Chlorproguanil-Dapsone: Effective Treatment for Uncomplicated Falciparum Malaria

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Pyrimethamine-sulfadoxine, the first choice for uncomplicated falciparum malaria in Africa, exerts strong selection pressure for resistance because of its slow elimination. It is likely that resistance will emerge rapidly, and there is no widely affordable replacement. Chlorproguanil-dapsone is cheap, rapidly eliminated, more potent than pyrimethamine-sulfadoxine, and could be introduced in the near future to delay the onset of antifolate resistance and as "salvage therapy" for pyrimethamine-sulfadoxine failure. A total of 448 children were randomly allocated (double blind) to either a single dose of pyrimethamine-sulfadoxine or to one of two chlorproguanil-dapsone regimens: a single dose or three doses at 24-h intervals. Reinfections are clinically indistinguishable from recrudescence and are more likely after treatment with rapidly eliminated drugs; we measured the incidence of parasitemia in 205 initially aparasitemic children to allow comparison with the three treatment groups. The patients and a community surveillance group were followed up for 28 days. At the study end point, 31.2% (95% confidence interval, 24.9–38.0) of the community surveillance group subjects were parasitemic, compared with subjects in the treatment groups, whose rates of parasitemia were 40.8% (32.9–49.0; relative risk [RR], 1.31 [0.99–1.73]) after triple-dose chlorproguanil-dapsone, 19.7% (13.5–27.2; RR, 0.63 [0.43–0.93]) after pyrimethamine-sulfadoxine, and 65.6% (57.5–73.0; RR, 2.10 [1.66–2.65]) after single-dose chlorproguanil-dapsone. Pyrimethamine-sulfadoxine and triple-dose chlorproguanil-dapsone were effective treatments. Pyrimethamine-sulfadoxine provided chemoprophylaxis during follow-up because of its slow elimination. Triple-dose chlorproguanil-dapsone should now be developed in an attempt to reduce the rate of emergence of antifolate resistance in Africa and for affordable salvage therapy in cases of pyrimethamine-sulfadoxine failure.

About 90% of global malaria, 2 million deaths annually, is borne by Africa (1). Chloroquine no longer achieves adequate cure rates, but pyrimethamine-sulfadoxine is nearly as cheap as chloroquine (17) and has become the first choice of therapy in many parts of Africa. Alternatives, such as halofantrine, mefloquine, pyronaridine (24), atovaquone-proguanil (14) and artemisinins are considerably more expensive. Pyrimethamine-sulfadoxine is eliminated slowly (half-lives of 81 and 116 h, respectively [10, 25]), providing chemoprophylaxis after treatment, but also favoring the selection of pyrimethamine-resistant parasites (20) (it is likely that sulfadoxine resistance [5, 19] is also selected). Widespread use of pyrimethamine-sulfadoxine in Africa will probably result in clinical failure in the near future; there is already strong evidence of this in Tanzania (18). The mechanism of clinical failure is not known: resistance to both drugs individually has been reported (5, 7, 12, 13, 15), but their respective importance remains unclear.

Rapidly eliminated antifolate drugs are very likely to exert less resistance selection pressure (20). Furthermore, *Plasmodium falciparum* resistant to pyrimethamine retains sensitivity to other dihydrofolate reductase inhibitors (2, 6, 7, 26). Chlorcycloguanil (the active metabolite of chlorproguanil) combined with dapsone is more potent in vitro than pyrimethamine-sulfadoxine (26), has efficacy in vivo (21), and is eliminated rapidly (22, 23). Furthermore, the mutation thought to confer

resistance to chlorcycloguanil occurs at low frequency in Africa (2, 13), whereas that for pyrimethamine is common. Consequently, we hypothesize that chlorproguanil-dapsone is at least as effective as pyrimethamine-sulfadoxine, in terms of curing the presenting infection, and that it will retain efficacy as pyrimethamine-sulfadoxine failure emerges. The present trial was designed to test the first of these hypotheses.

