Journal of Antimicrobial Chemotherapy (2002) **50**, 751–754
DOI: 10.1093/jac/dkf200

JAC

Evidence for the efficacy of artesunate in asymptomatic *Plasmodium malariae* infections

Steffen Borrmann[1,2]*, Nicole Szlezák[1,3], Ronald K. Binder[1], Michel A. Missinou[1,2], Bertrand Lell[1,2] and Peter G. Kremsner[1,2]

[1]*Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, B. P. 118, Gabon;* [2]*Department of Parasitology, Institute of Tropical Medicine, University of Tübingen, Wilhelmstrasse 27, D-72074 Tübingen, Germany;* [3]*John F. Kennedy School of Government, Harvard University, Cambridge, MA 02138, USA*

Received 10 January 2002; returned 16 May 2002; revised 29 May 2002; accepted 12 August 2002

This study evaluated the efficacy and safety of a 3-day course of artesunate (4 mg/kg/day) for asymptomatic *Plasmodium malariae* infections. The parasitological cure rates on days 7 and 56 in the group treated with artesunate were 100% and 83%, respectively, compared with no cure in the placebo group ($P < 0.0001$).

Keywords: *Plasmodium malariae*, artesunate, chemotherapy, malaria, Gabon

Introduction

The resistance of *Plasmodium falciparum* to chloroquine has been documented in all parts of sub-Saharan Africa.[1] To a lesser extent, but still alarmingly, resistance of *Plasmodium vivax* has also been reported and raises the gloomy prospect that *Plasmodium malariae* and *Plasmodium ovale* might also develop resistance to chloroquine. Only a few anti-malarial drugs have been evaluated for their efficacy against *P. malariae* infections.[2,3] Artesunate monotherapy is highly effective and well tolerated for the treatment of falciparum malaria, and combination with standard anti-malarials is strongly advocated.[4] Large WHO-coordinated multi-centre trials to assess the efficacy and tolerability of 3-day courses of combinations of artesunate with chloroquine, amodiaquine and sulphadoxine/pyrimethamine have been successfully conducted or are still underway in many African countries, including Gabon.[5–7] To the best of our knowledge, no published data exist on the efficacy of artesunate against *P. malariae*. Therefore, we studied the efficacy and tolerability of a 3-day course of artesunate for the clearance of asymptomatic *P. malariae* infections as part of a double-blind, randomized trial investigating the efficacy and safety of artesunate and praziquantel for the treatment of *Schistosoma haematobium* infections in Gabon.

Materials and methods

The study was carried out from October 2000 to February 2001 in the Province Moyen-Ogooué, Gabon, and was part of a double-blind, randomized, placebo-controlled trial including 300 schoolchildren in three different villages to investigate the efficacy of artesunate and praziquantel for the treatment of *S. haematobium* infections.[8] The study area is highly endemic for *P. falciparum* and *S. haematobium*.[8,9] The study described below was undertaken in a subset of participants of the *S. haematobium* study in one of the three study villages (Nombakélé) where baseline screening revealed a high prevalence of infections with *P. malariae*. Community consent and written informed consent of the parents or guardians of the children were obtained. Ethical clearance was obtained from the Ethics Committees of the International Foundation of the Albert Schweitzer Hospital in Lambaréné, Gabon, and the Medical Faculty of the University of Tübingen, Germany.

At baseline, children were screened for plasmodial infections. Giemsa-stained thick blood films were prepared by the Lambaréné method as described previously,[10] and asexual parasitaemia was assessed per microlitre. Briefly, 10 μL of peripheral blood was evenly spread in an area of 18 mm by 10 mm on a clean slide. Slides were subsequently dried and stained

