COMPARATIVE PHARMACOKINETICS OF CO-TRIFAMOLE AND CO-TRIMOXAZOLE TO 'STEADY STATE' IN NORMAL SUBJECTS

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- 1 The plasma and urine concentrations of trimethoprim (TMP) and sulphonamide obtained using co-trimoxazole and co-trifamole (TMP and sulphamoxole) were estimated in 10 patients in a two way cross-over study using the manufacturers' recommended dosage schedules.
- 2 The pharmacokinetics of TMP using either preparation were comparable with those reported elsewhere; as were the kinetics of sulphamethoxazole. The values obtained for sulphamoxole, not previously reported, were $t_{1/2}$ 8.0 ± 2.8 h, k_{el} 0.096 ± 0.031 h⁻¹, CL_s 0.9 ± 0.4 l h⁻¹ and V_d 9.4 ± 2.0 l.
- 3 Studies in plasma showed sulphamoxole to have a lower volume of distribution than sulphamethoxazole. The half-lives of the two did not differ significantly; steady state plasma concentrations were achieved more rapidly (≈ 25 h) for co-trifamole than for co-trimoxazole (≈ 49 h) as a result of the differences in recommended dosage schedule.
- 4 Differences in urinary concentration of TMP and sulphonamide obtained using the two preparations were a reflection of the dosage regimens rather than the pharmacokinetics of the individual drugs. Urinary concentrations of TMP greatly exceeded the MIC for sensitive organisms in all cases. Sulphonamide concentrations fell below the MIC for both drugs and in neither case were optimum synergistic ratios obtained in urine.
- 5 The figures obtained suggest that the use of sulphamoxole offers no advantage over sulphamethoxazole so far as the plasma and urinary concentrations are concerned, and suggest that in the treatment of urinary tract infections the necessity for combination therapy with TMP and a sulphonamide is open to question.

Introduction

Co-trimoxazole, a fixed dosage combination of trimethoprim (TMP) with sulphamethoxazole (SMZ), has been extensively used for the treatment of a diverse range of bacterial infections, in particular those of the urinary and respiratory tracts. *In vitro* tests have demonstrated that at optimum concentration ratios these two components act synergistically (Bushby & Hitchings, 1968). The synergistic effect is attributed to sequential blockade of the first two steps of bacterial folic acid synthesis. However, several studies have shown that following co-trimoxazole therapy optimum concentration ratios are not achieved in the urine, bronchial aspirate and cerebrospinal fluid (Greenwood & O'Grady, 1976; Reeves & Wilkinson, 1979; Bach *et al.*, 1973).

Recently new TMP-sulphonamide combinations, such as co-trimazine (TMP with sulphadiazine) and co-trifamole (TMP with sulphamoxole (SDMO)) have been introduced on the basis that the pharmacokinetic characteristics of these sulphonamides are more compatible with those of TMP, thus ensuring optimal concentration ratios in tissues and fluids.

In this study we investigated the serum and urinary concentrations of TMP and sulphonamide obtained following oral administration of the recommended standard dosing schedules for co-trimoxazole and co-trifamole in normal subjects. In particular, we wished to determine the pharmacokinetics of SDMO regarding which there is a paucity of data; we also wished to determine whether the ratios of TMP to sulphonamide achieved in serum and urine during treatment were optimal for synergy, i.e. between 1 to 10 and 1 to 40 (Ekstrom *et al.*, 1979).

Methods

Subjects

Five male and five non-pregnant female subjects aged 18 to 25 years participated in a two-way crossover study of co-trimoxazole and co-trifamole. All had normal body weight (range 58 to 80 kg), blood pressure, haematological and biochemical profiles.

Treatment

Standard co-trimoxazole treatment consisted of two tablets (each containing 80 mg TMP and 400 mg SMZ) orally every 12 h for nine doses.

Co-trifamole treatment consisted of a loading dose of two tablets (each containing 80 mg TMP and 400 mg SDMO) orally followed by maintenance doses of one tablet every 12 h for eight further doses; 100 ml of water was taken with the initial dose.

Study designs

The order of administration of treatment was randomized and balanced. There was a three week wash-out period between each leg of the trial.

