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Multiple-dose pharmacokinetics and *in vitro* antimalarial activity of dapsone plus pyrimethamine (Maloprim®) in man

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1 The multiple-dose kinetics of dapsone (DDS), its major metabolite monoacetyldapsone (MADDS) and pyrimethamine (PYR) were studied in six healthy adult male volunteers following weekly administration of Maloprim® (100 mg DDS plus 12.5 mg PYR).

2 After the last maintenance dose of Maloprim, the following kinetic parameters (mean values) were determined for DDS and PYR, respectively: maximum plasma concentration (C_{max}) = 1,134 and 116 ng ml^{-1}; elimination half-life ($t_{½}$) = 23 and 105 h; plasma clearance (CL) = 37.6 and 15.9 ml h^{-1} kg^{-1} and apparent volume of distribution (V_{ss}) = 1.20 and 2.29 l kg^{-1}. The mean $t_{½}$ of MADDS was 22 h.

3 The mean whole blood to plasma (B/P) and erythrocyte to plasma (E/P) concentration ratios for DDS were 1.04 and 1.09, respectively. MADDS had a B/P ratio of 0.69 and an E/P ratio of 0.33. The B/P and E/P ratios for PYR were 0.98 and 0.54, respectively.

4 The drug combination was assessed *in vitro* by measuring inhibition of re-invasion of two *Plasmodium falciparum* isolates grown in the presence of volunteers' sera. The chloroquine (CQ)- and PYR-sensitive FC-27 isolate was completely inhibited by the sera but the drug combination was ineffective against the CQ- and PYR-resistant K1 strain. The *in vitro* findings suggest that Maloprim may not be effective against strains of *P. falciparum* with a high level of resistance to pyrimethamine.

Keywords dapsone pyrimethamine kinetics malaria Maloprim

Introduction

Maloprim® contains two mutually potentiating drugs, dapsone (DDS) and pyrimethamine (PYR), which inhibit the folic acid metabolism of malaria parasites. Since its introduction in 1968 Maloprim has been widely used as a chemoprophylactic agent in South East Asia, the South West Pacific and Africa.

Maloprim alone and in combination with chloroquine is recommended for weekly chemoprophylaxis in areas where chloroquine resistance to *Plasmodium falciparum* is prevalent (Biddulph, 1987; Gilles, 1984; Spracklen & Monteagudo, 1986). Although Maloprim has been used for over two decades there is no published information on the multiple-dose kinetics of the Maloprim components, DDS-100 mg and PYR-12.5 mg. There are also no data on the antimalarial activity of steady-state concentrations of Maloprim components against falciparum malaria. Such information may be of value in determining if a breakthrough during Maloprim prophylaxis is due to low circulating blood drug concentrations or parasite resistance to the drug combination.

The present study deals with the multiple-dose kinetics of dapsone, its principal metabolite monoacetyldapsone (MADDS) and pyrimethamine following weekly administration of Maloprim to healthy volunteers. The *in vitro* antimalarial activity of the components of

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Maloprim was determined by measuring the inhibition of re-invasion of two *P. falciparum* isolates (FC-27 and K1) grown in the presence of volunteers' sera. Concentrations of DDS, MADDS and PYR in plasma, serum, whole blood and erythrocytes were measured by high performance liquid chromatography (h.p.l.c.).

Methods

Volunteers and dose regimen

Six healthy male volunteers (Caucasian, aged 25–33 years, weighing 67–91 kg) participated in the study after they had given their informed consent. Prior approval for the study had been obtained from the Ethics Committee of the Royal Australian Army Medical Corps. The volunteers were judged healthy on the basis of normal biochemical and haematological values. No other drugs were taken by the volunteers 1 week before and during the study. Each volunteer ingested one tablet of Maloprim (Burroughs Wellcome) at weekly intervals for 8 consecutive weeks. The final dose on the eighth week was preceded by an overnight fast.

Sample collection

Venous blood samples (20 ml) were collected prior to the first dose and at 0, 1, 2, 3, 4, 6, 8, 24, 48, 72, 120 and 168 h after the final dose. During the first 8 h after the last drug administration blood was withdrawn through an indwelling cannula which was inserted into a forearm vein and kept patent with heparinised saline. Subsequent blood samples were collected by venepuncture. From these 20 ml blood samples, 10 ml was transferred to a plain tube and allowed to clot and 10 ml was placed in a heparinised tube. From the latter tube 3 ml of whole blood was transferred to a plastic container, the remainder was centrifuged at 1,200 *g* for 15 min and the plasma was separated. The buffy coat was then removed and the erythrocytes were collected. The clotted blood was also centrifuged at 1,200 *g* for 15 min and the serum was transferred to a plastic container. All samples were stored at −25° C until analysed.