*Corresponding author. Tel: +49-7071-29-80234; Fax: +49-7071-29-4684; E-mail: steffen.borrmann@uni-tuebingen.de

with Giemsa (20%, pH 7.2, 20 min). Parasites were counted in at least 100 high-powered fields (HPFs; 1000× magnification), and the number of parasites per microlitre was calculated by multiplying the mean number of parasites per HPF by 600. This factor was previously determined for the study microscope and depends on the magnification and the size of the HPF of a particular microscope.[10] The factor was calculated according to the following formula: area of blood on slide/(area of HPF at 1000× magnification × volume of blood on slide). Thin blood smears were dried, fixed with methanol, stained for 30 min with Giemsa (20%, pH 7.2) and then examined microscopically for at least 30 min (1000×). Standard morphological characteristics were used to distinguish parasite species. Specifically, diagnosis of *P. malariae* infections was made if at least one of the following criteria was fulfilled: (i) identification of *P. malariae* schizonts on the thick blood film and (ii) identification of characteristic *P. malariae* stages (late trophozoites with band forms, schizonts or gametocytes) on the thin blood smear. All blood smears were read by two independent and experienced microscopists. PCR analysis to confirm the species specificity of microscopic diagnosis was not carried out. Haemoglobin was measured on days 0 and 56 with a portable photometric analyser (DHT Hb 523; Developing Health Technologies, UK). Reagent strips (Roche, Germany) were used for the semi-quantitative assessment of proteinuria. The treatment regimens were allocated sequentially according to the randomization code of the trial evaluating artesunate and praziquantel for the treatment of asymptomatic *S. haematobium* infections. The details of the randomization procedures have been decribed previously.[8]

One hundred and eight eligible children with asymptomatic *S. haematobium* infections were included in the study in Nombakélé. On study day 0, 40 children received artesunate (Sanofi, France) 4 mg/kg/day for 3 days and praziquantel placebo (Medochemie, Cyprus), 35 received artesunate placebo (Sanofi) and praziquantel (Medochemie), 23 received artesunate (identical dose) combined with praziquantel and 10 received placebo. For our analysis, we pooled all children who received artesunate in one group ($n = 63$) and the remaining children in the comparator group ($n = 45$). To the best of our knowledge, praziquantel has never been demonstrated to be effective against plasmodia, including negative results from analyses of our own unpublished data, and thus, for the purpose of this study on the efficacy of artesunate in asymptomatic *P. malariae* infections, the comparator group was considered as the placebo group for artesunate. Efficacy was assessed on days 7 and 56 by means of methodology identical to that used for screening. Proportional data were compared using Fisher's exact test, and exact binomial confidence intervals were calculated (Stata v7; Stata Corporation, College Station, TX, USA).

Results

Table 1 summarizes the baseline characteristics, including the malarial indices, of the study cohort. All children were asymptomatic during the whole study period. Positive blood smears were found in 102 children (94%). In particular, the high prevalence of infections with *P. malariae* is remarkable (60 of 108 children, 56%). Sixty asymptomatic children with *P. malariae* infections (including 18 *P. malariae* monoinfections) received artesunate ($n = 37$) or artesunate placebo ($n = 23$; Table 1). The urine analysis did not reveal any evidence of glomerulonephritis caused by *P. malariae* infection as

Table 1. Demographic, clinical and laboratory characteristics of asymptomatic children at baseline [data are given as arithmetic mean (standard deviation), unless otherwise indicated, or number and percentage of children]

	Artesunate ($n = 63$)	Placebo ($n = 45$)
Characteristics of children		
male/female	34/29	20/25
age, years (S.D.)	9.9 (2.4)	10.9 (2.8)
temperature, °C (S.D.)	36.6 (0.6)	36.7 (0.7)
haemoglobin, g/dL (S.D.)	11.4 (1.2)	11.9 (1.5)
children with >30 mg protein/dL urine (%)	9 (14)	4 (9)
Parasitology		
P. falciparum monoinfection	23	19
geometric mean parasite density/μL (95% CI)	240 (110–490)	130 (60–240)
P. malariae monoinfection	9	9
geometric mean parasite density/μL (95% CI)	120 (20–570)	300 (100–910)
mixed infection with *P. falciparum* and *P. malariae*	26	13
geometric mean parasite density/μL (95% CI)	250 (150–420)	760 (320–1800)
mixed infection with *P. falciparum*, *P. malariae* and *P. ovale*	2	1
mean parasite density/μL	1250	2400

measured by proteinuria. Only two children infected with *P. malariae*, compared with one uninfected child, had ~500 mg protein/dL urine, and no difference in the geometric mean levels of protein in the urine for infected versus uninfected children was detected (data not shown).

