Inadequate Efficacy of a New Formulation of Fosmidomycin-Clindamycin Combination in Mozambican Children Less than Three Years Old with Uncomplicated *Plasmodium falciparum* Malaria

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The combination of fosmidomycin and clindamycin (F/C) is effective in adults and older children for the treatment of malaria and could be an important alternative to existing artemisinin-based combinations (ACTs) if proven to work in younger children. We conducted an open-label clinical trial to assess the efficacy, safety, and tolerability of F/C for the treatment of uncomplicated *P. falciparum* malaria in Mozambican children <3 years of age. Aqueous solutions of the drugs were given for 3 days, and the children were followed up for 28 days. The primary outcome was the PCR-corrected adequate clinical and parasitological response at day 28. Secondary outcomes included day 7 and 28 uncorrected cure rates and fever (FCT) and parasite (PCT) clearance times. Fifty-two children were recruited, but only 37 patients were evaluable for the primary outcome. Day 7 cure rates were high (94.6%; 35/37), but the day 28 PCR-corrected cure rate was 45.9% (17/37). The FCT was short (median, 12 h), but the PCT was longer (median, 72 h) than in previous studies. Tolerability was good, and most common adverse events were related to the recurrence of malaria. The poor efficacy observed for the F/C combination may be a consequence of the new formulations used, differential bioavailability in younger children, naturally occurring variations in parasite sensitivity to the drugs, or an insufficient enhancement of their effects by naturally acquired immunity in young children. Additional studies should be conducted to respond to the many uncertainties arising from this trial, which should not discourage further evaluation of this promising combination.

Treatment of malaria has become increasingly complex due to the emergence and spread of drug-resistant strains of *Plasmodium*. Current recommendations for malaria treatment include the use of combination therapies based on artemisinin derivatives (29) with the aim of enhancing activity and delaying the development of resistance. Artemisinins are considered the most effective and fast-acting drugs (24) and have replaced other existing drugs in the majority of countries where malaria is endemic, particularly for the treatment of *Plasmodium falciparum*. In the context of decreasing malaria incidence worldwide (32) and renewed impetus in malaria elimination efforts (12), the role played by artemisinin-based combinations (ACTs) in reducing the global malaria burden and its associated morbidity and mortality is expected to be essential. However, the first evidence of decreased *P. falciparum* sensitivity to artemisinins (19), detected in Southeast Asia, a region traditionally considered the birthplace of previous emergence of resistance to other antimalarial drugs, is a matter of serious concern, as to date no clear alternatives exist to replace these compounds should they become ineffective (11). In response to this, WHO has launched an ambitious surveillance and containment plan for the area (28), but it is now clearer than ever that new drugs with unrelated mechanisms of action need to be developed (13, 16).

Fosmidomycin, isolated in the 1970s as a natural antibiotic from *Streptomyces lavendulae* and currently produced by chemical synthesis, has been considered a promising antimalarial agent and a possible alternative to artemisinins since the discovery of its inhibitory action on the nonmevalonate pathway of isoprenoid biosynthesis. This pathway, also referred to as the DOXP pathway,

is employed by *P. falciparum*, in contrast to mammals, where isoprenoid biosynthesis follows the mevalonate pathway. Specifically, fosmidomycin blocks *de novo* nonmevalonate isoprenoid biosynthesis inside the apicoplast of *P. falciparum* by selective inhibition of the enzyme DOXP reductoisomerase (26, 33).

Fosmidomycin does not display the delayed-kill kinetic effect observed with classical antibiotics and thus shows a rapid onset of action. Its relatively weak activity can be improved by combining it with clindamycin, a potent, albeit slower-acting, antibiotic with antimalarial activity. Furthermore, *in vitro* synergistic activity between fosmidomycin and clindamycin against various strains of *P. falciparum* has been demonstrated (27). Different clinical trials have confirmed the safety and efficacy of fosmidomycin alone (15) or combined with clindamycin for the treatment of uncomplicated *P. falciparum* both in adults (17, 18, 20, 23) and in older children (3, 5, 6, 20). However, data on the efficacy of this combination in younger children, the age group most affected by malaria worldwide, are scarce. Here, we present the results of an open-label study conducted in a semirural area of southern Mozambique designed to assess the safety and efficacy of the fosmidomy-

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cin-clindamycin (F/C) combination for the treatment of uncomplicated *P. falciparum* malaria in children less than 3 years of age.

