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Artesunate + amodiaquine and artesunate + sulphadoxine–pyrimethamine for treatment of uncomplicated malaria in Democratic Republic of Congo: a clinical trial with determination of sulphadoxine and pyrimethamine-resistant haplotypes

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Summary

We undertook a trial of artesunate + amodiaquine (AS + AQ) and artesunate + sulphadoxine–pyrimethamine (AS + SP) in 180 children of age 6–59 months with uncomplicated malaria in Democratic Republic of Congo. Children were randomly allocated to receive 3 days observed treatment of AS + AQ ($n = 90$) or 3 days of AS + SP ($n = 90$). Primary efficacy outcomes were 28-day parasite recurrence rates, and recrudescence rates were adjusted by genotyping to distinguish new infection and recrudescence. In addition, we determined the prevalence of molecular markers of resistance to sulphadoxine and pyrimethamine. Day 28 parasite recurrence rates were 16.9% (14/83; 95% CI: 9.5–26.7) in the AS + AQ group and 34.6% (28/81; 95% CI: 24.3–46.0) in the AS + SP group ($P = 0.009$). After PCR correction, recrudescence rates were 6.7% (5/74; 95% CI: 2.2–15.1) for AS + AQ and 19.7% (13/66; 95% CI: 10.9–31.3) for AS + SP ($P = 0.02$). There was no significant difference between the two arms in time to parasite clearance, fever clearance and gametocyte clearance. Parasite genotyping showed high frequencies of dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) molecular SP-resistance markers, with 57% of the samples showing more than three mutations linked to SP resistance, and 27% with triple-*dhfr*/double-*dhps* haplotype, confirming that SP treatment failure rates are likely to be high. AS + AQ had significantly higher efficacy than AS + SP. These results contributed to the subsequent change to AS + AQ as first-line regimen in the country. Efforts to properly implement the new protocol and maintain adherence at acceptable levels should include health staff and patient sensitization. The 6.8% recrudescence rate indicates that AS + AQ should be monitored closely until a more effective artemisinin combination therapy regimen is needed and can be introduced.

keywords malaria, efficacy, sulphadoxine–pyrimethamine, amodiaquine, artesunate, resistance haplotype, Democratic Republic of Congo

Introduction

Malaria remains a major health problem in the tropics, and *Plasmodium falciparum* resistance to common antimalarials poses a formidable obstacle for malaria control. Currently, new treatment policies are being developed and are in the process of implementation in many countries. However, many national health care systems in Africa lack the resources to respond adequately to this demand without international assistance. In areas such as eastern Democratic Republic of Congo (DRC), which recently experienced prolonged civil war and population displacement, primary health care has deteriorated and is not readily accessible to the whole population.

Resistance to chloroquine (CQ) has been documented in the DRC as early as 1983 (Delacolette *et al.* 1983) and reached levels of 29–80% (WHO 2005a). During 2001–2002, the DRC Ministry of Health (MOH) conducted studies in seven sentinel sites in various DRC health zones. These showed an emerging resistance to

sulphadoxine–pyrimethamine of up to 10% (Programme National de Lutte contre le Paludisme (PNLP) 2002; WHO 2005a). A multisite assessment showed 14-day sulphadoxine–pyrimethamine (SP) failure rates of 19.2% in the eastern region (Kazadi *et al.* 2003). A survey in southeast DRC showed an alarmingly low 49% efficacy of SP at day 14 (J .M. Escriba, unpublished data, MSF-Spain in Pweto, Katanga in 2002). Results of a recent assessment of the prevalence of SP resistance markers in central and eastern DRC also suggested high resistance to pyrimethamine alone or combined with sulphadoxine (Cohuet *et al.* in press).

In 2001, the WHO recommended the use of artemisinin-based combinations [artesunate + sulphadoxine–pyrimethamine (AS + SP), artesunate + amodiaquine (AS + AQ), artemether–lumefantrine)] as first-line treatment for uncomplicated *falciparum* malaria in response to reduced effectiveness of CQ and SP monotherapy, as seen in the DRC. In 2003, the DRC national malaria programme changed the protocol from CQ to SP monotherapy as an interim therapy, to be followed by an artemisinin combination therapy (ACT) in 1–2 years. In February 2005, the MOH of DRC made its choice for a modified national protocol, replacing SP with AS + AQ therapy. Although no trials on the efficacy of AQ or AQ + AS in the DRC were completed prior to this study, trials in various other parts of Africa had shown the safety and efficacy of AS + AQ (Adjuik *et al.* 2002) even in areas of moderate resistance to amodiaquine.