MATERIALS AND METHODS

This study was based in the Kenya Medical Research Institute's unit at Kilifi District Hospital on the Kenyan coast. Febrile children (6 to 71 months of age inclusive) were recruited from the clinic if they were well enough for outpatient management and fulfilled all of the following criteria: (i) residence within a previously defined study area (9, 16), (ii) capillary hemoglobin level of 5 g% or greater, and (iii) pure *P. falciparum* parasitemia above 2,000 but below 250,000 per µl. Children were excluded if there was (i) concurrent infection(s), (ii) allergy to sulfonamides, (iii) treatment with pyrimethamine-sulfadoxine or pyrimethamine-sulfalene within 2 months, or (iv) treatment with chloramphenicol, co-trimoxazole, erythromycin, or tetracycline within the last week. Chloroquine treatment was not an exclusion criterion. After written consent was obtained, patients were weighed and examined, and venous blood was drawn for measurement of chloroquine and sulfadoxine (high-performance liquid chromatography) (11, 25). Opaque randomization envelopes were then opened by a laboratory technician who took no part in patient assessment, but who acted as the drug dispenser throughout the trial.

Single-dose antimalarial treatment is practical, but chlorproguanil-dapsone is very rapidly eliminated. Therefore, we studied both a group given a single dose of chlorproguanil-dapsone (1.2 and 2.4 mg kg of body weight^{-1}, respectively) followed by placebo on days 1 and 2 (CD1 group) and a group given three doses at 24-h intervals (CD3 group). Another group was given pyrimethamine-sulfadoxine (1.25 and 25 mg kg^{-1}) as a single dose on day 0 and placebo on days 1 and 2 (PS group). Drugs were prepared as suspensions, as previously described (22, 25). The placebo comprised a suspension of calcium lactate. The first dose was taken under supervision, and patients were observed for 1 h. Mothers were then given the remaining two doses to administer, and empty containers were re-

TABLE 1. Status of patients on entry into the trial

Treatment group (n subjects)[a]	Median age (mo) [quartiles]	Male/female ratio	Geometric mean parasitemia (μl^{-1}) [95% CI]	Mean hemoglobin level (g/dl^{-1}) [95% CI]	No. (%) of patients with chloroquine:		Mean concn ($ng\ ml^{-1}$) of cloroquine in whole blood [95% CI]
					Taken during this illness	Detected	
CD1 (154)	34.0 [20,48]	93:61	21,340 [18,116–25,139]	81. [7.8–8.4]	60/154 (39)	66/150 (44)	135 [102–180]
CD3 (152)	28.0 [16,47]	77:75	17,985 [15,246–21,215]	8.2 [7.9–8.4]	64/152 (42)	71/151 (47)	118 [94–148]
PS (142)	27.0 [17,43]	85:57	21,837 [18,239–26,146]	8.1 [7.8–8.4]	50/142 (35)	40/137 (44)	143 [114–180]
Community surveillance (205)	37.0 [22,55]	130:75	None detectable	9.7 [9.4–10.0]	23/205 (11)	26/181 (14)	200 [99–302]

[a] CD1, chlorproguanil-dapsone single dose; CD3, chlorproguanil-dapsone triple dose; PS, pyrimethamine-sulfadoxine single dose. Note that the communit, surveillance group was given no treatment.

turned for counting. All patients were given paracetamol syrup (125 to 250 mg every 6 h). If the first dose of study medication was vomited within 1 h, patients were redosed; further vomiting caused withdrawal (and treatment with intramuscular pyrimethamine-sulfadoxine). Patients were seen on days 2, 7, 14, 21, and 28, when blood was drawn for measurement of hemoglobin and parasite count. Patients completed the study if (i) they were aparasitemic up to day 28, (ii) they were unfit for outpatient care by day 2, (iii) parasitemia persisted beyond day 7, (iv) new parasitemia developed during follow-up, (v) consent was withdrawn, or (vi) protocol violations occurred. Patients with parasitemia at the end point were given halofantrine.

In such an area of intense transmission, there is a high risk of acquiring a new infection over the 28 days following treatment for malaria, particularly with rapidly eliminated drugs, and reinfections cannot be clinically distinguished from recrudescence. Therefore, we estimated the monthly incidence of *P. falciparum* parasitaemia in a community surveillance group of initially aparasitemic children. Children were identified by random selection of households from computerized census data (9). Children were excluded if their slide was positive (they were given pyrimethamine-sulfadoxine); the other inclusion and exclusion criteria were those described above. Community surveillance children were seen on days 14 and 28, when they were weighed and examined, and gave further blood for measurement of hemoglobin and parasite count. Children completed the study when (i) they were aparasitemic up to day 28, (ii) they developed parasitemia between days 14 and 28 inclusive, (iii) consent was withdrawn, or (iv) protocol violation had occurred. Children with parasitemia at the end point were given pyrimethamine-sulfadoxine.

Statistical comparisons were made blind to drug treatment by an author (J.H.) not clinically involved in the trial. Proportions were calculated for each treatment group and compared by χ^2 analysis. The average levels of parasitemia and hemoglobin and the chloroquine concentration on entry were compared between treatment groups and the community surveillance group by analysis of variance.

