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Single dose kinetic study of the triple combination mefloquine/sulphadoxine/pyrimethamine (Fansimef®) in healthy male volunteers

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A single dose pharmacokinetic study of a combined antimalarial formulation of mefloquine, sulphadoxine and pyrimethamine (Fansimef®) has been performed in 10 healthy adult male Malaysian volunteers. The dose consisted of two tablets containing 250 mg mefloquine base, 500 mg sulphadoxine base and 25 mg pyrimethamine base each. Plasma concentrations of mefloquine and pyrimethamine were measured by GC-ECD, those of sulphadoxine by h.p.l.c. Time to peak concentrations (mean ± s.d. for mefloquine (5.70 ± 0.95 h), sulphadoxine (3.75 ± 2.03 h) and pyrimethamine (3.30 ± 1.98 h) were similar to those observed by others after administration of the single compounds. This was also true for elimination half-lives ($t_{1/2}$). The $t_{1/2}$s for mefloquine, sulphadoxine and pyrimethamine were 387 ± 98 h, 255 ± 61 h and 114 ± 42 h, respectively.

Keywords malaria pharmacokinetics mefloquine sulphadoxine pyrimethamine

Introduction

The emergence and proliferation of falciparum malaria resistant initially to chloroquine and subsequently to pyrimethamine, proguanil and other antimalarials, stimulated interest in the development of combination products which would decrease the risk of inducing resistance yet provide adequate protection against malaria infections (Tin & Nyunt Hlaing, 1977).

In response to this need, a triple combination antimalarial product containing mefloquine, pyrimethamine and sulphadoxine has been developed. Mefloquine, a quinoline methanol derivative which is a potent blood schizontocide, is highly active against various forms of human malaria infections (Trenholme *et al.*, 1975; Clyde *et al.*, 1976; Pearlman *et al.*, 1977, 1980). Pyrimethamine and sulphadoxine act as reversible inhibitors of tetrahydrofolate dehydrogenase (dihydrofolate reductase) and plasmodial dihydropteroate synthetase, respectively.

Since these two enzymes operate in sequence in the folate biosynthesis pathway of the plasmodia, their inhibition by pyrimethamine and sulphadoxine could be synergistic (Weidekamm *et al.*, 1982). The triple combination was developed in the belief that the activity of the components could be complementary. In addition, research in animal models has suggested that the development of plasmodial resistance against these antimalarials may be retarded in the simultaneous presence of all three compounds (Peters & Robinson, 1982). Clinical and field trials with the triple combination indicate high efficacy against falciparum malaria (WHO 1984; de Souza *et al.*, 1987; Harinasuta *et al.*, 1987; Nosten *et al.*, 1987).

There have been several pharmacokinetic studies on mefloquine alone (Desjardins, 1979; Schwartz *et al.*, 1980; Riviere *et al.*, 1985) as well as on pyrimethamine in combination with

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sulphadoxine (Fansidar®) (Weidekamm *et al.*, 1982). A pharmacokinetic study involving the concurrent administration of mefloquine and sulphadoxine/pyrimethamine has been reported (Weidekamm & Schwartz, 1983).

With the development of the triple combination in a galenic formulation, it became necessary to study the pharmacokinetic behaviour of this new formulation in man. The objective of this study was to establish the pharmacokinetic profile of the triple combination after a single dose.

Methods

Subjects

Ten male volunteers aged 22–30 years and weighing 51–60 kg, were selected for the study. The study protocol was approved by the Institutional Ethics Committee. The investigations were carried out in accordance with the guidelines of the Helsinki Declaration for Clinical Trials and informed written consent was obtained from all the subjects. A complete physical and laboratory examination was carried out to ensure that all of the subjects were healthy and free from renal, hepatic or gastrointestinal disorders. No other drugs or any alcohol were taken 7 days prior to or during the clinical trial. All subjects were residents of a non-malarious area of Malaysia and had therefore not taken any antimalarial drugs for several months.

Medication

Two tablets of Fansimef® each containing 250 mg mefloquine base, 500 mg sulphadoxine base and 25 mg pyrimethamine base were administered to each subject with 150 ml of water following an overnight fast. A normal breakfast was served 2 h later.

