TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1997) 91, 468–472

Plasmodium falciparum: sensitivity *in vivo* to chloroquine, pyrimethamine/sulfadoxine and mefloquine in western Myanmar

F. M. Smithuis[1], F. Monti[1], M. Grundl[1], A. Zaw Oo[2], T. T. Kyaw[2], O. Phe[2] and N. J. White[3,4] *[1]Artsen Zonder Grenzen, Médecins Sans Frontières–Holland, Yangon, Myanmar; [2]Vector Borne Disease Control, Sittwe, Myanmar; [3]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [4]Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, Oxford, UK*

Abstract

In Rakhine State, on the western border of Myanmar, the efficacy of chloroquine (CQ) and pyrimethamine/sulfadoxine (PS), the current treatments for uncomplicated *Plasmodium falciparum* malaria in this area, was evaluated in an open comparative study of 289 patients, stratified prospectively into 3 age groups. Chloroquine treatment was associated with more rapid clinical recovery (P=0·03), but the overall cure rates were worse than for PS treatment; failure to clear parasitaemia or recrudescence within 14 d occurred in 72% (102/141) of cases treated with CQ compared to 47% (69/148) of those who received PS (P<0·0001, adjusted for age). Failure rates at day 28 increased to 82% (116/141) in the CQ group and 67% (99/148) in the PS group (P=0·003). The risk of treatment failure was significantly higher in children under 15 years old than in adults for both CQ (relative risk [RR]=2·6; 95% confidence interval [95% CI] 1·3–5·2) and PS (RR=2·2; 95% CI 1·4–3·3). Mefloquine (15 mg base/kg) proved to be highly effective as a treatment for CQ and PS resistant *P. falciparum*; only 2 of 75 patients (3%) had early treatment failures (≤day 7), and the overall failure rate by day 42 was 7%. There is a very high level of chloroquine and PS resistance in *P. falciparum* on the western border of Myanmar, but mefloquine was effective in the area.

Keywords: malaria, *Plasmodium falciparum*, chemotherapy, drug resistance, chloroquine, pyrimethamine/sulfadoxine, mefloquine, Myanmar

Introduction

Antimalarial drug sensitivity testing *in vitro* and *in vivo* in Myanmar has indicated a growing level of resistance to chloroquine (CQ) and pyrimethamine/sulfadoxine (PS), and more recently mefloquine, which is most serious on the eastern Thai–Myanmar border. Low-grade resistance to CQ emerged in the mid 1970s, and resistance to both CQ and PS has worsened in the 1980s (TIN *et al.*, 1982; TIN & HLAING, 1984; Vector Borne Diseases Control, Myanmar, unpublished reports, 1994). High-grade mefloquine resistance also occurs on the eastern border of Myanmar contiguous with the malarious areas of western Thailand, and the extent to which these highly resistant parasites have spread is uncertain (WHITE, 1992). In order to determine the efficacy of these drugs *in vivo* we conducted a controlled clinical trial, comparing CQ and PS in the treatment of uncomplicated falciparum malaria in adults and children in western Myanmar.

Patients and Methods

Study sites and population

The trial was carried out in 2 small villages, Dabine and Kuntaung, 22 km apart and located in the townships of Sittwe and Ponnagyun, in Rakhine State, western Myanmar. Malaria transmission in this area is seasonal with peaks at the beginning of the rainy season (April to June) and at the end of the monsoon (October to December). *Plasmodium falciparum* is responsible for approximately 80% of malaria infections in this area. Malariometric indices (spleen and parasite rates) obtained from schoolchildren in September 1995 suggested that Dabine is a mesoendemic area and Kuntaung is hyperendemic (F. Monti, unpublished observations). From July to November 1995, all people in these villages with complaints of fever were invited to attend the rural health centres (RHC) before taking drugs, where doctors and microscopists from *Artsen Zonder Grenzen (Médecins Sans Frontières*–Holland) and the state Vector Borne Disease Control (VBDC) team joined the RHC health personnel to conduct this prospective study. The study protocol was approved by the VBDC of Myanmar and the health authorities of Rakhine State.

