Hindawi Publishing Corporation
Malaria Research and Treatment
Volume 2012, Article ID 749479, 5 pages
doi:10.1155/2012/749479

Clinical Study

Clinical Efficacy of Artemether-Lumefantrine in Congolese Children with Acute Uncomplicated Falciparum Malaria in Brazzaville

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Received 10 October 2012; Accepted 23 November 2012

Academic Editor: Sasithon Pukrittayakamee

The Republic of the Congo adopted artemisinin-based combination therapies (ACTs) in 2006: artesunate-amodiaquine and artemether-lumefantrine as the first-line and second-line drugs, respectively. The baseline efficacy of artemether-lumefantrine was evaluated between March and July 2006 in Brazzaville, the capital city of Congo. Seventy-seven children aged between 6 months and 10 years were enrolled in a nonrandomized study. The children were treated under supervision with 6 doses of artemether-lumefantrine and followed up for 28 days in accordance with the 2003 World Health Organization guideline. Pretreatment (i.e., day 0) and recrudescent *Plasmodium falciparum* isolates between day 14 and day 28 were compared by the polymerase chain reaction to distinguish between true recrudescence and reinfection. The overall cure rate on day 28 was 96.9% after PCR correction. Reported adverse effects included pruritus and dizziness. Artemether-lumefantrine was highly efficacious in Brazzaville.

Approximately 30% of the Congolese population reside in Brazzaville, the capital city. The epidemiology of malaria in the city of Brazzaville is heterogeneous [1]. Depending on the district, malaria transmission is low or intense. In general, malaria is meso- to hypoendemic in the city centre and hyperendemic in the periphery [2]. In terms of malaria burden, there are twice as many malaria-infected patients consulting health centres in the periphery, as compared with health centres in the city centre [3]. Surveys conducted in the main hospital in Brazzaville have shown that malaria is the first cause of admission in the department of paediatrics, mostly in children aged less than 4 years old [4, 5].

Due to the high levels of clinical resistance to chloroquine, amodiaquine, and sulphadoxine-pyrimethamine [6, 7], the Congolese Ministry of Public Health changed the national antimalarial drug policy in 2006. Two artemisinin-based combination therapies (ACTs) were adopted: artesunate-amodiaquine and artemether-lumefantrine for the first-line and second-line treatment of uncomplicated malaria, respectively. Before the drug policy change, only a single clinical study on the efficacy of artesunate-amodiaquine and artemether-lumefantrine had been conducted in a rural area in Congo [8]. The present nonrandomized study was conducted between March and July 2006

to provide the baseline data of artemether-lumefantrine efficacy in an urban area where the majority of the Congolese population reside.

The study was conducted in Tenrikyo health centre located in Makélékélé district, which is in the southern part of Brazzaville. The patients consulting the health centre reside in either the neighboring district of Bacongo (low transmission city centre) or the district of Makélékélé itself (high transmission peripheral area) [2, 3].

Febrile children aged between 6 months old and 10 years old were enrolled after a written informed consent was obtained from the parents or the legal guardian. The inclusion criteria were as follows: *P. falciparum* monoinfection with parasitaemia between 2,000 and 200,000 asexual parasites/μL of blood, axillary temperature >37.5°C, haematocrit >15%, absence of concomitant febrile illness, and easy accessibility of the residence for home visits [9]. Febrile patients who received antimalarial drugs, mostly non-ACTs, prior to consultation were also enrolled. Patients with signs and symptoms of severe malaria were excluded.

Based on the recommendation of the drug manufacturer, the following numbers of artemether-lumefantrine (Coartem, Novartis Pharma) tablets were administered under supervision, for a total of six doses: 1 tablet per dose for 5–14 kg body weight, 2 tablets per dose for 15–24 kg body weight, 3 tablets per dose for 25–34 kg body weight, and 4 tablets per dose for ≥35 kg body weight. For small children, the tablets were crushed and mixed with milk before administration. After the initial dose (on day 0), the patients were observed for one hour for possible vomiting and were discharged. If the patient vomited during the observation period, another dose of artemether-lumefantrine was administered. If vomiting occurred again, the patient was withdrawn from the study and treated with parenteral quinine. The second dose (on day 0) was given 8 hours after the initial dose at home under supervision. The patients returned to the health centre in the morning of day 1 and day 2 for the third and fifth doses. The fourth (evening of day 1) and sixth (evening of day 2) doses were given at home by the research team. The study protocol and written consent forms (in French, English, and local dialects) were reviewed and approved by the Congolese Ministry of Health and WHO Secretariat Committee on Research Involving Human Subjects (SCRIHS).