Blood sampling

Blood (15 ml) was collected in EDTA-containing Vacutainers® prior to drug administration and at 0.5, 1.5, 3, 6, 10, 24, 32, 48, 75, 120, 168, 216, 264 h and 2, 3, 4, 5, 6 weeks after drug administration. The blood samples were collected by means of an in-dwelling cannula for the first 10 h, after which they were collected by venepuncture. The blood samples were centrifuged immediately after collection at 1000 *g* for 20 min and the plasma supernatant was stored in glass tubes at −20° C until analyzed.

Analytical methods

A Hewlett-Packard HP 5880A gas chromatograph with electron capture detection (15 mCi nickel-63) (GC-ECD) was used to measure mefloquine in human plasma (Heizmann & Geschke, 1984). Plasma (200 μl) spiked with mefloquine and internal standard (±-(2-pyridyl)-2,7-bis(trifluoromethyl-4-quinoline methanol)) was extracted with 7 ml of dichloromethane at pH 8 using TRIS buffer. The lower dichloromethane layer was transferred into a 5 ml Reactivial® and evaporated to dryness at 60° C under a gentle stream of nitrogen. To the dried organic residue was added 100 μl of a 10% solution of *N*-trimethyl-silyl-imidazole (MSI) for derivatization to the *O*-silyl forms. The resultant mixture was diluted with 500 μl of acetonitrile and 1 μl was injected into the GC-ECD. The detector used was set at 280° C. An OV-17, 3% on 100/120 chromosorb (Hewlett-Packard) column was used. Injector temperature was 320° C with a column oven temperature of 185° C and at a programme rate of 8° C per min. The carrier gas, nitrogen, was used at a flow rate of 30 ml min^{-1}.

The mean percentage recovery for mefloquine was 94.75 ± 1.26% for a concentration range of 39–10,000 ng ml^{-1}, while that for the internal standard was 93.5 ± 1.73% for concentrations ranging from 100 to 10,000 ng ml^{-1} (n = 6 for each concentration). The mean percentage variation of recovery for mefloquine was 3.9% with a range of 2.9 to 4.5% indicating good within day precision at concentrations of 25 to 200 ng ml^{-1} of mefloquine (n = 6 at each concentration). For day to day variation, the mean coefficient of variation was 3.40 ± 0.38% at concentrations of 100 to 1500 ng ml^{-1} (n = 6 for each concentration). The limit of assay for mefloquine was 5 ng ml^{-1}, using 0.20 ml plasma.

A GC-ED method was developed to measure pyrimethamine in human plasma. Two hundred μl of chloroquine (internal standard) solution in methanol was added into a 15 ml glass test tube together with 500 μl of plasma and 500 μl of borate buffer saturated with potassium chloride at a pH of 9.5. The mixture was vortexed for 30 s following which 6 ml of diethyl ether was added. The resultant mixture was then thoroughly vortexed for 2 min followed by centrifugation at 3000 rev min^{-1} for 10 min. The supernatant organic layer was aspirated into a 5 ml Reactivial® and evaporated to dryness at 60° C under a gentle stream of nitrogen gas. After removing the organic phase, a further 6 ml of diethyl ether was added and the extraction procedure was repeated. The dry extract residue was redissolved

with methanol and analyzed using a Hewlett-Packard HP 5880A gas chromatograph with an electron capture detector (15 mCi nickel-63). The chromatography was performed with a 3% OV-17 on a 100/120 chromosorb column at a detector temperature of 350° C, an injector temperature of 300° C and a column oven temperature of 220° C. The oven temperature programme rate was set at 10° C per min. Nitrogen was used as the carrier gas at a flow rate of 30 ml min^{-1}. The percentage recovery of pyrimethamine ranged from 92 to 95% with a mean of 93.75%. The coefficient of variation of the assay for pyrimethamine ranged from 3.67% to 4.96% with a mean of 4.04% at concentrations of 18.6 to 1900 ng ml^{-1}. Replicates of 5 at each concentration were assayed. The within day variation ranged from 3.58% to 4.94% (mean = 3.95%) at concentrations of 20 to 800 ng ml^{-1}. Replicates of 5 at each concentration were assayed. The day to day variation ranged from 3.65 to 3.88% (mean = 3.77%) at concentrations of 40 to 1600 ng ml^{-1} with replicates of 10 at each concentration. The limit of assay for pyrimethamine was 10 ng ml^{-1} using 0.5 ml plasma.