Chromatography

All assays were carried out on a Waters chromatographic system consisting of an M590 pump, M480 u.v. variable detector, Schoeffel Model FS 970 fluorometer and a WISP 710B autosampler. The integrator was a Spectra-Physics 4270.

Assay methods

DDS and MADDS Concentrations of DDS and MADDS in plasma, serum, whole blood and erythrocytes were measured by ion-suppression reversed-phase h.p.l.c. The internal standard was diacetyldapsone (DADDS). Chromatographic separation of the three compounds was performed on a Nova-Pak C18 (5 μm, 15 cm × 3.9 mm i.d.) column using a mobile phase consisting of acetonitrile : water : acetic acid (20 : 79.5 : 0.5 v/v) pumped at a flow rate of 0.75 ml min^{-1}. U.v. detection at 295 nm was used to measure the compounds in plasma and serum. Because of a u.v. absorbing endogenous peak found in erythrocytes and whole blood with a retention time similar to MADDS, concentrations of DDS and MADDS in both biological fluids were detected by fluorescence (excitation = 295 nm, emission filter = 418 nm). Using either detector the retention times for DDS, MADDS and DADDS were 6.7, 8.4 and 10.8 min, respectively. Calibration curves for DDS and MADDS were linear over the range of 10–1,500 ng ml^{-1} in plasma and serum, and from 20–1,000 ng 0.5 ml^{-1} in whole blood and erythrocytes with correlation coefficients of 0.99 or better. The interassay coefficients of variation (n = 6) of spiked plasma samples for DDS and MADDS were respectively: 5.7 and 4.2% at 20 ng ml^{-1}, and 3.2 and 3.2% at 1,000 ng ml^{-1}. The accuracy of the method for measuring DDS and MADDS plasma concentrations at 20 ng ml^{-1} was 5.8 and 3.3%, respectively (n = 6). The interassay coefficients of variation (n = 6) of DDS and MADDS in whole blood and erythrocytes ranged from 11.9 to 15.4% at 20 ng 0.5 ml^{-1} (accuracy: 7.5–11.7%) and from 3.6 to 8.4% at 500 ng 0.5 ml^{-1} (accuracy: 3.3–10.1%). Recovery (mean ± s.d.) of drugs from the biological fluids spiked with 200 ng of DDS and MADDS were respectively: 89 ± 5 and 92 ± 6% (plasma), 85 ± 4 and 60 ± 4% (whole blood), and 82 ± 6 and 65 ± 5% (erythrocytes). Assay limits at a signal to noise ratio of 3 to 1 for DDS and MADDS were 2 ng ml^{-1} in plasma and serum. In whole blood and erythrocytes the assay limits for DDS and MADDS were 5 and 10 ng ml^{-1}, respectively.

PYR Measurement of PYR in the four biological fluids was by ion-paired reversed-phase h.p.l.c. A 15 cm × 4.6 mm i.d., Spherisorb-5-Phenyl C18 column was used and the mobile phase consisted of acetonitrile : water (40 : 60

v/v) containing 5 mM pentane sulphonic acid. The flow rate was 1 ml min^{-1} and u.v. detection was at 270 nm. The internal standard was WR 99210 [4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(2,4,5 - trichlorophenoxypropyloxy) - 1,3,5 - triazine]. The retention times for PYR and WR 99210 were 5.2 and 8.6 min, respectively.

Calibration curves for PYR were linear over the range of 20–200 ng ml^{-1} in plasma and serum, and from 10–200 ng 0.5 ml^{-1} in whole blood and erythrocytes. The interassay coefficients of variation and accuracy (n = 6) of PYR estimations in plasma were respectively: 9.2 and 9.2% at 20 ng ml^{-1}, and 6.3 and 4.8% at 100 ng ml^{-1}. The interassay coefficients of variation (n = 6) of PYR in whole blood and erythrocytes ranged from 8.1 to 10.5% at 20 ng 0.5 ml^{-1} (accuracy: 9.2–10.8%) and 1.9 to 5.4% at 100 ng 0.5 ml^{-1} (accuracy: 1.8–4.8%). Recovery (n = 6) of 20 ng PYR spiked to plasma, whole blood and erythrocytes averaged 84 ± 7, 95 ± 12 and 88 ± 4%, respectively. The assay limit was 5 ng ml^{-1} for PYR in the biological fluids.

