

Running head : dual efficiency of artesunate against *Plasmodium* and
Schistosoma

Title : Anti-schistosome efficacy of artesunate combination therapies
when administered as curative treatments of malaria attacks

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Abstract

Artesunate is a highly effective anti-malarial and there is some evidence that it is also active against schistosome infections. Therefore, we investigated whether treatment with artesunate of acute malaria in Senegalese children had an impact on their level of infection with *Schistosoma haematobium*. Twenty seven children entered into a clinical trial of antimalaria treatment were excreting eggs of *S. haematobium* in their urine on the day of treatment, 15 of whom received the combination of a single dose of sulfadoxine/pyrimethamine together with three daily doses of artesunate (4 mg/kg), the remaining 12 children receiving three daily doses of amodiaquine and artesunate. The overall cure rate and reduction in the mean number of excreted eggs 28 days post-treatment were 92.6% and 94.5%, respectively. Our findings indicate that artesunate, in addition to being a very effective treatment for uncomplicated malaria, can also reduce sharply the *S. haematobium* loads harboured by pre-school African children.

Key-words : artesunate, *Schistosoma haematobium*, malaria, chemotherapy, Senegal.

Introduction

Provision of an effective anti-malarial for the treatment of uncomplicated malaria is the corner stone of antimalarial control programmes in Africa (WHO, 2000). Because of progressive loss of efficacy of previously widely used antimalarials such as chloroquine and sulphadoxine/pyrimethamine, most African national malaria control programmes and the World Health Organisation now recommend artemisinin-based combination therapies (ACTs) as the first line of treatment for uncomplicated malaria in Africa (Olliaro et al., 2001). Numerous clinical trials have demonstrated their safety and efficacy. Surprisingly, both artesunate and artemether have also proved to have activity against several species of schistosomes (Xiao, 2005). There is, however, debate about their use on a large scale to control the second most prevalent parasitic disease worldwide. Because of the overlapping distributions of schistosomiasis and malaria, the risk that widespread use of artemisinins for the treatment of schistosomiasis could favour the emergence of resistant strains of *Plasmodium* must be seriously considered (Menard et al., 2005; Utzinger et al., 2001). Nevertheless, there is a possibility that use of artemisinins to treat malaria could have, as a secondary effect, a beneficial effect on the burden of schistosomiasis (Utzinger et al., 2003) but there is no information so far on how effective artemisinins might be against schistosome infections when used in this way. Like in many other African countries, chloroquinoreistance has reached in Senegal such a level (Trape et al., 1998) that the National

Program had to consider a change of malaria case management. Therefore, we have taken advantage of a clinical trial of two different forms of ACTs conducted in a rural area to evaluate if artesunate, when used for the treatment of uncomplicated malaria, also has an impact on the schistosome burden of young Senegalese children.

Materials and Methods

Study area and population

The study took place between November 2003 and January 2004, at the end of the rainy season, in four villages located within the community of Niakhar site, situated 145 kms east from Dakar, where regular demographic surveillance has been maintained since 1963 (Delaunay et al., 2001). These villages are situated in an area where water collections, either temporary (marshy lands) or semi-permanent (ponds used for agricultural purposes), are potential sites for the breeding of *Anopheles* mosquito larvae and of *Bulinus* snails, resulting in a high level of both *Plasmodium falciparum* malaria and of urinary schistosomosis in the community (Briand et al., 2005). 518 children, aged between 15 and 74 months old at the start of the study, were eligible.

Detection of malaria

Malaria cases were detected through both passive surveillance at health centres and weekly domiciliary visits. Malaria was defined as an illness associated with an auricular temperature of 37.5° C or greater and/or a

history of fever or vomiting during the previous 24 hours, accompanied by the detection of asexual malaria parasites on blood film examination. Blood smears were performed 3, 7, 14 and 28 days post-treatment to detect any persistence of parasites after treatment.

Chemotherapy

Children with malaria were randomised to receive treatment with artesunate (AS, Sanofi, Paris, France) given either with sulfadoxine/pyrimethamine (SP) or amodiaquine (AQ), both provided by the Senegalese National Pharmaceuticals Company. SP (25 mg/kg sulfadoxine + 1.25 mg/kg pyrimethamine) was administered once on the first day of treatment. Amodiaquine was used at a dose of 10 mg/kg for the first 2 days, then 5 mg/kg on the third day. Artesunate (4 mg/kg) was given once daily for three consecutive days. All treatments were given under direct observation.

Detection of schistosome infection

As soon as a malaria case was microscopically confirmed, a field worker visited the child, started chemotherapy and left a container with the child's mother together with instruction to collect the urine from a whole micturition the following morning (Day 0). In the laboratory, the volume of the sample was recorded (range : 3 – 325 ml), the urine was filtered through a nylon disc filter (Nytrel®) with a 30 μ mesh and the numbers of eggs trapped on the filter counted under a dissecting microscope. The same procedure was repeated on Days 1, 2, 28, 29 and 30 after the start of

antimalarial treatment, before any re-infection larva could have become an adult fertile schistosome.

