

Relationship between age, molecular markers, and response to sulphadoxine–pyrimethamine treatment in Kampala, Uganda

Sarah G. Staedke¹, Hakim Sendagire², Steven Lamola¹, Moses R. Kanya², Grant Dorsey¹ and Philip J. Rosenthal¹

¹ Department of Medicine, San Francisco General Hospital, University of California, San Francisco, CA, USA

² Makerere University Medical School, Kampala, Uganda

Summary

Sulphadoxine/pyrimethamine (SP) has become the first-line treatment of uncomplicated malaria in a number of African countries. Molecular surveillance of resistance-mediating mutations in *Plasmodium falciparum* dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) has been proposed as a means of predicting SP treatment outcomes, but optimal methods of surveillance in different populations have not been well established. To investigate the relationship between molecular markers of SP resistance, host immunity, and response to therapy, we evaluated the association between the presence of five key *dhfr* and *dhps* mutations at enrollment and clinical outcome in children and adults treated with SP for uncomplicated malaria in Kampala, Uganda. Clinical treatment failure was 11% at 14 days, increasing to 30% at 28 days, after excluding new infections. Outcomes varied markedly based on the number of *dhfr* and *dhps* mutations and on the age of treated subjects. All infections with less than two *dhfr/dhps* mutations were successfully treated. Treatment failure associated with any two, three, or four *dhfr/dhps* mutations occurred in nine of 24 (38%) children up to 5 years, but not in older patients (0/20). In the presence of all five mutations, treatment failure occurred equally in children aged 5 years or younger [7/16 (44%)] and in older patients [8/16 (50%)]. Our results showed that age, a surrogate marker of antimalarial immunity, had a major impact on the relationship between polymorphisms in SP target enzymes and treatment outcomes. The use of molecular markers of SP resistance to predict treatment failure rates should take age into account.

keywords malaria, molecular markers, drug resistance, *dhfr*, *dhps*, Africa, Uganda, sulphadoxine–pyrimethamine

Introduction

Antimalarial drug resistance is a major problem in Africa (White *et al.* 1999). Chloroquine failure has led to widespread use of sulphadoxine–pyrimethamine (SP), with several African countries adopting SP as the recommended first-line therapy for uncomplicated malaria, either alone or in combination with other agents. Although the low cost, simple dosing, and wide availability of SP make it an attractive regimen, data suggest that SP resistance is rising in East Africa (Mberu *et al.* 2000; EANMAT 2003). The level of SP resistance is likely to be much greater than generally appreciated, as recent clinical studies using standard 14-day assessment may have systematically underestimated SP resistance (White 2002). A new antifolate combination, chlorproguanil–dapson (CD), will soon be available and is anticipated to have greater efficacy and a longer therapeutic lifespan because of its action against some SP-resistant parasites and its favourable pharmacokinetic profile (Winstanley 2001). However, SP

and CD share a similar mechanism of action and cross-resistance may occur in areas where SP is widely used (Nzila-Mounda *et al.* 1998). Monitoring antifolate resistance will be an important task for countries currently using SP and for those considering new antifolate treatment options.

Although clinical studies are currently the ‘gold standard’ for assessing antimalarial drug efficacy and for guiding drug policy, they can be costly and time consuming (Bloland & Ertling 1999). Evaluation of molecular markers of drug resistance may provide essential complementary information to *in vivo* studies, ideally detecting emerging resistance before clinical treatment failure is evident (Hastings *et al.* 2002a). *In vitro* studies have shown a clear association between SP resistance and point mutations in the genes encoding dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS), the target enzymes of pyrimethamine and sulphadoxine, respectively (Peterson *et al.* 1988; Wang *et al.* 1997). The accumulation of *dhfr* (108-Asn, 51-Ile, 59-Arg) and *dhps* (437-Gly,

540-Glu) mutations also appears to produce clinical SP resistance in Africa, although the relative contribution of mutations in these genes has been controversial (Sibley *et al.* 2001).

