-resistant strains that have become widespread in all endemic areas of the world, resistance to sulfadoxine/pyrimethamine has now emerged in many parts of Asia and Central South America, and is spreading to Africa [1]. There is thus an urgent need for a new, effective, and safe antimalarial drug [2].

Fosmidomycin, a phosphonic acid derivative, was formerly under development as an antibacterial agent with potent activity against gram-negative organisms. However, its early promise in the treatment of uncomplicated urinary tract infections was countered by its relative lack of effectiveness against recurrent infections, although the safety of the drug even in high doses (1.0 g every 6 hours for 7 days, per os) was established [3-5]. As a potent inhibitor of 1-deoxy-d-xylulose 5-phosphate (DOXP) reductoisomerase, an essential enzyme of the

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nonmevalonate pathway, fosmidomycin blocks the biosynthesis of isopentenyl diphosphate and the subsequent development of isoprenoids in *P. falciparum* [6]. In contrast, isoprenoids are derived from an alternative pathway, known as the mevalonate pathway, in mammals [7]. Hence, fosmidomycin exerts its antimalarial activity through a mechanism of selective toxicity that allows the biosynthesis of isoprenoids, which are essential for cellular function, to be maintained in mammalian hosts. *In-vitro* experiments have shown that fosmidomycin exhibits its full antimalarial potency when the parasites are exposed to the agent for a full replication cycle, leading to arrested development in the late schizont stage. In contrast with other antibiotics having antimalarial properties, such as doxycycline or clindamycin, it does not exert a delayed effect (unpublished data). Therefore, we conducted a clinical trial, to evaluate the efficacy of fosmidomycin in the treatment of uncomplicated *P. falciparum* infection.

The study was undertaken to evaluate the efficacy and safety of fosmidomycin in the treatment of acute uncomplicated *P. falciparum* infection. The primary efficacy variable was the cure rate on day 7. Secondary efficacy variables were parasite and fever clearance times, and cure rate on day 28**.** Drug safety was assessed by the frequency and severity of adverse events observed among patients, possibly or probably related to the drug.

Operational definitions: parasite clearance time was defined as the time from initiation of treatment to the first of at least two consecutive negative blood smears. PC_{50} and PC_{90} were defined as hours until clearance of 50 and 90% of initial parasitemia levels, respectively. Fever clearance time was calculated from the commencement of therapy to the time that temperature fell < 37.5°C and remained < 37.5°C for 48 hours. Adverse events were defined as any symptoms or signs that first occurred, or increased in severity, during the study period. Each adverse event was categorized according to seriousness (need for hospitalization), intensity (mild to severe), and causality (relationship to the study drug). A clinical assessment checklist contained the following signs and symptoms: weakness, chills or rigors, headache, myalgia, dizziness, abdominal pain, anorexia, nausea, vomiting, diarrhea, palpitations, insomnia, pruritus, coughing, and tinnitus. Abnormal laboratory values considered clinically relevant were also reported as adverse events.

Materials and Methods

Study design

The study was designed as an open-label, uncontrolled trial, and was conducted in Mae Sot General Hospital, in Tak Province, Thailand; Tak is a malaria-endemic area with seasonal transmission and a high prevalence of multi-drug-resistant falciparum malaria [1]. Adults living in this area have little or no immunity to *P. falciparum* malaria. The study was conducted April-June 2001. The protocol was approved by the Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand. The study was conducted in accordance with the Declaration of Helsinki.

Study population

Adult patients aged 18-50 years, bodyweight > 40 kg, who presented with symptoms of acute uncomplicated *P. falciparum* malaria and parasitemia levels of 1,000-50,000/µl, were invited to participate in the study. Written informed consent was obtained from all participants pre-enrollment. Subjects were excluded if they had mixed plasmodial infections, significant concomitant disease, hemoglobin count < 8 g/dl, or white cell count > 12,000/µl, or had received antimalarial treatment within the previous 28 days. Pregnant or breastfeeding women were also excluded.