Fingerprick capillary blood was collected to prepare Giemsa-stained thick blood films, measure the packed cell volume in a capillary tube, and store parasite DNA on Whatman 3 MM filter paper. Parasite density was determined by counting the number of asexual parasites against 200 leukocytes and expressed as the number of asexual parasites/μL of blood, assuming a leukocyte density of 8,000 per μL. In case of hyperparasitaemia, the parasite count was stopped after reaching 500 asexual parasites even if 200 leukocytes had not been reached.

The patients were followed up for 28 days, according to the WHO protocol [9]. The body temperature was measured and clinical examination was performed during each visit (i.e., before each of the 6 doses for the first 3 days (day 0, day 1, and day 2), then on days 3, 7, 14, 21, and 28). Blood smears were examined during each visit on days 2, 3, 7, 14, 21, and 28. Body temperature and parasite density were measured on any other day during an unscheduled visit if the patient was febrile during the 28-day period. Recrudescent parasites between day 14 and day 28 were collected and stored on Whatman 3MM filter paper.

Parasite DNA was extracted using QIAamp DNA blood mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Paired samples (day 0 and recrudescent parasites on or after day 14) were genotyped by analysing the highly polymorphic loci, the block 2 of merozoite surface protein-1 (*msp*-1), and the central region of merozoite surface protein-2 (*msp*-2), as previously described [10, 11]. Samples from patients responding with early treatment failure (i.e., on or before day 3) were not analysed by PCR and were considered as recrudescent or persistent parasitaemia. Paired samples were initially genotyped using *msp*-2 locus. If different bands were found, the reappearance of parasites was considered to be due to reinfection. If the *msp*-2 bands were similar, *msp*-1 locus was further compared in paired samples.

Before PCR adjustment, clinical outcomes were classified as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), and adequate clinical and parasitological response (ACPR) [9]. ETF was defined as (i) the development of danger signs or severe malaria on day 1, day 2, or day 3 in the presence of parasitaemia, (ii) parasitaemia on day 2 > initial parasitaemia on day 0, (iii) presence of parasitaemia on day 3 with fever, or (iv) parasitaemia on day 3 ≥ 25% of initial parasitaemia on day 0. LCF was defined as (i) the development of danger signs or severe malaria after day 3 in the presence of parasitaemia or (ii) presence of parasitaemia and fever on any day between day 4 and day 28, without previously meeting any of the criteria of ETF. LPF was defined as the presence of parasitaemia on day 28 in the absence of fever, without previously meeting any of the criteria of ETF or LCF. ACPR was defined as the absence of parasitaemia on day 28, with or without fever, without previously meeting any of the criteria of ETF, LCF, or LPF. PCR allowed further classification of late failures (LCF and LPF) into true recrudescence (persistence or reappearance of the same isolates as those present on day 0) and new infection (appearance of a new isolate, absent on day 0).

Due to the lack of previous data on the efficacy of artemether-lumefantrine in Brazzaville, the minimum sample size was determined to be 50 patients [9]. Clinical and parasitological data were analysed using the preprogrammed Excel spreadsheet provided by the Department of Global Malaria Programme, WHO (Geneva, Switzerland). Patients who withdrew from the study and those lost to follow up during the 28-day period were excluded from further analysis (per protocol analysis), and the proportions of ETF, LCF, LPF, and ACPR were calculated. The treatment failure rate was defined as the number of patients responding with ETF, LCF, or LPF divided by the total number of included patients who completed the 28-day followup. Statistical analysis was performed using Epi-info version 6.04 (Centres for Disease Control and Prevention, Atlanta, GA).

Table 1: Baseline characteristics of patients before treatment with artemether-lumefantrine.

