

Fosmidomycin, a Novel Chemotherapeutic Agent for Malaria

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In previous studies, fosmidomycin has been shown to possess activity against *Plasmodium falciparum* in vitro and in the mouse model. It has a novel mode of action through inhibition of 1-deoxy-D-xylulose 5-phosphate reductoisomerase, an enzyme of the nonmevalonate pathway of isoprenoid biosynthesis, which is absent in humans. In this open-label, uncontrolled trial, the efficacy and safety of fosmidomycin, in an oral dose of 1,200 mg every 8 h for 7 days, were evaluated in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in 20 adult subjects in Gabon and Thailand. Clinical assessments were performed and thick blood smears were evaluated every 8 h until parasite clearance and resolution of symptoms were achieved; assessments continued at weekly intervals thereafter for the duration of the 28-day followup period. All subjects were clinically and parasitologically cured on day 7 (primary end point). Parasite and fever clearance were rapid, with means of 44 and 41 h, respectively. On day 28, seven out of nine subjects (78%) were cured in Gabon and two out of nine subjects (22%) were cured in Thailand. The drug was well tolerated, although mild gastrointestinal side effects were recorded for five subjects. Analysis of hematological and biochemical parameters showed no clinically significant changes throughout the study. Fosmidomycin is an effective and safe antimalarial drug, although its use as a single agent is restricted by the occurrence of recrudescence infections. However, its role in combination therapy should be explored.

Development of drug resistance and, in some cases, concerns over safety highlight the urgent requirement for new antimalarial drugs. Primarily directed towards the treatment of multidrug-resistant *Plasmodium falciparum* malaria, such drugs should possess novel modes of action while exhibiting efficacy and safety within the constraints of therapeutic regimens not exceeding 3 days. They should also be affordable in the developing world.

Fosmidomycin was formerly under development as an antibacterial agent. As a phosphonic acid derivative with potent activity against gram-negative organisms, its role in the management of urinary tract infections was the focus of clinical studies that were undertaken during the 1980s. 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 h for 7 days, per os) was established (7, 8, 9).

As a potent inhibitor of 1-deoxy-D-xylulose 5-phosphate reductoisomerase, an essential enzyme of the nonmevalonate pathway, fosmidomycin blocks the biosynthesis of isopentenyl diphosphate and the subsequent development of isoprenoids in *P. falciparum* (5). In contrast, isoprenoids are derived from an alternative pathway, known as the mevalonate pathway, in mammals (1). 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 an arrest of development in the late schizont stage. In contrast to other antibiotics with antimalarial properties such as doxycycline or clindamycin, it does not exert a delayed effect (unpublished data).

Fosmidomycin, through a novel mode of action against the malarial parasite and with the support of evidence of efficacy in experimental models (5), offers a new concept in antimalarial drug development (12). The present study was undertaken to evaluate the efficacy and safety of fosmidomycin in the treatment of acute uncomplicated *P. falciparum* malaria.

MATERIALS AND METHODS

Study setting. The study was designed as an open-label, uncontrolled trial conducted at two geographically diverse centers in Gabon and Thailand in areas where malaria with variable drug resistance is endemic. The Albert Schweitzer Hospital in Lambaréné, Gabon, lies in a hyperendemic area with year-round malaria transmission (14). Chloroquine resistance is highly prevalent, and resistance to sulfadoxine-pyrimethamine is increasing (16). The Mae Sot General Hospital in Tak Province, Thailand, lies in an area with seasonal malaria transmission. Adults living in this area have little or no immunity against *P. falciparum* malaria, and the prevalence of multiresistant parasites is high (2). Enrollment in the study was planned to commence with patients from the semi-immune population in Gabon and then (subject to efficacy being established) to be continued in Thailand.

Study design. Subjects aged 18 to 50 years, each with a body weight in excess of 40 kg, attending as outpatients with symptoms of acute uncomplicated malaria and parasitemia levels between 1,000 and 50,000/ μ l, were invited to participate in the study. Subjects were excluded if they had a mixed plasmodial infection, significant concomitant disease, a hemoglobin count of <8 g/dl, or a white cell count of $>12,000/\mu$ l (to exclude severe disease), or had received antimalarial

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treatment within the previous 28 days. Women who were pregnant or breast-feeding were also excluded.

The protocol was approved by the Ethics Committee of the International Foundation of the Albert Schweitzer Hospital, Lambaréné, Gabon, and by the Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand. The study was conducted in accordance with the provisions of the Declaration of Helsinki, and informed consent was obtained from all participants.