A high-pressure liquid chromatography (h.p.l.c.) assay was developed to measure sulphadoxine in human plasma. To 500 μl of plasma was added 500 μl of phosphate buffer (pH 3.8) and the mixture was vortexed for 30 s. Six ml of a mixture of diethyl ether and ethyl acetate (1:1 ratio) was used to extract the sulphadoxine. The supernatant organic layer was removed and placed in a Reactivial®. The extraction procedure was then repeated with another 6 ml of diethyl ether and ethyl acetate. The pooled organic layer was evaporated at 60° C under a gentle stream of nitrogen. The drug residue was reconstituted in methanol and 20 μl of the solution was injected into an h.p.l.c. A Hewlett Packard HP 1090 h.p.l.c. was used with a reverse phase (C_{18}) Lichrosorb (5 μm) column (dimensions 125 mm × 4 mm). The mobile phase was methanol : 1% v/v acetic acid (27:73) at pH 3.0. The flow rate was 1.5 ml min^{-1}. An ultraviolet detector set at 230 nm wavelength was used. No internal standard was used in this method. The percentage recovery for sulphadoxine varied between 84 and 86% (mean = 85.1%) for concentrations of 39 to 10,000 ng ml^{-1} with replicates of 6 at each concentration. The coefficient of variation of the assay ranged from 3.74% to 4.42%. The within day variation ranged from 3.87 to 4.53% (mean = 3.95%) at concentrations of 100 to 16,000 ng ml^{-1}. The number of replicates for each concentration was 6. The day to day variation ranged from 3.77 to 3.92% (mean = 3.84%) at concentrations of 100 to 10,000 ng ml^{-1}. Replicates of 12 were assayed at each concentration. The limit of assay for sulphadoxine was 50 ng ml^{-1} using 0.5 ml plasma.

To ensure assay accuracy and precision during the analysis of sulphadoxine, blank plasma samples were spiked with 0.1 to 100 μg ml^{-1} of sulphadoxine, 25 to 200 ng ml^{-1} of mefloquine and 20 to 200 ng ml^{-1} of pyrimethamine. Replicates of 6 at each concentration were measured. These spiked samples were run together with the experimental blood samples to ensure that assay accuracy and precision were maintained. Mean percent recoveries of sulphadoxine were 82.6% at 0.1 μg ml^{-1} and 84.5% at 100 μg ml^{-1} ($n = 6$) while the coefficient of variation ranged from 4.65% at 0.1 μg ml^{-1} to 4.0% at 100 μg ml^{-1}. The presence of pyrimethamine and mefloquine did not interfere with the percent recovery or sensitivity of the assay.

Data analysis

The data were analyzed by the AUTOANNONLIN computer programme (Wagner, 1975) and were found to be best fitted by a two-compartment model according to:

$$C = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} - (C_1 + C_2) e^{-k_a t}$$

where C is the plasma drug concentration at time t, λ_1 and λ_2 are first-order disposition rate constants and k_a is an apparent first-order rate constant for absorption.

The concentration-time data were fitted by the equation with a weighting value of $1/C^2$ where C is the concentration observed at time, t.

The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule. The oral clearance, CL_o was then calculated by dividing dose administered by AUC. The half-life of elimination was calculated from the terminal slope of the log plasma drug concentration *vs* time curve.

Results

The plasma drug concentration-time profiles for mefloquine, sulphadoxine and pyrimethamine are shown in Figure 1. It was found that measurable concentrations of mefloquine and sulphadoxine were present in all samples taken up to 42 days after drug administration, but that the pyrimethamine plasma concentrations dropped below detectable levels by day 28.

Table 1 shows the pharmacokinetic parameters obtained for mefloquine, sulphadoxine and pyrimethamine.

