Fixed-Dose Pyronaridine-Artesunate Combination for Treatment of Uncomplicated Falciparum Malaria in Pediatric Patients in Gabon

Michael Ramharter,[1,3,4] Florian Kurth,[1,3] Annette C. Schreier,[1,3] Johannes Nemeth,[1,3,4] Isabelle von Glasenapp,[1,4] Sabine Bélard,[1,4] Meike Schlie,[1,4] Judith Kammer,[1,4] Philemon Koumba Koumba,[1] Badara Cisse,[2] Benjamin Mordmüller,[1,4] Bertrand Lell,[1,4] Saadou Issifou,[1,4] Claude Oeuvray,[5] Lawrence Fleckenstein,[6] and Peter G. Kremsner[1,4]

[1]Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon; [2]Département de Parasitologie, Faculté de Médecine, Université de Dakar, Sénégal; [3]Department of Medicine I, Division of Infectious Diseases and Tropical Medicine, Medical University of Vienna, Vienna, Austria; [4]Institute for Tropical Medicine, Department of Parasitology, University of Tübingen, Tübingen, Germany; [5]Medicines for Malaria Venture, Geneva, Switzerland; [6]College of Pharmacy, University of Iowa, Iowa City

Background. The development of novel artemisinin-combination therapies suitable for the treatment of pediatric patients suffering from malaria is a research priority. The aim of this study was to investigate a novel fixed-dose pyronaridine-artesunate combination for the treatment of uncomplicated falciparum malaria in Gabonese patients 2–14 years old.

Methods. The study was designed as an open-label dose-escalation study recruiting 60 pediatric patients sequentially in 4 treatment cohorts: study drugs were administered once daily for 3 days, as tablet coformulations (pyronaridine:artesunate ratios of 6:2, 9:3, and 12:4 mg/kg) and as a granule coformulation (pyronaridine:artesunate ratio of 9:3 mg/kg). The primary end points were tolerability, safety, and pharmacokinetics of pyronaridine-artesunate treatment. Efficacy was treated as a secondary outcome measure.

Results. The drugs had a good tolerability and safety profile, at all dose levels. Pharmacokinetic analysis revealed a dose-dependent increase in the maximum plasma/blood concentration and the area under the curve, as well as comparable relative bioavailability for the granule coformulation. Polymerase chain reaction–corrected cure rates at day 28 were 100% in per-protocol analysis, at all dose levels.

Conclusions. Pyronaridine-artesunate is a promising novel artemisinin-combination therapy for pediatric patients with uncomplicated *Plasmodium falciparum* malaria, and the development of both the tablet and the granule coformulations is warranted.

Trial Registration. Clinical Trials.gov identifier NCT00331136.

Because of *Plasmodium falciparum*'s increasing resistance to previously used first-line therapies, artemisinin-combination therapy has become the mainstay of antimalarial treatment in most regions where the disease is endemic [1]. Currently available artemisinin combinations show good efficacy and sustained high cure rates. However, tolerability, cost, and impaired patient compliance because of complicated dosing schedules and inappropriate drug formulations are major drawbacks [1]. Specific resistance against drugs currently partnered with artemisinin is rising, and novel partner drugs are therefore currently being developed to overcome these limitations [1–9].

The not-for-profit organization Medicines for Malaria Venture (Geneva, Switzerland) and the pharmaceutical company Shin Poong Pharmaceuticals (Seoul, Republic of Korea) have started a joint drug-development program to evaluate pyronaridine-artesunate combination therapy for the treatment of uncomplicated *P. falciparum* and *P. vivax* malaria. The objective is to provide a fixed-dose artemisinin-combination therapy that has high efficacy, a good tolerability and safety profile, low cost

Received 8 February 2008; accepted 25 March 2008; electronically published 11 August 2008.

Potential conflicts of interest: none reported.

Presented in part: 5th European Congress on Tropical Medicine and International Health, Amsterdam, The Netherlands, 24–28 May 2007 (abstract O26-10).

Financial support: Medicines for Malaria Venture; Shin Poong Pharmaceuticals; Fulcrum Pharmaceuticals (logistical support).

Reprints or correspondence: Michael Ramharter, Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon (michael.ramharter@meduniwien.ac.at).

The Journal of Infectious Diseases 2008; 198:911–9
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0022-1899/2008/19806-0018$15.00
DOI: 10.1086/591096

(less than $1 per adult-treatment course), long shelf life, and an easy dosing regimen of 1 daily dose over 3 days [10].

Pyronaridine, a Mannich base 1-aza-acridine structurally related to mepacrine, one of the earliest synthetic antimalarial drugs, was first developed in China >30 years ago [11]. It has been used successfully as antimalarial monotherapy in eastern Asia and currently is marketed, in both oral and injectable formulations, in China [12]. Available data on pediatric patients in Africa indicate that pyronaridine is effective in cases that are resistant to chloroquine [13]. There is evidence that resistance to pyronaridine may emerge when the latter is used as monotherapy on a large scale [14]. Therefore, the rationale for treatment with a fixed-dose pyronaridine-artesunate combination is to achieve both rapid symptomatic relief and high cure rates and to delay the development of drug resistance.