Data processing

Data were independently recorded by two data entry clerks and then checked for concordance. Aberrant values were identified and corrected. Levels of egg excretion were expressed as the number of eggs per 10 ml of urine. Malaria cases were considered as eligible for inclusion in the study if the results of at least two filtrations before and after treatment were available. At least one of the pretreatment samples had to be positive before a case was included in the efficacy analysis. Cure rate was expressed as the percentage of children who became completely negative after treatment. Geometric means minus 1 of the available daily counts plus 1 were used to calculate the mean levels of individual egg excretions before (mean of Days 0-1-2) and after (mean of Days 28-29-30) treatment. Group mean egg excretion was calculated as the geometric mean minus 1 of the individual mean counts plus 1.

Statistical analysis

Yates' corrected chi-2 was used to compare cure rates. Exact 95% confidence intervals (CI) were calculated for proportions. Mann-Whitney U test and Wilcoxon signed-rank test were run to assess the significance of the differences between means of unpaired and paired values, respectively.

Ethical clearance

Informed consent was obtained from the children's mothers and the global

protocol received approval from the Senegalese National Ethics Committee. All children who were still excreting schistosome eggs on either Day 28-29-30 were treated with praziquantel (BILTRICID®) at a 40 mg/kg dosage.

Results

108 cases of malaria meeting the study definition were detected among 518 children during the period of the survey. Twenty-seven of these children also met the study criterion for a diagnosis of urinary schistosomiasis, 12 and 15 in the AQ3+AS3 and SP1+AS3 groups respectively. Fifteen were male and their age varied from 26 to 74 months. All of them were successfully cured of *Plasmodium* infection, as shown by negative 3, 7, 14 and 28 day blood smears. The mean level of egg excretion before treatment was higher in the SP1+AS3 group than in the AQ3 +AS3 group (Table) but the difference between the groups was not significant ($p = 0.097$). Twenty-five children were totally negative for schistosome eggs after treatment, an overall cure rate of 92.6 %. Cure rates did not differ significantly between the two groups ($p = 0.84$). All children in the AQ3+AS3 were cured ($p < 0.005$). In the SP1+AS3 group, the mean number of eggs was reduced by more than 90% (pre- versus post-treatment means comparison, $p < 0.001$).

Discussion

The main goal of this study was to determine if artesunate, when

administered for the treatment of young children against malaria, also had an effect on *Schistosoma haematobium* egg output. So far, three randomised clinical trials have investigated the capacity of artesunate to cure established *S. haematobium* infections but with divergent results. The first one, done in Gabon using a dosage of 4 mg/kg/day of artesunate, once a day over three days (Borrmann et al., 2001), concluded that it was not effective at all. In contrast, 8 tablets of 50 mg over 5 days delivered in Senegal (De Clercq et al., 2002) or two doses of 6mg/kg administered two weeks apart in Nigeria (Inyang-Etoh et al., 2004) provided good evidence of efficacy with mean reductions of egg excretion ranging from 55 to 89%, 5 to 6 weeks after treatment. However, these three studies involved schoolchildren between 5 and 18 years who were harbouring heavy infections. Our study is the first undertaken in children less than 6 years old who had malaria at the time that they were treated. Both artesunate-containing combination therapies led to marked reductions in egg counts, above 90%. The association of artesunate and amodiaquine completely cured all children who received this combination which ranks number two behind artemether/lumefantrine in WHO recommendations as first line of treatment of uncomplicated malaria (Majori, 2004). The combination of SP with artesunate was slightly less effective but numbers were small. It is unlikely that the partners of artesunate contributed to the activity seen against schistosome infections and one can reasonably assume that artesunate was the active drug.

This pilot study has a number of weaknesses. Firstly, there was no control group and some of the negative counts obtained at the end of the trial could have resulted from natural fluctuations in egg excretion. However, these could not explain the very high levels of 28 day cure obtained and repeated filtrations of a whole micturition are a way to prevent false negatives.

Secondly, the study was conducted in an area of relatively low schistosome endemicity with an overall mean egg count of 0.5 eggs/10 ml of urine. It cannot be speculated that such satisfactory results would be obtained in children with much higher levels of infection but, in Sahelian countries, malaria strikes mainly during the first five years of age at a time when heavy schistosome loads are unfrequent. Finally, the number of children in the study was small, making comparisons between groups able to detect only major differences. Despite these reservations, our findings suggest that adoption of artemisinin-based combination therapies as first lines of treatment for malaria in many African countries, including Senegal, may have an added benefit by reducing the burden of schistosomiasis in the many areas where both infections are found. Moreover, such bitherapies are also strongly supported as a basis for intermittent preventive treatment, especially in children less than 5 years (Cisse et al., 2006). It would be of great value to assess if, within the framework of a global strategy, both curative and preventive, against malaria, artesunate could also reduce schistosomiasis impact. However, our study was only a pilot trial involving children under the age of five years participating in a malaria study. There

is a need to confirm our findings in other areas where ACTs are used as first line treatment for malaria. In addition, there is a need for studies in older children, in whom schistosome burdens are likely to be heavier than was the case in this study, and for trials in areas where *S. mansoni* is the dominant species.