Until the time of study (2004), ongoing conflict limited research in this region and delayed efforts to identify an appropriate alternative ACT regimen. *Médecins sans Frontières* (MSF) has managed health projects in the DRC since the 1980s and recently has played an active role in ACT implementation and ACT efficacy trials in other parts of Africa, such as Sierra Leone, Sudan and Uganda (van den Broek *et al.* 2005; Checchi *et al.* 2005; Piola *et al.* 2005). This experience was used to set up a study in a remote area of DRC, South Kivu, in order to compare the efficacy of two ACTs eligible for the national protocol.

Methods

Study site

The DRC is Africa's third largest country with an estimated population of 53 million in 2002. A prolonged civil war has led to large numbers of internally displaced persons and degradation of infrastructure, including the health delivery systems. Health indicators for the DRC are among the worst in Africa (WHO 2005b). The Shabunda Health Zone population is estimated at 588 000. The study site was the small town of Shabunda in the South Kivu province, mid-east DRC, very isolated and accessible only by plane or on foot.

Malaria is highly endemic and seasonal. Malaria transmission is intense and perennial throughout the DRC, with peaks during the low (March to May) and high (September to November) rainy season and higher levels in rural than in urban environments. The main vectors are *Anopheles gambiae* ss and *Anopheles funestus* (Coene 1993). The level of malaria transmission is not homogeneous over the country, with its large size and variations in climate and topography. Large-scale displacement of populations because of ongoing conflict has blurred the edges of these areas, and the transmission patterns are not predictable.

Study design and patients

Following WHO guidelines for monitoring antimalarial drug efficacy (WHO 2003), we recruited patients with uncomplicated *P. falciparum* malaria proved by blood film from the 'Divine Maitre' Health Centre of Shabunda. Nurses interviewed the parents or guardians of all febrile children who were routine users of the health centre and those with temperature ≥ 37.5 °C were referred to the study team. All referred patients were interviewed again and clinically examined to exclude concomitant infections. Duplicate thick and thin film blood smears were examined for the presence of malaria parasites. Blood samples for PCR genotyping analysis were collected on glass fibre filter paper. Haemoglobin (Hgb) was measured using the Lovibond technique (Assistant Co., Sondheim Rhon, Germany). Children were eligible for inclusion if they were of age 6–59 months, had symptoms suggestive of clinical malaria and *P. falciparum* parasitaemia of at least 2000 parasites per μl of blood, were able to take study drugs by the oral route, were able to attend the clinic on stipulated days for follow-up and if a parent or guardian provided written informed consent for the child. Exclusion criteria consisted of (1) presence of severe and complicated malaria as defined by WHO (WHO 2003), (2) a mixed plasmodial infection or concomitant disease that could mask the response to antimalarial treatment, (3) *P. falciparum* parasitaemia higher than 200 000 parasites per μl of blood, (4) or known hypersensitivity to any of the study drugs.

Children were randomly assigned to one of the two study regimens. Randomization in blocks of 12 was performed by computer before the study started, using a 1:1 ratio. To each inclusion number corresponded a sealed envelope containing the treatment allocation; each envelope was opened only after informed consent had been obtained. Neither patients nor clinicians were blinded to the treatment given. All treatments were given under direct observation, and patients were observed for 30 min

following drug ingestion. If vomiting occurred during the first 30 min, a repeat dose was administered. If this dose was also vomited, the child was withdrawn from the study and referred to the general reference hospital for appropriate management.

Study procedures during follow-up

Parents were asked to bring their children back to the clinic on days 1, 2, 3, 7, 14, 21 and 28 after the start of treatment or on any other day if the child was unwell. Children were examined by the study team and treated appropriately. Parents or guardians were asked for any potential side effects of the drug and the child's tolerability to the treatment. Children with early and late treatment failures were given quinine 10 mg/kg/day three times a day for 7 days. Patients with any sign of severe malaria were admitted to hospital and treated with intravenous quinine. Children who did not attend on planned clinic days were visited at their homes by a study tracer and were encouraged to attend the next day.