Data from phase I and phase II studies of adults have demonstrated that the pyronaridine-artesunate combination has a good safety profile. Because children are the population primarily affected by malaria, the present study investigated a population of pediatric patients to determine the most appropriate dose of pyronaridine-artesunate, in 3 different tablet formulations and in a novel granule formulation. The main objectives were to determine (1) the pharmacokinetics of the 3 fixed-dose pyronaridine-artesunate tablet coformulations, (2) the relative bioavailability of the granule coformulation, and (3) the safety and tolerability of all dose formulations.

MATERIALS AND METHODS

Study design and outcome measures. The study was designed as an open-label sequential dose–escalation study. Tablet coformulations of 3 different fixed-dose pyronaridine-artesunate combinations—with pyronaridine tetraphosphate:artesunate ratios of 6:2, 9:3, and 12:4 mg/kg—were evaluated. The primary outcome measures were safety, tolerability, and pharmacokinetics; pharmacodynamics was a secondary outcome measure and was classified according to World Health Organization recommendations [15]. The polymerase chain reaction (PCR)–corrected adequate clinical and parasitological response (ACPR) rate at day 28 served as the primary efficacy end point in per-protocol (PP) analysis and as the secondary efficacy outcome in modified intention-to-treat (ITT) analysis. Each of the 4 cohorts included 15 patients; this sample size was chosen in this descriptive study in order to obtain, for each dose level, at least 12 patients who would be evaluable in terms of the primary outcome measures.

After treatment with the 3 tablet coformulations of pyronaridine-artesunate had been concluded, a protocol extension was implemented to evaluate the granule coformulation. This drug formulation was specifically developed for the treatment of very young children, to increase the acceptability of the drug; on the basis of previous results, the 9:3-mg/kg pyronaridine:artesunate dose was evaluated in 15 patients. The primary aim of this protocol extension was the assessment of the relative bioavailability of the granule coformulation versus that of the tablet coformulations.

Study area and enrollment. The study was conducted during June–December 2006 at the Medical Research Unit of the Albert Schweitzer Hospital in Lambaréné, Gabon [16]. *P. falciparum* is transmitted perennially and shows both 100% resistance to chloroquine and reduced sensitivity to sulfadoxine-pyrimethamine [17–21].

Patients suffering from uncomplicated *P. falciparum* malaria were invited to participate in the study if the following inclusion criteria were met: *P. falciparum* monoinfection (parasitemia, 1000–200,000 parasites/μL blood), age 2–14 years, body weight 10–40 kg, absence of pregnancy and of severe malnutrition, and ability to tolerate oral medication. Exclusion criteria were signs or symptoms of severe malaria or severe diarrhea, evidence of significant disorders, other febrile conditions, hypersensitivity to the study drugs, intake of antimalarial drugs during the preceding 2 weeks, liver function–test results that were >3 times above normal levels, and significant renal impairment (plasma/blood creatinine level $\geq$2 mg/dL).

Written informed consent was obtained from parents or guardians, and, when appropriate, the assent of the pediatric patients was obtained. The study was approved by the Ethics Committee of the International Foundation for the Albert Schweitzer Hospital in Lambaréné.

Study drugs and study flow. At the first visit, the patient's medical history was recorded, a physical examination was performed, and hematological, biochemical, and parasitological characteristics were determined. The pyronaridine-artesunate combination (Pyramax; Shin Poong Pharmaceutical) was first administered at the lowest level, 6:2 mg/kg body weight, and, after a Safety Oversight Group had reviewed the patient's clinical and laboratory data, the dose was increased to 9:3 and then to 12:4 mg/kg; this study design allowed for assessment of the safety and tolerability of the pyronaridine-artesunate combination before the dose was increased. The tablet coformulations contained 48 mg of pyronaridine tetraphosphate (the dosing regimen was based on pyronaridine salt; 100 mg of salt is equivalent to 56.93 mg of free-base pyronaridine) and 16 mg of artesunate, for group A; 72 mg of pyronaridine tetraphosphate and 24 mg of artesunate, for group B; and 96 mg of pyronaridine tetraphosphate and 32 mg of artesunate, for group C. The granule coformulation used for group D was administered in a sachet that contained 60 mg of pyronaridine tetraphosphate and 20 mg of artesunate in taste-masked granules. All of the drug formulations were administered with liquid once daily for 3 days. Patients were hospitalized during the treatment period, and follow-up visits were scheduled until day 42.

Pharmacokinetics. For pharmacokinetic analysis of pyronaridine, artesunate, and the principal active metabolite dihydroartemisinin, 1 mL of blood was drawn at the following times:

Table 1. Characteristics of patients in the intention-to-treat population, stratified by pyronaridine:artesunate dose.