Study procedure

A blood film was prepared from all patients presenting to the clinics with an axillary temperature >37·5°C or a recent history of fever. As malaria transmission was intense in the area, and thus asymptomatic parasitaemias would be expected, it was decided prospectively that only patients with fever (or a recent history of fever) and more than 1000 asexual malaria parasites/mm^3 of blood would be eligible for inclusion in the study. Malaria parasites were counted relative to 200 leucocytes in a thick blood film stained with Giemsa's stain (pH 7·2), assuming a standard whole blood leucocyte count of 8000/mm^3. Infants, pregnant women, and patients with symptoms or signs of complicated malaria (WHO, 1990), or a history of antimalarial treatment within the previous 48 h, were also excluded. Patients were enrolled into the study only after fully informed consent was obtained from them or their relative or guardian. A clinical examination and symptom questionnaire were completed in each case. The patients were stratified prospectively for age and allocated randomly to receive either CQ (Pharmamed, Malta: 10 mg base/kg on days 0 and 1, and 5 mg/kg on day 2) or PS (Pharmamed, Malta: 1·25 mg/kg and 25 mg/kg, respectively) as a single supervised dose. If the axillary temperature exceeded 39°C, patients were also given oral paracetamol (15 mg/kg) before antimalarial treatment. All drug administrations were supervised.

Patients were seen on days 0, 1, 2, 3, 7, 14, 21, and 28 and at any other time if they felt unwell and/or developed a fever. At each follow-up visit, malaria blood films were prepared and clinical symptoms and temperature were noted on the study record. The haemoglobin concentration was measured at enrolment and on day 14. Treatment failures were categorized using the standard World Health Organization criteria for aminoquinoline resistance.

Re-treatment

Patients with treatment failures at the RI or RII levels were re-treated with the alternative drug (i.e., patients failing after treatment with CQ were given PS, and vice versa). Patients with RIII resistance were treated with

Address for correspondence: Dr F. M. Smithuis, AZG (MSF–Holland), c/o UNICEF EAPRO, Yangon Pouch for MSF–Holland, P.O. Box 2154, Bangkok 10200, Thailand.

mefloquine (Mephaquine®, Mepha Ltd, Aesch-Basel, Switzerland, 15 mg base/kg). However, when it became apparent early in the trial that high grade resistance to both CQ and PS was common, the original study plan was changed and all recrudescent infections were treated thereafter with mefloquine 15 mg/kg. Patients treated with mefloquine were followed for 42 d, and any falciparum malaria occurring within this time was considered to be a recrudescent infection. Treatment failures following mefloquine 15 mg/kg were re-treated with high dose mefloquine (25 mg/kg). Thus the study comprised 3 treatment groups: (i) initial treatment with either CQ or PS, (ii) re-treatment with the alternative drug following treatment failure, and (iii) mefloquine re-treatment as a second or third drug.

Statistical analysis

The proportions of patients with parasitaemia or symptoms are given only for those patients who attended follow-up on the specified days. Patients who missed an appointment and attended on the subsequent day were not included in these calculations. Continuous data were analysed by Student's *t* test and proportions were assessed by the χ^2 test with Yates's correction, or Fisher's exact test. The Mantel–Haenszel procedure was used for stratified analyses. Data were analysed with EpiInfo, version 6 (Centers for Disease Control and Prevention, Atlanta, Georgia, USA).

Results

Overall, 296 patients with uncomplicated falciparum malaria were recruited: 160 in Dabine and 136 in Kuntaung. Seven patients withdrew from the study, 3 for medical reasons and 4 because they did not attend for follow-up. No patient died. Thus 155 patients completed the treatment in Dabine (79 with CQ and 76 with PS) and 134 in Kuntaung (62 with CQ and 72 with PS). Baseline clinical and laboratory characteristics on admission are shown in Table 1. Geometric mean parasite densities on admission at the 2 sites were significantly different: 15 463/mm^3 (range 1000 to 97 720) in Dabine compared with 5024/mm^3 (range 1000 to 68 800) in Kuntaung where transmission was more intense ($P<0{\cdot}0001$), although there was no significant difference between the 2 treatment groups at either site. In Dabine, where malaria transmission was less intense, there was a significant difference in parasite densities in the 3 age groups with the highest density in children aged <5 years, and the lowest in adults. In Kuntaung, where malaria was hyperendemic, there was no significant difference in parasitaemia between the 3 age groups. Most children aged under 15 years were anaemic (haemoglobin ≤10 g/dL) on the day of enrolment: 75% (98/131) in Dabine and 86·5% (90/104) in Kuntaung; 29 of the children had haemoglobin concentrations ≤8 g/dL.