Recent clinical studies have demonstrated a strong association between the presence of the 'quintuple mutant' (*dhfr* 108-Asn, 51-Ile, 59-Arg plus *dhps* 437-Gly, 540-Glu) and 7-day parasitological failure with SP therapy in Kenya (Nzila *et al.* 2000) and Malawi (Kublin *et al.* 2002). Resistance to CD appears to be mediated by different mutations (*dhfr* 108-Thr, 16-Val), but high-level cross-resistance with SP occurs in the presence of *dhfr* 164-Leu, a mutation that has been rarely reported from Africa (Hastings *et al.* 2002b). Host immunity, which in endemic areas increases with age, is likely to modify the relationship between mutations and treatment outcome (Wernsdorfer 1994), but the relevance of age in assessments of molecular predictors of antifolate resistance has been little studied to date. To further investigate the relationship between the parasite, host immunity, and response to SP therapy, we examined the association between the number of parasite *dhfr* and *dhps* mutations present at enrollment and clinical outcome in different age groups.

Methods

Clinical study

This study took place between March and August 1999 in Kampala, Uganda. Full details of the clinical trial have been reported elsewhere (Kanya *et al.* 2001). Briefly, symptomatic patients with a positive screening thick blood smear were enrolled in the study if they met the following inclusion criteria: (1) age ≥ 6 months, (2) tympanic temperature ≥ 38.0 °C or history of fever in previous 48 h, (3) *Plasmodium falciparum* mono-infection with ≥ 2000 asexual parasites/ μ l, (4) absence of severe malaria or danger signs, and (5) absence of other causes of fever. For this study, subjects were limited to the 106 patients treated with SP. Treatment outcomes at 14 and 28 days were classified according to World Health Organization criteria (WHO 1996). Day 28 treatment outcomes were corrected using molecular genotyping based on merozoite surface protein-2 polymorphisms to distinguish recrudescence and new infections, as previously described (Cattamanchi *et al.* 2003). Recrudescence was defined as a late clinical treatment failure due to parasite strains present at enrollment. The clinical study was approved by the Institutional Review Boards of the University of California, San Francisco and Makerere University, Kampala.

Mutation analysis

Polymorphisms of interest in the *P. falciparum dhfr* and *dhps* genes were assessed using nested-polymerase chain reaction (PCR) amplification followed by mutation-specific restriction enzyme digestion. Blood was collected on filter paper each time a patient was diagnosed with malaria and at the time of clinical treatment failure. Parasite DNA was isolated from filter paper using the chelex extraction method (Plowe *et al.* 1995). Primers, enzymes, and amplification conditions for PCR assays to detect *dhfr* 108-Asn, 51-Ile, 59-Arg, and 164-Leu, and *dhps* 437-Gly and 540-Glu were as described in a prior report (Duraisingh *et al.* 1998) except that (a) *dhfr* first-round primers were modified (sense, 5'-TTTATGATGGAACAAGTCTGC-GACGTTTTTC-3' and antisense, 5'-AATTTGATACTCA-TTTTCATTTATTTCTGG-3') to yield a 622-bp fragment, (b) nested primers for codons 51 and 108 were modified (sense, 5'-GTCTGCGACGTTTTTCGATATTTATGC-3' and antisense, 5'-AAATTCTTGATAAACCAACGGAACC-TTTTA-3') to yield a 506-bp fragment, (c) Vent polymerase (New England Biolabs, Beverly, MA, USA) was used for the *dhfr* first-round reaction, and (d) annealing temperatures were changed to 54 °C for five cycles followed by 56 °C for 35 cycles for the first-round *dhfr* reaction and to 50 °C for five cycles followed by 54 °C for 35 cycles for both nested *dhfr* reactions (Kyabayinze *et al.* 2003).

The presence of mutations at each codon was determined using restriction enzyme digestion of nested PCR products using published methods (Duraisingh *et al.* 1998). Digestion products were visualized by electrophoresis and results classified as wild type, pure mutant, or mixed (both wild-type and mutant genotype present in the same infection). For additional analysis of one isolate of interest, the *dhfr* gene was amplified using primers spanning the coding region, the amplicon was cloned into a plasmid, bacteria were transfected with this plasmid and grown overnight, plasmid DNA was purified, and dideoxy sequencing of *dhfr* was performed in both directions. Investigators were blinded to clinical outcomes at the time of molecular analysis.

Statistical analysis

Clinical treatment outcomes at 14 days were dichotomized into success (adequate clinical response) and failure (early treatment failure and late treatment failure). For 28-day outcomes, the same classification schemes were used, with genotyping correction of late clinical treatment failures. Late treatment failures due to new infections were re-classified as treatment successes. All data were entered

and verified using Epi-Info 6.04 and SPSS. Analysis was performed using STATA statistical software. Associations between individual mutations and treatment outcomes were assessed using chi-squared or Fisher's exact tests, where appropriate. All confidence levels were set at 95%.