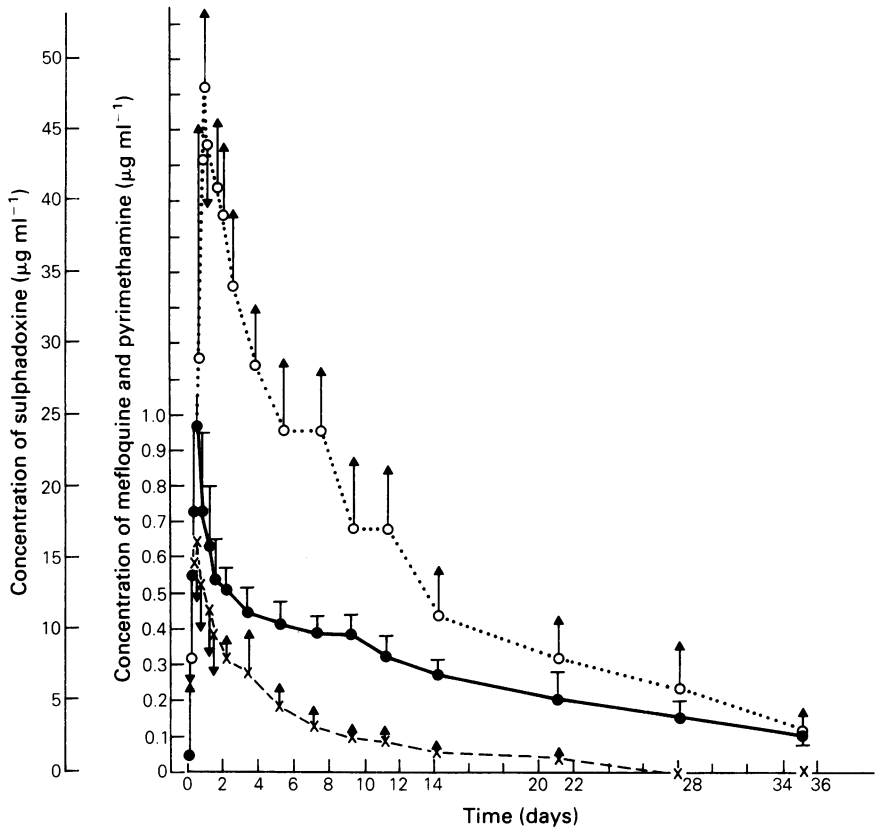

Figure 1 Mean (± s.d.) plasma concentrations of mefloquine (●——●), pyrimethamine (×------×) and sulphadoxine (○ · · · · ○) following administration of two tablets of Fansimef®. Each tablet contained 250 mg mefloquine base, 500 mg sulphadoxine base and 25 mg pyrimethamine base (n = 10).

Table 1 Means (± s.d.) of the pharmacokinetic parameters describing the fate of mefloquine, sulphadoxine and pyrimethamine following a single oral dose of Fansimef®

	Mefloquine	*Sulphadoxine*	*Pyrimethamine*
k_a (h^{-1})	0.53 ± 0.15	1.30 ± 1.10	1.31 ± 1.11
$t_{½,z}$ (h)	387 ± 98	255 ± 61.4	113.8 ± 41.5
C_{max} (mg l^{-1})	1.01 ± 0.23	52.20 ± 4.89	0.76 ± 0.15
t_{max} (h)	5.70 ± 0.95	3.75 ± 2.03	3.30 ± 1.98
AUC (mg l^{-1} h)	306 ± 85	12202 ± 2260	76 ± 22
CL_o (ml min^{-1})	28.9 ± 7.1	1.40 ± 0.22	12.1 ± 3.68

k_a = apparent absorption rate constant.
$t_{½,z}$ = half-life of the terminal elimination phase.
C_{max} = maximum concentration obtained.
t_{max} = time at which C_{max} was obtained.
AUC = area under the plasma drug concentration-time curve, and
CL_o = oral clearance.