MATERIALS AND METHODS

Study site and population. The study was conducted at the Centro de Investigação em Saúde de Manhiça (CISM), located in Manhiça, Maputo Province, southern Mozambique. The area has been described in detail elsewhere (2). Transmission of *P. falciparum* is perennial, with marked seasonality and moderate intensity, and has an entomological inoculation rate of 38 infective bites/person/year (1). CISM runs a demographic surveillance system (DSS) in its study area and a morbidity surveillance system at neighboring Manhiça District Hospital (MDH) and other health posts in the area through which standardized data on all pediatric outpatient visits and admissions to the hospital are collected.

Study design. An open-label uncontrolled clinical trial was designed to be conducted at MDH. Between January and March 2010, children 6 to <36 months old attending the health facilities with uncomplicated malaria were included in the study if they fulfilled the following inclusion criteria: body weight of >5 kg, microscopically confirmed *P. falciparum* mono-infection with asexual parasite densities between 1,000 and 200,000 parasites/ μ l, and fever (axillary temperature of $\geq 37.5^{\circ}\text{C}$) or history of fever in the preceding 24 h. Patients were not recruited if they met at least one of the following exclusion criteria: severe malaria according to WHO criteria (31) or other danger signs; acute malnutrition (weight for height of <70% of the median National Center for Health Statistics/WHO reference or clinical diagnosis of marasmus or kwashiorkor) or any other concomitant illness or underlying disease masking assessment of response, including sickle cell disease or severe cardiac, hepatic, or renal impairment; gastrointestinal disturbance associated with persistent vomiting (>3 episodes within the previous 24 h) and/or diarrhea (>5 loose stools in the preceding 24 h); inability to tolerate oral therapy; contraindication to receive the trial drugs or history of treatment with any anti-malarial drug or drug with anti-malarial activity (including cotrimoxazole prophylaxis) within the 7 days preceding enrollment; other concomitant plasmodial infections; or packed cell volume (PCV) on arrival of <25%. Patients satisfying the inclusion criteria were enrolled if the parent/guardian signed detailed written informed consent.

Administration of the investigational product. The study medication was supplied by Pharbil Pharma GmbH as complete treatment packs for allocation on an individual-subject basis at the time of enrollment in the study. Consisting of separate bottles containing granules of fosmidomycin sodium and clindamycin hydrochloride, it was reconstituted with water at concentrations of 250 mg/5 ml and 75 mg/5 ml, respectively, immediately prior to the administration of the first dose. It was stored at between 15 and 25°C under strict temperature control in accordance with the manufacturers' recommendations. The study drugs were consecutively administered under direct supervision during 3 consecutive days according to a targeted twice-a-day dosage of fosmidomycin at 30 mg/kg of body weight/dose and clindamycin at 10 mg/kg/dose, rounded to facilitate preparation according to the following weight groups: 5 to 8 kg, 5 ml of each syrup; 9 to 12 kg, 7.5 ml; 13 to 15 kg, 10 ml. In case of vomiting within the first hour, a full dose was repeated, and recurring vomiting (≥ 2 episodes with the same dose) resulted in exclusion from the study and administration of rescue treatment.

Treatment follow-up and clinical and laboratory procedures. All children were admitted to MDH's clinical-trial unit for the 3-day dosing period. The parent/guardian was asked to return with the child for scheduled visits on days 7, 14, 21, and 28 postrecruitment or at any time should new symptoms occur. Field workers traced patients who missed any visit using the permanent identification number from the DSS. For each visit, a physical examination was performed by the study clinicians, and vital signs, including temperature and heart and respiratory rates, were recorded. Adverse events (AEs) and serious adverse events (SAEs) were recorded and monitored throughout the study. An independent local

safety monitor reviewed all SAEs throughout the study. Rescue treatment for recurrent parasitemia or clinical malaria was done with artemether-lumefantrine according to the national guidelines.