Table 1. Admission variables of patients with falciparum malaria

	Dabine[a]		Kuntaung[a]	
	CQ	PS	CQ	PS
No. of patients	79	76	62	72
Age composition				
<5 years	37%	38%	26%	25%
5–14 years	47%	47%	55%	50%
>14 years	16%	15%	19%	25%
Mean age (years)[b]	9·5(1·2–55)	9·4(1·5–50)	10·3(1·5–61)	12·8(1·5–63)
Males (%)	61	62	47	50
Haemoglobin (g/dL)[b]	10·0(8–13)	10·0(7–14)	9·6(7–13)	9·7(5–14)
Geometric mean parasite density/mm^3	14713	16281	4950	5087

[a]CQ=chloroquine, PS=pyrimethamine/sulfadoxine.
[b]Range in parentheses.

Clinical and parasitological responses

Both CQ and PS were well tolerated. There was no serious adverse effect. The majority of patients given either antimalarial drug failed treatment: 86% (68/79) of those treated with CQ in Dabine failed, and 77% (48/62) of those in Kuntaung ($P=0{\cdot}2$). With PS, 70% (53/76) failed in Dabine and 64% (46/72) in Kuntaung ($P=0{\cdot}5$) (Table 2). High grade symptomatic (RIII) failures occurred in 12% (34/289) of patients overall: 22 of 141 (16%) in the CQ groups and 12 of 148 (8%) in the PS groups (relative risk [RR]=1·9, 95% confidence interval [95% CI] 1·0–3·7; $P=0{\cdot}048$). The overall cure rate assessed at 28 d was 18% (25/141) for CQ and 33% (49/148) for PS (RR=0·54, 95% CI 0·35–0·82; $P=0{\cdot}003$). Despite this inferior overall efficacy, the rate of clinical response was faster with CQ; 78% (71/91) of patients treated with CQ had improved clinically by day 2 compared to 64% (62/97) of those treated with PS ($P=0{\cdot}03$). This difference was evident only in the area with less transmission (Dabine), where 73% (44/60) of patients given CQ had improved by day 2 compared to 51% (29/57) of those receiving PS (RR=1·4, 95% CI 1·1–1·9; $P=0{\cdot}01$) (Table 2). The differences in rates of clinical response were most evident in those patients with partially sensitive infections (RII, RI or sensitive); in Dabine 83% (39/47) of these patients treated with CQ had improved by day 2 compared to 56% (28/50) of the PS recipients (RR=1·48, 95% CI 1·1–2·0; $P=0{\cdot}0004$). The failure rates in children <5 years old, and in children aged between 5 and 14 years, were similar but, compared with adults, children had significantly higher failure rates for both CQ ($P=0{\cdot}008$) and PS ($P<0{\cdot}01$). On the day of treatment failure or recrudescence, 45% (42/116) of patients receiving CQ, and 39%

Table 2. Clinical and parasitological response to initial antimalarial treatment

	Dabine[a]		Kuntaung[a]	
	CQ	PS	CQ	PS
Parasitological response				
RIII	14/79(18%)	8/76(11%)	8/62(13%)	4/72(6%)
RII	28/79(35%)	15/76(20%)	20/62(32%)	23/72(32%)
RI	26/79(33%)	30/76(39%)	20/62(32%)	19/72(26%)
Sensitive	11/79(14%)	23/76(30%)	14/62(23%)	26/72(36%)
Clinical response				
Clinically improved by day 2[b]	44/60(73%)	29/57(51%)	27/31(87%)	33/40(83%)
Symptomatic on day of failure[c]	33/68(49%)	23/52(43%)	19/48(40%)	16/46(35%)
Clinical failure[d]	30/79(38%)	15/76(20%)	16/62(26%)	13/72(18%)

