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The pharmacokinetics of pyrimethamine in the rat: effect of mefloquine

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The pharmacokinetics and tissue distribution of pyrimethamine have been determined in the rat. Following administration of pyrimethamine alone, drug concentrations declined biexponentially. By contrast, in the presence of mefloquine, the decline in pyrimethamine concentration more closely fitted a monoexponential pattern and the AUC_{0-6h} for pyrimethamine was significantly reduced. Significantly more pyrimethamine was recovered from the livers but less from the lungs of the mefloquine-dosed rats compared with control. This study outlines a potentially clinically relevant drug interaction.

Pyrimethamine, in combination with a sulphonamide or sulphone has been widely used in the suppression and treatment of chloroquine-resistant strains of *Plasmodium falciparum* malaria (Leimer 1981). Pyrimethamine/sulphadoxine (Fansidar) has recently been used together with the promising 4-quinoline methanol, mefloquine, in the preparation Fansimef (250 mg mefloquine, 25 mg pyrimethamine, 500 mg sulphadoxine), which is aimed at delaying the emergence of plasmodial resistance to mefloquine (Kofi-Ekue et al 1985). However a number of unexplained treatment failures (WHO 1983) and a lack of published pharmacokinetic data have been associated with this triple combination. Therefore in the present report, we wished to determine the effect of mefloquine on the disposition of pyrimethamine in the whole rat.

Methods

Male Wistar rats (200–250 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}) administered intraperitoneally. The left jugular vein, and right carotid artery were exposed and cannulated with polythene tubing (Portex, Hythe, Kent). The trachea was also exposed and cannulated to assist breathing, and heparin sodium was administered (400 units kg^{-1} i.v.). To a first group of animals (n = 5), pyrimethamine (2 mg kg^{-1}) was administered intraperitoneally (i.p.) dissolved in Hartmann's solution (Travenol Laboratories, Thetford, Norfolk) while a second animal group (n = 5) received pyrimethamine (2 mg kg^{-1} i.p.) concurrently with mefloquine (20 mg kg^{-1}, in Hartmann's solution, i.p.). Blood samples (150 µl) were removed from the carotid artery pre dose, then at 15, 30, 60, 120, 180, 240, 300 and 360 min. After centrifugation (1100*g*, 2 min) the plasma was removed and stored at −20 °C before assay for pyrimethamine and its 3-*N*-oxide metabolite by HPLC (Coleman et al 1984). This method was found to be free from chromatographic interference by mefloquine. An equal volume of heparinized saline was then administered via the jugular vein to replace blood volume removed by sampling. At the conclusion of each experiment, the animals were killed and the liver, kidneys, spleen and lungs removed and weighed. The soft organs were each homogenized in three times their weight of 1/15 molar phosphate buffer using a Teflon-in-glass homogenizer. The resulting 25% homogenates were then stored at −20 °C. Before assay for pyrimethamine and pyrimethamine 3-*N*-oxide by HPLC, standard curves for each compound were prepared in blank homogenate for each tissue.

Pharmacokinetic parameters for pyrimethamine, were calculated as previously described (Coleman et al 1985). However, the volume of distribution in the case of a biexponential decline was calculated with the formula $Vd = Dose/(AUC \times \beta)$. In the present study,

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Table 1. Pharmacokinetic determinates of pyrimethamine (2 mg kg^{-1}) after administration alone and in combination with 20 mg kg^{-1} mefloquine.

	AUC_{0-6h} μg h ml^{-1}	$AUC_{0-\infty}$ μg h ml^{-1}	Cl i.p. ml h^{-1}	Vd i.p. ml	$t_{\frac{1}{2}}$ h
Pyrimethamine alone	5·4 ± 1·0	22·3 ± 9·3	26·5 ± 13·1	429·6 ± 120·1	14·8 ± 6·3
Pyrimethamine plus mefloquine	4·7 ± 0·8	22·5 ± 16·9	28·5 ± 10·7	461·6 ± 193·6	17·4 ± 18·2
P	≤0·05	N/S	N/S	N/S	N/S

the systemic availability from the i.p. dosage site and hence systemic clearance, of unchanged pyrimethamine, could not be determined. Consequently, the clearance of drug after i.p. dosage (Cl i.p.) was used as a measure of elimination efficiency, and was calculated from the ratio of dose to $AUC_{0-\infty}$ (Rowland & Tozer 1980). Statistical data analysis was made by the Mann-Whitney U test, significance being accepted at $P \leq 0{\cdot}05$. Data are tabulated as mean ± s.d. and presented graphically as mean ± s.e.m.