Capillary or venous blood was taken at every visit. Thick and thin blood films were prepared, dried, and Giemsa stained, and parasite density was estimated using the Lamberé method, which counts parasites against a fixed known blood volume (21). All slides were double read, and discrepant ones were solved by a third reading. Hematocrit was determined by a finger prick sample on arrival. Samples for hematology (full blood count) and biochemistry (liver and renal function) were taken at enrollment, at days 7 and 28, and at any other visit if judged necessary by the clinician. For PCR analysis, three 50- μ l blood spots were collected on filter paper (Whatman; 3MM) at enrollment and at any visit after day 7. Each filter paper was dried and individually stored in a plastic bag containing silica gel. All filter papers were subsequently shipped to the Centre for International Health Research (CRESIB) (Barcelona, Spain), where centralized genotyping was conducted. DNA was extracted using a QIAamp DNA Mini Kit (Qiagen), and the three polymorphic genetic markers MSP1, MSP2, and GluRP were used to distinguish recrudescence from new infections, according to WHO recommended procedures (30) and as previously described by Snounou et al. (25). Recrudescence was defined as at least one identical allele for each of the three markers in the pretreatment and posttreatment samples. New infections were diagnosed when all alleles for at least one of the markers differed between the two samples.

Outcome classification. The primary efficacy endpoint was the PCR-corrected adequate clinical and parasitological response (ACPR) at day 28; the secondary efficacy endpoints included non-PCR-corrected cure rates at days 7 and 28, parasite (PCT) and fever clearance times, and presence and clearance of gametocytes. All standard safety outcomes, such as the incidence of adverse events, vital-sign variation, and incidence of hematology and clinical chemistry abnormalities, were also evaluated.

Data management and statistical analyses. Data were recorded using specifically designed standardized case report forms, which were independently monitored by a contracted external contract research organization and subsequently entered into an electronic database. Two populations were defined for the analysis. The intention-to-treat (ITT) population included any patient who had received at least one dose of the study medication. The safety analysis included abnormal laboratory data and adverse events for all subjects in this population. Efficacy was calculated in the according-to-protocol (ATP) population, which included all patients who had received the full treatment, with no protocol violations, and followed up with no more than one missed visit until day 28. Cure rates were calculated as the number of patients with clinical and parasitological cure by day 28 (or other endpoints) divided by the total number of patients who could be evaluated (ATP). Fever and parasite clearance times were calculated from the start of treatment until the first of two consecutive determinations not showing fever (axillary temperature, $< 37.5^{\circ}\text{C}$) or parasitemia, respectively. Statistical analyses were done with Stata 11 (Stata Corp., College Station, TX), and the statistical significance level was set at 5%.

Sample size calculations. Sample size calculations were based on the efficacy results obtained with this combination in a study previously performed in Gabonese children, where 54 children were recruited and the per-protocol, PCR-adjusted day 28 cure rate was 94% (46/49) (20). Based, therefore, on an expected 90% efficacy estimate, a sample size of 35 children would produce a two-sided 95% confidence interval with an estimated precision of 10%. Allowing for an attrition rate of 20% for non-evaluable subjects, the final sample size was set at 50 subjects.

Ethical considerations. The protocol was approved by the National Mozambican Ethics Review Committee and the Hospital Clinic of Barcelona Ethics Review Committee. The trial was conducted according to the International Conference on Harmonization's Good Clinical Practice guidelines. The clinical-trial identifier is NCT01464138 (<http://www.clinicaltrials.gov>).

FIG 1 Trial profile.

RESULTS

Trial profile and baseline characteristics. Between January and March 2010, 486 sick children were screened, and 52 were recruited into the trial. The ineligible patients comprised 416 children with a negative malaria slide, 6 whose parents refused to participate, and 12 excluded for other reasons (Fig. 1). Table 1 summarizes the baseline characteristics of the 52 study participants who received at least one dose of the study medication (the ITT population). Four participants were prematurely excluded because of repeated vomiting within the first 12 h and 1 because of severe deterioration, but the remaining 47 patients received the full course (six doses) of F/C. Ten children were excluded from the efficacy analysis: 6 participants had a definite parasite density outside the predefined protocol limits (following double reading), 2 had a false-positive malaria parasitemia diagnosed on day 7 and received antimalarial rescue treatment before this could be confirmed, and 2 were lost to follow-up. Consequently, 37 patients were included in the ATP population.