Characteristic	Group A: 6:2-mg/kg tablets (n = 14)	Group B: 9:3-mg/kg tablets (n = 15)	Group C: 12:4-mg/kg tablets (n = 15)	Group D: 9:3-mg/kg granules (n = 15)
Male sex, no. (%) of patients	8 (57.1)	8 (53.3)	6 (40.0)	8 (53.3)
Age, median (range), years	6 (2–11)	5 (2–14)	5 (2–10)	4 (2–10)
Height, mean ± SD, cm	110 ± 13	109 ± 19	112 ± 16	103 ± 14
Weight, mean ± SD, kg	19 ± 6	19 ± 7	19 ± 6	17 ± 5
Gametocytemia, no. (%) of patients	2 (14)	0 (0)	1 (7)	1 (7)
Plasmodium falciparum parasitemia, median (range), no./μL	8144 (1072–57890)	11090 (1152–58893)	7020 (1350–118500)	2971 (1096–174241)
Body temperature, mean ± SD, °C	37.6 ± 01.0	37.7 ± 0.9	37.1 ± 0.9	37.2 ± 0.9
Pyronaridine tetraphosphate dose, median (range), mg/kg	6.3 (4.2–7.2)	9.9 (7.2–11.6)	13.2 (10.1–16.3)	11.4 (9.1–13.0)
Artesunate dose, median (range), mg/kg	2.1 (1.4–2.4)	3.3 (2.4–3.9)	4.8 (3.0–6.1)	3.8 (3.0–4.3)

before administration of the study drug; at 0.25 (for artesunate or dihydroartemisinin only), 0.5, 1.0, 1.5, 2.5, 4.0, 8.0, and 12.0 h after intake of the first drug; before administration of the second drug; before administration of the third drug; 24 h after administration of the third drug (for pyronaridine only); at 168 (for pyronaridine only), 336 (for pyronaridine only), and 504 (for pyronaridine only) h after initiation of treatment; and if parasitemia reappeared (for pyronaridine only). Plasma concentrations of artesunate and of dihydroartemisinin were determined by liquid-chromatography mass-spectrometry analysis, and blood concentrations of pyronaridine were measured in whole-blood samples by liquid chromatography, as described elsewhere (<11.1% relative SD of quality-control samples) [22, 23].

Laboratory analysis. Results of electrocardiography (CT110 CardioConcept; SECA) were interpreted by both a study physician on site and an independent reviewer at a different location.

A sequential approach to distinguishing between recrudescent and newly acquired infections was performed by analysis of merozoite surface antigen (MSA)–2 length polymorphisms and gene-product sequencing. DNA was extracted from blood spots (FTA Classic Card; Whatman International) of anonymized samples (QIAmp DNA extraction; Qiagen). The *P. falciparum MSA-2* gene was amplified by use of a family-specific nested-

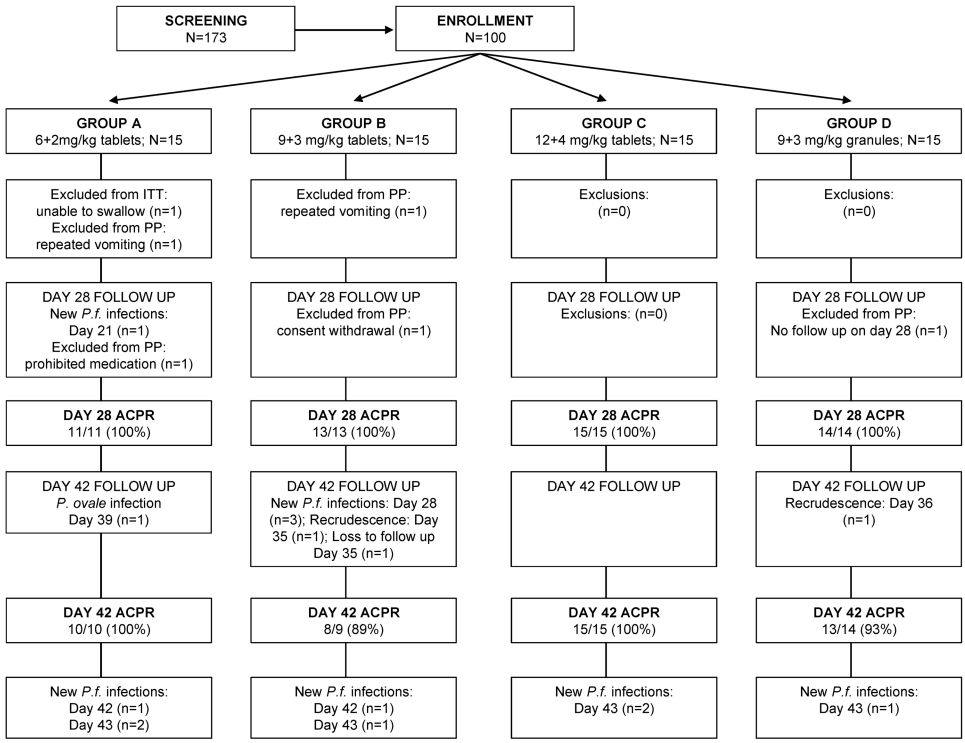

Figure 1. Patient flow.

Table 2. Patients experiencing any adverse event (AE) or any at least possibly drug-related AE during the course of the study, stratified by pyronaridine:artesunate dose.