Laboratory methods

Blood films were taken on day 0, 2, 3, 7, 14, 21 and 28, combining thick and thin smears on one slide. Thick film microscopy was used to determine parasite density and thin film microscopy to determine parasite species and stage. Slides were stained with 10% pre-filtered Giemsa solution for 30 min. Both asexual parasites and gametocytes were counted per 200–500 white blood cells (WBCs) and the density, expressed in parasites per μl of blood, was calculated assuming a standard of 8000 WBCs per μl. A slide was reported negative when examination of 100 fields of a thick smear showed no presence of asexual parasites. Microscopists unaware of treatment allocation read all slides. Internal quality control included a blind second reading of a proportion of the slides by a second microscopist: all slides taken during day 0 and day 3, all positive slides after day 3 and 20% of negative slides. All day 28 negative slides were reread. Discordances were resolved by a third, experienced reader. External controls were conducted by an MOH reference lab in the provincial capital. Clinical and parasitological outcomes were graded according to WHO 2003 guidelines (WHO 2003). Clinical failure was defined as parasites recorded in combination with clinical symptoms at any follow-up visit after day 2 of treatment; parasitological failure was defined as parasites recorded at the last day of follow-up, day 28, without presence of symptoms.

On day 0 (pre-treatment), and the day of failure endpoint if applicable, a dry blood spot was collected on glass fibre filter paper (Item No. 1205-401; Perkin-Elmer) for genotyping to distinguish recrudescence from reinfection in cases of treatment failure, estimation of the multiplicity of infection (MoI) and determination of the prevalence of molecular markers associated with SP resistance. Differentiation between recrudescence and new infections was based on protocols developed by Snounou *et al.* (1999). In brief, six separate nested PCR reactions were performed with oligonucleotide primer pairs specific for the three allelic families of *msp-1* (MAD20, K1 and R033), and two allelic families of *msp-2* (FC27 and IC). Size polymorphism was analysed by electrophoresis on agarose gel and visualized by UV transillumination. MoI was obtained by multiplying the number of alleles in each *msp1* and *msp2*. A conservative estimate was used, represented by the minimum number of genotypes (i.e. the highest number of genotypes within any one of the two markers).

Molecular determination of resistance to sulphadoxine and pyrimethamine was determined by a PCR approach using sequence-specific probes for the detection of known single-nucleotide polymorphisms conferring resistance to SP (Pearce *et al.* 2003). Parasite DNA from pre-treatment blood samples were amplified and screened using the PCR-SSOP (polymerase chain reaction using sequence-specific olignucleotide probes) technique for mutations associated with SP resistance. The samples were genotyped for mutations in codons 50, 51, 59 and 108 in dihydrofolate reductase (*dhfr*), conferring resistance to pyrimethamine and in codons 436, 437 and 540 of dihydropteroate synthase (*dhps*), known to further enhance resistance. PCR-amplified coding regions of *dhfr* and *dhps* genes were fixed on membrane and probed with sequence-specific oligonucleotide probes designed to detect each of the single-base-pair substitutions at the codons given in Table 1.

Sample size

The intent was to estimate the efficacy of the two combination treatments, accepting a risk of type I error of 5%, with a precision of 10%. Therefore, a sample size of at least 73 patients in each arm was planned with estimated 80% efficacy (Epi-info 6.0; Centre for Disease Control, Atlanta, GA, USA). Adding 15% to account for defaulters, 84 patients per arm had to be recruited, thus totalling at least 168.

Data analysis

Data was double-entered into Excel (Microsoft XP) and transferred to STATA (version 8.0; Stata Corporation, College Station, TX, USA) for further analyses. Statistical tests used were chi-square tests to compare categorical

Table 1 Mutant and wild-type amino acids at *dhfr* and *dhps* loci

	dhfr – codons				*dhps* – codons		
	50*	51	59	108	436*	437	540
Mutation	**R, CGT**	**I, ATT**	**R, CGT**	**N, AAC T, ACC**	**F, TTT A, GCT C, TGT**	**G, GGT**	**E, GAA**
Wild-type	C, TGT	N, AAT AAC	C, TGT	S, AGC	S, TCT	A, GCT	K, AAA

Letter codes show amino acids and codons; those that result from mutations are shown in bold.
*The numbers represent amino acid positions in *dhfr* and *dhps* where mutations occur.

data; Fisher exact when expected groups were smaller than $n = 5$. Continuous data were tested for normality (test for skewness, Shapira–Wilk test for normality). Normally distributed data were analysed with *t*-tests and ANOVA.