No concomitant medication was permitted for one week prior to and during the study. Alcohol was not permitted for two days prior to the commencement of, and during treatment periods. The subjects were fasted for 12 h prior to each study leg, food was allowed after 4 h and thereafter there were no further dietary or fluid restrictions.

Serum collection

Blood samples (10 ml) were drawn before the initial dose and thereafter at intervals of ½, 1, 2, 3, 4, 8 and 12 h on the first day. On the remaining days of the study blood was withdrawn immediately before the morning dose (08.00 h) and 2 h after that dose. The blood was allowed to clot and then centrifuged at 1400 g for 10 min, the serum was removed and stored in 1.5 ml aliquots at -20°C for subsequent analysis.

Urine collection

Urine was collected between 0–12 and 12–24 h on the first day and 24 h collections were made on subsequent days; specimens were collected without preservative and after measurement of volume, a sample of each collection was frozen at -20°C for subsequent analysis. The creatinine concentration in each urine sample was estimated in order to detect major errors in collection.

Adverse reactions

During the period of the study no adverse reactions to the drugs were reported.

Analytical methods

Levels of unmetabolised TMP were determined in serum and urine using a high performance liquid chromatographic assay (Watson *et al.*, 1980).

Total (bound and unbound) non-acetylated sulphonamides were measured in serum and urine by

the method of Rieder (1972).

All batches were controlled using serum containing a known (weighed-in) amount of TMP, and SDMO or SMZ; in addition one third of the samples were randomly selected and re-assayed in independent batches in order to confirm the precision of the assay procedure.

Calibration of the assays was achieved using weighed amounts of the appropriate drug volumetrically prepared in serum and subjected to the same storage and analytical conditions as the assays.

Acetylator status

The acetylator status of each subject was determined according to the procedure of Schroder (1972) after oral administration of 10 mg/kg sulphadimidine.

Pharmacokinetic analysis

Single dose (0–12 h data) The observed serum concentrations and corresponding times to peak were taken as representing peak serum concentration and time to peak respectively. The area under the serum concentration curve (AUC) was calculated from the 12 h data extrapolated to the sensitivity of the assay, using the trapezoidal rule.

The derived pharmacokinetic parameters were obtained by least square linear regression of logarithmic serum concentration against time data from the peak to 12 h following the first dose. First order open compartment kinetics and complete absorption were assumed. The following equations were used to calculate pharmacokinetic parameters

$$1. \quad t_{1/2} = \frac{0.693}{k_{el}}$$

$$2. \quad V_d = \frac{D}{AUC \times k_{el}}$$

$$3. \quad CL_s = \frac{D}{AUC}$$

where $t_{1/2}$ = Serum elimination half-life
 k_{el} = serum elimination rate constant
 V_d = apparent volume of distribution
 D = dose
 CL_s = serum clearance
 AUC = area under curve

Repeated dosing (0–98 h data) Time to 'steady state' was taken as the time at which the average of the 'peak' and 'trough' serum values for a 24 h period was within 10% of the average for one or more subsequent 24 h periods (using levels at 24 and 26 h, 48 and 50 h, 72 and 74 h and 96 and 98 h).

Results

Serum

Following administration of the initial dose of co-trifamole and co-trimoxazole, SDMO concentrations obtained over the 12 h period and peak SDMO concentrations were significantly higher ($P < 0.05$, ANOVA) than for SMZ (Figure 1b and Table 1). In contrast, no significant differences were observed between TMP concentrations obtained over the 12 h period and peak TMP concentration for the two preparations (Figure 1a and Table 1). The serum level profiles for both TMP and sulphonamide were similar for the two preparations.

The apparent volume of distribution was significantly less for SDMO than for SMZ ($P < 0.05$, t -test). There was no significant difference between the other pharmacokinetic parameters for TMP and the two sulphonamides of co-trifamole and co-trimoxazole (Table 1). The pharmacokinetics of TMP were comparable in the presence of either SDMO or SMZ.

As would be predicted from the different dosing regimens 'peak' serum TMP and sulphonamide concentrations obtained from 24 h onwards during multiple dosing with co-trifamole were significantly lower ($P < 0.05$, t -tests) than for co-trimoxazole except at 24 h and 26 h (Table 2).