	Group A: 6:2-mg/kg tablets ($n = 14$)	Group B: 9:3-mg/kg tablets ($n = 15$)	Group C: 12:4-mg/kg tablets ($n = 15$)	Group D: 9:3-mg/kg granules ($n = 15$)
Total data				
Any at least possibly AE	13 (93)	11 (73)	13 (87)	12 (80)
Drug-related AE	5 (36)	4 (27)	5 (33)	3 (20)
Serious AE	2 (14)	0 (0)	0 (0)	0 (0)
Any definite AE				
Infection	9 (64)	8 (53)	10 (67)	11 (73)
Gastrointestinal disorder	7 (50)	3 (20)	3 (20)	1 (7)
Headache	5 (36)	1 (7)	2 (13)	1 (7)
Fatigue or pyrexia	2 (14)	2 (23)	1 (7)	2 (13)
Cough	2 (14)	1 (7)	2 (13)	1 (7)
Splenomegaly or hepatomegaly	1 (7)	2 (13)	3 (20)	1 (7)
Anorexia	2 (14)	0 (0)	1 (7)	1 (7)
Any possibly drug-related AE				
Gastrointestinal disorders	3 (21)	2 (13)	2 (13)	1 (7)
Fatigue or pyrexia	1 (7)	1 (7)	0 (0)	1 (7)
Splenomegaly or hepatomegaly	0 (0)	1 (7)	2 (13)	0 (0)
Anemia	1 (7)	0 (0)	0 (0)	0 (0)
Anorexia	1 (7)	0 (0)	0 (0)	1 (7)
Headache	1 (7)	0 (0)	0 (0)	1 (7)
Hyperhidrosis	0 (0)	0 (0)	1 (7)	0 (0)

NOTE. Data are no. (%) of patients.

PCR protocol, and length polymorphisms of PCR products were analyzed by electrophoresis (Biometra Uno thermal cycler) [24]. When at least 1 PCR product from 1 family was of similar size at inclusion and at day of reappearance, the PCR products were sequenced by use of forward and backward PCR primers (BigDye chemistry 3100; Applied Biosystems).

Recrudescence was considered to have occurred if, at baseline and at the time of reappearing parasitemia, there were at least 2 amplified DNA fragments of the same size and at least 2 gene products with identical sequences; reinfection was considered to have occurred if at least 1 of these 2 conditions was not met. When PCR and sequence analysis could not determine the status, recrudescence was assumed to have occurred.

Statistical analysis. The accuracy of data was checked on the basis of a predefined data-validation plan. Standard descriptive statistics were used for quantitative variables, and rates and proportions were used for categorical variables (SAS version 8.2). ACPR was summarized by use of exact 95% Pearson-Clopper confidence intervals. Parasite-clearance times were analyzed by Kaplan-Meier survival analysis. Two populations were defined for statistical analysis: a modified ITT population (all patients received at least 1 dose of study medication) and a PP population (all patients received a full course of treatment; their PCR-corrected ACPR status at day 28 was known, and there were no major protocol violations). ITT analysis was performed to determine safety and tolerability, and PP analysis was performed to define the primary efficacy outcome.

Pharmacokinetic parameters were computed by noncompartmental analysis (WinNonlin Professional model 200, version 5.0; Pharsight). Peak plasma/blood concentrations were determined without interpolation, and the area under the curve (AUC) was computed by use of the linear trapezoidal rule. The log-linear proportion (at least 3 plasma concentrations for artesunate and for dihydroartemisinin and at least 4 blood concentrations for pyronaridine) was used to determine the terminal-phase hybrid constant λ_z. The volume of distribution was calculated as follows: dose/($\lambda_z \times AUC_{0-\infty}$). Total-body clearance for extravascular administration was calculated by dividing the dose by $AUC_{0-\infty}$. Pairwise comparisons of untransformed pharmacokinetic parameters were performed by use of 2-sided t tests, with $P < .05$ as the level of significance and with no adjustment for multiple testing.

RESULTS

A total of 60 patients were enrolled in this clinical study during June–December 2006; 15 patients were sequentially assigned to each dose level, and the baseline characteristics of the 4 groups were comparable (table 1). All patients were included in the ITT analysis, except for 1 patient in group A who was unable to tolerate oral medication and therefore was removed from the study

population. During the course of the study, 11 patients were excluded from the PP analysis because they either required antimalarial treatment of reappearing parasitemia during follow-up ($n = 4$), developed *P. ovale* infection ($n = 1$), vomited redosed study medications ($n = 2$), withdrew consent ($n = 1$), ingested prohibited medication ($n = 1$), and were lost to follow-up ($n = 2$); these patients were classified, in ITT analysis, as treatment failures. The disposition of the patients during the course of this clinical trial is shown in figure 1.

Safety, tolerability, and evolution of laboratory data. In the 4 treatment groups, 73%–93% of patients experienced at least 1 adverse event (AE) during the course of this study (table 2), and one-third (20%-36%) of these cases were judged as being at least possibly related to the study drugs. The majority of AEs that were related to the study drugs were gastrointestinal disorders, including vomiting ($n = 4$), abdominal pain ($n = 4$), diarrhea ($n = 1$), and nausea ($n = 1$); other AEs that were at least possibly related to the study drugs were rare. No abnormal electrocardiographic result associated with the study drugs occurred in this clinical trial.