Results

Treatment outcomes

Of 106 patients treated with SP for uncomplicated malaria, 93 (88%) completed 14 days and 87 (82%) completed 28 days of follow-up. Of these patients, successful genotyping results were obtained for 84 (97%) for 28-day clinical outcome (three samples were missing). Treatment failure, adjusted by genotyping, was much more common when follow-up was extended to 28 days, with clinical failure increasing from 11% to 30%. At 28 days, young children (aged 5 years or less) were more likely to experience treatment failure than older patients [39% *vs.* 20%; RR = 1.93; 95% CI 0.94–3.98; $P = 0.06$], although the difference was of borderline statistical significance.

Prevalence of *dhfr* and *dhps* mutations

Molecular assays were performed on all pre-treatment samples for six *dhfr* and *dhps* mutations that are known to impact on response to antifolate treatment (*dhfr* 108-Asn, 51-Ile, 59-Arg, and 164-Leu, and *dhps* 437-Gly and 540-Glu). Mutation analysis was successful for 81 (96%) of the 84 patients with available 28-day clinical outcome adjusted by genotyping. All pre-treatment samples were wild type at *dhfr* codon 164. Results are presented for the other five *dhfr/dhps* codons evaluated. Considering isolates collected at the time of initial diagnosis, mutations in *dhfr* 108-Asn (94%) and 51-Ile (91%) were very common (Figure 1). The prevalence of pure mutant *dhfr* 59-Arg (40%), *dhps* 437-Gly (24%) and *dhps* 540-Glu (43%) was lower, with

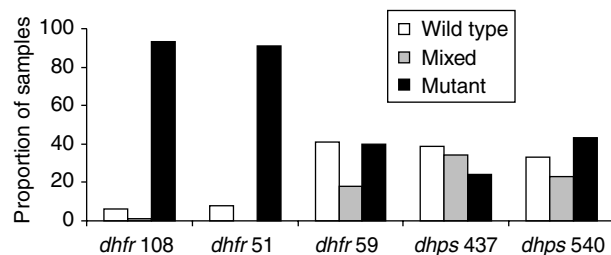


Figure 1 Prevalence of *dhfr* and *dhps* polymorphisms at enrollment.

samples frequently showing a mixed pattern (18%, 35%, and 23%, respectively).

Although the 164-Leu mutation was not found in any pre-treatment samples, on analysis of post-treatment samples (data not shown) the 164-Leu mutation was isolated from an asymptomatic patient on day 21 of follow-up. The isolate, classified as a new infection by genotyping, also contained mutations in *dhfr* 108, 51, and 59 and had a mixed pattern for *dhps* 437 and 540. The presence of the *dhfr* mutations was verified by sequencing of the *dhfr* gene in both directions. As this patient remained asymptomatic, no additional antimalarial treatment was provided, and the parasitaemia cleared without specific treatment. The patient was classified as a clinical success on day 28.

Association between *dhfr* and *dhps* mutations and clinical treatment failure

As the *dhfr* 108-Asn and 51-Ile mutations were highly prevalent, they were not studied for association with clinical outcome. The association between *dhfr* 59-Arg, and *dhps* 437-Gly and 540-Glu mutations present at enrollment and clinical treatment failure at 14 and 28 days was investigated. Each mutation was independently associated with clinical failure, and the strength of the association increased as follow-up was extended to 28 days (Table 1).

Impact of age on clinical outcome

The association between the total number of *dhfr* and *dhps* mutations present at enrollment and clinical outcome at 28 days, stratified by age, was investigated (Table 2). Considering the five relevant *dhfr/dhps* mutations (*dhfr* 108-Asn, 51-Ile, and 59-Arg; *dhps* 437-Gly and 540-Glu), only five (6%) pre-treatment samples had no mutations or a single mutation, and all of these infections were treated successfully, regardless of age. Treatment failure associated with the presence of any two, three, or four *dhfr/dhps* mutations was more common, but only occurred in children aged 5 years or less (nine of 16 failures in this age group). Failure in young children occurred commonly in the presence of either four or five mutations. Older patients experienced clinical failure only in the presence of five mutations. There was no predominant mutation pattern in the nine younger children who failed treatment in the presence of less than five *dhfr/dhps* mutations. The relationship between the total number of *dhfr/dhps* mutations present at enrollment and 28-day clinical outcome was further investigated in the two age groups. In patients older than 5 years, the maximum difference in risk of clinical failure was obtained by setting a threshold of five