Primary efficacy outcomes were 28-day true failure rates, adjusted by genotyping to distinguish new infection and recrudescence. Secondary endpoints included gametocyte clearance rates, changes in Hgb concentration and levels of molecular SP resistance.

Ethics review

The ethics committee of the DRC National Malaria Programme and the external Ethics Review Board used by MSF reviewed and approved the study protocol. The study was discussed with and approved by community leaders before its start. Parents or guardians of children were asked for informed written consent before inclusion of their child in the study.

Results

Between 1 April and 13 May 2004, 435 children were screened for inclusion. Of these, 180 were recruited into the study and 256 children were excluded: 137 slide negative, 33 with parasitaemia <2000/μl, 14 with parasitaemia >200 000/μl, 44 with serious concomitant infections, 22 with presence of non-*falciparum* infection and 6 living too far from the study site. Microscopy results from 251 screened positive patients showed that 91.2% (229) were pure *P. falciparum* infections, 6.4% (16) *P. falciparum* + *Plasmodium malariae*, 0.4% (1) *P. falciparum* + *Plasmodium ovale*, 1.6% (4) *P. malariae* and 0.4% (1) *P. ovale*.

The 180 children who met entry criteria were randomly assigned to one of the two drug combinations. Baseline characteristics (Table 1) were similar across treatment groups. After inclusion, five were withdrawn (one because of incorrect inclusion, two for intake of non-study antimalarials and two for vomiting the treatment dose twice). Eleven children were lost to follow-up (1 because of family movement, 10 by an inability to complete a day 28 visit after evacuation of the study team because of political insecurity). There was no significant difference between cases lost to follow-up or withdrawn in the AS + SP (9/90) and AS + AQ group (7/90; $P = 0.21$).

The results of external quality control of 73 slides revealed three disagreements in the presence of *P. falciparum* parasites, which were all at a density of less than 50 parasites per μl. Additionally, three slides had different findings for *P. falciparum* gametocytes, and in one slide the reference laboratory reported the presence of *P. ovale* parasites. None of the discrepancies changed the outcome of treatment.

Primary outcomes

Parasitological and clinical outcomes were available from 164 children at day 28 (Table 2). By day 14, one child in the AS + SP group experienced late clinical failure and was re-treated. Within the 28 days of follow-up, 42 children were re-treated: 16.9% (14/83) in the AS + AQ group and 34.6% (28/81) in the AS + SP group. Table 3 shows the classification of treatment outcome at day 28 before correction by PCR analysis. Two samples were removed from the AS + SP group because of indeterminate PCR results. PCR genotyping identified 9 of 14 recurrent parasitemias in the AS + AQ group and 13 of 26 in the AS + SP group as new infections, and these were removed from final analysis as per protocol (WHO 2003). After correction by PCR, final analysis showed a 6.8% recrudescent rate in the AS + AQ group and a 19.7% recrudescent rate in the AS + SP group (Table 3, bottom row).

Secondary outcomes

Fever clearance was complete within 2–3 days for both therapies and showed no significant difference between the

Table 2 Baseline characteristics included children, by treatment group

	AS + AQ ($n = 90$)	AS + SP ($n = 90$)	Total ($n = 180$)	*P*-value
Female, *n* (%)	45 (50)	36 (40)	81 (45%)	0.20
Age (months), mean ± SD	23.7 ± 12.9	23.7 ± 14.5	23.7 ± 13.6	0.71
Haemoglobin (Hgb, g/dl), mean ± SD	9.8 ± 1.5	9.7 ± 1.7	9.8 ± 1.6	0.95
Moderate anaemia (Hgb 5 to <8 g/dl), *n* (%)	9 (10)	15 (17)	24 (14%)	0.27
Mild anaemia (Hgb 8 to <11 g/dl), *n* (%)	64 (71)	56 (63)	120 (67%)	0.27
Temperature (°C), Mean ± SD	38.8 ± 0.8	38.7 ± 0.9	38.8 ± 0.9	0.59
Parasite density (per μl blood), geometric mean (range)	27 392 (2200–180 560)	21 360 (2040–179 200)	24 492 (2040–180 560)	0.20
Gametocytaemic, *n* (%)	11 (12.2)	12 (13.5)	23 (12.8)	0.80
Gametocytaemia (per μl blood), mean (range)	255 (40–1920)	87 (40–200)	167(40–1920)	