RESULTS

Between July 1993 and April 1995, 511 patients were randomized to treatment, but 63 were withdrawn (20 from group CD1, 20 from group CD3, and 23 from group PS) because of vomiting of the first dose of study medication (13 of 511; evenly distributed between the three groups), early deterioration in clinical state (1 of 511), protocol violation (43 of 511), detectable sulfonamide in day 0 plasma (2 of 511), and failure to attend for follow-up (6 of 511). A total of 448 patients remained in the trial (154 in group CD1, 152 in group CD3, and 142 in group PS). Between the same dates, 542 children were sampled for the community surveillance group: 239 of 542 had patent parasitemia on day 0. Of the 303 children remaining, 98

were withdrawn (60 had violated the protocol at recruitment, 36 missed follow-up, and 2 withdrew consent), leaving 205 in the community surveillance group. Table 1 gives the baseline data: the three patient groups were similar, but the community surveillance group differed in age, prior chloroquine treatment, and entry level of hemoglobin.

Two empty drug containers were returned by all mothers. By day 2, slides were negative in 100 of 153 (65.4%; 95% confidence interval [CI], 57.3 to 72.9) group CD1 subjects, 119 of 151 (78.8% [71.4 to 85.0]) group CD3 subjects, and 89 of 142 (62.7% [54.2 to 70.6]) group PS subjects. Where slides remained positive on day 2, counts were very low, and there was no difference in parasitemia between the three treatment groups. On direct questioning, 100, 99, and 98% of mothers in groups CD1, CD3, and PS, respectively, said that their child had improved by day 2. No adverse drug reactions were seen. Axillary temperature was 37.5°C or below in 121 of 153 (79.1% [71.8 to 85.2]) group CD1 subjects, 122 of 152 (80.3% [73.0 to 86.3]) group CD3 subjects, and 109 of 142 (76.8% [68.9 to 83.4]) group PS subjects. Two children (both from group PS) were judged too ill on day 2 to continue as outpatients (both had fever and low parasitemia); they were admitted to the hospital and recovered without sequelae. By day 7, slides were negative in 142 of 152 (93.4% [88.2 to 96.8]) group CD1 subjects, 145 of 147 (98.0% [95.2 to 99.8]) group CD3 subjects, and 136 of 137 (99.3% [96.0 to 100.0]) group PS subjects. Axillary temperature was 37.5°C or below in 139 of 150 (92.7% [87.3 to 96.3]), 136 of 145 (93.8% [88.5 to 97.1]), and 127 of 129 (98.4% [94.5 to 99.8]) subjects of groups CD1, CD3, and PS, respectively. The mean hemoglobin level was lower on day 2 than on day 0 in all treatment groups and recovered thereafter (Table 2).

In comparison with the community surveillance group, the relative risk of new parasitemia over 28 days of follow-up was 1.31 (95% CI, 0.99 to 1.73) after three daily doses of chlorproguanil-dapsone, 0.63 (0.43 to 0.93) after one dose of pyrimethamine-sulfadoxine, and 2.10 (1.66 to 2.65) after one dose of chlorproguanil-dapsone. Table 3 shows the status of patients and members of the community surveillance group at the end

TABLE 2. Hematological outcome for subjects in this study

Treatment group[a]	Mean hemoglobin level ($g\ dl^{-1}$) [95% CI] in subjects (n) on study day:					
	0	2	7	14	21	28
CD1	8.1 [7.8–8.4] (154)	7.4 [7.1–7.7] (149)	8.0 [7.8–8.3] (141)	8.8 [8.6–9.1] (131)	9.2 [8.9–9.5] (109)	9.7 [9.3–10.0] (81)
CD3	8.2 [7.9–8.4] (152)	7.4 [7.1–7.7] (147)	7.9 [7.6–8.2] (128)	8.9 [8.7–9.2] (129)	9.3 [9.0–9.6] (116)	9.3 [9.0–9.6] (120)
PS	8.1 [7.8–8.4] (142)	7.7 [7.4–8.0] (138)	8.2 [7.9–8.6] (118)	8.8 [8.5–9.1] (121)	9.4 [9.1–9.6] (121)	9.6 [9.4–9.9] (121)
Community surveillance	9.7 [9.4–10.0] (205)	Not done	Not done	9.9 [9.6–10.2] (186)	Not done	10.1 [9.8–10.4] (186)

[a] CD1, chlorproguanil-dapsone single dose; CD3, chlorproguanil-dapsone triple dose; PS, pyrimethamine-sulfadoxine single dose. Note that the community surveillance group was given no treatment.