All participants received the full course of the study regimen under the direct supervision of the study physicians. Two children in the artesunate group were excluded by day 7: one child had not received the full treatment course and the other child was mistakenly given the wrong treatment regimen. All other children were followed up until day 56. The treatment regimens were well tolerated, and no differences in adverse events by day 7 could be detected between the groups (data not shown).

For all parasitaemic children at baseline, the results comparing efficacy of artesunate treatment against placebo are given in Table 2. In the artesunate group, all children had cleared *P. malariae* infections by day 7, and six of 35 children had reappearance of parasites of *P. malariae* on day 56 (a cure rate of 83%) compared with ongoing infections in all children in the placebo group on days 7 and 56 ($P < 0.0001$ for the comparisons on days 7 and 56; Table 2). There was no difference in the cure rate between children who received artesunate and praziquantel or artesunate and placebo (19 of 23, 83%, versus 10 of 12, 83%; $P = 0.96$). Artesunate cleared *P. falciparum* parasitaemia in all children except one by day 7 (98%), but the cure rate fell to 38% on day 56 (Table 2). Both treatment regimens cleared *P. ovale* infections (two infections in the artesunate group and one in the placebo group).

The level of haemoglobin in children infected with *P. malariae* at baseline increased from day 0 to day 56 in the group receiving artesunate, compared with the group who did not receive artesunate, but this difference did not reach significance (0.8 versus 0.1 g/dL; $P = 0.1$ with paired *t*-test). Results on the efficacy of praziquantel and artesunate in *S. haematobium* infections have been presented previously.[8] Children with plasmodial or *S. haematobium* infections at the end of the study received curative treatment.

Discussion

Our study shows for the first time that a short course of artesunate is effective and well tolerated for asymptomatic *P. malariae* infections. Treatment with a 3-day course of artesunate led to a parasitological cure rate of 83% by day 56 in a semi-immune population and an increase in haemoglobin of 0.8 g/dL over the study period. In addition, our results indicate that praziquantel is not effective against *P. malariae* nor does it interfere with the efficacy of artesunate against *P. malariae*.

The reported cure rate has not been corrected for by PCR, which would have enabled us to distinguish recrudescent from new infections. Assuming the possibility of reinfections occurring during the 56 day follow-up period, our results are therefore likely to underestimate the exact cure rate. Nonetheless, a parasitological cure rate of 83% compares favourably with findings for *P. falciparum* where 3-day regimens of artesunate are associated with high recrudescence rates by day 28 in Thailand.[11] No studies evaluating the efficacy of the 3-day regimen in Africa have been published so far. We hypothesize that the difference is primarily due to parasite species-related drug sensitivities. In addition, the different levels of initial parasitaemia—*P. malariae* infections do not produce the high parasite load seen with *P. falciparum*—and differences in the level of host immune responses may also play an important role. Additional studies are now needed to confirm the efficacy of a short course of artesunate alone, or preferably in combination, for curing clinical episodes of *P. malariae* infections in non-immune and semi-immune populations.

Table 2. Efficacy of artesunate versus placebo for asymptomatic *P. malariae* infections in children

	Day 7		Day 56	
	artesunate	placebo	artesunate	placebo
P. malariae	($n = 35$)	($n = 23$)	($n = 35$)	($n = 23$)
cured (95% CI)	35[a] (100%; 91–100)	0 (0%; 0–15)	29[a] (83%; 66–93)	0 (0%; 0–15)
treatment failures	0	23	6	23
geometric mean parasite density/μL (95% CI)	–	520 (250–1100)	470 (60–3900)	620 (280–1400)
P. falciparum	($n = 49$)	($n = 33$)	($n = 49$)	($n = 33$)
cured (95% CI)	48[a] (98%; 90–100)	0 (0%; 0–11)	19[b] (38%; 25–54)	1 (3%; 1–16)
treatment failures	1	33	30	32
geometric mean parasite density/μL (95% CI)	67 (–)	570 (290–1100)	250 (120–530)	440 (220–870)

[a] $P < 0.0001$ for the comparison with placebo.
[b] $P = 0.0001$ for the comparison with placebo.