[a]CQ=chloroquine, PS=pyrimethamine/sulfadoxine.
[b]Clinical improvement among febrile patients (day 0 axillary temperature >38°C) was defined as decrease of the temperature to <37·5°C on day 2.
[c]Patients were considered symptomatic if the axillary temperature was >37·5°C or there was a recent history of fever with or without other symptoms.
[d]Clinical failure: RIII/RII or RI resistance within 14 d of onset of treatment together with symptoms (axillary temperature >37·5°C or recent history of fever).

Table 3. Parasitological response to antimalarial treatment stratified by age

	Response			
	S	RI	RII	RIII
Chloroquine				
Age <15 years	16/116(14%)	33/116(28%)	45/116(39%)	22/116(19%)
Age ≥15 years	9/25 (36%)	13/25 (52%)	3/25 (12%)	0/25 (0%)
pyrimethamine/sulfadoxine				
Age <15 years	32/119(27%)	46/119(39%)	30/119(25%)	11/119 (9%)
Age ≥15 years	17/29 (59%)	3/29 (10%)	8/29 (28%)	0/29 (3%)

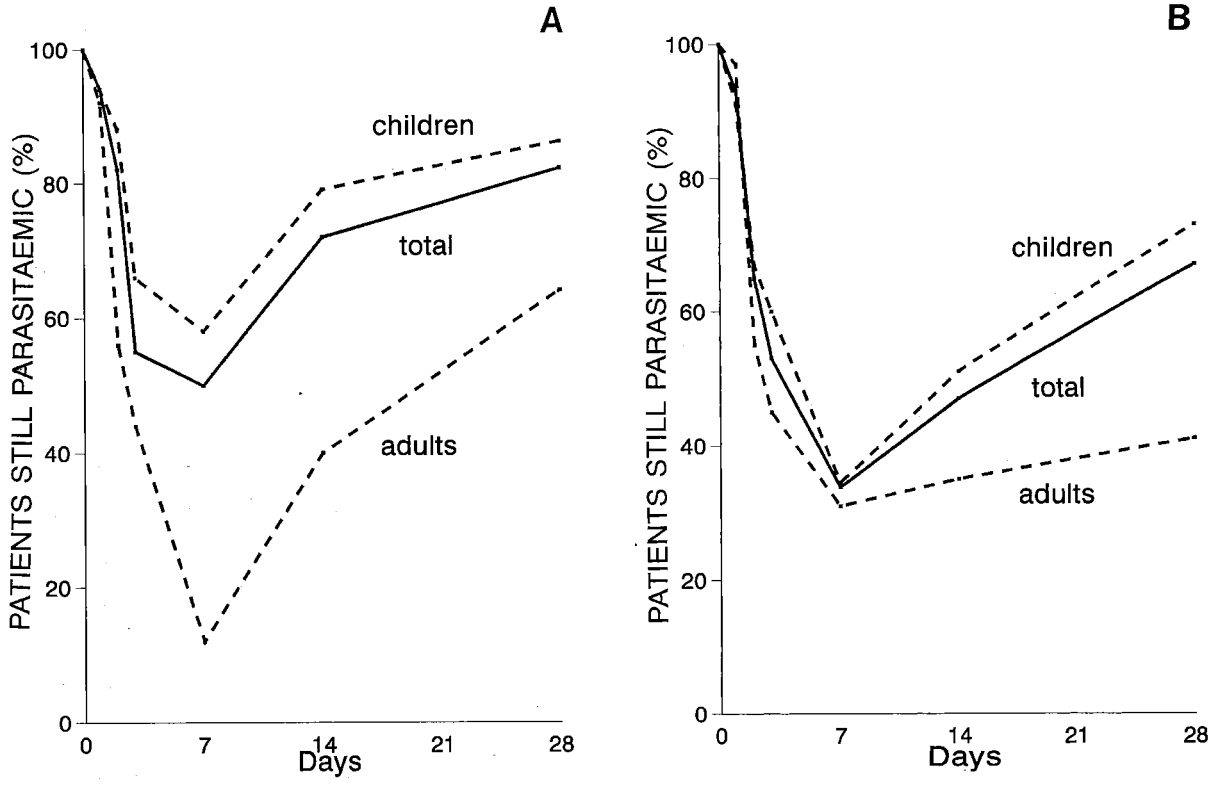

Fig. 1. Proportion of adults and children parasitaemic following (A) chloroquine and (B) pyrimethamine/sulfadoxine treatment of acute falciparum malaria.