TABLE 3. Status of patients and community surveillance group at trial end point

Treatment group[a]	Mean no. (%) of patients with outcome [95% CI]:			Geometric mean parasitemia (μl^{-1}) [95% CI]
	Parasitemic	Parasitemic with symptoms	Parasitemic and admitted to hospital	
CD1	101/154 (65.6) [57.5–73.0]	50/154 (32.5) [25.1–40.5]	0/154	3,472 [2,331–5,171]
CD3	62/152 (40.8) [32.9–49.0]	34/152 (22.4) [16.0–29.8]	1/152	4,219 [2,487–7,158]
PS	28/142 (19.7) [13.5–27.2]	14/142 (9.9) [5.5–16.0]	3/142	3,494 [1,508–8,093]
Community surveillance	64/205 (31.2) [24.9–38.0]	28/205 (13.7) [9.3–19.1]	0/205	1,358 [811–2,276]

[a] CD1, chlorproguanil-dapsone single dose; CD3, chlorproguanil-dapsone triple dose; PS, pyrimethamine-sulfadoxine single dose. Note that the community surveillance group was given no treatment.

point. When an intention-to-treat analysis was done, assuming that all children withdrawn became parasitemic, the relative risks of new parasitemia were 0.89 (0.74 to 1.08) for group CD3, 0.58 (0.45 to 0.76) for group PS, and 1.30 (1.13 to 1.50) for group CD1. Intention-to-treat analysis, assuming that no subjects who had withdrawn developed new parasitemia, gave relative risks of 1.71 (1.27 to 2.29), 0.80 (0.54 to 1.20), and 2.75 (2.14 to 3.53) for groups CD3, PS, and CD1, respectively.

DISCUSSION

Widespread pyrimethamine-sulfadoxine failure will be devastating for Africa because of Africa's enormous caseload and the lack of an affordable replacement. We are trying to develop both a means of postponing antifolate resistance and an affordable salvage therapy.

We recruited symptomatic young children with high parasitemia who were well enough for outpatient follow-up: this is the largest patient group attending African hospitals for malaria. Only children from within a defined study area were recruited, partly to increase the uniformity of the study population and partly to aid follow-up. The three patient groups had comparable baseline clinical and laboratory data, but the community surveillance group was significantly older—an unanticipated difference which occurred by chance. As expected, because the patients had malaria, their hemoglobin concentration was lower than that of the community surveillance group; similarly, because the patients had febrile illness, a greater proportion had detectable chloroquine in the blood. It is impossible to quantify the chemoprophylactic effect of chloroquine in this population. Although RIII resistance is still comparatively rare in Kenya (3), resistance to chloroquine at all levels on the Kenyan coast was 71% in 1988 (4), and prevalence was probably higher in 1993 as a result of continuing chloroquine use. National guidelines ceased to recommend chloroquine for children in 1992 because of poor efficacy. Very few children had a history of prior treatment with pyrimethamine-sulfadoxine or pyrimethamine-sulfalene, and sulfonamide was rarely detectable in predose blood, reflecting the current rarity of these drugs in the shops, which are the main source of drug treatment in this area (8).

The three patient groups were comparable on day 2: most patients had cleared parasitemia (a slightly larger proportion of group CD3), but mean hemoglobin levels had fallen. By day 7, there was no difference between the patient groups in terms of symptoms, temperature, parasitemia, or hemoglobin. However, during follow-up, the data suggest a high risk of recrudescence after a single dose of chlorproguanil-dapsone and a small degree of chemoprophylaxis after pyrimethamine-sulfadoxine. In contrast, after three daily doses of chlorproguanil-dapsone, the risk of new parasitemic illness did not differ significantly from that of the community surveillance group.

The lack of chemoprophylaxis after triple-dose chlorproguanil-dapsone could be a disadvantage—but no more than after treatment with any rapidly eliminated drug. Even so, we have recently started a second clinical trial to compare the annual incidences of symptomatic malaria in children treated with either pyrimethamine-sulfadoxine or triple-dose chlorproguanil-dapsone. Furthermore, the present chlorproguanil-dapsone regimen maintains effective synergistic concentrations for no longer than 6 days (23). This period could be prolonged in three ways: (i) by giving further doses of the present combination (which may reduce compliance), (ii) by formulating the present combination for extreme sustained release (which may be technically impossible and expensive), or (iii) by increasing the drug doses. The final possibility would be practicable, and we are therefore investigating the tolerability of higher chlorproguanil doses than those used in the present study.