Results

Within 30 min of the administration of pyrimethamine alone, maximum measured plasma drug concentrations were attained (Fig. 1). Drug levels were then assumed

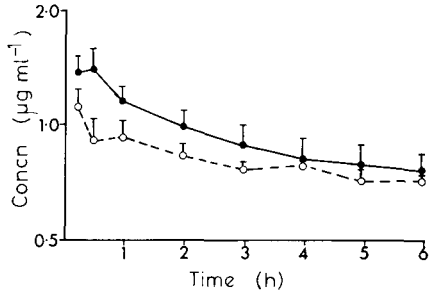

FIG. 1. Semilogarithmic plot of pyrimethamine plasma concentrations (+ s.e.m.) following 2 mg kg^{-1} pyrimethamine alone i.p. (●——●) and concomitant mefloquine 20 mg kg^{-1} i.p. (○——○), n = 5

to decay biexponentially, with an elimination half life of 14·8 ± 6·3 h; AUC was calculated to be 22·3 ± 9·3 μg h ml^{-1} (Table 1). By contrast, on concomitant mefloquine administration, pyrimethamine absorption was much more rapid (Fig. 1); maximum measured drug levels were determined 15 min post dose. Plasma pyrimethamine concentrations decayed monoexponentially, with no clearly defined distribution phase. Although there were no significant differences between the treatment groups regarding AUC, $t_{\frac{1}{2}}$, Cl or Vd, the AUC_{0-6h} for pyrimethamine was significantly reduced in the presence of mefloquine.

At the conclusion of the study (6 h), pyrimethamine concentrations were determined in the liver, lungs, spleen and kidney; the greatest proportion of unchanged drug being recovered from the liver. However, significantly more unchanged pyrimethamine was recovered from the livers (Fig. 2) and significantly less drug located in the lungs of the mefloquine dosed animals. There were no significant differences between groups regarding pyrimethamine concentrations in the spleens and kidneys. The expression of pyrimethamine soft tissue recovery in the ratio of percentage dose per gram tissue (Fig. 3) indicated pyrimethamine accumulation to be most avid in the lungs after the administration of pyrimethamine alone. Pyrimethamine 3-*N*-oxide was only intermittently detectable in plasma at the relatively low dose of pyrimethamine used in this study.

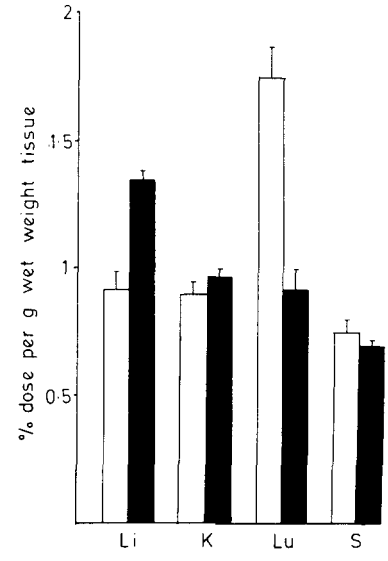

FIG. 2. Soft tissue distribution of (□) pyrimethamine and (■) mefloquine plotted as percentage of dose per organ (+ s.e.m.). Li, liver; K, kidney; Lu, lung; S, spleen.

Discussion

The administration of mefloquine exerted a marked effect on the pharmacokinetics and tissue distribution of pyrimethamine. The significant fall in the AUC_{0-6h} for pyrimethamine in the presence of mefloquine was largely accounted for by the absence of the clearly defined distribution phase seen in the animals dosed with pyrimethamine alone. This may have been due to a delay in the absorption of pyrimethamine into the

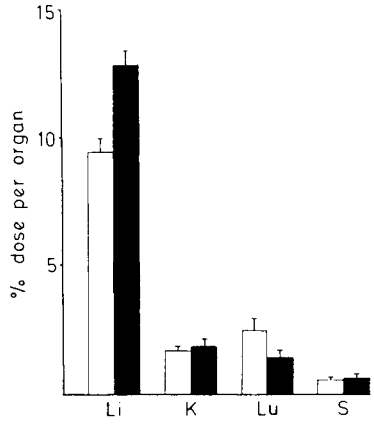

FIG. 3. Soft tissue distribution of (□) pyrimethamine and (■) mefloquine potted as percentage of dose per gram wet tissue weight (+ s.e.m.). Key as in Fig. 1.

hepatic portal circulation from the i.p. dosage site, caused by simultaneous mefloquine administration. However it might also be speculated that, through an intracellular binding interaction, mefloquine may have retarded pyrimethamine movement through the liver to the systemic circulation. This hypothesis is supported by the significant increase in the recovery of pyrimethamine from the livers of the animals which received concurrent mefloquine.

Pyrimethamine and other 2,4-diaminopyrimidines have been shown in previous studies to accumulate unchanged almost exclusively in soft, well perfused tissues, most notably the lungs (Nichol et al 1977; Cavallito et al 1978; Coleman et al 1985). Hence in the present study, the fall in the pyrimethamine AUC_{0-6h} in the presence of mefloquine was reflected most clearly in the lungs, rather than the spleen and kidneys.

Previous reports have indicated that the plasma pharmacokinetics and tissue distribution of both mefloquine and pyrimethamine were unaffected by route of administration (Rozman et al 1978; Coleman et al 1986). Hence the i.p. dosage site employed in this study was an acceptable substitute for oral dosage. Further oral pharmacokinetic studies are required in larger animals and ultimately in man to determine if these alterations in pyrimethamine disposition in the presence of mefloquine are relevant to the chemotherapy of malaria.

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