Table 1 Association between *dhfr* and *dhps* mutations and clinical treatment failure

Codons	14-day treatment			28-day treatment		
	Risk difference	95% CI	P-value	Risk difference	95% CI	P-value
59	13.7	2.6–24.7	0.05	30.2	12.8–47.7	0.003
437	12.9	2.0–23.9	0.08	25.3	7.2–43.4	0.02
540	15.9	6.8–24.9	0.03	34.9	19.0–50.9	0.0009

Table 2 Age-stratified association between total number of *dhfr/dhps* mutations present at enrolment and clinical outcome at 28 days, adjusted by genotyping

Total number of <i>dhfr/dhps</i> mutations*	Age ≤ 5 years		Age > 5 years	
	Success (%)	Failure (%)	Success (%)	Failure (%)
0	0	0	4/4 (100)	0
1	1/1 (100)	0	0	0
2	3/4 (75)	1/4 (25)	8/8 (100)	0
3	8/9 (89)	1/9 (11)	6/6 (100)	0
4	4/11 (36)	7/11 (64)	6/6 (100)	0
5	9/16 (56)	7/16 (44)	8/16 (50)	8/16 (50)

* The five mutations evaluated were *dhfr* 108-Asn, 51-Ile, and 59-Arg, and *dhps* 437-Gly and 540-Glu.

mutations. Older patients with five mutations were at 50% (95% CI: 26–74%) greater risk of experiencing treatment failure than those with 0–4 mutations ($P = 0.0002$). For children aged 5 years or less, a threshold of four mutations produced the maximum risk difference. The risk of treatment failure was 38% (95% CI: 11–64%) higher in children with four or more mutations than in those with fewer mutations ($P = 0.04$). In contrast, the risk of treatment failure was only 8% (95% CI: –23–38%) higher in children with five mutations compared with those with 0–4 mutations ($P = 0.75$).

Discussion

In this study, the presence of *dhfr* and *dhps* mutations prior to therapy was associated with SP resistance as measured by clinical outcome at 28 days. As previously observed in Kampala, *dhfr* 108-Asn and 51-Ile mutations were present in over 90% of samples and were not useful predictors of clinical outcome (Kyabayinze *et al.* 2003). However, *dhfr* 59-Arg and *dhps* 437-Gly and 540-Glu mutations were less common and were independently associated with 28-day treatment failure.

The finding of *dhfr* 164-Leu in a post-treatment sample is a matter of concern, although this isolate was not clinically significant. Until recently, 164-Leu, which confers high-level pyrimethamine resistance rendering SP and CD ineffective, had only been isolated from Southeast Asian and South American isolates (Plowe *et al.* 1997). Recently, however, 164-Leu was identified in yeast that

had been transfected with *dhfr* genes from Tanzanian samples, a technique designed to identify even rare populations in clinical isolates (Hastings *et al.* 2002b). In addition, the 164-Leu mutation was identified in isolates from four travellers from Africa who presented with malaria in Sweden (Farnert *et al.* 2002). The identification of 164-Leu in isolates from several sites in Africa suggests that high-level pyrimethamine resistance is emerging in the face of increased drug pressure (Hastings *et al.* 2002a). Selection of this highly resistant genotype could threaten the efficacy of CD, as well as SP, and severely limit the affordable antimalarial armamentarium available for Africa (Sibley *et al.* 2001). Continued monitoring for the 164-Leu mutation and SP efficacy will be essential.

Successful antimalarial treatment depends on the complex interaction between the drug regimen, parasite, and infected host (Wernsdorfer 1994). Young age has previously been identified as an important risk factor for treatment failure (Fontanet & Walker 1993; Dorsey *et al.* 2000). In endemic areas, malaria-specific immunity is acquired with repeated exposure and increases with age (Rogier *et al.* 1999). In our study, older patients, presumably with greater acquired immunity, had superior outcomes compared with younger patients. Consistent with the observation that partially immune hosts can clear drug-resistant parasites (Cravo *et al.* 2001), in older patients only highly resistant parasites lead to treatment failure. Specifically, patients over 5 years of age failed SP treatment only in the presence of five *dhfr/dhps* mutations (the 'quintuple mutant'), while clinical failure in children aged

S. G. Staedke *et al.* **Age and molecular markers of SP resistance**

5 years or less occurred with fewer mutations. Interestingly, once all five mutations were present, the risk of failure was the same in both age groups. One explanation of this finding is that as parasite resistance increases, the relative role of host immunity becomes less important.