Discussion

In general, the pharmacokinetic parameters estimated in this study for the mefloquine component of Fansimef® were in close agreement with those reported in studies where mefloquine was administered by itself. The apparent absorption half-life values obtained in our study were between 0.94 and 2.43 h. Schwartz *et al.* (1982) obtained apparent absorption half-life values of 0.36 to 2.0 h when mefloquine was administered alone. The mean elimination half-life of mefloquine (mean ± s.d. = 16.1 ± 4.1 days) was similar to the values of 13.9 ± 5.3 days and 15.5 ± 10.4 days reported by Desjardins *et al.* (1979) and Riviere *et al.* (1985), respectively, following a single dose of mefloquine alone. Weidekamm *et al.* (1987) observed similar results with an elimination half-life for mefloquine of 18.8 ± 5.4 days when administered alone and 18.2 ± 6.7 days when administered as Fansimef®.

The mean (± s.d.) elimination $t_{½}$ of pyrimethamine was 113.8 h (± 41.5 h), which is higher than the 80 to 100 h obtained by Cavallito *et al.* (1978), Jones & Overnell (1979) and Stickney *et al.* (1973) for pyrimethamine administered alone. However, the value does lie within the range of 94.0 h and 87.7 h obtained by Weidekamm *et al.* (1987) for pyrimethamine when administered as Fansidar® and Fansimef®, respectively.

The mean t_{max} for pyrimethamine obtained was 3.30 h when Fansimef® was administered and was similar in magnitude to that obtained by Weidekamm *et al.* (1987) which was 3.4 h.

The elimination half-life reported by Weidekamm *et al.* (1987) was 182.9 h for sulphadoxine when Fansimef® was administered which was shorter than that obtained in our study (255 h). Our mean value was also greater than that reported by Peck *et al.* (1975) (194 h), Hall (1976) (200 h) and Bohni *et al.* (1979) (179 h). However, these differences do not seem to be significant.

The results of the present study suggest that the pharmacokinetics of mefloquine, pyrimethamine and sulphadoxine are not significantly altered when administered as a triple combination.

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References

Bohni, E., Fust, B., Rieder, J., Schoerer, K. & Havas, L. (1969). Comparative toxicological chemotherapeutic and pharmacokinetic studies with sulphamethoxine and other sulphonamides in animals and man. *Chemother.*, **14**, 195–226.

Cavallito, J. C., Nichol, C. A., Brenchman, W. D., De Angelis, L., Stickney, D. R., Simmons, W. S. & Sigel, C. W. (1978). Lipid-soluble inhibitors and dihydrofolate reductase. I. Kinetics, tissue distribution and extent of metabolism of pyrimethamine, metoprine and etoprine in rat, dog and man. *Drug Metab. Disposition*, **6**, 329–337.

Clyde, D. F., McCarthy, V. C., Miller, R. M. & Hormick, R. B. (1976). Suppressive activity of mefloquine in sporozoite-induced human malaria. *Antimicrob. Agents Chemother.*, **9**, 384–386.

de Souza, J. M., Sheth, U. K., Wernsdorfer, W. H., Trigg, P.I. & Doberstyn, E. B. (1987). A phase II/III double-blind, dose-finding clinical trial of a combination of mefloquine, sulphadoxine and pyrimethamine (Fansimef) in falciparum malaria. *Bull. Wld. Hlth. Org.*, **65**, 357–361.

Desjardins, R. E., Pamplin, C. L., Von Bredow, J., Barry, K. G. & Canfield, C. J. (1979). Kinetics of a new antimalarial, mefloquine. *Clin. Pharmac. Ther.*, **26**, 372–379.

Hall, A. P. (1976). The treatment of malaria. *Br. med. J.*, **1**, 323–328.

Harinasuta, T., Bunnag, D., Vanijanond, S., Charoenlarp, P., Suntharasamai, P., Chitamas, S., Sheth, U. K. & Wernsdorfer, W. H. (1987). Mefloquine, sulphadoxine and pyrimethamine in the treatment of symptomatic falciparum malaria: a double-blind trial for determining the most effective dose. *Bull. Wld. Hlth. Org.*, **65**, 363–367.

Heizmann, P. & Geschke, R. (1984). Determination of the antimalarial mefloquine in human plasma by gas chromatography with electron capture detection. *J. Chromatogr.*, **311**, 411–417.