(39/99) of those treated with PS, were symptomatic (axillary temperature >37·5°C or a recent history of fever) (P=0·63). Haemoglobin concentrations rose from a mean value of 9·9 g/dL (SD=1·2) in patients treated with CQ (n=141) to 10·1 g/dL (SD=1·2) at 14 d in the 83 patients who had not already failed. Corresponding figures for the PS recipients were 9·9 g/dL (SD=1·3) (n=148), rising to 10·3 g/dL (SD=1·2) (n=110) at day 14. These rises in haemoglobin concentration were not significantly different (P>0·2).

Results of re-treatment

Patients with RI and RII resistance (i.e., those who initially responded) and who were re-treated with the alternative drug, had a high rate of failure within 7 d: 59% (41/69) of re-treatments failed, 17/23 (74%) with CQ re-treatment and 24/46 (50%) with PS. This was unacceptable and, as a result, mefloquine re-treatment was instituted for all failures. Mefloquine proved highly effective: of the 75 patients who were followed for 42 d, 92% were cured, 2 (3%) failed within 7 d (RII), and 3 (4%) had late recrudescences (RI) on days 21, 28, and 42, respectively.

Discussion

This study showed that *P. falciparum* strains which are highly resistant to CQ and PS are prevalent in Rakhine State on the western border of Myanmar. Complete lack of response (RIII) occurred in 16% of patients given CQ and 8% of those receiving PS, and the overall cure rate assessed at day 28 was only 18% (25/141) after CQ and 33% (49/148) after PS treatment. Despite the different levels of malaria transmission reflected in different malariometric indices in the 2 studied populations, the overall cure rates were similar at both sites. However, the rate of clinical and parasitological response to CQ was significantly faster than that to PS, and this difference was most evident in the site with less transmission (Dabine), where 90% of such patients given CQ improved symptomatically, compared to only 56% of PS recipients. This discrepancy between the rate of clinical response and overall efficacy (measured as cure rate) for CQ and PS has been noted recently in The Gambia (ONYIORAH *et al.*, 1996), and probably reflects differences in the stage specificity of drug action (TER KUILE *et al.*, 1993), or possibly the anti-inflammatory effects of chloroquine (KWIATKOWSKI & BATE, 1995). It may give the misleading impression that the drug is still effective. Treatment failure rates were particularly high in children aged under 15 years—the population most at risk. On the day of treatment failure, 42% of patients were symptomatic (45% with CQ and 39% with PS). If

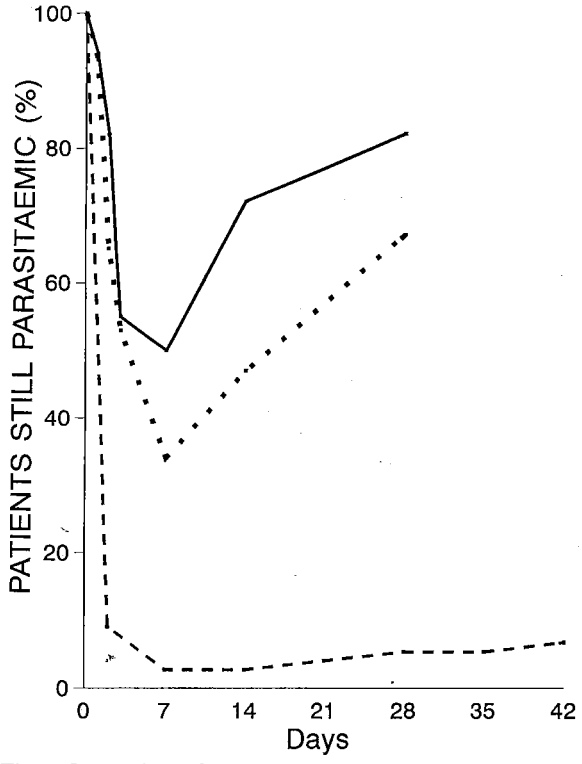

Fig. 2. Comparison of responses to antimalarial treatment with chloroquine (——), pyrimethamine/sulfadoxine (···) and mefloquine (---).