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 closely monitored in the hospital and received 1,200 mg of fosmidomycin orally every 8 h for 7 days. This regimen had been shown to be well tolerated in previous studies (9). The study drug (fosmidomycin sodium batch no. 008003) was provided by Jomaa Pharmaka GmbH. The drug's stability was confirmed prior to the commencement of the study. Parasite counts were performed, and vital signs (temperature and pulse) were monitored every 8 h during the acute stage of the infection and then once daily for the duration of this phase. Clinical examinations were also carried out daily, with subjects being queried from a standard list of symptoms commonly associated with malaria. Blood samples were obtained for routine hematology and clinical chemistry testing prior to drug administration and on days 2, 4, and 7. A pretreatment blood sample was also taken for parasite genotyping in the event of recurrent parasitemia. Urinalysis was carried out at screening and on days 2, 4, and 7.

During the follow-up phase, subjects were reviewed weekly on days 14, 21, and 28, when clinical assessments and thick blood smears were performed. 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 and the subject was withdrawn from the study and treated with standard antimalarials—sulfadoxine-pyrimethamine in Gabon and mefloquine-artesunate in Thailand.

Variables analyzed. The primary efficacy variable was the cure rate on day 7. Secondary efficacy variables were the parasite and fever clearance times and the cure rate on day 28. The parasite clearance time was defined as the time from initiation of treatment to the first of at least two consecutive negative blood smears. PC₅₀ and PC₉₀ were defined as hours until clearance of 50 and 90% of the initial parasitemia levels, respectively. The fever clearance time was calculated from the commencement of therapy to the time that the temperature fell below 37.5°C and remained below 37.5°C for 48 h. Adverse events were defined as any symptoms or signs which 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 to be clinically relevant, were also reported as adverse events.

Parasite genotyping was performed in order to differentiate between reinfection and recrudescence infections. Established procedures were employed using the PCR for the amplification of the merozoite surface protein 1 (MSP-1) and MSP-2 (10).

Statistical analysis. A sample size of 20 evaluable subjects was used to estimate the day 7 cure rate with appropriate accuracy. A 70% cure rate would have 95% confidence intervals of 46 to 88%. 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. The efficacy was evaluated based on results within the per-protocol population. Efficacy analysis was performed by calculating the cure rates and by analyzing descriptive statistics of parasite and fever clearance times. Calculations of confidence intervals were 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 the paired *t* test. A *P* value below 0.05 was considered significant.

RESULTS

A total of 26 subjects, 11 in Gabon and 15 in Thailand, participated in the study. Table 1 shows characteristics of the subjects on admission. One subject from Gabon withdrew his consent prior to receiving the study drug. In Thailand, one subject withdrew her consent after receiving nine doses of the

TABLE 1. Characteristics on admission

Site	<i>n</i> ^a	Age (yr) ^b	Wt (kg) ^b	Male/female ^a	No. of parasites/μl (95% CI) ^c	Temp (°C) ^b
Gabon	11	29 (9)	69 (18)	7/4	10,017 (3,064–32,754)	37.9 (1.0)
Thailand	15	28 (9)	53 (4)	11/4	11,307 (5,330–23,990)	38.0 (1.3)
Total	26	28 (9)	59 (14)	18/8	10,773 (5,916–19,617)	37.9 (1.2)

^a Values are numbers of subjects.

^b Values are means (standard deviations).

^c Values are geometric means.

study drug, one was withdrawn because of a rapid increase in parasitemia level between the time of screening and the administration of the first dose of the study drug, one was withdrawn because of an equivocal pregnancy test result, and two were withdrawn because they had not received the full course of treatment.

The cure rate on day 7 (primary end point) was evaluable in 20 subjects, and all were found to have negative blood smears at this time point. Parasite clearance was rapid, with a mean (\pm standard deviation) of 44 (\pm 18) h. PC₅₀ and PC₉₀ times were 21 (\pm 11) and 28 (\pm 13) h, respectively. No differences in results were found between the study sites. Fever was cleared after a mean time of 41 (\pm 25) h. A significant difference in results between the study sites was found, with fever being cleared faster in Gabon (28 h) than in Thailand (52 h) (*P* = 0.02).

During follow-up, two subjects were excluded, one being unavailable for follow-up and the other developing *P. vivax* parasitemia. Eighteen subjects were evaluable on day 28, and nine subjects (50%) experienced a reappearance of *P. falciparum* parasitemia (Table 2). A clear difference between the study sites was apparent, there being only two subjects in Gabon compared to seven subjects in Thailand who experienced recrudescence. The time to recrudescence was shorter in subjects from Thailand than in subjects from Gabon (mean, 21 versus 26 days). Parasite genotyping revealed that all cases of treatment failure were true recrudescences and not reinfections.

Gametocytes were observed in the initial blood films of 2 subjects and were observed in an additional 13 subjects on at least one occasion. Gametocytes present on admission were not cleared during the first 7 days. The levels of gametocytes ranged between 2 and 11,280/μl with a geometric mean of 198/μl. On days 7 and 14, 11 (55%) and 9 (47%) subjects had gametocytes, respectively.