All AEs in the present study were of mild or moderate intensity; none was severe or life-threatening. Overall, there was no observable increase in the rate or severity of AEs during the dose-escalation procedure. The 2 serious AEs that did occur during the course of the present study—specifically, a soft-tissue infection of the right thumb, which necessitated surgical treatment, and hospitalization for observation of an unspecified febrile episode with abdominal pain and no evidence of parasitemia during the third week of follow up (both of these AEs occurred in group A)—were not related to the study drugs.

Hematological analysis, biochemical analysis, and urinalysis were performed for all patients, at enrollment and on days 3 and 7 (table 3). There was a modest decrease in median hemoglobin levels during the first 72 h of treatment, with complete recovery to initial levels by day 7. In 1 patient, there was a clinically significant, 2.5-g/dl decrease in hemoglobin during the first 24 h, but, without further intervention, the hemoglobin level subsequently increased until, by day 7, it was 7.7 g/dL, which exceeded the baseline value. A general rise in platelet count and eosinophil count was noted during the study. Abnormalities revealed by urinalysis were caused by concomitant *Schistosoma haematobium* infections. There were no clinically significant changes in the biochemical parameters during the course of the study (table 3).

Pharmacokinetics. Pharmacokinetic characteristics of pyronaridine, artesunate, and dihydroartemisinin were evaluated in all patients (table 4). For artesunate, both the mean maximum plasma concentration (C_{max}) and the mean $AUC_{0-\infty}$ increased in a dose-dependent manner, from 93 ng/mL to 287 ng/mL/h and from 104 to 232 ng/mL/h, respectively; the time to C_{max} was 0.5–1.0 h, and the terminal half-life was 0.5–1.2 h. Dihydroartemisinin, the major active metabolite of artesunate, showed a linear increase both in C_{max}, from 479 to 1186 ng/mL, and in AUC, from 1055 to 2961 ng/mL/h; the time to C_{max} was 1.3–1.7 h after administration. Dihydroartemisinin showed a relatively short half-life of 0.9–1.2 h. The blood concentration of pyronaridine also increased in a dose-dependent pattern: both C_{max} and AUC rose, from 86 ng/mL to 339 ng/mL and from 17,623 to 35,360 ng/mL/h, respectively; the time to C_{max} was 2.4–3.2 h, and the elimination half-life was 6.6–9.0 days (table 4). The pharmacokinetic parameters of the granule coformulation were compared with those of the tablet formulation of the same dose (i.e., the pyronaridine:artesunate ratio of 9:3 mg/kg); there were no statistically significant differences, except for a higher C_{max} (168 ng/mL vs. 119 ng/mL [$P < .05$]; table 4) of pyronaridine in the patients receiving the granule coformulation.

Efficacy. All patients showed a satisfactory initial response to the study drugs, and no early treatment failure was observed during the course of the trial. A total of 14 patients—4, 6, 2, and 2 in groups A, B, C, and D, respectively—had reappearing *P. falciparum* parasitemia during follow-up, and 1 patient in group A had *P. ovale* infection at day 39. The earliest reappearance of *P. falciparum* infection was observed at day 21, whereas all other reappearing parasitemias occurred after day 28.

By day 28, 53 patients had reached the primary efficacy end point in the PP analysis (table 5), and all evaluable patients had experienced ACPR (100% cure rate [95% confidence interval 93%–100%] when data from all groups are pooled). Two patients were classified, on the basis of genotyping, as true treatment failures (on days 35 and 36 in the 9:3 mg/kg tablet and granule coformulation groups, respectively). The PCR-corrected efficacy at day 42 was 89%–100% in the 4 groups. Parasite-clearance times were remarkably short, with a median of 16, 16, 8, and 8 h in groups A, B, C, and D, respectively. There was thus a statistically significant trend toward faster parasite clearance in the higher-dose groups ($P < .05$ for group A vs. group C, by Tukey-Kramer honestly significant difference test). The results of the ITT analysis are provided in table 5.

DISCUSSION

Young children in sub-Saharan Africa suffer disproportionately from malaria-related morbidity and mortality and therefore should be the primary target population for treatment with novel antimalarial regimens [25, 26]. Although the introduction of artemisinin-combination therapies in most regions where malaria is endemic has led to sustained and unparalleled high cure rates, there is a lack of suitable pediatric drug formulations [1, 27]. Therefore, therapeutic management of patients who are most in need of effective treatment is often suboptimal, and the development of pediatric drug formulations is a research priority.

In the present study, pyronaridine-artesunate therapy had a good tolerability profile at all doses used. Most AEs were of mild or moderate intensity, with gastrointestinal symptoms occur-

Table 3. Evolution of laboratory parameters and vital signs, stratified by pyronaridine:artesunate dose.

