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The disposition of oral and intramuscular pyrimethamine/sulphadoxine in Kenyan children with high parasitaemia but clinically non-severe falciparum malaria

P. A. WINSTANLEY[1,3,4], W. M. WATKINS[1,2,4], C. R. J. C. NEWTON[1,3], C. NEVILL[5], E. MBERU[2], P. A. WARN[1,3], C. M. WARUIRU[1,2], I. N. MWANGI[1,2], D. A. WARRELL[3] & K. MARSH[1,3]
[1]Kenya Medical Research Institute, Kilifi Research Unit, P.O. Box 428, Kilifi, Kenya, [2]Kenya Medical Research Institute, Clinical Research Centre, Nairobi, Kenya, [3]Nuffield Department of Clinical Medicine, the University of Oxford, UK, [4]Department of Pharmacology and Therapeutics, the University of Liverpool, UK and [5]African Medical and Research Foundation, Nairobi, Kenya

1 H.p.l.c. methods are described for the measurement of pyrimethamine and sulphadoxine in small volumes of plasma dried on filter paper strips.
2 Pyrimethamine/sulphadoxine (Fansidar, Hoffman LaRoche) was given by mouth and by intramuscular injection to children with uncomplicated falciparum malaria but with high parasitaemia (n = 8 for both routes; pyrimethamine 1.25 mg kg^{-1}, sulphadoxine 25 mg kg^{-1}).
3 Plasma concentrations of pyrimethamine and sulphadoxine associated with synergistic effects against pyrimethamine-resistant strains of *Plasmodium falciparum in vitro* were achieved within 1 h of administration and were maintained beyond the end of sampling.
4 After both oral and parenteral administration the plasma concentrations of both compounds were lower than those predicted by data from healthy subjects.
5 Areas under the plasma concentration-time curves of sulphadoxine after oral and i.m. administration did not differ significantly, although maximum plasma drug concentrations were higher after the i.m. route (P = 0.03).
6 The AUC values of pyrimethamine did not differ significantly between the two routes of administration. However, after i.m. administration AUC(0,24 h) values were smaller (P = 0.03), and the time to maximum plasma drug concentration (t_{max}) was longer (P = 0.004) than when the drug was given orally.

Keywords pyrimethamine sulphadoxine non-severe malaria pharmacokinetics

Introduction

With the spread of chloroquine-resistant falciparum malaria in Africa, Fansidar® (Hoffman LaRoche) (PSD), an oral formulation of pyrimethamine (PM) and sulphadoxine (SD) is used increasingly for the treatment of non-severe malaria (Salako *et al.*, 1990). PSD is highly effective in much of the Continent (Salako *et al.*, 1990; Watkins *et al.*, 1988),although sporadic resistance has been reported from some areas (Gubler, 1988; Lege-Oguntoye *et al.*, 1990; Pierce *et al.*, 1988; Timmermans *et al.*, 1982).

There is considerable clinical experience with oral PSD (Nevill & Watkins, 1990; Schapira *et al.*, 1986; Spencer *et al.*, 1984; Watkins *et al.*, 1988), and its pharmacokinetics have been described in healthy subjects (Edstein, 1987; Weidekamm *et al.*, 1982), during malaria prophylaxis (Hellgren *et al.*, 1990a) and in parasitaemic children (Hellgren *et al.*, 1990b). There is less clinical experience with the parenteral formulation of PSD (Harinasuta *et al.*, 1988), although it has been used for about 20 years in francophone West Africa (Farrero *et al.*, 1973; Niang *et al.*, 1975), and more recently in Nigeria (Salako *et al.*, 1990). It is possible that this formulation will be used in severely ill patients (Niang *et al.*, 1975; Salako *et al.*, 1990), but its pharmacokinetics have been described in neither healthy subjects nor in patients with malaria. The time to achieve and the duration of synergistic drug concentrations (Berenbaum, 1973) need to be determined for the

Correspondence: Dr P. A. Winstanley, Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool

parenteral formulation, and compared with those of the oral drug. Because malaria is primarily a paediatric problem in our area, and since age can affect drug disposition, we chose to study the kinetics of the two drugs in children.