A total of 46 adverse events were recorded throughout the study period, and 20 subjects (80%) had at least one adverse event. All adverse events were nonserious and mild or moderate in intensity. Ten adverse events were categorized as possi-

TABLE 2. Efficacy of fosmidomycin at two end points^a

Site	End point 1 (day 7)			End point 2 (day 28)		
	<i>n</i>	No. (%) positive	Cure rate (%)	<i>n</i>	No. (%) positive	Cure rate (%) (95% CI) ^b
Gabon	10	0 (0)	100	9	2 (22%)	78 (40–97)
Thailand	10	0 (0)	100	9	7 (78%)	22 (3–60)
Total	20	0 (0)	100	18	9 (50%)	50 (26–74)

^a *n*, number of subjects.

^b CI, confidence interval.

TABLE 3. Laboratory values on admission and on days 7 and 28

Parameter	Value upon admission (day 0) ^a	Mean change in value from:	
		Day 7 to day 0	Day 28 to day 0
Hemoglobin (g/dl)	12.7 (2.7)	-1.2 ^b	-0.9
Hematocrit (%)	40.2 (8.1)	-4.9 ^b	-3.9 ^b
Leukocytes (/nl)	6.3 (2.1)	0.2	0.0
Neutrophils (%)	60 (13)	-15.2 ^b	-9.7
Lymphocytes (%)	32 (12)	12.9 ^b	3.8
Monocytes (%)	6 (4)	-2.6	-0.6
Eosinophils (%)	2 (3)	4.7 ^b	6.7 ^b
Thrombocytes (/nl)	134 (83)	166 ^b	61 ^b
ALT (U/liter)	34 (18)	22	-1
Albumin (g/dl)	3.8 (0.4)	-0.2 ^b	0.1
Bilirubin (μmol/liter)	25 (14)	-13	-7
Creatinine (μmol/liter)	100 (23)	-13	-7
Glucose (mmol/liter)	5.7 (1.2)	-1.0 ^b	-1.2 ^b
Total protein (g/dl)	7.4 (0.4)	-0.1	0.2
Urea (mmol/liter)	5.6 (2.2)	-2.5 ^b	-2.0 ^b

^a Values are means (standard deviations).

^b $P < 0.05$.

bly being related to the study medication, and gastrointestinal events were the most frequent of these. These were recorded in five subjects (20%) and consisted of loose stools (three subjects), diarrhea (two subjects), and flatulence (two subjects). Elevations in alanine aminotransferase (ALT) levels were recorded for two subjects (peak levels of 124 and 130 U/liter), and dizziness was reported in one subject as an adverse event. Vital signs remained within normal limits throughout the study.

Table 3 summarizes the laboratory data on admission and throughout the study. Significant differences over time (days 0, 2, 4, 7, 28, or at withdrawal) were found for most hematological parameters, reflecting in all cases changes normally encountered during a malarial infection. Specifically, these were reductions in hemoglobin and hematocrit measurements within the first days of treatment followed by a return to normal values and a fall in the numbers of eosinophils and thrombocytes during infection. Significant differences over time were found for levels of glucose, urea, albumin, and total protein. None of these differences was considered clinically significant. Elevation of ALT levels over 60 U/liter was found in five subjects. All subjects had an ALT level within the normal range by day 28 or upon withdrawal.

DISCUSSION

The present study establishes the efficacy of fosmidomycin as an antimalarial drug in the initial clearance of asexual parasitemia at both study centers. The mean parasite clearance time of 44 h is comparable to that of conventional antimalarials and surpasses that of other antibacterial drugs with antimalarial activity, including doxycycline, clindamycin, and tetracycline (3, 6, 13). Similarly, the resolution of clinical symptoms was rapid, with a mean fever clearance time of 41 h.

Following confirmation from parasite genotyping that the recurrent parasitemias represented recrudescence infections, the radical cure rates of 78% in Gabon and 22% in Thailand revealed a marked variation in the overall response between

the two centers. While this 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 intrinsic sensitivity of the parasite, may be involved. A pooled estimate of cure rates should therefore be viewed with caution. The high prevalence of gametocytes for prolonged periods suggests that fosmidomycin is devoid of gametocytocidal activity.

A regimen of one dose every 8 h was chosen in this study because of the short half-life of fosmidomycin, which has been estimated to be 1.6 h (7, 8). 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 and curtailing the overall duration of treatment.

Due to the lack of a comparator arm in our study, interpretation of adverse events should be exercised with caution. Fosmidomycin appears to be well tolerated. In common with the adverse-effect profile of the earlier studies, gastrointestinal symptoms of loose stools, flatulence, and diarrhea were most commonly reported (9). These symptoms may be the result of poor absorption of the drug, previously found to be between 20 and 40% (7), and of the prolonged treatment regimen of 7 days. Therefore, alteration in the intestinal flora may have contributed to the development of diarrhea (4). The frequency of diarrhea was comparable to that found with other antibiotics (17). 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.

The rapid and effective action of fosmidomycin in achieving clinical and short-term parasitological cures, in addition to its 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 (15). 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 the mouse model (5, 11). 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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