Table 3 Efficacy at day 28 days

	AS + AQ			AS + SP			
Endpoint	%	*n*	95% CI	%	*n*	95% CI	Chi-square
ACPR	83.1	69	73.3–90.5	65.4	53	54.0–75.6	$P = 0.009$
ETF	0	0	0–4.3	0	0	0–4.5	
LCF	10.8	9	5.1–19.5	17.3	14	9.8–27.3	$P = 0.24$
LPF	6.0	5	2.0–13.5	17.3	14	9.8–27.3	$P = 0.02$
Failure rate							
Before PCR confirmation	16.9	14/83	9.5–26.7	34.6	28/81	24.3–46.0	$P = 0.009$
After PCR confirmation	6.8	5/74	2.2–15.1	19.7	13/66	10.9–31.3	$P = 0.02$

ACPR, adequate clinical and parasitological response; ETF, early treatment failure; LCF, late clinical failure; LPF, late parasitological failure.

two arms. Parasite clearance was fast in both treatment arms with all children parasite-free by day 3. The two treatment groups did not show a significant difference in gametocyte clearance rates. The proportion of cases with gametocytes in the blood increased during the first 2 days of treatment. Thirteen percentage had gametocytes at the day of inclusion and a further 23% of cases without gametocytes at enrolment developed detectable gametocytemia. This percentage dropped in both treatment groups after treatment, with more than 95% of cases free from gametocytes by day 21. The percentage of patients with mild (Hgb 8–10.9 g/dl) and moderate anaemia (Hgb 5–7.9 g/dl) dropped from 67% (116/173) and 14% (24/173) at the time of recruitment to 48% (71/148) and 3% (5/148) by day 28, respectively. There were no adverse side effects reported by parents, and drug regimens were well tolerated.

Genotyping of the pre-failure samples ($n = 42$) showed that patients were infected by multiple *P. falciparum* strains at the time of screening, with an MoI of 3.0 (126 strains in 42 infections).

Molecular markers of sulphadoxine–pyrimethamine resistance

PCR-SSOP identified the sensitive and single-, double- or triple-mutant *dhfr* and *dhps* haplotypes commonly recorded in African populations. Of 217 samples screened for *dhps*, 177 single and majority genotype infections could be deduced for this locus; excluded from analysis were 11 PCR-negative samples and 29 mixed infections. Of 182 samples screened for *dhfr*, 158 could be analysed; excluded from analysis were 7 PCR-negative samples and 17 mixed infections.

A high prevalence of key mutations for SP resistance at *dhfr* and *dhps* were found (Table 4). At *dhps*, four different alleles were identified: 30% of parasites had the sensitive haplotype, 24% the single-mutant S436A, 1% the single-mutant A437G and 45% the double-mutant A437G K540E allelic haplotype. Five *dhfr* alleles were identified: 8% of parasites had the sensitive *dhfr* allele, 3% had a single S108N mutation, 39% had a double-mutant N51I, S108N allele, 4% a C59R, S108N double-mutant allele

Table 4 Haplotype frequencies in *dhfr* and *dhps* loci in *P. falciparum* infections

	Genotype*	Count	%
dhfr ($n = 158$)			
Sensitive	CNCS	12	7.6
Single-mutant 108	CNC**N**	5	3.2
Double-mutant 51, 108	C**I**C**N**	62	39.2
Double-mutant 59, 108	CN**R**N	6	3.8
Triple-mutant 51, 59, 108	C**IR**N	73	46.2
dhps ($n = 177$)			
Sensitive	SAK	53	30.0
Single-mutant 436A	**A**AK	43	24.3
Single-mutant 437	SG**K**	1	0.6
Double-mutant 437, 540	SG**E**	80	45.2
dhfr + *dhps* ($n = 0$ 133)			
Sensitive		4	3.0
Single		3	2.3
Double		22	16.7
Triple		28	21.1
Quadruple		40	30.1
Quintuple – highly resistant		36	27.1