The importance of *P. malariae* for the development of complications of falciparum malaria (e.g. severe anaemia) remains so far unknown. However, morbidity from *P. malariae* infections contributes to the excessive but preventable disease burden in sub-Saharan African countries. Co-infections of *P. malariae* with *P. falciparum* are common, and the diagnostic repertoire to differentiate between the species is limited. Treatment decisions in sub-Saharan Africa are overwhelmingly based on clinical criteria, and light microscopy plays a negligible role. An ideal anti-malarial will thus be cheap and well tolerated as well as highly effective not only against *P. falciparum* but also against *P. malariae* and *P. ovale*. Pending the confirmation of our results in symptomatic *P. malariae* infections, our study adds support to the use of a 3-day course of artesunate in combination with other anti-malarials in future malaria control programmes.

Acknowledgements

We would like to acknowledge the crucial support of Mr Phaedon Ellinas, Medochemie, and Dr Jean-Pascal Ducret, Sanofi, for providing the study medication. We are indebted to the study children, their parents and schoolteachers. We thank Dr Adrian Luty for carefully reviewing the manuscript. We also thank the local health workers Brigitte Migombet and Jean-Roger Avera. In addition, we thank Swissair for the transport of study material.

References

1. Marsh, K. (1998). Malaria disaster in Africa. *Lancet* **352**, 924.

2. Radloff, P. D., Philipps, J., Hutchinson, D. & Kremsner, P. G. (1996). Atovaquone plus proguanil is an effective treatment for *Plasmodium ovale* and *P. malariae* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 682.

3. Ringwald, P., Bickii, J., Same-Ekobo, A. & Basco, L. K. (1997). Pyronaridine for treatment of *Plasmodium ovale* and *Plasmodium malariae* infections. *Antimicrobial Agents and Chemotherapy* **41**, 2317–9.

4. White, N. J. & Olliaro, P. (1998). Artemisinin and derivatives in the treatment of uncomplicated malaria. *Médecine Tropicale* **58**, 54–6.

5. Adjuik, M., Agnamey, P., Babiker, A., Borrmann, S., Brasseur, P., Cisse, M. *et al.* (2002). Amodiaquine-artesunate versus amodiaquine for uncomplicated *Plasmodium falciparum* malaria in African children: a randomised, multicentre trial. *Lancet* **359**, 1365–72.

6. Olliaro, P., Taylor, W. R. & Rigal, J. (2001). Controlling malaria: challenges and solutions. *Tropical Medicine and International Health* **6**, 922–7.

7. von Seidlein, L., Milligan, P., Pinder, M., Bojang, K., Anyalebechi, C., Gosling, R. *et al.* (2000). Efficacy of artesunate plus pyrimethamine-sulphadoxine for uncomplicated malaria in Gambian children: a double-blind, randomised, controlled trial. *Lancet* **355**, 352–7.

8. Borrmann, S., Szlezák, N., Faucher, J. F., Matsiegui, P. B., Neubauer, R., Binder, R. K. *et al.* (2001). Artesunate and praziquantel for the treatment of *Schistosoma haematobium* infections: a double-blind, randomized, placebo-controlled study. *Journal of Infectious Diseases* **184**, 1363–6.

9. Wildling, E., Winkler, S., Kremsner, P. G., Brandts, C., Jenne, L. & Wernsdorfer, W. H. (1995). Malaria epidemiology in the province of Moyen Ogoov, Gabon. *Tropical Medicine and Parasitology* **46**, 77–82.

10. Planche, T., Krishna, S., Kombila, M., Engel, K., Faucher, J. F., Ngou-Milama, E. *et al.* (2001). Comparison of methods for the rapid laboratory assessment of children with malaria. *American Journal of Tropical Medicine and Hygiene* **65**, 599–602.

11. Bunnag, D., Viravan, C., Looareesuwan, S., Karbwang, J. & Harinasuta, T. (1991). Clinical trial of artesunate and artemether on multidrug resistant falciparum malaria in Thailand. A preliminary report. *Southeast Asian Journal of Tropical Medicine and Public Health* **22**, 380–5.