Characteristic	Group A: 6:2-mg/kg tablets (n = 14)	Group B: 9:3-mg/kg tablets (n = 15)	Group C: 12:4-mg/kg tablets (n = 15)	Group D: 9:3-mg/kg granules (n = 15)
Hematology				
Hemoglobin, g/dL				
Baseline	10.0 (7.4–12.9)	10.5 (8.6–12.3)	10.2 (8.5–12.5)	9.5 (8.2–10.6)
Day 3	9.7 (6.5–13.1)	10.4 (8.3–12.3)	9.9 (8.3–12.2)	9.1 (7.6–10.3)
Day 7	10.1 (7.7–12.1)	10.6 (9.6–12.0)	9.7 (7.4–11.9)	10.0 (7.3–10.7)
Red blood cells, 10^6/mm^3				
Baseline	4.4 (3.6–6.0)	4.4 (3.4–5.1)	4.4 (3.3–4.9)	3.8 (3.4–6.0)
Day 7	4.4 (3.6–5.7)	4.5 (3.6–4.9)	4.0 (3.1–4.6)	4.0 (3.0–5.6)
Platelets, 10^3/mm^3				
Baseline	244 (94–350)	210 (122–411)	219 (113–319)	196 (116–264)
Day 7	336 (151–685)	370 (141–409)	271 (110–688)	324 (48.6–591)
White blood cells, 10^9/L				
Baseline	7.6 (5.0–21.3)	7.9 (4.7–12.3)	9.3 (5.2–13.4)	7.5 (4.0–15.6)
Day 7	7.9 (4.8–13.2)	9.7 (5.2–12.5)	9.4 (6.8–13.8)	8.7 (4.3–17.9)
Neutrophils, %				
Baseline	43 (18–77)	41 (19–74)	29 (23–77)	31 (17–67)
Day 7	27 (24–46)	29 (24–37)	26 (22–55)	31 (11–43)
Lymphocytes, %				
Baseline	37 (13–56)	35 (16–60)	42 (11–56)	44 (18–58)
Day 7	50 (40–62)	40 (28–53)	43 (29–57)	45 (30–59)
Monocytes, %				
Baseline	11 (6–16)	11 (6–17)	9 (5–17)	13 (7–28)
Day 7	8 (5–12)	9 (6–13)	8 (5–13)	10 (6–18)
Eosinophils, %				
Baseline	6 (1–35)	7 (1–31)	5 (1–11)	9 (1–23)
Day 7	10 (4–27)	13 (7–34)	15 (2–34)	14 (5–21)
Basophils, %				
Baseline	1 (0–1)	1 (1–2)	1 (0–1)	1 (1–2)
Day 7	1 (1–1)	1 (1–1)	1 (1–2)	1 (1–4)
Biochemistry				
Total bilirubin, μmol/L				
Baseline	9.4 (5.3–44.3)	10.9 (1.6–27.9)	11.6 (2.6–45.8)	13.6 (2.9–31.9)
Day 7	4.1 (0.3–26.3)	7.4 (3.1–16.4)	8.1 (4.1–12.5)	7.0 (2.6–16.2)
Albumin, g/dL				
Baseline	3.3 (2.7–4.1)	4.0 (3.3–4.5)	3.6 (3.0–4.2)	3.5 (3.1–4.4)
Day 7	3.4 (0.0–4.5)	3.8 (3.2–4.8)	3.7 (3.1–4.4)	3.5 (2.8–4.2)
Alanine aminotransferase, IU/L				
Baseline	19.0 (7.0–57.0)	16.6 (13.0–28.0)	23.0 (10.0–70.0)	21.0 (12.0–32.0)
Day 7	17.0 (2.0–48.0)	14.5 (10.0–22.0)	23.0 (8.0–112.0)	18.0 (11.0–27.0)
Aspartate aminotransferase, IU/L				
Baseline	36.0 (19.0–57.0)	28.0 (18.0–38.2)	29.0 (22.0–71.0)	36.0 (19.0–48.0)
Day 7	30.0 (23.0–73.0)	25.0 (20.0–44.0)	29.0 (17.0–66.0)	33.0 (22.0–45.0)
Creatine kinase, IU/L				
Baseline	72.0 (30.0–159.0)	87.0 (37.0–136.0)	79.0 (46.0–196.0)	61.0 (23.0–88.0)
Day 7	72.0 (34.0–276.0)	88.0 (57.0–124.0)	71.0 (30.4–330.0)	67.0 (33.0–166.0)
Alkaline phosphatase, IU/L				
Baseline	262 (154–397)	274 (162–422)	206 (127–303)	188 (114–254)
Day 7	254 (178–487)	298 (173–636)	172 (108–297)	166 (123–222)
Urea, mmol/L				
Baseline	2.8 (1.0–7.2)	2.6 (1.3–6.0)	3.4 (2.1–6.2)	3.4 (1.2–5.9)
Day 7	2.2 (0.4–4.4)	4.0 (0.9–6.1)	2.9 (1.9–4.9)	2.3 (1.3–4.0)

(continued)