*The three-letter codes for *dhps* show amino acids at positions 436, 437 and 540. Four-letter codes at *dhfr* show amino acids at positions 50, 51, 59 and 108. Amino acids that result from mutations are shown in bold.

and 46% the triple-mutant N51I, C59R, S108N allele (numbers indicate positions at dhfr locus and letters the amino acid at this position before and after mutation). Analysis of the combined *dhfr* and *dhps* genotypes showed that only 3% of all parasites were sensitive at both loci, while the majority, 78%, had three or more mutations linked to SP resistance. The genotype that is most closely associated with SP treatment failure *in vivo* is the triple-mutant *dhfr* N51I, C59R, S108N in combination with double-mutant *dhps* A437G K540E. This genotype was present in 27% of parasites.

Discussion

Here we report results from one of the first ACT drug efficacy trials in eastern DRC. We found that the combination AS + AQ performed better than AS + SP in the 28 days after observed treatment, with AS + SP failing in 20% of patients after 28 days. Both treatment groups had similarly positive secondary treatment outcomes, including rapid fever and parasite clearance, effect on gametocytaemia and recovery of Hgb values. Regarding implementation of the new national treatment protocol, AS + AQ is confirmed to be the more effective option in this region.

Molecular genetic analysis of day 0 samples showed high prevalence of SP-resistant parasites among the patient cohort. Resistance mutations were common in both *dhfr* and *dhps* genes and 27% of the parasites were shown to have a combination of both the triple-mutant *dhfr* and the double-mutant *dhps*, which has been demonstrated to be predictive of treatment failure (Kublin *et al.* 2002; Kyabayinze *et al.* 2003; Omar *et al.* 2005). The triple-mutant *dhfr* alone is refractory to SP (Plowe *et al.* 1998) and this was found in almost half of parasites. Cohuet *et al.* in press recently reported similar molecular results (*P. falciparum* from Equator, Oriental and Katanga provinces in the DRC), highlighting the need to discontinue the use of SP, whether in monotherapy or in combinations in this region. These study results add to the growing body of knowledge that AS + SP is disappointingly ineffective where the level of SP resistance is high (WHO 2005b).

Amodiaquine has a metabolism, structure and mode of action very different from SP and problems of amodiaquine resistance are less widespread regionally (East African Network for Monitoring Antimalarial Treatment (EANMAT) 2004).

Hence, the combination AS + AQ is not only more effective than AS + SP at present but also may remain effective for an extended period of time. Combinations with AS have proved very efficacious and could, in the right circumstances, protect drugs from the progressive development of resistance, as has been shown with implementation of ACT elsewhere (Nosten & Brasseur 2002). AS + AQ has now been adopted as first-line antimalarial treatment in 15 countries in Africa (WHO 2006). The combination AS + AQ was evaluated positively in a number of recent studies in Africa, showing 28-day corrected efficacies >90% (range 90.3–100%) in most locations (Barennes *et al.* 2004; Rwagacondo *et al.* 2004; Staedke *et al.* 2004; van den Broek *et al.* 2005; Guthmann *et al.* 2005; Hamour *et al.* 2005; Martensson *et al.* 2005; Yeka *et al.* 2005; S. Cohuet, M. Bonnet, Epicentre/MSF unpublished data). In some locations, however, the efficacy of this combination was below 90% (range 73.0–86.7; Rwagacondo *et al.* 2004; Grandesso *et al.* in press).

The gametocidal effect is important for ACT capacity to reduce malaria transmission (Price *et al.* 1996). In our study, gametocytes developed later in a quarter of the patients. This occurred mainly during the first 2 days, after one or two doses of the AS course had been taken; this pattern was similar in both ACT groups. Artemisinins act through suppressing the development of new gametocytes (Pukrittayakamee *et al.* 2004) and might therefore not affect gametocytes that are already (nearly) formed. In patients treated with non-artemisinin antimalarial drugs or untreated (asymptomatic) persons, gametocytes develop for a longer period after asexual parasite stages are cleared (Bousema *et al.* 2004). Drug-resistant strains also show higher gametocytaemia than sensitive strains (Sowumni &

Fateye 2003). Gametocyte development might be an important secondary treatment outcome to incorporate in monitoring ACT efficacy, although it can only be regarded within the local and seasonal context of malaria transmission.