Methods

The study was approved by the Ethics Committee of the Kenya Medical Research Institute (KEMRI). Parents gave written informed consent.

Patients

Children were studied if they had clinical malaria with parasitaemia between *circa* 10^4 and 10^6 μl^{-1}, and were excluded if they had clinically severe malaria (Warrell *et al.*, 1990); a history of treatment with PSD or Metakelfin (PM/sulphametopyrazine; Farmitalia); or a history of sensitivity to antibiotics.

Clinical care, dosing and sampling

Patients were treated in the KEMRI intensive care ward of Kilifi District Hospital. Children were weighed and venous access was obtained using Teflon® cannulae (these were used for blood sampling until discharge; thereafter blood was drawn by venepuncture). Blood was drawn for full blood count, glucose and blood culture. Plasma was separated for the measurement of chloroquine. Blood films were done 6 hourly after treatment until parasites had cleared.

The doses of PM and SD were 1.25 mg kg^{-1} and 25 mg kg^{-1}, respectively. PSD was given orally to eight children as a suspension (prepared extemporaneously by suspending powdered Fansidar tablets in syrup; PM 2.5 mg ml^{-1}; SD 50 mg ml^{-1}) by syringe. Patients who vomited within 1 h of oral dosing were given a further dose of PSD, and removed from the study. PSD was given i.m. (Fansidar Parenteral; Hoffman LaRoche; PM 10 mg ml^{-1}, SD 200 mg ml^{-1}] to a further eight children by deep injection into the anterior thigh.

Blood (1.0 ml) was drawn for the measurement of PM and SD pre-dose and at the following times after dosing: 1, 2, 4, 6, 12, 24, 48, 72, 96, 120 and 240 h. Samples were transferred to plastic lithium heparin tubes and centrifuged (1000 *g* for 5 min) within 8 h. Plasma was transferred in duplicate to filter paper strips (200 μl for measurement of PM and 50 μl for SD), dried, and stored at room temperature protected from sunlight. Additional samples (1.0 ml) were drawn 6 h after dosing for measurement of whole blood drug concentrations.

Chemicals and reagents

Filter paper strips (Whatman chromatography paper grade 17) were a gift from Dr F. Churchill (Centres for Disease Control, USA). Antigen and antibody for the chloroquine enzyme-linked immunoassay (ELISA), were gifts from Dr T. Eggelte (Royal Tropical Institute, Amsterdam, The Netherlands). Acetonitrile (h.p.l.c. grade), glacial acetic acid, orthophosphoric acid, ethyl acetate, ammonia (SG 0.91) and methanol (all AnalaR grade) were obtained from BDH Chemicals (Poole, UK). Methanol and ethyl acetate were redistilled before use. 1-octane sulphonic acid, PM and sulphisoxazole (SS) were obtained from Sigma Chemicals (St Louis, USA). SD was obtained from Hoffman LaRoche (Nutley, USA), while proguanil (PG) was supplied by ICI (Cheshire, UK).

Drug assays

Sulphadoxine The filter paper strips were cut up, incubated in acetate buffer (0.1 M; pH 3.48; 5 min) containing internal standard (SS; 5 μg) and extracted, by vortex mixing (30 s), into hexane/ethyl acetate (1:1, v/v; 5.0 ml) in silanised glass culture tubes. After centrifugation (2000 *g*, 15 min) the organic phase was transferred to clean tubes and evaporated to dryness under N_2 (37° C). Samples were reconstituted in mobile phase (120 μl) and 50 μl aliquots injected into the chromatograph. Calibration samples were prepared identically, from filter paper blots of drug-free plasma to which was added SD in known concentrations.