Extraction procedure for DDS, MADDS and PYR To a plasma or serum sample (1 ml) in a silanised glass tube were added 100 µl DADDS (5 µg ml^{-1}) and 100 µl WR 99210 (10 µg ml^{-1}), 1 ml 1M sodium hydroxide and 8 ml dichloromethane. The contents of the tube were mixed for 20 min. After centrifugation (1,200 g × 10 min) the aqueous phase was discarded. The remaining dichloromethane phase was then transferred to a clean silanised glass tube and evaporated to dryness at 40° C under a steady stream of instrument grade air. The residue was reconstituted in 150 µl of acetonitrile : water (30 : 70 v/v) and an aliquot (25–50 µl) was injected onto a column. Drug concentrations in whole blood and erythrocytes were analysed as above after diluting the samples (0.5 ml) with an equal volume of water.

Pharmacokinetic calculations

Pharmacokinetic analysis was by model-independent methods. The maximum concentration (C_{max}) and the time taken to reach C_{max} (t_{max}) were obtained directly from the plasma concentration-time data. The elimination rate constant (k) was determined by least-squares regression analysis of the plasma drug concentration-time curve and the elimination half-life ($t_{½}$) from the ratio of 0.693/k. The area under the plasma drug concentration-time curve (AUC) from t = 0 h (day 56) to t = 168 h (day 63) was calculated using the trapezoidal rule. These time limits correspond to the weekly dosing interval following the last (8th) dose. Assuming complete systemic availability, plasma clearance (CL) was estimated from the ratio of dose/AUC (0,168) and the apparent volume of distribution (V_{ss}) from the ratio of CL/k. The accumulation factor (R) was calculated according to the equation:

$$R = \frac{1}{1 - e^{-k\tau}}$$

where τ = dosing interval.

The number of doses (n) required to reach 95% of the steady-state drug concentration was calculated from the equation:

$$1 - 2^{-n\tau/t_{½}} = 0.95 \text{ (Welling, 1986)}$$

Acetylation phenotype

Slow acetylators of DDS were defined as volunteers with plasma MADDS/DDS concentration ratios of < 0.30 and rapid acetylators were those with a ratio of > 0.35 (Reidenberg *et al.*, 1973). Because the MADDS/DDS ratio is achieved rapidly and remains constant following DDS administration (Gelber *et al.*, 1971) the AUC values for MADDS and DDS were used to determine this ratio.

Minimal inhibitory concentration (MIC)

The MICs of PYR were determined by measuring the inhibition of re-invasion of two *P. falciparum* isolates grown *in vitro* in the presence of serum containing the drug (Scott *et al.*, 1988). The culture wells contained 80 µl of serum and 20 µl of a 10% RBC suspension of parasitised erythrocytes (0.4% parasitaemia) in plain RPMI LPLF medium. The cultures were incubated at 37° C for 48 h.

The MIC was taken to be the lowest PYR concentration which inhibited parasite re-invasion of erythrocytes by at least 95% relative to the controls. The *P. falciparum* isolates were FC-27 (originating in Papua New Guinea and sensitive to chloroquine (CQ) and PYR) and K1 (originating in Thailand and resistant to CQ and PYR) (Scott *et al.*, 1987). The MICs of PYR for the two isolates were determined in triplicate from pre-dosed plates spiked with known drug concentrations. For the assessment of *in vitro* antimalarial activity of the components of Maloprim the parasites were cultured in the presence of volunteers' sera collected after the last maintenance dose.

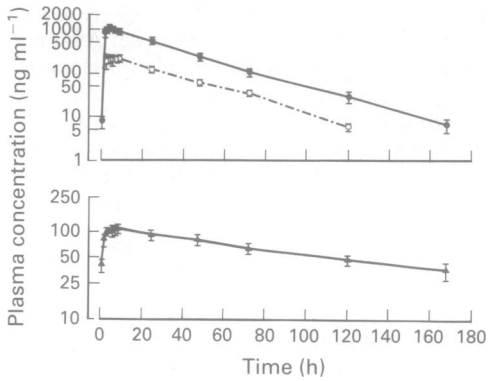

Figure 1 Plasma concentration-time curves (mean ± s.e. mean) of DDS (•), MADDS (○) and PYR (▲) following the administration of the eighth weekly dose of Maloprim (1 tablet) to five healthy volunteers (i.e. slow acetylators).