As discussed by Mutabingwa *et al.* (2005), there is a concern that efficacy data are not a realistic measure of the effectiveness of a drug in operational practice. While this can be true, effective sensitization efforts will improve drug adherence, especially if in collaboration with an easily dispensed and consumed drug formula, as seen when using WHO packaging of Coartem (Fogg *et al.* 2004; Mutabingwa *et al.* 2005). To this end, there must be an ongoing push for the introduction of an AS + AQ co-formulation in blister pack, which is foreseen to become available in 2006. Importantly, there is also a need to establish a better system of diagnostics, in order to prevent unnecessary use of ACTs, which masks its effectiveness to the users. It will take a major shift in mindset and practice to make the African health care providers see that fever does not equal malaria (Malenga *et al.* 2005).

In 1990, the WHO withdrew amodiaquine for the treatment of malaria after reports of rare but severe toxic effects associated with its use as prophylaxis. Subsequent to systematic review of trials (Olliaro *et al.* 1996) showing the safety of amodiaquine when given as 3-day treatment, the WHO modified its recommendations to reinstate amodiaquine for the treatment of *falciparum* malaria. In the DRC, this information has not yet penetrated to all remote rural health centres and, therefore, health staff, community health care volunteers and patients will need to be sensitized to these recommendations in efforts to ensure AS + AQ is effectively accepted and implemented.

In countries such as the DRC, with a per capita gross domestic product of US$352 and a total health expenditure per capita of US$12 (WHO 2005b), local capacity to provide ACT is very limited. The cost of treatment has to be considered, as this is a limiting factor to the success of implementation (Whitty *et al.* 2004). Price for patients has to be kept as low as possible and preferably free. In the DRC, this will require the ongoing assistance of external donors. The Global Fund as well as some other donors has shown a willingness to make available extra funds for this treatment at a regional or national level (Global Fund 2004; WHO 2006).

This article highlights the difficult predicament many African countries continue to face today. With the high cost of effective ACT, many African countries are forced to rely on ineffective antimalarials. Although AS + AQ was shown to be highly effective in our study in the DRC, a failure rate of 6.8% is worthy of concern. Our results support the change in national protocol to AS + AQ as first-line treatment of uncomplicated malaria, with the proviso that the monitoring of both efficacy and effectiveness must continue.

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Artésunate + Amiodaquine et Artésunate + Sulfadoxine–Pyriméthamine dans le traitement de l'accès palustre simple en République Démocratique du Congo : étude clinique avec détermination des haplotypes de résistance de la sulfadoxine et de la pyriméthamine

