J Trop Med Parasitol 2008;31:34-40.

ORIGINAL ARTICLE

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Evaluation of Fosmidomycin, in Combination with Clindamycin, in Adult Patients 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 that are resistant to the commonly used antimalarials. It has been shown that fosmidomycin can be used alone to treat uncomplicated *P. falciparum* infection. However, a high rate of recrudescence has been noted. In Thailand, fosmidomycin alone gave a 100% cure rate at 7 days' follow-up, but only 22% at 28 days. In Gabon, fosmidomycin yielded a 100% cure rate at 7 days' follow-up and 78% at 28 days. Therefore, there is a need to find suitable combinations to treat *P. falciparum* and prevent recrudescence. Clindamycin has been shown to be synergistic in isobolograms. The potentiation of fosmidomycin by clindamycin has also been observed *in vivo* in a *P. vinckei* mouse model, in which co-administration resulted in a significantly higher cure rate than individual administration. Enhanced activity was also evident when the drugs were co-administered in the presence of high parasitemia. Combination therapy with fosmidomycin and clindamycin was highly effective with 100% cure rate 28 days' follow-up. No serious adverse effect was found. Only some cases of diarrhea, vomiting and dizziness were reported.

Keywords: falciparum malaria, fosmidomycin, clindamycin

Introduction

Malaria is one of the leading causes of morbidity and mortality in the tropics, with an annual estimated 500 million clinical cases and 2 million deaths [1]. The treatment of malaria is becoming increasingly difficult due to *Plasmodium falciparum* strains resistant to the commonly used antimalarials. In addition to the high-grade chloroquine-resistant strains found in high frequencies in all endemic areas of the world, resistance to sulphadoxine/pyrimethamine is now common in large parts of Asia and South America, and is spreading to Africa. The development of drug resistance highlights the urgent need for new

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antimalarial drugs. Primarily directed towards the treatment of multi-drug resistant *P. falciparum*, such drugs should possess novel modes of action while being of proven efficacy and safety.

Now, most combination treatments for *P. falciparum* is artemisinin combination therapy. Fosmidomycin is a phosphonic acid derivative previously investigated as an antibacterial agent and later on has been shown to be an effective malarial blood schizonticide. It acts as a potent inhibitor of 1-deoxy-*D*-xylulose *5*-phosphate (DOXP) reducto-isomerase, an essential enzyme of the non-mevalonate pathway, and therefore selectively blocks the biosynthesis of isopentenyl diphosphate and the subsequent development of isoprenoids in *P. falciparum* [2-5]. The mevalonate independent biosynthesis pathway for isoprenoids takes place in special organelles of the parasites, the apicoplasts. These possess, like mitochondria or the chloroplasts of green plants, their own genome and can replicate independently of the cell nucleus. Metabolic processes in the apicoplast are similar to those found in bacteria and plants and differ fundamentally from those of animal organisms. Fosmidomycin should, therefore, be very safe for humans in whom isoprenoids are synthesized through a completely different pathway. In *in vitro* and *in vivo* animal studies, the drug has been shown to be a potential antimalarial, but the development of recrudescence in the early clinical-trial phase precludes mono-therapeutic use [6]. Drug combinations are considered superior to monotherapy due to their pharmacodynamic synergistic effects and, in addition, the delay in resistance to individual components. Clindamycin emerged as a potential combination following the demonstration of *in vitro* and *in vivo* synergistic activity [7]. A subsequent clinical study in Gabon confirmed the effectiveness of this combination in children with asymptomatic malaria to be superior to fosmidomycin and clindamycin, when administered as monotherapy, both in the initial clearance of parasitemia and in the prevention of recrudescent infections [8,9]. It is not known, however, whether concurrent administration of fosmidomycin and clindamycin would affect the pharmacokinetics of the individual drugs.

The study was conducted to determine the efficacy and safety of fosmidomycin and clindamycin combination therapy for acute uncomplicated multidrug-resistant falciparum malaria.

Materials and Methods

Study design

This study was an open-label, uncontrolled clinical trial conducted in Thailand in April-August 2002. The study protocols were approved by the Ethics Committees of the Ministry of Public Health, Thailand and the Secretariat Committee for Research Involving Human Subjects (SCRIHS) of the World Health Organization, Geneva, Switzerland.

Methods

A total of 18 patients with acute uncomplicated *P. falciparum* malaria who fulfilled the enrollment criteria were recruited from the Outpatient Department of Mae Sot Hospital, Tak Province, Thailand. The study was held on April-August 2002. Patients were treated with combination therapy with fosmidomycin (900 mg every 12 hours for 7 days) and clindamycin (600 mg every 12 hours for 7 days). Efficacy assessments included clinical and parasitological cure rate on days 7 and 28. Safety and tolerability were assessed based on clinical and laboratory investigations.