Method

The study drug (fosmidomycin sodium batch no. 008003) was provided by Jomaa Pharmaka GmbH. The drug's stability was confirmed prior to study commencement.

The study had two phases: a treatment phase lasting 7 days and a follow-up phase of 21 days. During the treatment phase, the subjects were admitted to hospital and given fosmidomycin 1,200 mg orally every 8 hours for 7 days. This regimen was shown to be well-tolerated in previous studies [5]. The subjects were monitored closely in the hospital. Parasite counts were performed, and vital signs (temperature and pulse) were monitored every 8 hours during the acute stage of infection, then once daily for the duration of this phase. Clinical examinations were also conducted daily, with subjects being asked about common malaria-associated symptoms. Blood samples were obtained for routine hematology and clinical chemistry testing, prior to drug administration, and on days 2, 4, and 7. A pre-treatment blood sample was also taken for parasite genotyping in the event of recurrent parasitemia. Urinalysis was conducted at screening, and on days 2, 4, and 7. During the follow-up phase, clinical assessments and thick blood smears were performed weekly on days 14, 21, and 28. Additional blood samples for hematology and clinical chemistry studies were taken on day 28. In the event of recurrent parasitemia, a second blood sample was taken for PCR analysis; the subject was then withdrawn from the study and treated with standard antimalarials; artesunate-mefloquine. Parasite genotyping was performed to differentiate between re-infections and recrudescent infections. Established procedures were employed using the PCR for the amplification of the merozoite surface protein 1 (MSP-1) and MSP-2 [8].

Statistical analysis

Efficacy was evaluated based on results from the per-protocol population, and analyzed by calculating cure rates and descriptive statistics of parasite- and fever-clearance times. No comparison of the cure rate with internal or external controls was planned. Adverse events and tolerability assessments were based on results from the intention-to-treat population, defined as all individuals having taken at least one dose of the drug. Calculation of confidence intervals was based on the exact binomial distribution. Changes in laboratory values were assessed by multiple measurement variance analysis. Differences among day 7, day 28, and baseline data were assessed by paired *t* test. A P-value < 0.05 was considered significant.

Results

A total of 15 patients participated in the study; 11 males and 4 females, mean (± SD) age 28 (± 9) years, bodyweight 53 (± 4) kg, body temperature on admission 38.0 (± 1.3 °C), and geometric mean asexual parasite count 11,307 (5,330-23,990)/µl (Table 1). Five subjects withdrew from the study: 1 withdrew consent after receiving 9 doses of the study drug, 1 showed a rapid increase in parasitemia level between screening and administration of the first dose of the study drug, 1 withdrew because of an equivocal pregnancy test result, and 2 did not receive the full treatment course.

Table 2 shows the efficacy and tolerability results of this study. The cure rate on day 7

Table 1 Demographic profile and admission data of patients. Data are presented as mean ± SD, median (range) or number of patients, where appropriate.

Characteristics	(N = 15)
Age (year)	29 (18-28)
Body weight (kg)	53 ± 4
Gender: male/female	11/4
Body temperature (°C)	38.0 ± 1.3
Baseline asexual form parasitemia (GM/µl)	11,307 (5,330-23,990)

(primary endpoint) was evaluable in 10 of the study subjects; all had negative blood smears at this time-point, so yielding a 100% cure rate. However, only 2 of the 9 evaluable subjects remained cured at day 28 (22%). Parasite clearance was rapid, with a mean (± standard deviation) of 44 (± 18) hours. PC_{50} and PC_{90} times were 21 (± 11) and 28 (± 13) hours, respectively. Fever was cleared after a mean time of 41 (± 25) hours. A total of 16 adverse events were recorded during the study period, and all were non-serious and mild-moderate in intensity. Laboratory parameters showed elevations in alanine aminotransferase (ALT) levels in 2 subjects (peaks of 124 and 130 U/liter). Hematological parameters showed hemoglobin and hematocrit reductions in the first phase of treatment, followed by a return to normal values. There was a fall in the numbers of eosinophils and thrombocytes during infection. Significant within-subject differences over time (days 0, 2, 4, 7, 28, or withdrawal) were found for all laboratory parameters measured, which reflected changes normally encountered during malaria infection.