Chromatography was performed using an Isochrom solvent delivery system (No. A0099-314; Spectra Physics; San Jose, USA) connected to a Rheodyne valve injector (50 μl injection loop). A stainless steel column packed with Ultrasphere ODS (5 μm particle size, 15 cm × 4.6 mm internal diameter) preceded by a CN guard column (Waters Ass; Milford, USA) was used. The mobile phase comprised water:methanol: acetonitrile (70:19:11 v/v; premixed and de-gassed ultrasonically) containing 1-octane sulphonic acid (4.6 mmol l^{-1}) at a flow rate of 2.0 ml min^{-1}. The column effluent was monitored with a variable wavelength u.v. absorbance detector (No. A0099-307; Spectra Physics) set at 274 nm.

Pyrimethamine The filter paper strips were cut up, incubated in ammonia (sg 0.91; 1.0 ml, 5 min) containing internal standard (PG, 50 ng) and extracted into ethyl acetate (5 ml) by vortex mixing (30 s) in silanised glass culture tubes. Subsequent sample processing was as above.

Chromatography was performed using a model SP8700 solvent delivery system equipped with a dynamic mixer (SP 8500) and organizer module (SP8750; all Spectra Physics, San Jose, USA) and connected to a Rheodyne valve injector (50 μl injection loop). A plastic column (Microbondapak phenyl; 10 μm particle size) in a radial compression module (Z-module) preceded by a CN guard column (all Waters Ass., Milford, USA) was used. The mobile phase comprised water:acetonitrile:methanol (60:30:10 v/v) containing 1-octane sulphonic acid (5.0 mmol l^{-1}) adjusted to pH 2.5 with orthophosphoric acid and flowing at 3.0 ml min^{-1}. The column effluent was monitored using a variable wavelength u.v. absorbance detector (SP8773; Spectra Physics) set at 254 nm.

The intra-assay and inter-assay (weekly for 6 weeks) reproducibility of both methods was assessed using drug-free plasma to which was added SD (5 and 100 μg ml^{-1}) or PM (50 and 600 ng ml^{-1}), and which was then transferred to filter paper strips and treated in the same way as the unknowns.

Chloroquine Chloroquine was measured by ELISA using the method of Shenton *et al.* (1988).

Calculations

Values of C_{max} and t_{max} were noted directly from the plasma drug concentration data. All AUC values were estimated using the linear trapezoidal rule with extrapolation to infinity using the value of the terminal elimination rate constant determined from the concentration data points. The rate of decline of parasitaemia was calculated by non-linear regression of all data points between, and including, maximum parasitaemia ($Para_{max}$) and the last available data point. Where the correlation coefficient was significant at the 5% level, or better, the slope of the line was obtained and the apparent parasite elimination rate constant (k_{para}) was calculated from 2.303 × the slope.

The Mann-Whitney U test was used for between group comparisons.

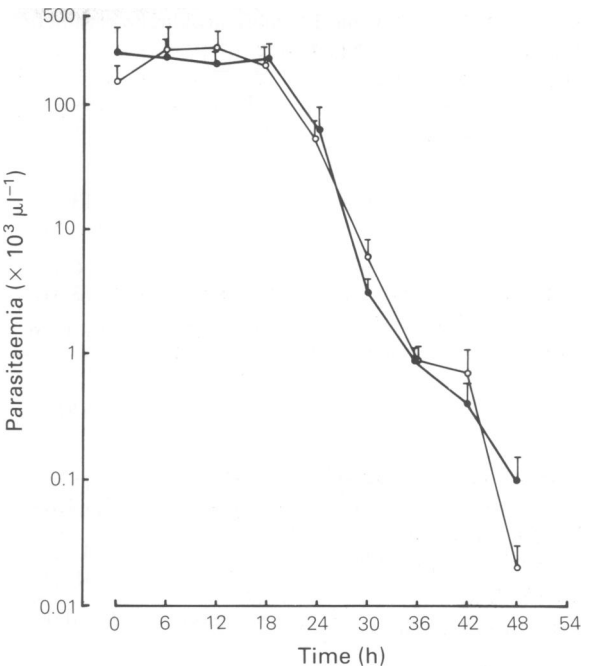

Figure 1 Parasite count ($\times 10^3$ μl^{-1}; mean ± s.d.) *vs* time (h after administration of the drug) in patients given oral (●) and i.m. (○) pyrimethamine/sulphadoxine.