Inclusion criteria: adult patients aged 18-50 years, bodyweight > 40 kg, attending as outpatients with signs/symptoms of acute uncomplicated falciparum malaria, with parasitemia levels 1,000-50,000/µl. Study participants were enrolled after given written informed consent. Subjects were excluded if they were pregnant or breast-feeding, or had a mixed infection, or significant concomitant disease, or hemoglobin < 8.0 g/dl, or white cell count > 12,000/µl (to exclude severe disease), or had received antimalarial treatment within the previous 28 days.

Pre-treatment investigations consisted of clinical assessments (general medical history, demographic data, drug sensitivity and allergy,

significant medical history, previous drug administration, physical examination, monitoring of vital signs and signs and symptoms of malaria), laboratory assessments (thick and thin blood smears for parasite identification/quantification, urine test for chloroquine [10] and sulphonamides [11], routine hematology, serum biochemistry, urinalysis, and stool examination for parasites and ova.

All patients were admitted to the hospitals for 7 days and requested to complete follow-up visits on days 10, 14, 21, and 28, or until recrudescence occurred.

Drug administration

Patients were treated with oral fosmidomycin concurrently with clindamycin. Fosmidomycin was given at a dose of 900 mg every 12 hours for 7 days. Fosmidomycin capsules (batch no. FK 150–02007) were supplied by Alphamed PHARBIL Arzneimittel GmbH; each capsule contained 150 mg of fosmidomycin. Clindamycin was given at a dose of 600 mg every 12 hours for 7 days. Clindamycin film-coated tablets (batch number 16843-007) were supplied by Alphamed PHARBIL Arzneimittel GmbH; each tablet contained 150 mg of clindamycin.

The patients fasted over night (8-10 hours) and emptied their bladders immediately before drug administration. The drugs were administered orally with 250 ml of water and a standard hospital meal (20-25% fat content) under the supervision of assigned study staff. After ingestion of the drug tablets, patients were observed for 1 hour to ensure retention of the drug. Patients who vomited any oral dose of fosmidomycin or clindamycin were excluded from data analysis. A meal was provided at 4 hours' post-dosing. Juices and water were freely available during hospitalization. All drugs necessary for the welfare of the patients were allowed during the study period.

Efficacy assessment

Efficacy assessments included clinical and parasitological evaluations. Clinical signs/symptoms of malaria, including body temperature, were monitored on days 0, 1, 2, 3, 4, 5, 6, 7, 10, 14, 21, and 28. Finger-prick blood smears were examined at 6-hourly intervals until negative 3 consecutive times, then daily during hospitalization, and on days 10, 14, 21, and 28. The films were stained with Giemsa and parasite numbers determined by counting the number of asexual parasites per 1,000 red blood cells on a thin film or per 200 white blood cells on a thick film. Analysis of the response to treatment was adapted from the World Health Organization [12]. Efficacy was assessed using the following parameters: (i) 28-day cure rate: the proportion of patients with clearance of asexual parasites within 7 days of treatment initiation without subsequent recrudescence during 28-day follow-up; (ii) 7-day cure rate: the proportion of patients with clearance of asexual parasites within 7 days of treatment initiation; (iii) parasite clearance time (PCT): defined as time from first dose to continued clearance of asexual parasite forms, which remained for at least a further 48 hours; and (iv) fever clearance time (FCT): time from first dose until the first time body temperature < 37.5°C and remained < 37.5°C for at least a further 48 hours.

Safety and tolerability assessment

The safety and tolerability of fosmidomycin/clindamycin combination therapy were assessed based on clinical findings or abnormal laboratory (hematology and biochemistry) tests that first occurred or increased in intensity within 7 days of treatment initiation, and during the follow-up period, on days 14, 21, and 28, in accordance with the Common Toxicity Criteria grade [13].

Results

Eighteen patients were enrolled. Demographics, clinical and laboratory data for the patients on admission are presented in Table 1. Most patients had baseline laboratory parameters outside the normal ranges, which is common for patients with acute falciparum malaria, and all were mild or moderate in severity. Concomitant medication included paracetamol, dimenhydinate, diphenhydramine, and chlorpheniramime.

Table 1 Demographics and clinical and laboratory data of patients on admission. Data are presented as median (95% CI) or number of patients and percentage values.

Characteristics	(N = 18)
Age (year)	29 (18-28)
Body weight (kg)	56 (40-75)
Gender: male/female	13/5
Ethnic:	
Thai	10
Karen	3
Burmese	5
Body temperature (°C)	38.0 (37.3-39.9)
Baseline asexual form parasitemia/µl	30,600 (944-67,640)
Hemoglobin (g/dl)	12.6 (8.1-15.0)
Hematocrit (%)	39.8 (25.5-45.7)
WBC (x 10^9/l)	6.0 (3.0-8.9)
Platelet count (x 10^9/l)	80 (21-225)
Total bilirubin (mg/dl)	1.3 (0.1-2.6)
ALT (iu/l)	24 (8-103)
Creatinine (mg/dl)	1.1 (0.7-1.9)
BUN (mg/dl)	14 (7-43)
Total protein (g/dl)	6.0 (5.0-8.0)
Albumin (g/dl)	3.6 (2.9-4.5)
Glucose (mg/dl)	110 (79-205)

Efficacy

Combination therapy with fosmidomycin and clindamycin was proven highly effective with 100% cure rate on day 7 follow-up and 100% on 28 days follow-up. The fever clearance time and parasite clearance time [median (range)] were 56 (8-80) hours and 40 (16-80) hours, respectively (Table 2).