Table 3. ***(Continued.)***

Characteristic	Group A: 6:2-mg/kg tablets (n = 14)	Group B: 9:3-mg/kg tablets (n = 15)	Group C: 12:4-mg/kg tablets (n = 15)	Group D: 9:3-mg/kg granules (n = 15)
Creatinine, μmol/L				
Baseline	35.8 (11.5–49.4)	38.6 (19.1–61.9)	24.8 (18.9–43.1)	29.0 (5.1–43.0)
Day 7	27.9 (2.0–58.0)	36.0 (13.2–68.3)	31.0 (17.5–40.7)	29.2 (0.2–79.0)
Sodium, mmol/L				
Baseline	136 (133–138)	137 (133–140)	138 (132–139)	138 (133–141)
Day 7	138 (136–142)	138 (136–142)	138 (134–140)	140 (137–142)
Potassium, mmol/L				
Baseline	4.0 (3.1–5.0)	4.1 (3.6–4.7)	4.1 (3.5–4.5)	3.9 (3.3–4.5)
Day 7	4.3 (3.9–7.4)	4.6 (4.0–5.7)	4.3 (3.6–5.3)	4.4 (3.9–4.9)
Vital signs				
Blood pressure, mmHG				
Systolic				
Baseline	100 (75–130)	100 (80–120)	95 (80–110)	95 (70–110)
Day 7	100 (85–115)	90 (75–115)	95 (80–120)	90 (70–110)
Diastolic				
Baseline	65 (50–80)	60 (40–80)	60 (40–90)	55 (35–70)
Day 7	60 (45–80)	65 (50–90)	60 (40–80)	50 (40–60)
Heart rate, beats/min				
Baseline	110 (80–128)	100 (84–156)	96 (60–150)	120 (80–144)
Day 7	95 (73–114)	94 (80–124)	94 (76–112)	96 (84–132)

NOTE. Data are median (range).

ring most frequently. Most important, the treatment produced no dose-dependent increase in AEs and no dose-limiting side effects. Given the inherent difficulty in differentiating between side effects of the study drugs and symptoms of malaria in uncontrolled phase II studies, the tolerability of pyronaridine-artesunate will be fully established only by larger randomized controlled trials and postmarketing surveillance. Despite these limitations of the present study and the relatively modest number of patients that it considered, it is reassuring that no safety concerns occurred during the dose-escalation procedure.

In the present study, the standard pharmacokinetic parameters of artesunate were comparable with previous findings in adults [28, 29] and children [30, 31], despite methodological and dose differences. Artesunate showed a rapid biotransformation to dihydroartemisinin, with overall C_{max} levels that were lower than those observed for its metabolite dihydroartemisinin. Interestingly, artemisinin derivatives generally show marked interpatient variability, with an exceptionally wide range of C_{max} levels [31, 32]. However, in the present study, there was no obvious relationship between variation in C_{max} levels and pharmacodynamic efficacy.

Similar to what previously had been reported on pyronaridine treatment in adults, the elimination half-life in the pediatric population in the present study was in the range of 1 week [23]. Antimalarial drugs with a long half-life may be particularly vulnerable for development of drug-resistant *P. falciparum* isolates [1]. Given the comparatively shorter half-life of pyronaridine, compared with that of combination partners such as piperaquine, treatment with pyronaridine-artesunate may therefore prove to be particularly useful in regions where malaria has a high rate of transmission.

By day 28, the cure rates were 100% in the PP analysis, for all dose levels and for both the tablet and granule coformulations; however, they showed a dose-dependent increase in the ITT analysis. There was no indication that the pharmacokinetic parameters in the 2 patients who were classified as treatment failures were significantly different from those in the other patients. Similarly, despite a dose-dependent increase in both the C_{max} and the AUC of the active metabolites, the different doses showed no difference in PCR-corrected cure rates. Although not designed as a dose-finding study to detect efficacy differences between groups, the present clinical trial found that pyronaridine-artesunate therapy has high intrinsic activity in central Africa.

A major aim of this drug-development program is to make an appropriate and registered pediatric drug formulation available for the treatment of young children. Therefore, a granule coformulation of pyronaridine-artesunate was developed, which considerably facilitated drug intake by young children. The tolerability, safety, and efficacy of this coformulation were similar to those of the 3 tablet coformulations, and, except for a trend toward higher C_{max} levels of pyronaridine, all relevant pharmacokinetic parameters showed that the relative bioavailability of the granule coformulation was comparable to those of the tablet coformulations. Despite its statistical significance in unadjusted analysis, the granule coformulation's trend toward higher C_{max}

Table 4. Pharmacokinetic characteristics of artesunate, dihydroartemisinin, and pyronaridine, stratified by pyronaridine:artesunate dose.