Results

Clinical progress

Sixteen children were studied (eight given oral, and eight i.m. PSD; Table 1). One patient had *Salmonella* septicaemia. All had detectable plasma chloroquine concentrations on admission, and there was no difference in chloroquine concentration between the groups (Table 1). All patients achieved clinical cure and recovered without sequelae. No adverse reactions were recorded.

Parasite kinetics

There was no significant difference in $Para_{max}$ between the orally and parenterally treated groups ($P = 0.75$). In both groups a 'plateau phase' (White & Krishna, 1989) of about 18 h was observed, during which the parasite concentration was constant or increased (Figure 1). This was followed by a log-linear decline in 14 patients ($n = 6$ and 8 for oral and i.m. treatment, respectively). There was no significant difference in k_{para} between the orally and i.m. treated patients (0.21 ± 0.06 h^{-1} after oral PSD, and 0.25 ± 0.15 h^{-1} after i.m. PSD; $P = 0.55$). No patients had parasitaemia on the tenth day after treatment.

Assay specifications

Sulphadoxine SD and SS (internal standard) were resolved to baseline, and were free of interference from endogenous compounds, PM, chloroquine, quinine, PG, cycloguanil, chlorproguanil, chlorcycloguanil, dapsone and sulphamethoxazole. The lower assay limit for SD from 50 µl plasma was 1.0 mg l^{-1} which, at 0.005 absorbance units full scale (AUFS), gave a peak > ×4 background. Intra-assay coefficients of variation (CV) were 3.6 and 4.3% at 5 and 100 mg l^{-1}, respectively; inter-assay CVs were 9.9 and 2.2%, respectively. Calibration curves were linear ($r^2 > 0.98$) within the range 5–100 µg ml^{-1}.

Pyrimethamine PM and PG (internal standard) were resolved to baseline and were free of interference from

Table 1. Admission variables

Age (months)	*Parasitaemia (μl^{-1})*	*Haemoglobin (g dl^{-1})*	*Plasma chloroquine (ng ml^{-1})*
Oral PSD			
41*	259,263	8.1	261
(23)	(346,383)	(1.8)	(59)
range 12–72	28,200–1,025,200	5.5–10.2	169–330
i.m. PSD			
31.9	153,230	8.4	257
(21.4)	(156,596)	(3.2)	(76)
range 5–72	18,200–276,100	4.4–14.1	173–351

*Figures are mean (± s.d.); $n = 8$ in each group.

Table 2 Pharmacokinetic parameters (mean ± s.d.) of pyrimethamine and sulphadoxine

	C_{max} ($mg\ l^{-1}$)	t_{max} (h)	AUC ($mg\ l^{-1}\ h$)		$t_{1/2,z}$ (h)
Sulphadoxine					
Oral	79	13.5	20013		116
	(22)	(15.5)	(12218)		(65)
i.m.	115	6.4	24253		126
	(30)	(3.8)	(11432)		(38)
	$P = 0.03$	$P = 0.19$	$P = 0.20$		$P = 0.20$
	C_{max} ($\mu g\ l^{-1}$)	t_{max} (h)	AUC ($\mu g\ l^{-1}\ h$)	$AUC(0,24h)$ ($\mu g\ l^{-1}\ h$)	$t_{1/2,z}$ (h)
Pyrimethamine					
Oral	533	12.0	62573	9652	81
	(242)	(14.9)	(38867)	(5964)	(32)
i.m.	288	41.1	61423	3898	124
	(46)	(18.1)	(21169)	(2174)	(36)
	$P = 0.06$	$P = 0.004$	$P = 0.77$	$P = 0.03$	$P = 0.09$

endogenous compounds, quinine, SD, SS, sulphamethoxazole, dapsone, chloroquine, chlorproguanil, cycloguanil and chlorcycloguanil. The lower limit for PM from 200 µl plasma was 20 µg l^{-1}, which at 0.002 AUFS gave a peak > ×4 background. Intra-assay CVs were 8.9 and 2.7% at 50 and 600 µg l^{-1}, respectively; inter-assay CVs were 8.9 and 3.3%, respectively. Calibration curves were linear within the range 50–600 ng ml^{-1} ($r^2 > 0.98$).