Chlorproguanil-dapsone is likely to select less readily than pyrimethamine-sulfadoxine for resistant parasites, and the study needed to substantiate or refute this hypothesis is currently in progress. Perhaps even more important, chlorproguanil-dapsone is likely to retain activity against infections where pyrimethamine-sulfadoxine has failed. The in vitro potency of chlorcycloguanil against the Kenyan K39 strain (pyrimethamine resistant) of *P. falciparum* was 430-fold greater than that of pyrimethamine (26), and there is strong evidence that chlorproguanil-dapsone remains efficacious in an area of rural Tanzania where resistance to pyrimethamine-sulfadoxine is emerging (18). Mefloquine, halofantrine, quinine, artemisinins, pyronaridine, and atovaquone-proguanil are all potential salvage treatments for malaria resistant to pyrimethamine-sulfadoxine, but the main advantage of chlorproguanil-dapsone is likely to be its very low cost (probably no more than pyrimethamine-sulfonamide combinations), which is of immense importance in an African setting.

REFERENCES

1. **Anonymous.** 1995. Twelfth programme report of the UNDP-World Bank-WHO Special Programme for Research and Training in Tropical Diseases (TDR). World Health Organization, Geneva, Switzerland.
2. **Basco, L. K., P. Eldin de Pecoulas, C. M. Wilson, J. Le Bras, and A. Mazabraud.** 1995. Point mutations in the dihydrofolate reductase-thymidylate synthase gene and pyrimethamine and cycloguanil resistance in *Plasmodium falciparum*. Mol. Biochem. Parasitol. **69:**135–138.

3. **Bloland, P. B., E. Lackritz, P. Kazembe, J. B. O. Were, R. Steketee, and C. C. Campbell.** 1993. Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy. J. Infect. Dis. **167:** 932–937.

4. **Brandling-Bennett, A. D., A. Ollo, W. M. Watkins, D. A. Boriga, D. Kariuki, and W. E. Collins.** 1988. Chloroquine treatment of falciparum malaria in an area of Kenya of intermediate chloroquine resistance. Trans. R. Soc. Trop. Med. Hyg. **82:**833–837.

5. **Brooks, D. R., P. Wang, M. Read, W. M. Watkins, P. F. Sims, and J. E. Hyde.** 1994. Sequence variation of the hydroxymethyldihydropterin pyrophosphokinase: dihydropteroate synthetase gene in lines of the human malaria parasite *Plasmodium falciparum*, with differing resistance to sulfadoxine. Eur. J. Biochem. **224:**397–405.

6. **Foote, S. J., D. Galatis, and A. F. Cowman.** 1990. Amino acids in the dihydrofolate reductase-thymidylate synthase gene of *Plasmodium falciparum* involved in cycloguanil resistance differ from those involved in pyrimethamine resistance. Proc. Natl. Acad. Sci. USA **87:**3014–3017.

7. **Hyde, J. E.** 1990. The dihydrofolate-thymidylate synthetase gene in the drug resistance of malaria parasites. Pharmacol. Ther. **48:**45–59.

8. **Mwenesi, H., T. Harpham, and R. W. Snow.** 1995. Child malaria treatment among mothers in Kenya. Soc. Sci. Med. **49:**1271–1277.

9. **Nevill, C. G., E. S. Some, V. O. Mungala, W. Mutemi, L. New, K. Marsh, C. Lengeler, and R. W. Snow.** 1996. Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. Trop. Med. Int. Health **1:**139–146.

10. **Newton, C. R. J. C., P. A. Winstanley, W. M. Watkins, I. N. Mwangi, C. M. Waruiru, E. K. Mberu, N. Peshu, P. A. Warn, and K. Marsh.** 1993. Single dose intramuscular pyrimethamine-sulfadoxine as an adjunct to quinine in the treatment of severe malaria: pharmacokinetics and efficacy. Trans. R. Soc. Trop. Med. Hyg. **87:**207–210.

11. **Patchen, L. C., D. L. Mount, I. K. Schwartz, and F. C. Churchill.** 1983. Analysis of filter-paper-absorbed finger-stick blood samples for chloroquine and its major metabolites using high performance liquid chromatography with fluorescence detection. J. Chromatogr. **278:**81–89.