Statistical analysis

Values in the text and tables are presented as mean ± s.d. and statistical comparisons were made by Student's paired *t*-test with a significance level of 5%.

Results

Five of the volunteers were slow acetylators of DDS with MADDS/DDS concentration ratios ranging from 0.21 to 0.29 (mean 0.25). The sixth volunteer was a rapid acetylator with a MADDS/DDS ratio of 0.58. Plasma concentration-time curves of DDS, MADDS and PYR in the five slow acetylators of DDS are shown in Figure 1. The multiple-dose kinetics of DDS and PYR estimated after the last maintenance dose of Maloprim in the six healthy volunteers are presented in Table 1. The maximum plasma concentration (C_{max}) of DDS and the time at which it was reached (t_{max}) averaged 1,134 ng ml^{-1} and 1.7 h, respectively. The mean C_{max} of MADDS in the slow acetylators was 273 ng ml^{-1} (185–375) with a t_{max} of 3.6 h. The rapid acetylator had a C_{max} of 719 ng ml^{-1} and a t_{max} of 1 h for MADDS. The C_{max} of PYR ranged from 87 to 145 ng ml^{-1} with a mean of 116 ng ml^{-1} and these values were reached between 1 and 8 h after drug administration.

The mean elimination half-lives ($t_{½}$) of DDS and PYR were 22.6 and 104.9 h, respectively. The $t_{½}$ of MADDS in the slow acetylators of DDS averaged 22.1 h whilst the rapid acetylator had a $t_{½}$ of 21 h for MADDS. DDS with a $t_{½}$ of 22.6 h does not accumulate following the weekly administration of Maloprim. The plasma trough (C_{min}) concentration of DDS at 0 h–day 56 (7.5 ± 4.4 ng ml^{-1}) and 168 h–day 63 (6.7 ± 4.3 ng ml^{-1}) were similar. PYR with a longer $t_{½}$ than DDS does accumulate following the dosage regimen with a mean accumulation factor of 1.5 (1.3–1.9). Since the mean trough concentration of PYR at 0 h (42 ng ml^{-1}) was similar to the concentration of the next trough (168 h–37 ng ml^{-1}), steady-state concentrations of PYR appeared to have been reached after eight doses. Furthermore, based on a $t_{½}$ for PYR of 105 h and a weekly dosage regimen, the theoretical number of doses required to reach 95% of the steady-state concentration for PYR was calculated to be 2.7 doses. The mean plasma clearances (CL) of DDS and PYR were 37.6 and 15.9 ml h^{-1} kg^{-1}, respectively. The apparent volume of distribution (V_{ss}) was 1.20 l kg^{-1} for DDS and 2.29 l kg^{-1} for PYR.

Table 1 Multiple-dose kinetics (mean ± s.d.) of DDS and PYR derived from plasma data following the last maintenance dose of Maloprim (1 tablet) in six healthy volunteers

Parameters	*DDS*	*PYR*
C_{max} (ng ml^{-1})	1,134.0 ± 118.4	116.2 ± 26.7
C_{min} (ng ml^{-1})	7.5 ± 4.4	41.5 ± 12.7
t_{max} (h)	1.7 ± 1.0	3.7 ± 3.4
$t_{½}$ (h)	22.6 ± 2.8	104.9 ± 30.3
AUC (μg ml^{-1} h)	35.0 ± 8.0	10.7 ± 2.9
CL (ml h^{-1} kg^{-1})	37.6 ± 8.6	15.9 ± 5.4
V_{ss} (l kg^{-1})	1.20 ± 0.15	2.29 ± 0.56

The whole blood to plasma (B/P) and erythrocyte to plasma (E/P) concentration ratios of DDS and PYR at 4, 24 and 72 h after the last dose are shown in Table 2. Because MADDS concentrations in several of the volunteers were below the assay limit at 72 h after dosing, the B/P and E/P ratios for MADDS were based on samples collected at 4 and 24 h after drug administration. The B/P and E/P ratios of DDS, MADDS and PYR in the volunteers were relatively constant between the sampling times after dosing. DDS concentrations in whole blood and erythrocytes were similar to those found in plasma with a mean B/P ratio of 1.04 and E/P ratio of 1.09. The B/P and E/P ratios of MADDS averaged 0.69 and 0.33, respectively. The B/P ratio of PYR was close to unity (0.98) with an E/P ratio of 0.54.

No significant difference ($P > 0.05$) was observed between serum and plasma concentrations of DDS, MADDS and PYR measured at 1, 3, 8, 24, 48, 72 and 168 h after the last dose.