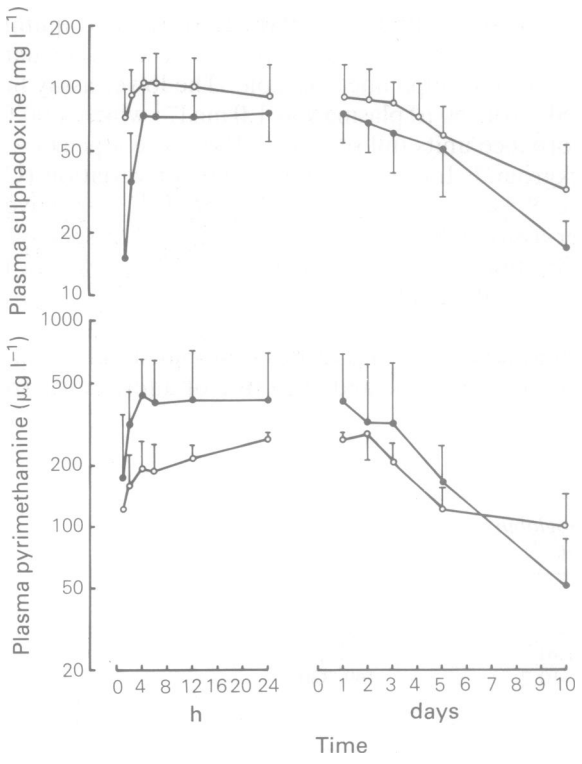

Figure 2 Plasma concentrations (mean ± s.d.) of sulphadoxine (upper graphs; mg l^{-1}) and pyrimethamine (lower graphs; µg l^{-1}) in patients given oral (•) and i.m. (○) pyrimethamine/sulphadoxine.

Pharmacokinetics of SD and PM

The maximum plasma concentration (C_{max}) of SD after i.m. administration was greater than that after oral dosing ($P = 0.03$, Figure 2), but there was no difference in AUC ($P = 0.20$, Table 2). At the end of sampling (240 h) plasma SD was 17 ± 6 and 33 ± 21 mg l^{-1} after oral and i.m. dosing, respectively (mean ± s.d.). The blood:plasma concentration ratio of SD, 6 h after dosing, was 0.43 ± 0.32, and was below unity in all samples ($n = 8$).

PM appeared to be more slowly absorbed after i.m. than oral administration (Figure 2), since t_{max} was longer (41.1 *vs* 12.0 h; $P = 0.004$; Table 2) and AUC(0,24 h) was less ($P = 0.03$). However, there was no significant difference in total AUC between the two routes of administration. The terminal phase half-life ($t_{1/2,z}$) was longer after i.m. administration (124 *vs* 87 h), but the difference was not statistically significant ($P = 0.09$). At the end of sampling plasma PM was 52 ± 39 and 103 ± 43 µg l^{-1} after oral and i.m. administration, respectively. The blood:plasma concentration ratio of PM at 6 h after dosing was 1.09 ± 0.46 ($n = 8$; range 0.39 to 1.96).

Discussion

Patients without manifestations of severe malaria (Warrell *et al.*, 1990) are often very unwell. In our series of 500 cases of non-severe malaria about 3% have developed 'severe' disease after admission despite treatment with chloroquine, and three of them have died despite the administration of parenteral quinine (unpublished data). Falciparum malaria in young children is always life-threatening and the prompt achievement of adequate concentrations of an effective drug is essential. Unfortunately, resistance to the first line drug, chloroquine, is increasing in East Africa

(Brandling-Bennett *et al.*, 1988); PSD is the most likely alternative for the treatment of non-severe malaria because of the high cost of mefloquine and halofantrine and because the use of quinine is impractical.