'Steady state' conditions were also achieved earlier for both components of co-trifamole than for co-trimoxazole: (significantly so for SDMO, $P < 0.01$, sign test). Median times to peak were 25 h (range 25–49 h) and 49 h (25–73 h) respectively; this is consistent with the dosing regimens.

Figure 1 Mean ($\pm$ s.d.) serum concentrations of:
(a) trimethoprim,
(b) sulphonamide, following a single dose.
—●— co-trimoxazole (160 mg TMP, 800 mg SMZ)
---○--- co-trifamole (160 mg TMP, 800 mg SDMO)

Table 1 Observed and derived pharmacokinetic parameters for TMP, SMZ and SDMO following dosing with co-trimoxazole and co-trifamole.

Drug	Preparation	$t_{\max}$ (h)		$C_{\max}$ mg l^{-1}	k_{el} (h^{-1})	$t_{1/2}$ (h)	AUC mg h l^{-1}	V_d (l)	CL_s (l h^{-1})
TMP	Co-trifamole	Median = 2	Mean	1.73	0.067	10.5	31.8	77.8	5.1
		Range = 0.5–3.0	$\pm$ s.d.	$\pm$ 0.35	$\pm$ 0.007	$\pm$ 1.0	$\pm$ 4.77	$\pm$ 16.6	$\pm$ 0.8
	Co-trimoxazole	Median = 2	Mean	1.86	0.071	11.0	37.1	71.3	5.0
		Range = 0.5–4.0	$\pm$ s.d.	$\pm$ 0.28	$\pm$ 0.024	$\pm$ 4.2	$\pm$ 16.1	$\pm$ 15.6	$\pm$ 1.7
SDMO	Co-trifamole	Median = 2	Mean	88	0.096	8.0	1030	9.4	0.9
		Range = 1.0–4.0	$\pm$ s.d.	$\pm$ 21	$\pm$ 0.031	$\pm$ 2.8	$\pm$ 393	$\pm$ 2.0	$\pm$ 0.4
SMZ	Co-trimoxazole	Median = 2	Mean	68	0.082	9.1	874	12.0	1.0
		Range = 2.0–4.0	$\pm$ s.d.	$\pm$ 11	$\pm$ 0.027	$\pm$ 2.6	$\pm$ 210	$\pm$ 2.3	$\pm$ 0.2

Table 2 (a) Lowest observed serum concentrations (mg/l) of TMP, SMZ and SDMO following multiple dosing with co-trimoxazole and co-trifamole (mean $\pm$ s.d.) $n = 10$.
 (b) 'Peak' serum concentrations (mg/l) of TMP, SMZ and SDMO following multiple dosing with co-trimoxazole and co-trifamole (mean $\pm$ s.d.) $n = 10$.

		<i>Time after commencement of treatment (h)</i>			
<i>Drug</i>	<i>Preparation</i>	<i>24</i>	<i>48</i>	<i>72</i>	<i>96</i>
TMP	Co-trifamole	0.91 ± 0.20	0.85 ± 0.18	0.83 ± 0.26	0.81 ± 0.24
	Co-trimoxazole	1.37 ± 0.41	1.93 ± 0.45	2.05 ± 0.65	2.05 ± 0.49
SDMO	Co-trifamole	38 ± 17	42 ± 19	42 ± 21	41 ± 20
SMZ	Co-trimoxazole	48 ± 7	65 ± 12	70 ± 16	70 ± 13

		<i>Time after commencement of treatment (h)</i>			
<i>Drug</i>	<i>Preparation</i>	<i>26</i>	<i>50</i>	<i>74</i>	<i>98</i>
TMP	Co-trifamole	1.73 ± 0.34	1.50 ± 0.27	1.51 ± 0.39	1.40 ± 0.39
	Co-trimoxazole	3.34 ± 0.76	3.58 ± 0.49	3.83 ± 0.95	3.66 ± 0.94
SDMO	Co-trifamole	76 ± 24	75 ± 27	75 ± 31	72 ± 24
SMZ	Co-trimoxazole	79 ± 21	104 ± 20	113 ± 26	110 ± 29