Nous avons mené une étude sur l'Artésunate + Amiodaquine (AS + AQ) et Artésunate + Sulfadoxine–Pyriméthamine (AS + SP) chez 180 enfants âgés de 6 à 59 mois présentant un accès palustre simple en Republique Democratique du Congo (RDC). Les enfants recevaient de façon randomisée 3 jours de traitement supervisé d'AS + AQ ($n = 90$) ou 3 jours d'AS + SP ($n = 90$). Les critères de jugement principaux étaient les taux de réinfection au 28éme jours et les taux de rechutes ajustés selon le génotype permettant de différencier une nouvelle infection d'une rechute. De plus, nous avons déterminé la prévalence des marqueurs moléculaires de résistance à la sulfadoxine et à la pyriméthamine. Les taux de reinfection au 28éme jours étaient de 16.9% (14/83; IC 95%: [9.5; 26.7]) dans le groupe AS + AQ et 34.6% (28/81; IC 95% [24.3; 46.0]) dans le groupe AS + SP ($P = 0{,}009$). Apres correction par PCR, les taux de rechute étaient de 6.7% (5/74; IC 95% [2.2; 15.1]) pour l'AS + AQ et 19.7% (13/66; IC 95% [10.9–31.3]) pour l'AS + SP ($P = 0.02$). Il n'y avait pas de différence statistiquement significative entre les deux bras en terme de delai de négativation de la parasitémie, de disparition de la fièvre et de disparition des gamétocytes. Le génotypage parasitaire montrait une fréquence élevée des marqueurs de résistance moléculaire à la SP pour la *dhfr* et la *dhps* avec 57% des échantillons montrant plus de 3 mutations liées a la résistance à la SP et 27% avec l'haplotype triple-*dhfr*/double-*dhps*, confirmant que le taux d'échec du traitement par SP est probablement élevé. AS + AQ avait de façon significative une efficacité supérieure en comparaison à AS + SP. Ces résultats ont favorisé un changement de traitement pour l'association AS + AQ en première ligne de traitement dans le pays. Les efforts pour mettre en place correctement le nouveau protocole et maintenir l'adhérence à des niveaux acceptables devraient inclure la sensibilisation aussi bien du personnel de santé que du patient. Le taux de rechute de 6,8% indique que le traitement par AS + AQ devrait être surveillé de façon rapprochée jusqu'à ce qu'une nouvelle Association Combine d'Artemisine (ACT) plus efficace soit disponible et puisse être utilisée.

mots clefs paludisme, efficacité, sulfadoxine–pyriméthamine, amiodaquine, artésunate, haplotype de résistance, République Démocratique du Congo

Artesunato + Amodiaquina y Artesunato + Sulfadoxina–Pirimetamina para el tratamiento de la malaria no complicada en la República Democrática del Congo: una prueba clínica con determinación de los haplotipos resistentes a la sulfadoxina y pirimetamina

Llevamos adelante un ensayo de Artesunato + Amodiaquina (AS + AQ) y Artesunato + Sulfadoxina-Pirimetamina (AS + SP) entre 180 niños de 6 a 59 meses de edad con malaria no complicada en la República Democrática del Congo (RDC). Los niños fueron asignados de forma aleatoria para recibir un tratamiento controlado de 3 días de AS + AQ ($n = 90$) o 3 días de AS + SP ($n = 90$). Los resultados de eficacia primaria eran de tasas de recurrencia de parásitos de 28 días, y las tasas de recrudescencia fueron ajustadas por genotipificación para distinguir nuevas infecciones de las recrudescentes. Sumado a ello, determinamos la prevalencia de marcadores moleculares de resistencia a la sulfadoxina y la pirimetamina. Las tasas de recurrencia de parásitos al día 28 eran de 16.9% (14/83; 95% CI: 9.5–26.7) en el grupo AS + AQ y 34.6% (28/81; 95% CI: 24.3–46.0) en el grupo AS + SP ($P = 0.009$). Después de la corrección PCR, las tasas de recrudescencia eran 6.7% (5/74: 95% CI: 2.2–15.1) para AS + AQ y 19.7% (13/66; 95% CI: 10.9–31.3) para AS + SP ($P = 0.02$). No hubo diferencia significativa entre los dos grupos al momento de la eliminación de los parásitos, la eliminación de la fiebre y la eliminación de los gametocitos. La genotipificación del parásito mostró altas frecuencias de marcadores moleculares de resistencia SP *dhfr* y *dhps*, con un 57% de las muestras mostrando más de 3 mutaciones ligadas a resistencia SP, y 27% con haplotipo triple-*dhfr*/double-*dhps*, confirmando que las tasas de fracaso del tratamiento SP serán probablemente altas. AS + AQ tiene significantemente mayor eficacia que AS + SP. Estos resultados contribuyeron al subsiguiente cambio a AS + AQ como primera línea de tratamiento en el país. Los esfuerzos para implementar apropiadamente los nuevos protocolos y para mantener la adherencia en niveles aceptables debería incluir la sensibilización de los pacientes y del personal sanitario. La tasa de recrudescencia del 6.8% indica que el AS + AQ debería ser monitoreado estrechamente hasta el momento en el que sea necesaria una Terapia de Combinación de Artemisinina (TCA), y esta terapia pueda ser introducida.

palabras clave malaria, eficacia, sulfadoxina–pirimetamina, amodiaquina, artesunato, haplotipo resistente, República Democrática del Congo