12. **Peterson, D. S., W. K. Milhous, and T. E. Wellems.** 1990. Molecular basis of differential resistance to cycloguanil and pyrimethamine in *Plasmodium falciparum* malaria. Proc. Natl. Acad. Sci. USA **87:**3018–3022.

13. **Plowe, C. V., A. Djimde, T. E. Wellems, B. Kouriba, and O. K. Doumbo.** 1996. Community pyrimethamine use and prevalence of resistant *Plasmodium falciparum* genotypes in Mali: a model for deterring resistance. Am. J. Trop. Med. Hyg. **55:**467–471.

14. **Radloff, P. D., J. Phillips, M. Nkeyi, D. Hutchinson, and P. G. Kremsner.** 1996. Atovaquone and proguanil for *Plasmodium falciparum* malaria. Lancet **347:**1511–1514.

15. **Snewin, V. A., S. M. England, P. F. Sims, and J. E. Hyde.** 1989. Characterisation of the dihydrofolate reductase-thymidylate synthetase gene from human malaria parasites highly resistant to pyrimethamine. Gene **76:**41–52.

16. **Snow, R. W., J. R. M. Armstrong, N. Peshu, D. Forster, C. R. J. C. Newton, P. A. Winstanley, I. Mwangi, C. Waruiru, P. A. Warn, C. Newbold, and K. Marsh.** 1993. Periodicity and time-space clustering of severe childhood malaria on the Kenyan coast. Trans. R. Soc. Trop. Med. Hyg. **87:**386–390.

17. **Sudre, P., J. G. Breman, D. McFarland, and J. P. Koplan.** 1992. Treatment of chloroquine-resistant malaria in African children: a cost-effectiveness analysis. Int. J. Epidemiol. **21:**146–154.

18. **Trigg, J. K., H. Mbwana, O. Chambo, E. Hills, J. Curtis, S. Issifou, M. Duraisingh, and C. F. Curtis.** 1996. Resistance in Tanzanian *Plasmodium falciparum* to pyrimethamine-sulfadoxine and a test of chlorproguanil-dapsone. Ann. Trop. Med. Parasitol. **90:**408. (Abstract.)

19. **Triglia, A., and A. F. Cowman.** 1994. Primary structure and expression of the dihydropteroate synthetase gene of *Plasmodium falciparum*. Proc. Natl. Acad. Sci. USA **91:**7149–7153.

20. **Watkins, W. M., and M. Mosobo.** 1993. Treatment of *Plasmodium falciparum* malaria with pyrimethamine and sulphadoxine: a selective pressure for resistance in a function of long elimination half-life. Trans. R. Soc. Trop. Med. Hyg. **87:**75–79.

21. **Watkins, W. M., A. D. Brandling-Bennett, C. G. Nevill, J. Y. Carter, D. A. Boriga, R. E. Howells, and D. K. Koech.** 1988. Chlorproguanil/dapsone for the treatment of non-severe *Plasmodium falciparum* malaria in Kenya: a pilot study. Trans. R. Soc. Trop. Med. Hyg. **82:**398.

22. **Watkins, W. M., J. D. Chulay, D. G. Sixsmith, H. C. Spencer, and R. E. Howells.** 1987. A preliminary pharmacokinetic study of the antimalarial drugs proguanil and chlorproguanil. J. Pharm. Pharmacol. **39:**261–269.

23. **Winstanley, P., W. Watkins, D. Muhia, I. S. F. Szwandt, E. Amukoye, and K. Marsh.** 1997. Chlorproguanil-dapsone for uncomplicated falciparum malaria in young children: pharmacokinetics and therapeutic range. Trans. R. Soc. Trop. Med. Hyg. **91:**322–327.

24. **Winstanley, P. A.** 1996. Pyronaridine—a promising drug for Africa? Lancet **347:**2–3.

25. **Winstanley, P. A., W. M. Watkins, C. R. J. C. Newton, C. Nevill, E. Mberu, P. A. Warn, C. M. Waruiru, I. N. Mwangi, D. A. Warrell, and K. Marsh.** 1992. The disposition of oral and intramuscular pyrimethamine/sulfadoxine in Kenyan children with high parasitaemia but clinically non-severe falciparum malaria. Br. J. Clin. Pharmacol. **33:**143–148.

26. **Winstanley, P. A., E. K. Mberu, I. S. F. Szwandt, A. M. Breckenridge, and W. M. Watkins.** 1995. The in vitro activities of novel antifolate drug combinations against *Plasmodium falciparum* and human granulocyte CFUs. Antimicrob. Agents Chemother. **39:**948–952.