The ratios of serum sulphonamide to TMP concentrations through the first 12 h were consistently higher during co-trifamole treatment than with co-trimoxazole (Figure 2), a situation that continued throughout the remainder of the study. Statistically significant differences were noted at 26, 50, 74 and 98 h ($P < 0.05$, t -tests). The lowest concentration ratio observed during co-trifamole and co-trimoxazole treatment were 12 and 9 respectively; and the highest individual ratios 116 and 64 respectively. In general the ratios for co-trifamole but not co-trimoxazole were outside the quoted optimum ranges for synergy.

Acetylator phenotyping

Using sulphadimidine, four of the nine subjects were found to be fast acetylators and five were slow acetylators. The acetylator status for one subject could not be determined. For the fast acetylator group the mean (range) half-life of SDMO and SMZ were 7.3 h (4.9–13.2 h) and 8.7 h (4.7–12.6 h) respectively and for the slow acetylator group 9.0 h (6.6–11.0 h) and 9.6 h (7.6–13.9 h) respectively. No

consistent correlation between acetylator status and sulphonamide half-lives was observed.

Urine

Urinary TMP and sulphonamide concentrations achieved during the first 12 h following the initial dose were not significantly different for the two preparations. However, TMP concentrations achieved during the 12 to 96 h period were significantly lower during co-trifamole treatment as would be predicted (Table 3a, $P < 0.05$, t -tests). SDMO levels were lower than SMZ over the same period (Table 3a, $P < 0.05$, t -test). Urinary sulphonamide to TMP concentration ratios for both co-trimoxazole and co-trifamole were similar with mean ratios ranging between 1.2 and 2.4 throughout the study (Table 3b). All individual ratios achieved during both treatments were below the minimum ratio of 10 reported to be required for optimal synergy, the highest individual ratio obtained being 3 (co-trifamole—72–96 h collection).

The mean cumulative excretion from 0–108 h following co-trifamole and co-trimoxazole therapy

Figure 2 Mean ($\pm$ s.d.) ratio in serum of TMP: sulphonamide following a single dose.

—●— co-trimoxazole
 —○— co-trifamole
 ----- optimum synergistic range

Table 3 (a) Urinary TMP, SMZ and SDMO concentrations (mg/l) during the dosage with co-trimoxazole and co-trifamole (mean $\pm$ s.d.) $n \pm 10$.

(b) Urinary concentration ratios of sulphonamide/trimethoprim for co-trimoxazole and co-trifamole throughout the dosing regime.

a

		Time after commencement of treatment (h)				
Drug	Preparation	0-12	12-24	24-48	48-72	72-96
TMP	Co-trifamole	66 ± 20	102 ± 28	80 ± 15	74 ± 15	70 ± 17
	Co-trimoxazole	73 ± 27	162 ± 49	134 ± 37	155 ± 45	135 ± 32
SDMO	Co-trifamole	155 ± 47	151 ± 62	134 ± 75	145 ± 81	136 ± 77
	Co-trimoxazole	179 ± 110	185 ± 89	188 ± 75	175 ± 84	205 ± 104

b

SDMO/TMP	Co-trifamole	2.4 ± 0.5	1.5 ± 0.6	1.6 ± 0.7	2.0 ± 1.1	2.0 ± 1.1
SMZ/TMP	Co-trimoxazole	2.2 ± 0.8	1.3 ± 0.8	1.5 ± 0.7	1.2 ± 0.5	1.6 ± 0.9

for SDMO and SMZ were 791.5 mg (393.2–1296.2 mg) and 1046.9 mg (619.8–1545.0 mg) i.e. 18.0% (8.9–29.5%) and 13.1% (7.7–19.3%) respectively of the administered dose; the corresponding figures for TMP were 425.7 mg (305.7–504.4 mg) and 750.4 mg (579.9–944.1 mg) i.e. 48.4% (34.7–57.3%) and 46.9% (36.2–59.0%) respectively of the administered dose. As would be expected from the dose regimens there were statistically significant differences in amount of drug excreted as a cumulation for TMP and for SDMO and SMZ ($P < 0.05$, t -test).