Table 2 Whole blood to plasma (B/P) and erythrocyte to plasma (E/P) concentration ratios (mean ± s.d.) of DDS, MADDS and PYR following Maloprim dosing in six healthy volunteers

Concentration ratios	*Time after last Maloprim dose* 4 h	24 h	72 h	*All samples (mean ± s.d.)*
B/P—DDS	1.07 ± 0.18	0.96 ± 0.16	1.09 ± 0.13	1.04 ± 0.16
E/P—DDS	1.07 ± 0.27	1.01 ± 0.31	1.10 ± 0.21	1.09 ± 0.22
B/P—MADDS	0.78 ± 0.07	0.61 ± 0.11	N.D.	0.69 ± 0.12
E/P—MADDS	0.38 ± 0.09	0.32 ± 0.12	N.D.	0.33 ± 0.10
B/P—PYR	1.00 ± 0.11	0.94 ± 0.15	1.01 ± 0.21	0.98 ± 0.16
E/P—PYR	0.51 ± 0.10	0.55 ± 0.12	0.54 ± 0.10	0.54 ± 0.10

N.D.—Not determined

The correlation coefficients (r) between serum and plasma drug concentrations were 0.970 for DDS (n = 40), 0.975 for MADDS (n = 34) and 0.911 for PYR (n = 39). The average trough serum concentration (C_{min}) was 5.8 ± 3.8 ng ml^{-1} for DDS and 47.0 ± 19.4 ng ml^{-1} for PYR (n = 6).

The MICs of PYR against the FC-27 and K1 isolates ranged from 4 to 6 ng ml^{-1} and from 1,200 to 1,800 ng ml^{-1}, respectively. Serum samples collected from the volunteers after the last dose of Maloprim (0–168 h) did not inhibit the growth of the K1 isolate but completely inhibited the FC-27 parasites.

Discussion

The h.p.l.c. methods described in this paper are simple, sensitive and selective for the measurement of DDS, MADDS and PYR in plasma, serum, whole blood and erythrocytes. The internal standards, DADDS and WR 99210, were selected because of similar chromatographic and structural properties to the parent drugs. Acetylation of DDS to DADDS does not occur to any significant extent (Zuidema *et al.*, 1986). By comparison with authentic DADDS we were unable to detect this metabolite in plasma following Maloprim dosing. The analytical methods, requiring a single-step liquid–liquid extraction and a run time of less than 12.5 min, appear suitable for routine monitoring of the Maloprim components.

The multiple-dose kinetics of DDS, MADDS and PYR in six healthy volunteers were estimated after eight, weekly doses of Maloprim. The elimination half-lives ($t_{½}$) of DDS (22.6 h) and MADDS (21.9 h) were similar to those reported by Pieters & Zuidema (1986) for DDS (16.7 h) and MADDS (16.9 h) following a single oral dose of 100 mg DDS to healthy male volunteers. The inter-individual variation in the $t_{½}$ of PYR, assessed after the last maintenance dose, was large and ranged from 78 to 158 h with a mean value of 105 h. This large inter-individual variation in the $t_{½}$ of PYR was also observed by Jones & Ovenall (1979) in healthy volunteers following a single oral dose of Maloprim (35–174 h). Similarly, Ahmad & Rogers (1980) noted a large variation in $t_{½}$ of PYR with a mean value of 83.2 ± 30.3 h following the coadministration of 100 mg DDS with 25 mg PYR.

The steady-state values of plasma CL and V_{ss} for DDS of 37.6 ml h^{-1} kg^{-1} and 1.20 l kg^{-1}, respectively, were in close agreement with that reported by Ahmad & Rogers (1980) following a single oral dose of 100 mg DDS to healthy volunteers (CL = 38.4 ml h^{-1} kg^{-1}; V_d = 1.53 l kg^{-1}). Pieter & Zuidema (1986) also estimated the V_d for DDS at 1.06 l kg^{-1}. The longer $t_{½}$ of PYR observed in the present study is reflected in the plasma CL of 15.9 ml h^{-1} kg^{-1} for PYR. This is in good accordance with an estimated CL of 19.1 ml h^{-1} kg^{-1} (Weidekamm *et al.*, 1987) and 14.0 ml h^{-1} kg^{-1} (Schwartz *et al.*, 1987) for PYR obtained in healthy volunteers following the administration of Fansidar® (sulphadoxine + PYR) and Fansimef® (mefloquine + sulphadoxine + PYR), respectively. Although Ahmad & Rogers (1980) reported a larger CL for PYR, there was no significant difference between PYR given alone (24.8 ml h^{-1} kg^{-1}) and in combination with DDS (25.8 ml h^{-1} kg^{-1}).