Efficacy. The uncorrected cure rate at day 7 was 94.6% (35/37)

TABLE 1 Baseline characteristics of enrolled patients (safety population; $n = 52$)

Characteristic ^a	Value for F/C ($n = 52$)
Girls [no. (%)]	29 (55.8)
Median age (IQR) (mo)	23.5 (15.0–30.8)
6 to <12 mo [no. (%)]	10 (19.2)
12 to <24 mo [no. (%)]	18 (34.6)
24 to <36 mo [no. (%)]	24 (46.2)
Mean (SD) body wt (kg)	10.4 (2.3)
Mean (SD) ht (cm)	77.3 (8.2)
Mean (SD) temp (°C)	37.8 (1.3)
Mean (SD) respiratory rate	37 (5.5)
Mean (SD) heart rate	136 (22)
Median parasite density (IQR) (per μ l)	39106 (27,992–184,243)
Positive gametocytes [no. (%)]	1 (1.9)
Mean hemoglobin (SD) (g/liter)	93.3 (12.3)
Mean platelet count (SD) (10^9 /liter)	149.8 (89.2)
Mean ALT (SD) (U/liter)	27.8 (16.7)

^a IQR, interquartile range; SD, standard deviation; ALT, alanine aminotransferase.

TABLE 2 Efficacy results (PCR corrected and non-PCR corrected) in the ATP population

PCR correction	Time point (days)	Statistic ^a	Value for F/C
Uncorrected	7	<i>N</i>	37
		<i>n</i> (%) cured	35 (94.6)
		95% CI (%)	(87.3–100)
	28	<i>N</i>	37
		<i>n</i> (%) cured	15 (40.5)
		95% CI (%)	(24.7–56.3)
PCR corrected	28 ^b	<i>N</i>	37
		<i>n</i> (%) cured	16 (43.2)
		95% CI (%)	(27.2–59.2)
Parasite clearance time		<i>N</i>	29
		Mean (SD) time (h)	121 (97)
		Median (IQR) time (h)	72 (72–168)
Fever clearance time		<i>N</i>	39
		Mean (SD) time (h)	23.3 (30.7)
		Median (IQR) time (h)	12 (0–36)

^a *N*, ATP population; *n*, number of cured subjects; CI, confidence interval.

^b Participants with unclear or missing PCR results were considered not cured.

in the ATP population, while at day 28, it decreased to 40.5% (15/37) (Table 2). When the PCR correction was applied to all recurrent parasitemia cases in the ATP population, only two cases could be classified as new infections, yielding a day 28 PCR-corrected cure rate (primary endpoint) of 45.9% (17/37). Because of the poor results obtained, we also performed PCR to detect submicroscopic infections among those who were negative by microscopy (theoretical treatment successes; $n = 17$). A further 9 patients were confirmed to be still positive for *P. falciparum* parasites by PCR, 3 of them identical to their initial infection, 2 proving to be a new submicroscopic infection, and 4 for which the result was inconclusive because not all the markers amplified during posttreatment PCR, and among those that amplified, some alleles were identical to the pretreatment markers. However, and despite the overall poor efficacy shown by the combination, the median fever clearance time was short (12 h; evaluated in 39 patients), whereas both mean (121 h) and median (72 h; evaluated in 29 patients) parasite clearance times were significantly longer than expected.

Safety. Fosmidomycin coadministered with clindamycin was well tolerated, with the majority of clinical and/or laboratory AEs of mild and moderate severity and consistent with symptoms attributable to malaria (first or recurrent infection). Table 3 summarizes the AEs that occurred. Four out of the 52 children initially recruited (7.7%) were excluded after repeated vomiting, which also occurred in 6 other patients throughout follow-up (total incidence of vomiting, 19% [10/52]). Thrombocytopenia (40.4%) and anemia (28.8%) were frequently observed, and respiratory symptoms (cough, 28.8%; bronchitis, 40.4%) were also commonly reported. Two children experienced SAEs. A 22-month-old boy developed severe anemia with signs of heart decompensation 48 h after the last dose of F/C in the context of low but persisting parasitemia without fever. He was treated with intravenous quinine and transfused whole blood and recovered fully. The second SAE was in a 19-month-old boy recruited with mild diar-