Jones, C. R. & Overnell, S. M. (1979). Determination of plasma concentrations of dapsone, monoacetyl dapsone and pyrimethamine in human subjects dosed with maloprim. *J. Chromatogr.*, **163**, 179–185.

Nosten, F., Imvithaya, S., Vincenti, M., Delmas, G., Lebihan, G., Hausler, B. & White, N. (1987). Malaria on the Thai-Burmese border: treatment of 5192 patients with mefloquine-sulphadoxine-pyrimethamine. *Bull. Wld. Hlth. Org.*, **65**, 891–896.

Pearlman, E. J., Thiemanum, W. & Doberstyn, E. B. (1977). The suppression of *Plasmodium falciparum* parasitaemias by mefloquine. *Trop. med. parasit.*, **28**, 269.

Pearlman, E. J., Doberstyn, E. B., Sudsok, S., Thiemanum, W., Kennedy, R. & Canfield, C. J. (1980). Chemosuppressive field trials with mefloquine in Thailand. *Am. J. trop. med. Hyg.*, **29**, 1131–1137.

Peck, C. C., Lewis, A. N. & Joyce, B.E. (1975).

Pharmacokinetic rationale for a malaria suppressant administered once monthly. *An. trop. Med. Parasit.*, **69**, 141–146.

Peters, W. & Robinson, B.L. (1982). Value of a triple mefloquine combination in delaying the development of drug resistance in rodent malaria. Paper presented at the *5th International Congress of Parasitology*, Toronto.

Riviere, J. H., Back, D. J., Breckenbridge, A. M. & Howells, R. E. (1985). The pharmacokinetics of mefloquine in man: lack of effect of mefloquine on antipyrine metabolism. *Br. J. clin. Pharmac.*, **20**, 469–474.

Schwartz, D. E., Weber, B., Richard-Lenoble, D. & Gentilini, M. (1980). Kinetic studies of mefloquine and one of its metabolites, RO 21-5104, in the dog and man. *Acta Tropica*, **37**, 238–242.

Schwartz, D. E., Eckert, G., Hartmann, D., Weber, B., Richard-Lenoble, D., Ekue, J. M. K. & Gentilini, M. (1982). Single dose kinetic of mefloquine in man. *Chemother.*, **28**, 70–84.

Stickney, D. R., Simmons, W. S., De Angelis, R. L., Rundles, R. W. & Nichol, C. A. (1973). Pharmacokinetics of pyrimethamine and 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine relevant to meningeal leukemia. *Proc. Am. Assoc. Cancer. Res.*, **14**, 522.

Tin, F. & Nyung Hlaing. (1977). Resistance to antimalarials by *Plasmodium falciparum* in Burma. *S.E.A. J. trop. med. pub. hlth.*, **8**, 104–112.

Trenholme, G. M., Williams, R. L., Desjardins, R. E., Frischer, H., Carson, P. E., Rieckmann, K. H. & Canfield, C. J. (1975). Mefloquine (WR 142,490) in the treatment of human malaria. *Science*, **190**, 792–794.

Wagner, J. G. (1975). *Fundamentals of clinical pharmacokinetics*, first edition, Drug Intelligence Publication Inc. USA.

Weidekamm, E. & Schwartz, D. E. (1983). Pharmacokinetik und Dosierung der neuen Antimalariakombination, Fansidar/Mefloquine RO 13-112. Paper presented at the *Meeting of the German-speaking Societies for Tropical Medicine in Garmisch-Partenkirchen*, Federal Republic of Germany.

Weidekamm, E., Plozza-No Hebrock, H., Forgo, J. & Duback, U. S. (1982). Plasma concentrations of pyrimethamine and sulphadoxine and evaluation of pharmacokinetic data by computerized curve fitting. *Bull. Wld. Hlth. Org.*, **60**, 115–122.

Weidekamm, E., Schwartz, D. E., Dubach, U. C. & Weber, B. (1987). Single dose investigation of possible interaction between the components of the antimalarial combination Fansimef®. *Chemother.*, **33**, 259–265.

World Health Organization (1984). Advances in malaria chemotherapy. *WHO Technical Report Series*, 711, Geneva.

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