a clinical failure rate of 25% within 14 d after administration of a first line antimalarial treatment represents a threshold for a change in recommended drug regimen (WHO, 1994), then, in this study, CQ exceeded the threshold, and PS was close to it. Furthermore, these figures represent underestimates as patients were re-treated for parasitological failure, whether or not they were symptomatic. If it is assumed that most patients with parasitological failures would have become symptomatic, then both CQ and PS have already exceeded the threshold for a change in recommended antimalarial drug regimen. Both these drugs gave only temporary respite, at best, to the majority of patients. The lack of efficacy of the 2 first line drugs for antimalarial treatment probably contributes to the high levels of malaria transmission in this area of western Myanmar, and may be a significant contributor to morbidity, notably the anaemia found in the present study, and to impairment of the growth and development of children (SHIFF *et al.*, 1996).

In many tropical countries, cost is a major factor influencing the change to alternative, more effective drugs. Mefloquine treatment is at least 10 times as expensive as CQ and PS, although this calculation does not take into account the economic costs of protracted malaria morbidity or re-treatment associated with the latter drugs. In this study mefloquine proved highly effective at both sites and in all age groups, and it would be a satisfactory first line treatment, particularly for high-risk patients such as children and pregnant women. However, there were still early treatment failures with mefloquine which may indicate that the highly mefloquine-resistant strains of *P. falciparum*, prevalent on the eastern border of Myanmar, have already spread to the west (WHITE, 1992). Therefore, if mefloquine were to be deployed, it might be preferable to combine it with oral artesunate from the outset. This would accelerate the initial therapeutic response, improve overall cure rates, reduce transmission, and thus reduce the rate at which resistance develops (NOSTEN *et al.*, 1994; PRICE *et al.*, 1996).

Are there effective available and affordable alternatives to mefloquine? Amodiaquine is considerably cheaper than mefloquine and has proved effective against most moderately chloroquine-resistant strains of *P. falciparum* (see OLLIARO *et al.*, 1996). However, amodiaquine proved disappointing over 10 years ago in Thailand (LOOAREESUWAN *et al.*, 1985), and was less effective than CQ in The Philippines (WATT *et al.*, 1987). Whether amodiaquine, or the related compound pyronaridine, could be an effective alternative to mefloquine as a replacement for CQ and PS also remains to be determined. Further studies to determine the optimum treatment of multi-drug resistant falciparum malaria in this area are needed.

Acknowledgements

We are grateful to the staff of the Vector Borne Disease Control (DOH), the staff of *Artsen Zonder Grenzen, Médecins Sans Frontières* (MSF)–Holland, Sittwe, in particular Dr Sanda, Dr T. T. Zin and C. Rogers, for their assistance. We thank Supida Kaewsuksombat for typing the manuscript. This study was funded by *Artsen Zonder Grenzen* (MSF–Holland). Professor N. J. White is a Wellcome Trust Principal Fellow.

References

Kwiatkowski, D. & Bate, C. (1995). Inhibition of tumour necrosis factor (TNF) production by antimalarial drugs used in cerebral malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **89**, 215–216.

Looareesuwan, S., Phillips, R. E., White, N. J., Karbwang, J., Benjasurat, Y., Attanath, P. & Warrell, D. A. (1985). Intravenous amodiaquine and oral amodiaquine/erythromycin in the treatment of chloroquine-resistant falciparum malaria. *Lancet*, **ii**, 805–808.

Nosten, F., Luxemburger, C., ter Kuile, F. O., Woodrow, C., Pa Eh, J., Chongsuphajaisiddhi, T. & White, N. J. (1994). Optimum artesunate–mefloquine combination for the treatment of multi-drug resistant *Plasmodium falciparum* malaria. *Journal of Infectious Diseases*, **170**, 971–977.