Adverse effects

Fosmidomycin combination therapy with clindamycin was well tolerated with no serious adverse events. No neutropenia or decreased hemoglobin was observed, as previously reported in children [8]. Six (33.3%), one (5.5%), one (5.5%), six (33.3%), three (16.7%) and one (5.5%) cases experienced diarrhea, dizziness, epistaxis, headache, anorexic-macular rash, and myalgia respectively. In addition, elevated ALT and hypokalemia were found in four (22.2%) and one (5.5%) cases, respectively (Table 2). All adverse events were categorized as mild or moderate in severity. 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 a malaria infection.

Discussion

This study showed an excellent cure rate could be achieved for acute uncomplicated *P. falciparum* malaria with combination therapy of fosmidomycin and clindamycin in the area of highest resistant parasite while monotherapy with fosmidomycin alone gave only 22% in day 28 in the same area. This suggests intrinsically low sensitivity

Table 2 Efficacy and tolerability of fosmidomycin and clindamycin combination in 18 acute uncomplicated *P. falciparum* malaria patients. Values are expressed as no. (%) or median (range), where appropriate.

Parameter	No. (%) or Median (range)
Efficacy:	
Cure rate at Day 7	18 (100)
Cure rate at Day 28	18 (100)
Fever clearance time (FCT) (hour)	56 (8-80)
Parasite clearance time (PCT) (hour)	40 (16-80)
Adverse events:	
Diarrhea	6 (33.3)
Dizziness	1 (5.5)
Epistaxis	1 (5.5)
Headache	6 (33.3)
Anorexia	3 (16.7)
Macular rash	1 (5.5)
Myalgia	1 (5.5)
Elevated ALT	4 (22.2)
Hypokalemia	1 (5.5)

to fosmidomycin among some parasite strains in Thailand, and that fosmidomycin monotherapy may not be effective in treating multidrug-resistant falciparum malaria. The improved efficacy of the fosmidomycin and clindamycin combination might be explained by the pharmacodynamic synergistic interaction between fosmidomycin and clindamycin on parasite enzyme DOXP reducto-isomerase. Clindamycin targets the prokaryote-like ribosomes of the apicoplast and so inhibits self-replication of the organelle [14,15]. As a consequence of this mechanism, the drug displays a typical delayed-kill kinetic effect, the growth of parasites being unaffected until the second replication after drug exposure. Under such conditions, the *in vitro* growth of *P. falciparum* is inhibited with an IC_{50} and IC_{90} of approximately 25 and 50 nM, respectively. As a consequence of its high activity but slow onset of activity, clindamycin is recommended for the treatment of asymptomatic malaria or in combination with other antimalarials. It has been suggested that time-dependent antimicrobial agent levels should remain above the MIC of pathogens for at least 3 malaria cycles of 7 days. Unlike fosmidomycin, recent studies have suggested that clindamycin displays concentration-dependent bactericidal activity [16,17] while exhibiting an *in vitro* post-antibiotic effect (PAE) for a certain period. *In vivo*, the PAE is generally longer and due to effects such as post-antibiotic leukocyte enhancement and post-antibiotic sub-MIC effect. A frequent dosing interval for a clindamycin regimen is therefore unnecessary. The recommended oral dosing of clindamycin for bacterial infection was 600 mg every 6 hours or 900 mg every 8 hours. However, it was later shown that the pharmacokinetics and concentration-time profiles were comparable with a dosing regimen of 1,200 mg every 12 hours, which is more practical for clinical application. This may explain the sustained antimalarial efficacy noted when clindamycin was used with fosmidomycin, despite low minimum plasma concentrations of fosmidomycin at steady-state. Whether the antimalarial activity of clindamycin bioactive metabolites should also be considered when

performing a pharmacokinetic/pharmacodynamic evaluation has not yet been elucidated. In humans, the formation of *N*-desmethyl clindamycin and its excretion in urine and feces have been confirmed [18], but the metabolite could not be detected in patient plasma [19]. The currently suggested dose regimens are based on parent compound concentrations only, and the presence of metabolite activity (if any) would only amplify the positive therapeutic outcome. Further study is required for optimization of the appropriate ratio of fosmidomycin and clindamycin in combination, including dosing interval and shorter treatment course for multi-drug resistant falciparum malaria, to ensure good compliance.

Acknowledgements

This study received financial support from the UNICEF-UNDP-World Bank-WHO Special Programme for Research and Training in Tropical Diseases. We are grateful to the Product Development Team, Dr Win Gutteridge and Dr Deborah Kioy, for their technical support during the study. We thank Director Dr Kanoknart Siripaibul and staff of Mae Sot General Hospital for their support with blood sample collection, and Jomaa Pharma GmbH, Germany, for the supply of fosmidomycin and clindamycin.

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