TABLE 3 Cumulative adverse events reported during the follow-up period (safety population; $n = 52$)

AE ^a	No. (%) for F/C ($n = 52$)
At least one AE	50 (96.1)
Fever	18 (34.6)
Malaria	25 (48.1)
Anorexia	1 (1.9)
Vomiting	10 (19.2)
Diarrhea	6 (11.5)
Cough	15 (28.8)
Bronchitis	21 (40.4)
Upper respiratory tract infection	7 (13.5)
Allergic reaction	0 (0)
Abnormal WBC count ($<5 \times 10^9$ or $> 18 \times 10^9$ /liter)	14 (26.9)
Anemia (Hb < 70 g/liter)	15 (28.8)
Thrombocytopenia (platelet count $< 100 \times 10^9$ /liter)	21 (40.4)
High liver enzymes (ALT > 60 U/liter)	11 (21.2)

^a A patient presenting the same sign/symptom/diagnosis more than once contributed only once to the proportion. WBC, white blood cell; ALT, alanine aminotransferase; Hb, hemoglobin.

rhea who developed moderate dehydration secondary to persistent diarrhea after receiving one dose of F/C. He was therefore withdrawn from the study, admitted to the hospital, and managed with intravenous fluids and intravenous quinine, with full recovery in 24 h. No fatal cases occurred during the study follow-up.

DISCUSSION

The disappointing results of this trial, showing a PCR-corrected efficacy for the fosmidomycin-clindamycin combination below 50%, are an unexpected but nevertheless important finding that needs to be made public. Indeed, while the idea of using fosmidomycin monotherapy had already been disregarded, because of both its poor efficacy when used alone (3) and the general WHO recommendation of using combination therapy for the treatment of malaria (29), its combination with clindamycin had been shown to work adequately in both adults (20) and children (3, 5, 6, 20), with cure rates at day 28 consistently above 85 to 90%.

A subgroup analysis performed among 8 Gabonese children under the age of 3 participating in another study and treated with the same combination (administered as crushed tablets rather than aqueous solutions but with an identical daily dose), demonstrated a decrease in efficacy to 62% (5/8), a finding whose significance remained unknown because of the small sample size but which was the basis for the specific assessment of the efficacy of the combination among young children in our study. Our results seem to confirm that trend. Furthermore, the unacceptably low efficacy results in our trial seemed to be associated with an increased PCT. Indeed, the mean PCT in our study was 121 h, a significant 3- to 5-fold increase over what had been shown to occur in previous studies (6, 20) involving African children (~25 h), although this did not seem to be associated with a parallel delay in the fever clearance time (12 h), which was surprisingly shorter than what had been previously described (range, 38 to 46 h) (6, 20).

Several factors need to be taken into consideration when attempting to understand the reason for such discrepancies and the poor performance of the F/C combination. The first explanation could be related to the change in formulation introduced in this

study. For the first time, a solution reconstituted from water-soluble granules was prepared as a formulation theoretically better suited than crushed tablets to the treatment of young children. Reconstitution was performed immediately prior to treatment, and the prepared solutions were maintained under strict temperature control (between 15 and 25°C) during the entire duration of each child's therapy. The significantly longer parasite clearance times in this study suggest that the delayed antimalarial effect observed may have derived almost exclusively from the slowly acting antimalarial, i.e., from clindamycin, largely unsupported by fosmidomycin. However, biochemical analyses performed by Jomaa Pharma in Hamburg, Germany, of the remaining contents of bottles used for the study confirmed that the active principles could be found at their adequate concentrations (data not shown); thus, degradation of the pharmacological activity of the drugs seems unlikely.

Alternatively, the high volumes required to reach the necessary doses of each of the two drugs forming the combination (in some cases adding up to 20 ml) may have represented an unnecessary risk factor for inadequate tolerability and may explain the number of children who had to be excluded from the trial due to repeated vomiting. Alternatively, younger children may have lower absorption, accelerated metabolism, or proportionally higher distribution volume than older children, and this may have contributed to poorer bioavailability of the drugs administered at the current dosing recommendations calculated for older children. Specific pharmacokinetic data for our patients, unfortunately unavailable, could have contributed to understanding whether the decreased efficacy could be related to these factors.