Analytical precision

The precision for serum and urinary TMP was 6.3% and 1.1% and for sulphonamides 3.2% and 7.6% respectively.

Discussion

Following oral administration of multiple doses of co-trifamole and co-trimoxazole tablets to normal subjects, mean serum TMP concentrations obtained were similar to those reported previously whilst mean serum SDMO and SMZ concentrations were consistently higher in our study. In the study by Reeves *et al.* (1980) mean peak serum SDMO and SMZ concentrations of 50 and 41 mg/l respectively were obtained following oral administration of two capsules of each combination (160 mg TMP with 800 mg sulphonamide); in our study respective values were 88 and 68 mg/l. The observed differences may be due to differences in bioavailability as Kuhne *et al.* (1976) following a dosage regimen using tablets very similar to ours, reported mean nadir serum concentrations of SDMO of 46 mg/l at steady state. We found the mean nadir serum concentration of SDMO at steady state to be 42 mg/l.

The TMP concentrations in urine for co-trifamole and co-trimoxazole compare with other reports, as do the urinary SMZ concentrations. The urinary SDMO concentrations we found are about 20% lower than those reported by Kuhne *et al.* (1976). The assay used by us is claimed to be more accurate than the standard Bratton Marshall procedure (Reider, 1972)—we have confirmed this observation (unpublished work).

The distribution and pharmacokinetic parameters calculated for TMP and SMZ agree with other reports (Kasenan *et al.*, 1978; Welling *et al.*, 1973). Although Reeves *et al.* (1980) were the first to examine the pharmacokinetics of co-trifamole in a study similar in design to our own, they did not report any calculated pharmacokinetic parameters except SDMO half-life

ranging from 7.2–9.8 h. We found a mean of 8.0 h, which is in contrast to the half-life of 11 h reported by Seydel (1980). Thus the half-life of SDMO does not match TMP half-life.

The pairing of SDMO with TMP therefore offers no advantage over the pairing of SMZ with TMP on pharmacokinetic grounds.

Both the preparations used in this study have been formulated to produce TMP to sulphonamide ratios optimal for synergistic activity in serum. The synergistic ratios were determined *in vitro*, whereas extrapolation *in vivo* does not allow for the problems of protein binding, the presence of pus, or folate antagonists in urine. The approximate percentages of free (unbound) TMP, SDMO and SMZ in the serum have been reported as 55, 10 and 20% respectively (Seydel, 1980). Therefore, for both co-trifamole and co-trimoxazole the respective mean serum ratios achieved during treatment were between 7 and 15, which is within the lower range for optimal synergy to occur *in vitro*. However, synergy in serum is not usually relevant to treatment of the infected site as the degree and rate of penetration from the plasma water for the drugs may well differ and is reflected in reported ratios for TMP: sulphonamide for sputum (1:1.25) (Reeves & Wilkinson, 1979) and cerebrospinal fluid (1:1.6) (Bach *et al.*, 1973).

Considered independently, urinary TMP values far exceeded the minimum inhibitory concentration (MIC) for sensitive organisms of 2 mg/l (Brumfitt *et al.*, 1973). The urinary concentrations of both SDMO and SMZ did not consistently exceed the MIC of 200 mg/l for sensitive organisms (Brumfitt *et al.*, 1973) nor did the concentration ratio ever approach that consistent with optimum synergy. The relevance of synergy as a mechanism in antimicrobial therapy in urine is therefore open to question.

TMP alone has been successfully used to treat urinary (Kasenan *et al.*, 1978) and respiratory tract infections (Lacey *et al.*, 1980) and is as effective as co-trimoxazole in the treatment of such infections (Lacey *et al.*, 1980; Trimethoprim Study Group, 1981). Co-trifamole is as effective as co-trimoxazole (Nijessen, *et al.*, 1976) in urinary and respiratory tract infection. As most side effects in combined TMP/sulphonamide therapy are attributable to the sulphonamide component (Lacey *et al.*, 1980) and the evidence for *in vivo* synergy between TMP and SMZ or SDMO is poor, the use of TMP alone in the treatment of appropriate infections may be preferred.

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