Conflicts of interest statement

The authors declare to have no known conflict of interest. The sponsors and the artesunate provider (Sanofi) did not interact in the management of the study nor in the data analysis.

Acknowledgments

We would like to specially thank the population of the Niakhar site for its willingness to participate to the study and the IRD Service Unit 009 for maintenance of the Niakhar database. Skillful participation of Ernest Faye, Ibou Cissé and Agnès Bakhoun is greatly acknowledged. This work was funded by the French Ministry of Research and Technology (PAL+ contract #2829), by the University Cheikh Anta Diop of Dakar, by the Bill and Melinda Gates Foundation and by the Institut de Recherche pour le Développement. Sanofi donated artesunate tablets.

BIBLIOGRAPHY

- Borrmann, S., Szlezak, N., Faucher, J.F., Matsiegui, P.B., Neubauer, R., Binder, R.K., Lell, B. & Kremsner, P.G., 2001. Artesunate and praziquantel for the treatment of *Schistosoma haematobium* infections: a double-blind, randomized, placebo-controlled study. *J. Infect. Dis.*, 184, 1363-1366.
- Briand, V., Watier, L., Le Hesran JY, Garcia, A. & Cot, M., 2005. Coinfection with *Plasmodium falciparum* and *Schistosoma haematobium*: protective effect of schistosomiasis on malaria in senegalese children? *Am. J. Trop. Med. Hyg.*, 72, 702-707.
- Cisse, B., Sokhna, C., Boulanger, D., Milet, J., Ba, E., Richardson, K., Hallett, R., Sutherland, C., Simondon, K., Simondon, F., Alexander, N., Gaye, O., Targett, G., Lines, J., Greenwood, B. & Trape, J.-F., 2006. Seasonal intermittent treatment with artesunate and sulfadoxine-pyrimethamine for prevention of malaria in Senegalese children: a randomised, placebo-controlled, double-blind trial. *Lancet*, 367, 659-67.
- De Clercq, D., Vercruyssen, J., Kongs, A., Verle, P., Dompnier, J.P. & Faye, P.C., 2002. Efficacy of artesunate and praziquantel in *Schistosoma haematobium* infected schoolchildren. *Acta Trop.*, 82, 61-66.
- Delaunay, V., Etard, J.F., Preziosi, M.P., Marra, A. & Simondon, F., 2001. Decline of infant and child mortality rates in rural Senegal over a 37-year period (1963-1999). *Int. J. Epidemiol.*, 30, 1286-1293.

- Inyang-Etoh, P.C., Ejezie, G.C., Useh, M.F. & Inyang-Etoh, E.C., 2004. Efficacy of artesunate in the treatment of urinary schistosomiasis, in an endemic community in Nigeria. *Ann. Trop. Med. Parasitol.*, 98, 491-499.
- Majori, G., 2004. [Combined antimalarial therapy using artemisinin]. *Parassitologia*, 46, 85-7.
- Menard, D., Matsika-Claquin, M.D., Djalle, D., Yapou, F., Manirakiza, A., Dolmazon, V., Sarda, J. & Talarmin, A., 2005. Association of failures of seven-day courses of artesunate in a non-immune population in Bangui, Central African Republic with decreased sensitivity of *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.*, 73, 616-621.
- Olliaro, P., Taylor, W.R. & Rigal, J., 2001. Controlling malaria: challenges and solutions. *Trop. Med. Int. Health*, 6, 922-927.
- Trape, J.F., Pison, G., Preziosi, M.P., Enel, C., Desgrees du Lou, A., Delaunay, V., Samb, B., Lagarde, E., Molez, J.F. & Simondon, F., 1998. Impact of chloroquine resistance on malaria mortality. *C. R. Acad. Sci. III*, 321, 689-697.
- Utzing, J., Keiser, J., Shuhua, X., Tanner, M. & Singer, B.H., 2003. Combination chemotherapy of schistosomiasis in laboratory studies and clinical trials. *Antimicrob Agents Chemother*, 47, 1487-95.
- Utzing, J., Xiao, S., Keiser, J., Chen, M., Zheng, J. & Tanner, M., 2001. Current progress in the development and use of artemether for chemoprophylaxis of major human schistosome parasites. *Curr Med Chem*, 8, 1841-60.

WHO, 2000. WHO Expert Committee on Malaria. World Health Organ. Tech. Rep. Ser., 892, i-v, 1-74.

Xiao, S.H., 2005. Development of antischistosomal drugs in China, with particular consideration to praziquantel and the artemisininins. *Acta Trop.*, 96, 153-167.

Table. Cure rates and mean egg excretions in children undergoing two distinct artesunate combination therapies.

	Group	AQ3+AS3	SP1+AS3	Both AS3
Cure rates	n° of included children	12	15	27
	n° of cured children	12	13	25
	% cure rate	100%	86.7%	92.6%
	95% CI	73.5-100.0% ^a	59.5-98.3%	75.7-99.1%
Mean egg counts ^b	before treatment	0.233	0.403	0.325
	after treatment	0.000	0.033	0.018
	% reduction	100%	91.9%	94.5%

^a : 97.5% CI (one-sided)

^b : geometric means