A further possible explanation may have to do with the choice of the partner drug to accompany fosmidomycin. When fosmidomycin has been combined with a fast-acting antimalarial, such as artesunate, it has proven highly efficacious (4). Clindamycin, although shown to have good *in vitro* synergistic effect when combined with fosmidomycin (27), has a slow onset of clinical action and usually requires a long time (5 days) to effectively act on parasites when given as monotherapy (14). It is possible that the shorter course of clindamycin used in this study may have insufficiently complemented fosmidomycin and that, as a result, parasite clearance may have been incomplete or delayed. However, neither the good cure rates observed on day 7 (35/37; 94.6%) nor the good results obtained with the F/C combination elsewhere (3, 5, 6, 18, 20, 23) support this hypothesis, and as previously mentioned, the significantly prolonged parasite clearance times instead support the hypothesis that clindamycin worked better than fosmidomycin. The roles of other partner drugs, such as piper-quine, with longer half-lives and the prospect of requiring shorter dosing courses should be explored.

Younger children born in areas where malaria is endemic naturally develop throughout their first years of life a certain degree of immunity to malarial disease. It is possible that children under the age of 3 may have not yet acquired the immunity necessary to enhance the effect of antimalarial drugs and that without this, their response to the drugs remains insufficient to clear the originating infection, as has previously been shown (7, 10). This could be an alternative explanation for the decreased efficacy and prolonged PCT found in our study or in the subgroup of younger Gabonese children. However, and in response to this argument, the efficacy evaluated by age group (6 to <12 months, 12 to <24 months, and 24 to <36 months) did not seem to show any partic-

ular increasing trend, and the drug combination performed similarly inadequately in the different groups (data not shown).

A third possible explanation is related to naturally occurring geographical differences between parasites in Mozambique and those in Gabon. A higher prevalence in Manhiça of mutations conferring resistance to fosmidomycin, clindamycin, or both could explain the lower efficacy of these drugs among Mozambican patients than in Gabon. As markers for resistance have recently been proposed for both drugs (8, 9), it should be possible to assay for them in recrudescence parasites to confirm this hypothesis. Likewise, if the situation regarding resistance is not a matter of differential prevalence according to geography but rather a factor related to worsening in recent years (the studies in Gabon were performed more than 5 years ago), this could be an alternative explanation. Repeating the study at this time in Gabon and Mozambique simultaneously in a group of older children, using the conventional tablets and coupled with a thorough pharmacokinetic evaluation and assessment of proposed markers of drug resistance among recrudescence cases, could help to shed light on the reasons for our unexpected findings.

PCR revision of recurrent parasitemias did not significantly correct the low observed cure rates in our study, confirming that all but two of these cases were recrudescences of the original infection. Unusually, we performed PCR diagnosis on all patients who reached the 28-day follow-up endpoint, irrespective of the presence or absence of parasites detected by microscopy. Surprisingly, nine new cases (19% of our ATP population) of recurrent parasitemia, undetected by microscopy, were identified, a finding that further confirms the poor performance of the F/C combination and that may be an indication of the need to expand PCR diagnosis to all patients participating in drug trials and not only those with microscopic detection of recurrence. In drugs with a potential to increase the gametocyte carriage, such as fosmidomycin or sulfadoxine-pyrimethamine (22), there is reasonable uncertainty as to whether PCR methodologies may mistakenly identify the gametocyte's DNA as recrudescence infection. The low prevalence of concurrent gametocytemia among recrudescence cases in our study (5/21 [24%]) contradicts this hypothesis and confirms that PCR on this occasion correctly identified all cases where the drugs did not manage to fully clear the asexual infection.

In conclusion, the combination of fosmidomycin with clindamycin was poorly effective in the treatment of uncomplicated *P. falciparum* malaria in this group of Mozambican children aged 6 to 36 months. Further studies with this combination should be conducted to respond to the many uncertainties arising from this small trial. In a time when the risk of artemisinin resistance is seen as the sword of Damocles for control and elimination efforts, unexpectedly and prematurely abandoning the clinical development of a promising non-ACT combination may represent an important drawback in the therapy of malaria. Efforts should be devoted to understanding the causes of this inadequate efficacy and to continuing the development of fosmidomycin as an antimalarial drug, either in combination with clindamycin or in association with other partner drugs.

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