Category	Group A: 6:2-mg/kg tablets	Group B: 9:3-mg/kg tablets	Group C: 12:4-mg/kg tablets	Group D: 9:3-mg/kg granules
Patients tested, no.	13	14	15	15
Artesunate				
C_{max}, ng/mL	93 ± 85	154 ± 166	287 ± 480	171 ± 119
Time to C_{max}, h	0.6 ± 0.4	0.7 ± 0.5	1.0 ± 0.4	0.5 ± 0.6
$AUC_{0-\infty}$, ng/mL, h[a]	104 ± 65	154 ± 92	232 ± 245	179 ± 66
Volume of distribution, kg/L[a]	29 ± 18	35 ± 51	25 ± 17	41 ± 35
Clearance, kg/L/h[a]	28 ± 17	30 ± 26	29 ± 12	25 ± 15
Terminal half-life, h[a]	0.8 ± 0.6	1.1 ± 2.3	0.5 ± 0.3	1.2 ± 1.0
Dihydroartemisinin				
C_{max}, ng/mL	479 ± 300	940 ± 549	1186 ± 359	792 ± 488
Time to C_{max}, h	1.4 ± 0.7	1.7 ± 1.9	1.7 ± 0.6	1.3 ± 1.0
$AUC_{0-\infty}$, ng/mL, h[a]	1055 ± 468	1989 ± 1092	2961 ± 979	2245 ± 1241
Volume of distribution, kg/L[a]	3.2 ± 1.8	3.3 ± 3.4	1.6 ± 0.3	4.2 ± 4.3
Clearance, kg/L/h[a]	2.3 ± 1.0	2.3 ± 2.0	2.7 ± 1.8	2.4 ± 2.0
Terminal half-life, h[a]	1.0 ± 0.3	0.9 ± 0.2	1.2 ± 0.8	1.2 ± 0.5
Pyronaridine				
C_{max}, ng/mL	86 ± 51	119 ± 53	339 ± 172	168 ± 57
Time to C_{max}, h	2.7 ± 1.9	3.2 ± 2.4	3.1 ± 1.2	2.4 ± 1.3
$AUC_{0-\infty}$, ng/mL, h[a]	17,623 ± 7202	21,787 ± 6797	35,360 ± 11,163	25,325 ± 8286
Terminal half-life, h[a]	9.0 ± 2.5	7.0 ± 1.9	6.6 ± 2.0	6.7 ± 1.8

NOTE. Data are mean ± SD, except in the case of the no. of patients tested. AUC, area under the curve; C_{max}, mean maximum plasma/blood concentration.

[a] $n = 12$ for group A, $n = 12$ for group B, $n = 10$ for group C, and $n = 13$ for group D.

levels of pyronaridine is unlikely to cause difficulties, given that, even at the higher C_{max} levels seen in the group treated with the 12:4 mg/kg tablet-coformulation, the study drug showed a good tolerability profile. In light of the favorable clinical and pharmacokinetic results observed for the granule coformulation, it has the potential, through improved acceptability and compliance by patients, to significantly ameliorate the management of patients in regions where malaria is endemic.

Table 5. Polymerase chain reaction (PCR)–corrected adequate clinical and parasitological response (ACPR) to artesunate-pyronaridine treatment, in per-protocol and intention-to-treat analysis, stratified by pyronaridine:artesunate dose.

PCR-corrected ACPR	Group A: 6:2-mg/kg tablets ($n = 14$)	Group B: 9:3-mg/kg tablets ($n = 15$)	Group C: 12:4-mg/kg tablets ($n = 15$)	Group D: 9:3-mg/kg granules ($n = 15$)
Per-protocol population				
Day 14	11/11 (100 [72–100])	13/13 (100 [75–100])	15/15 (100 [78–100])	14/14 (100 [77–100])
Day 28	11/11 (100 [72–100])	13/13 (100 [75–100])	15/15 (100 [78–100])	14/14 (100 [77–100])
Day 42	10/10 (100 [69–100])	8/9 (89 [52–100])	15/15 (100 [78–100])	13/14 (93 [66–100])
Intention-to-treat population				
Cure rate				
Day 14	13/14 (93 [66–100])	13/15 (87 [60–98])	15/15 (100 [78–100])	15/15 (100 [78–100])
Day 28	11/14 (79 [49–95])	13/15 (87 [60–98])	15/15 (100 [78–100])	14/15 (93 [68–100])
Day 42	11/13 (85 [55–98])[a]	8/11 (73 [39–94])[b]	15/15 (100 [78–100])	14/15 (93 [68–100])[c]

NOTE. Data are no. of observations/no. of evaluable patients (% [range]).

[a] One patient with a new infection on day 21 was considered not cured at day 28 and was not taken into account for the day-42 analysis.

[b] Three patients with a new infection on day 28 were considered to be cured at day 28 and were not taken into account for the day-42 analysis. One patient was not taken into account for the day-42 analysis because he was lost to follow-up on day 35.

[c] One patient did not come for the day-28 visit but returned for further follow-up visits up to day 42; this patient was classified as "treatment failure" for the day-28 analysis and as "cured" for the day-42 analysis.

In summary, this pediatric phase II study has demonstrated that, at all doses considered, pyronaridine-artesunate combination therapy has a good tolerability and safety profile in 2–14-year-old patients in Gabon. Given the high efficacy of this drug combination, both the tablet coformulations and the granule coformulation warrant further development.

Acknowledgments

We thank the patients and their families, for their willingness and motivation to participate in this clinical trial, and Sarah Arbe-Barnes, Eric Didillon, Robert Miller, Lise Riopel, and Carl Craft, for their fruitful collaboration.

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