Discussion

The present study establishes fosmidomycin as an efficacious antimalarial drug for the initial clearance of asexual parasitemia. The mean parasite clearance time of 44 hours is comparable to conventional antimalarials, and surpasses other antibacterial drugs with antimalarial activity, including doxycycline, clindamycin, and tetracycline [9-11]. Similarly, the resolution of clinical symptoms was rapid, with a mean fever-clearance time of 41 hours. However, there was a high rate (78%) of recurrent parasitemia, which represented recrudescent infections following confirmation of parasite genotyping. We also observed prolonged persistent gametocytemia, which suggested that fosmidomycin is devoid of gametocytocidal activity. A regimen of one dose every 8 hours was chosen in this study, because of the estimated short half-life of fosmidomycin (1.6 hours) [3,4]. For practical purposes, this regimen is too complex for outpatient use. Therefore, additional studies are under way to investigate the response to reducing the number of daily doses

Table 2 Efficacy and tolerability of fosmidomycin in patients with acute uncomplicated *P. falciparum* malaria. Values are expressed as No. (%), mean ± SD or median (range) where appropriate.

Parameter	No. (%) or Median (range)
Efficacy:	
Cure rate at Day 7 (N = 10)	10 (100%)
Cure rate at Day 28 (N = 9)	2 (22%)
Fever clearance time (FCT) (hour)	41 ± 25
Parasite clearance time (PCT) (hour)	44 ± 18
PC_{50}	21 ± 11
PC_{90}	28 ± 13
Adverse events:	
All events	16
Headache	5
Epistaxis	1
Vertigo	2
Diarrhea	2
Vomiting	1
Elevated ALT	4
Hypokalemia	1

and curtailing the overall duration of treatment.

Due to the lack of a comparator arm in our study, adverse events should be interpreted with caution. Fosmidomycin appears to be well tolerated. In common with the adverse-effect profile of earlier studies, gastrointestinal symptoms of loose stools, flatulence, and diarrhea, were most commonly reported [5]. These symptoms may be the result of poor drug absorption, previously found to be 20-40% [3], and the prolonged treatment regimen (7 days). Therefore, a change in intestinal flora may have contributed to diarrhea [12]. The frequency of diarrhea was comparable with other antibiotics [13]. The small study size does not allow conclusions to be drawn from the less frequent side effects. Monitoring of vital signs and laboratory parameters showed no clinically significant changes.

A similar study was conducted in the Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon. The result of treatment showed a higher 28-day cure rate (78%), but was similar in terms of adverse effects, and parasite and fever clearance times. The marked difference in the radical cure rates of 78% in Gabon and 22% in Thailand probably reflects the difference in immunity between a population from Central Africa where malaria is hyperendemic, and a population from Asia, where malaria is hypoendemic. Other factors, including the intrinsic sensitivity of the parasite, may be involved.

The rapid and effective action of fosmidomycin in achieving clinical and short-term parasitological cure, and the drug's tolerability, are properties that should be exploited. Therefore, research should be directed towards the identification of a partner drug that would enhance its activity through potentiation, while serving to protect it against the development of resistance [14]. Furthermore, this study encourages the development of new inhibitors of the nonmevalonate pathway with increased bioavailability and enhanced antimalarial activity. A derivative of fosmidomycin, FR900098, and a prodrug of this compound, have been shown to be more effective than fosmidomycin in a mouse model [6, 15]. Since fosmidomycin represents a simple molecule, which can be synthesized from cheap raw materials, cost-efficient, large-scale production seems possible.

In summary, the advent of fosmidomycin as an inhibitor of the nonmevalonate pathway represents a novel approach to antimalarial chemotherapy. In view of the paucity of new leads emerging over the past decades, this signifies an important development.

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