Number of patients	77
Age (years), mean ± SD (range)	4.5 ± 2.4 (8 months–10 years)
Number of patients <5 years old	42 (54.5%)
Number of patients aged 5–10 years old	35 (45.5%)
Weight (kg), mean ± SD (range)	15.9 ± 5.4 (8–28)
Sex ratio (F/M)	39/38 (1.03)
Number of patients who took an antimalarial drug before inclusion	14 (18.2%)*
Body temperature, mean ± SD (range)	38.1 ± 0.8 (36.0–40.3)**
Parasite density (asexual parasites/μL)	
Overall geometric mean (range), (95% CI)	33,300 (2,450–381,000), [28,200–38,500]
Number of patients with parasite density > 200,000 asexual parasites/μL	2 (2.8%)
Geometric mean (range), (95% CI) in patients <5 years old	35,000 (3,480–213,000) [14,100–55,200]
Geometric mean (range) (95% CI) in patients 5–10 years old	33,700 (2,450–381,000) [8,990–58,400]
Haematocrit (%) mean (range), (95% CI)	32.6 (20–40) [31.6–33.8]

SD: standard deviation; 95% CI: 95% confidence intervals; *eight patients received chloroquine; 6 received quinine; **two patients had fever within 24 hr before consultation but were no longer febrile at the time of consultation.

From March to July 2006, there were 1,355 febrile patients consulting the Tenrikyo health centre. Of 1,355 patients, 285 (21.0%) received antimalarial drugs before consultation, mostly due to self-medication: chloroquine (115 patients), quinine (62), amodiaquine (32), sulphadoxine-pyrimethamine (32), artemisinin derivatives (32), ACT (10), and halofantrine (2). Of 1,355 febrile patients, 313 (23.1%) had positive thick smears and 204 (15.0%) were aged <10 years old. Seventy-seven febrile patients aged ≤10 years old were eligible and enrolled. Among these 77 eligible patients, 14 (18.2%) received an antimalarial drug (self-medication) prior to enrollment. The geometric mean parasite density (95% confidence intervals (95% CI)) was 33,300 (28,200–38,500) asexual parasites/μL. The characteristics of these 77 patients are summarized in Table 1. Two patients with uncomplicated malaria associated with parasitaemia >200,000 asexual parasites/μL were recruited after the approval of the medical staff. During the follow-up, 4 children were excluded (1 for repeated vomiting, 1 for the development of pneumonia, and 2 for protocol violation (1 received erythromycin and another self-medicated with an antimalarial drug)) and 4 were lost to follow up.

The therapeutic efficacy of artemether-lumefantrine is summarized in Table 2. After PCR adjustment, 62 of 64 patients (96.9% (95% CI, 89.2–99.6%)) responded with ACPR on day 28. If reinfection ($n = 5$) is considered as ACPR, 67 of 69 patients (97.1% (95% CI, 89.9–99.6%)) had ACPR on day 28. Fever and parasite clearance was rapid. The mean (±SD) body temperatures were 38.2 ± 0.9°C on day 0 (before treatment), 36.4 ± 0.6°C on day 1 (24 hr after the first dose), 36.4 ± 0.6°C on day 2, and 36.4 ± 0.5°C on day 3. On day 2, 5 patients were still febrile and only 3 had positive smears at low parasitaemias (53–161 asexual parasites/μL). On day 3, 3 patients were still febrile and none had a positive blood smear. None of the patients, including 3 patients presenting gametocytaemia on day 0, had gametocytaemia between day 2 and day 28. One patient had an aggravation of signs and symptoms with repeated vomiting and asthenia despite a decrease of parasitaemia from 119,380 asexual parasites/μL on day 0 (axillary temperature, 40.3°C) to 50,000 asexual parasites/μL on day 1 (axillary temperature, 38°C). This clinical outcome was considered as ETF, and the child was referred to the district hospital for parenteral treatment with quinine on day 2, according to the national guidelines for the management of severe and complicated malaria.

The following adverse effects were reported by the patients aged >5 years old themselves or parents between day 0 and day 7: asthenia (30%), diarrhea (18%), abdominal pain (12%), vomiting (12%), headache (12%), skin rash (9%), dizziness (3%), and anorexia (3%). On day 3, 3% of patients reported skin rash, abdominal pain, diarrhoea, and vomiting and 6% reported asthenia. From day 3 to day 7, 3% of patients had diarrhoea and asthenia. None of these adverse effects was reported beyond day 7. No severe adverse effect was observed during the 28-day follow up period.