Olliaro, P., Nevill, C., Le Bras, J., Ringwald, P., Mussano, P., Garner, P. & Brasseur, P. (1996). Systematic review of amodiaquine treatment in uncomplicated malaria. *Lancet*, **348**, 1196–1201.

Onyiorah, E., van Hensbroek, M. B., Jah, M. S. & Greenwood, B. (1996). Early clinical failures after pyrimethamine/sulfadoxine treatment of uncomplicated falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **90**, 307–308.

Price, R. N., Nosten, F., Luxemburger, C., ter Kuile, F., Paiphun, L., Chongsuphajaisiddhi, T. & White, N. J. (1996). The effects of artemisinin derivatives on malaria transmissability. *Lancet*, **347**, 1654–1658.

Shiff, C., Checkley, W., Winch, P., Premji, Z., Minjas, J. & Lubega, P. (1996). Changes in weight gain and anaemia attributable to malaria in Tanzanian children living under holoendemic conditions. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **90**, 262–265.

Ter Kuile, F., White, N. J., Holloway, P., Pasvol, G. & Krishna, S. (1993). *Plasmodium falciparum: in-vitro* studies of the pharmacodynamic properties of drugs used for the treatment of severe malaria. *Experimental Parasitology*, **76**, 86–95.

Tin, F. & Hlaing, N. (1984). Comparative drug trial of a sulfadoxine/pyrimethamine and a sulfalene/pyrimethamine combination against *Plasmodium falciparum* infections in semi-immune populations of Burma. *Southeast Asian Journal of Tropical Medicine and Public Health*, **15**, 238–248.

Tin, F., Hlaing, N. & Lasserre, R. (1982). Single-dose treatment of falciparum malaria with mefloquine: field studies with different doses in semi-immune adults and children in Burma. *Bulletin of the World Health Organization*, **60**, 913–917.

Watt, G., Long, G. W., Padre, L., Alban, P., Sangalang, R., Ranoa, C. P. & Laughlin, L. W. (1987). Amodiaquine less effective than chloroquine in the treatment of falciparum malaria in the Philippines. *American Journal of Tropical Medicine and Hygiene*, **36**, 3–8.

White, N. J. (1992). Antimalarial drug resistance: the pace quickens. *Journal of Antimicrobial Agents and Chemotherapy*, **30**, 571–585.

WHO (1973). *Chemotherapy of Malaria and Resistance to Anti-*

malarials. Geneva: World Health Organization, Technical Report Series, no. 529.
WHO (1990). Severe and complicated malaria, 2nd edition. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **84**, supplement 2.
WHO (1994). *Antimalarial drug policies: data requirements, treatment of uncomplicated malaria and management of malaria in pregnancy. Report of an informal consultation.* Geneva: World Health Organization, mimeographed document WHO/MAL/94.1070.

Received 29 August 1996; revised 26 November 1996; accepted for publication 27 November 1996

Announcements

Second International Conference on *Mycobacterium ulcerans*
University of Melbourne, Australia: 1–3 April 1998

The conference will mark the 50th anniversary of the publication by MacCallum *et al.* describing the disease and the bacterium; the paper originated from the University and the Alfred Hospital, Melbourne.

The conference will be followed by a two days' bus tour of the two most important endemic areas in Victoria, incidentally passing through some of the most beautiful scenery in southern Australia.

Further information can be obtained from A/Professor John Hayman, Department of Pathology, Box Hill Hospital, Box Hill, VIC 3128, Australia; phone +61 (0) 3 9895 3146, fax +61 (0) 3 9899 6132.

Hepatology, Gastroenterology and Infectious Diseases
Cairo, Egypt: 16–19 December 1997

A Congress organized by the Egyptian Society of Hepatology, Gastroenterology and Infectious Diseases.

Further information can be obtained from Professor Kabil S. M., Cairo GIT and Liver Centre, 41 Talaat Harb Street, Central Cairo, Egypt; phone +20 2 3915115, fax +20 2 3938723.