Rates of decline of parasitaemia in the present study were comparable with those in our patients treated with quinine (Pasvol *et al.*, 1991). Chloroquine was detectable in the plasma of patients from both the present study, and that of Pasvol *et al.* (1991), but its contribution to antimalarial effect in this area of extensive chloroquine-resistance cannot be determined. The assumption that similar rates of peripheral parasite clearance necessarily indicate equivalent therapeutic efficacy of two drugs is false (White & Krishna, 1989). In particular, the point of action of antifolate drugs in the life cycle of the parasite is probably too late to interfere with sequestration (White & Krishna, 1989). However, the demonstration of rapid parasite decline after PSD together with favourable clinical comparisons of PSD with aminoquinolines in non-severe malaria (Keuter *et al.*, 1990; Salako *et al.*, 1990; Schapira *et al.*, 1986) support the wider use of this drug.

Plasma concentrations of SD and PM observed in the present study were lower than expected. After healthy adults were given oral SD (about 7 mg kg^{-1}) C_{max} was about 60 mg l^{-1} (Weidekamm *et al.*, 1982), whereas in the present study a dose of 25 mg kg^{-1} was described with mean C_{max} values of only 79 (oral) and 115 mg l^{-1} (i.m.). In the study of Weidekamm *et al.* (1982) healthy adults given oral PM (0.4 mg kg^{-1}) had a mean C_{max} value of 0.2 mg l^{-1}, whereas in the present study 1.25 mg kg^{-1} was associated with a mean C_{max} of only 0.53 (oral) and 0.29 mg l^{-1} (i.m.). Likewise, although the apparent elimination half-lives of PM in this study were similar to those reported by Weidekamm *et al.* (1982), those for SD (116 and 126 h) were shorter than previous values (Hellgren *et al.*, 1990a,b; Weidekamm *et al.*, 1982). The cause of these differences in PM and SD disposition is unknown, but they may reflect the effects of malaria. Age differences alone do not explain the disparity since PSD kinetics have been reported in children, and plasma drug concentrations were similar to those in adults (Hellgren *et al.*, 1990b). These same children did have parasitaemia but counts ranged only from 430–11,500 μl^{-1}; all of the subjects were well enough to attend school and the patients' temperatures were not reported. It is likely that none of these children had clinical malaria.

Synergistic concentrations effective against PM-resistant parasites (K39 strain; 15 $\mu g\ l^{-1}$ PM, 60.0 mg l^{-1} SD; Chulay *et al.*, 1984) were exceeded within 1 h irrespective of route of drug administration, and were maintained beyond the end of sampling. SD achieved higher C_{max} values after i.m. than after oral dosing, although the extent of absorption, as indicated by total AUC, was not significantly different. In contrast, over the first 24 h, PM appeared to be absorbed more slowly and less extensively when given i.m. than orally. However, there was no significant difference in total AUC values of PM between the oral and i.m. groups. The apparent terminal $t_{1/2,z}$ was slower after i.m. dosing, although this difference did not reach significance ($P = 0.09$). PM is poorly soluble in water (Martindale, 1989), and those observations may be explained by absorption rate-limited elimination.

The drug recommended by the World Health Organisation for the treatment of severe malaria in areas of extensive chloroquine resistance is quinine (Warrell *et al.*, 1990). However, management strategies in the tropics are often dictated by practical necessities. For example, the i.m. administration of chloroquine was considered dangerous for several years (WHO, 1984), but continued to be widely used (Phillips *et al.*, 1987; White *et al.*, 1989). Quinine is not uniformly available in African hospitals, and doctors faced with the problem of increasing resistance to chloroquine will be tempted to use i.m. PSD, if it is available, to treat severe malaria. In this aspect the lower rate of absorption of PM after i.m. dosing may be of concern. None of the patients in this series had severe malaria (Warrell *et al.*, 1990), and the absorption of PM could be further perturbed in such patients, especially if limb blood flow were reduced. Although not the only consideration, the pharmacokinetic results of the present study counsel caution in the adoption of i.m. PSD as sole treatment for severe malaria until its disposition in such patients has been studied.

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