Surveillance for key mutations in parasite genes may be an important tool for monitoring antimalarial resistance, particularly before clinical failure is apparent. A model based on the prevalence of a single point mutation, *pfcr* T76, has been developed for surveillance of chloroquine resistance, and a similar model, using the combination of 59-Arg + 540-Glu, has been proposed for monitoring SP resistance (Djimde *et al.* 2001; Kublin *et al.* 2002). However, the greater complexity of SP resistance, involving two genes and multiple mutations, may limit the predictive capacity of a simplified model. To be reliable, predictive models may need to account for the age of the patients as well as the number of mutations present. Additional studies in differing epidemiological settings will be needed to validate the relationship between molecular markers of SP resistance and treatment outcome.

Acknowledgements

We thank Daniel Kyabayinze, Byoung-Kuk Na, and Sunil Parikh for laboratory assistance; the clinical study team of Juliet Babirye, Anne Gasasira, B.M. Karakire, Sam Nyole, Sam Nsobya, Moses Kiggundu, Christopher Bongole, Regina Nakafero; and the study participants and their parents/guardians. Financial support was provided by the Fogarty International Center/National Institutes of Health (TW00007, TW01506 and AI43301) and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

References

- Bloland PB & Ertling M (1999) Making malaria-treatment policy in the face of drug resistance. *Annals of Tropical Medicine & Parasitology* **93**, 5–23.
- Cattamanchi A, Kyabayinze D, Hubbard A, Rosenthal PJ & Dorsey G (2003) Distinguishing recrudescence from reinfection in a longitudinal antimalarial drug efficacy study: comparison of results based on genotyping of *m*sp-1, *m*sp-2, and *glurp*. *American Journal of Tropical Medicine and Hygiene* **68**, 133–139.
- Cravo P, Culleton R, Hunt P, Walliker D & MacKinnon MJ (2001) Antimalarial drugs clear resistant parasites from partially immune hosts. *Antimicrobial Agents and Chemotherapy* **45**, 2897–2901.
- Djimde A, Doumbo OK, Steketee RW & Plowe CV (2001) Application of a molecular marker for surveillance of chloroquine-resistant *falciparum* malaria. *Lancet* **358**, 890–891.
- Dorsey G, Kanya MR, Ndeezi G *et al.* (2000) Predictors of chloroquine treatment failure in children and adults with *falciparum* malaria in Kampala, Uganda. *American Journal of Tropical Medicine and Hygiene* **62**, 686–692.
- Duraisingh M, Curtis J & Warhurst D (1998) *Plasmodium falciparum*: detection of polymorphisms in the dihydrofolate reductase and dihydropteroate synthetase genes by PCR and restriction digestion. *Experimental Parasitology* **89**, 1–8.
- EANMAT (2003) The efficacy of antimalarial monotherapies, sulphadoxine-pyrimethamine and amodiaquine in East Africa: implications for sub-regional policy. *Tropical Medicine & International Health* **8**, 860–867.
- Farnert A, Tengstam K, Palme IB *et al.* (2002) Polyclonal *Plasmodium falciparum* malaria in travelers and selection of antifolate mutations after proguanil prophylaxis. *American Journal of Tropical Medicine and Hygiene* **66**, 487–491.
- Fontanet AL & Walker AM (1993) Predictors of treatment failure in multiple drug-resistant *falciparum* malaria: results from a 42-day follow-up of 224 patients in Eastern Thailand. *American Journal of Tropical Medicine and Hygiene* **49**, 465–472.
- Hastings IM, Watkins WM & White NJ (2002a) The evolution of drug-resistant malaria: the role of drug elimination half-life. *Philosophical Transactions of the Royal Society of London B* **357**, 505–519.
- Hastings MD, Bates SJ, Blackstone EA, Monks S & Sibley CH (2002b) Highly pyrimethamine-resistant alleles of dihydrofolate reductase in isolates of *Plasmodium falciparum* from Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **96**, 674–676.
- Kanya MR, Dorsey G, Gasasira A *et al.* (2001) The comparative efficacy of chloroquine and sulfadoxine-pyrimethamine for the treatment of uncomplicated *falciparum* malaria in Kampala, Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **95**, 50–55.
- Kublin JG, Dzinjalama FK, Kamwendo DD *et al.* (2002) Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of *Plasmodium falciparum* malaria. *Journal of Infectious Diseases* **185**, 380–388.
- Kyabayinze D, Cattamanchi A, Kanya MR, Rosenthal PJ & Dorsey G (2003) Validation of a simplified method for using molecular markers to predict sulfadoxine-pyrimethamine treatment failure in African children with *falciparum* malaria. *American Journal of Tropical Medicine and Hygiene* **69**, 247–252.
- Mberu EK, Mosobo MK, Nzila AM *et al.* (2000) The changing in vitro susceptibility pattern to pyrimethamine/sulfadoxine in *Plasmodium falciparum* field isolates from Kilifi, Kenya. *American Journal of Tropical Medicine and Hygiene* **62**, 396–401.
- Nzila AM, Mberu EK, Sulo J *et al.* (2000) Towards an understanding of the mechanism of pyrimethamine-sulfadoxine resistance in *Plasmodium falciparum*: genotyping of dihydrofolate reductase and dihydropteroate synthase of Kenyan parasites. *Antimicrobial Agents and Chemotherapy* **44**, 991–996.
- Nzila-Mounda AM, Mberu EK, Sibley CH *et al.* (1998) Kenyan *Plasmodium falciparum* field isolates: correlation between