This nonrandomized study on artemether-lumefantrine efficacy is the first trial conducted in Brazzaville. The results of the present study demonstrated its high efficacy and are in agreement with other studies conducted in African countries. Its high efficacy was, in particular, in agreement with the results reported from countries sharing common borders with Congo, that is, Angola and Cameroon [12, 13]. Elsewhere in Sub-Saharan African countries, the cure rate (i.e., the proportion of ACPR) on day 28 after artemether-lumefantrine treatment was reported to be >95.5% (a single study in Malawi showed a cure rate >93%) [14]. Moreover, in our study, most reported adverse effects were mild and were difficult to attribute to malaria infection itself or to drug intake, with the exception of skin rash and dizziness.

Artemether-lumefantrine paediatric formulations (syrup, dispersible tablet) have become available in more recent years. These formulations are much more convenient than tablets that had to be crushed and mixed with milk to treat

Table 2: Artemether-lumefantrine efficacy in children in Brazzaville.

	Number	Percentage	95% CI
No. of patients	77		
Responses on day 14			
Withdrawn or loss to follow up	4	5.2	1.4–12.8
Eligible	73	94.8	87.2–98.6
ETF	1	1.4	0–7.4
ACPR	72	98.6	92.6–100
Responses on day 28 (PCR uncorrected)			
Withdrawn or loss to follow up	8	10.4	4.6–19.4
Eligible	69	89.6	80.6–95.4
Treatment failure	7	11.3	4.2–19.8
ETF	1	1.5	0.05–7.8
LCF	2	2.9	0.4–10.1
LPF	4	5.5	1.6–14.2
ACPR[†]	62	89.9	80.2–95.8
Responses on day 28 (PCR corrected)			
Withdrawn or loss to follow up	8	16.9	9.3–27.1
Eligible	69	86.6	80.6–5.4
Treatment failure	2	2.9	0.4–10.0
ETF	1	1.4	0.04–7.8
Recrudescence	1	1.4	0.04–7.8
ACPR[‡]	67	97.1	89.9–99.6
PCR analysed isolates			
Recrudescence	1	16.7	0.4–64.1
Reinfection	5	83.3	35.9–99.6

95% CI: 95% confidence intervals; ETF: early treatment failure; LCF: late clinical failure; LPF: late parasitological failure; ACPR: adequate clinical and parasitological response.

[†]Of these patients, 86.5% (95% CI, 71.2–95.5%) under 5 years old had ACPR response, while 93.8% (95% CI, 79.2–99.2%) of the patients aged between 5 and 10 years old responded with ACPR. The difference in ACPR proportions between these two subpopulations of patients was not significant ($P = 0.6$, chi-square test).

[‡]Of the patients responding with ACPR, 94.1% (95% CI, 80.3–99.3%) were aged <5 years old and 100% (95% CI, 88.4–100%) were aged between 5 and 10 years old. The difference in ACPR proportions between the two subpopulations of patients was not significant ($P = 0.5$, chi-square test).

small children in the present study. Moreover, for unsupervised treatment, these novel formulations are expected to increase patient compliance and possibly improve drug tolerance in children, as compared with crushed tablets.

At the time when the present study was conducted, artemether-lumefantrine combination was one of the most expensive antimalarial drugs sold in Congolese pharmacies (US$10 for 24 adult tablets). This is the main reason why this ACT was not used for self-medication. Since 2008, artemether-lumefantrine has been available free of charge in the public sectors as second-line antimalarial drug, as in the health centre where the present study was performed, and an increasing number of malaria-infected Congolese patients is expected to be treated with this ACT. The drug is still available at approximately the same cost as in 2006, that is, US$ 10 for 24 tablets, in private pharmacies. The dispersible paediatric formulation costs US$ 2 for 12 tablets. These costs are far above the goal of less than US$ 1 per treatment, considered to be an affordable cost for the majority of African patients.

Further clinical studies comparing the efficacy of artesunate-amodiaquine and artemether-lumefantrine, which are current drugs of choice for the treatment of uncomplicated falciparum malaria, in different epidemiological strata in Congo, including rural and urban endemic areas, are required to monitor their efficacy in the country.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

The authors are grateful to the medical staff of Tenrikyo health centre for their aid in screening patients. The authors thank Dr. Pascal Ringwald of WHO for his advice and support. This study was supported by the A 20789 Project of the Special Programme for Research and Training in Tropical Diseases (TDR, World Health Organization, Geneva,

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