DDS and PYR are blood schizontocides (Sturchler, 1984) which act on the intra-erythrocytic stage of the malaria parasite. Because erythrocytes are the host cells of the parasite, information on the concentration of the Maloprim components in whole blood and, more particularly, in erythrocytes is of importance. This study showed that whole blood and erythrocyte

concentrations of DDS were similar to corresponding plasma concentrations. At physiological pH, DDS (pKa of 1, Merck, 1968) is predominantly unionised and, therefore, freely distributes across the erythrocytic membrane. In contrast to DDS which is moderately protein bound (71–75%), MADDS is almost entirely (>98%) bound to plasma proteins (Peters *et al.*, 1981). MADDS is also 20 to 25-fold more tightly bound to plasma proteins than DDS (Glazko *et al.*, 1969). Presumably, the small fraction of unbound MADDS and its strong binding to plasma proteins contributes to the low MADDS concentrations found in erythrocytes (E/P = 0.33).

Although the B/P ratio of PYR was close to unity, the concentration of PYR in erythrocytes was about 50% of the corresponding plasma concentrations. Kinetic studies of the components of Fansimef (Weidekamm *et al.*, 1987) and Fansidar (Edstein, 1987) have shown E/P ratios for PYR of below 0.5 and 0.36, respectively. Mean B/P and E/P ratios of 0.78 and 0.45, respectively, for PYR have been demonstrated in *in vitro* studies using [^{14}C]-pyrimethamine (E. Weidekamm, personal communication). Detailed examination of the distribution of PYR into other blood fractions (e.g. white blood cells) was not undertaken in this study.

The relationship between serum and plasma drug concentrations of the components of Maloprim was determined because it has been shown that for chloroquine (Bergqvist & Domeij-Nyberg, 1983) and quinidine (Ooi *et al.*, 1980) serum drug concentrations are higher than corresponding plasma concentrations. Our findings show that DDS, MADDS and PYR concentrations are similar in plasma and serum. Thus, either plasma, serum or whole blood may be used to measure DDS and PYR concentrations following Maloprim administration in healthy volunteers. In the present study, it was also important to measure serum drug concentrations as sera from the volunteers was used to assess the *in vitro* antimalarial activity of Maloprim.

Assessment of *P. falciparum* susceptibility to antimalarial drugs can be evaluated both *in vivo* and *in vitro*. The morphological *in vitro* assay is technically simple to perform, suitable for field application and provides a result within 50 h which may be useful in a clinical situation. Assessment of PYR sensitivity alone using the *in vitro* test has been demonstrated to correlate well with the *in vivo* response of the sulphadoxine/pyrimethamine combination (Fansidar) (Lamont & Darlow, 1982; Spencer *et al.*, 1984). The MIC values of PYR, obtained in the present study, show that the FC-27 isolate was sensitive to PYR whereas the K1 isolate was highly resistant to PYR. The MIC of DDS, however, was not estimated since it has been shown that for sulphones and sulphonamides, which are slow acting blood schizontocides, a longer incubation period (66 h) is required for the accurate determination of drug inhibition (Milhous *et al.*, 1985).

Based on the MICs of PYR against the two *P. falciparum* isolates the combination of DDS plus PYR was expected to inhibit the growth of the FC-27 parasites. When this prediction was tested, the volunteers' trough serum drug concentrations of DDS, PYR and their metabolites completely inhibited the FC-27 isolate. These same concentrations, however, had no effect against the K1 parasites. Furthermore, serum from volunteers on Maloprim, sampled at or near the t_{max} did not inhibit the K1 isolate. Although these findings suggest that Maloprim is an effective prophylactic agent against a PYR-sensitive isolate the drug combination is unlikely to provide adequate protection against strains of *P. falciparum* that are highly resistant to PYR. Since many parasites encountered in endemic areas are less resistant than parasites of the K1 isolate, peak drug concentrations may be effective against such parasites. However, complete suppression of parasitaemia may not be achieved because of inadequate trough concentrations of the drug.

In conclusion, the elimination half-lives and clearance of DDS and PYR in healthy volunteers following multiple dosing of Maloprim are in close agreement with those reported from single dose studies. The drug concentration data obtained in this study may be useful in assessing whether a breakthrough during malaria prophylaxis with Maloprim is due to parasite resistance or to inadequate blood concentration of the drugs.

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