S. G. Staedke *et al.* **Age and molecular markers of SP resistance**

- pyrimethamine and chlorcycloguanil activity in vitro and point mutations in the dihydrofolate reductase domain. *Antimicrobial Agents Chemotherapy* **42**, 164–169.
- Peterson DS, Walliker D & Wellems TE (1988) Evidence that a point mutation in dihydrofolate reductase-thymidylate synthase confers resistance to pyrimethamine in falciparum malaria. *Proceedings of the National Academy of Sciences, USA* **85**, 9114–9118.
- Plowe CV, Djimde A, Bouare M, Doumbo O & Wellems TE (1995) Pyrimethamine and proguanil resistance-conferring mutations in *Plasmodium falciparum* dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. *American Journal of Tropical Medicine and Hygiene* **52**, 565–568.
- Plowe CV, Cortese JF, Djimde A *et al.* (1997) Mutations in *Plasmodium falciparum* dihydrofolate reductase and dihydropteroate synthase and epidemiologic patterns of pyrimethamine-sulfadoxine use and resistance. *Journal of Infectious Diseases* **176**, 1590–1596.
- Rogier C, Tall A, Diagne N *et al.* (1999) *Plasmodium falciparum* clinical malaria: lessons from longitudinal studies in Senegal. *Parassitologia* **41**, 255–259.
- Sibley CH, Hyde JE, Sims PF *et al.* (2001) Pyrimethamine-sulfadoxine resistance in *Plasmodium falciparum*: what next? *Trends in Parasitology* **17**, 582–588.
- Wang P, Read M, Sims PF & Hyde JE (1997) Sulfadoxine resistance in the human malaria parasite *Plasmodium falciparum* is determined by mutations in dihydropteroate synthase and an additional factor associated with folate utilization. *Molecular Microbiology* **23**, 979–986.
- Wernsdorfer WH (1994) Epidemiology of drug resistance in malaria. *Acta Tropica* **56**, 143–156.
- White NJ (2002) The assessment of antimalarial drug efficacy. *Trends in Parasitology* **18**, 458–464.
- White NJ, Nosten F, Looareesuwan S *et al.* (1999) Averting a malaria disaster. *Lancet* **353** 1965–1967.
- Winstanley P (2001) Chlorproguanil-dapsone (LAPDAP) for uncomplicated falciparum malaria. *Tropical Medicine & International Health* **6**, 952–954.
- World Health Organization (1996) *Assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria in areas with intense transmission*. WHO Division of Control of Tropical Diseases, Geneva.

Authors

Philip J. Rosenthal (corresponding author), **Sarah G. Staedke**, **Steven Lamola** and **Grant Dorsey**, University of California San Francisco, Box 0811, San Francisco, CA 94143, USA. Tel.: +1-415-206-8845; Fax: +1-415-648-8425; E-mail: rosenth@itsa.ucsf.edu, staedke@itsa.ucsf.edu, slamola@itsa.ucsf.edu, grantd@itsa.ucsf.edu

Hakim Sendagire and **Moses R. Kamya**, Department of Biochemistry, Makerere University, P.O. Box 7072, Kampala, Uganda. E-mail: hsendagire@yahoo.com, mkamya@infocom.co.ug