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Note

Bioavailability of sulphamethoxazole-trimethoprim spheroidal granules

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Summary

The comparative bioavailability of a new formulation of sulfamethoxazole (SMZ) and trimethoprim (TMP), which was produced as reported previously (Athanassiou et al., *Int. J. Pharm.*, 72 (1991) 141–147) and a reference product (Septrin, Wellcome), was assessed following administration to a group of healthy, male volunteers. Each volunteer received the test (T) and reference (R) formulation. Plasma concentrations of SMZ and TMP were monitored over a period of 12 h, after drug administration, using a new and sensitive HPLC method. All pharmacokinetic parameters showed wide intersubject variation. Maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}) and the area under the curve up to the last measurement (AUC_0^{last}) and infinity (AUC_0^{∞}), were compared by analysis of variance or *t*-test and were found not to be significantly different among the two formulations.

Co-trimoxazole, a 1:5 (w/w) mixture of trimethoprim (TMP) and sulfamethoxazole (SMZ), is widely used for the treatment of a variety of infections caused by Gram-positive and Gram-negative bacteria, particularly those of the urinary and respiratory tracts (Goodman and Gilman, 1980).

While both TMP and SMZ are mainly bacteriostatic when used alone, in combination their effect is bactericidal. Synergism is due to sequential blockade at two separate steps in bacterial folate metabolism, resulting in inhibition of deoxyribonucleic acid synthesis (Wilson and Gisvold, 1982).

Optimal bactericidal activity is observed at SMZ concentrations 5–40-times greater than those of TMP, which is consistent with their minimum inhibitory concentration (MIC) values against susceptible bacteria (Patel and Welling, 1980).

As previously reported (Athanassiou et al., 1991), spheroidal granules of SMZ and TMP were produced and then encapsulated at a 5:1 weight ratio into hard gelatin capsules (400 mg SMZ and 80 mg TMP), using the method of wet granulation of powders in a rotating pan. Before encapsulation, TMP spheres were coated with Eudragit E 12.5% solution, in order to avoid

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interaction between the molecules of SMZ and TMP (Giordano et al., 1977).

The purpose of the present study was to evaluate the bioavailability and bioequivalence of the above formulation compared with a reference formulation (Septrin tablets, 400 mg SMZ and 80 mg TMP, Wellcome).

Bioequivalence of the two products was assessed based on the plasma concentration-time data obtained following oral administration to a group of healthy volunteers (McGilveray, 1991).

The study was performed in accordance with the Declaration of Helsinki and approved by the local ethics committee. All subjects read the protocol and gave written informed consent for their participation.

The subjects were selected from healthy male volunteers, aged 28–35 years, with standard weight to height ratio. Each volunteer was required to be free of cardiovascular, hepatic, renal, gastrointestinal or urinary diseases, as assessed by physical examination and review of medical history. Finally, five subjects were entered in the study. All of them had medical history free of severe diseases and the results of their clinical laboratory tests were within the normal ranges.

All subjects avoided using other drugs for at least 10 days prior to the study and were required to abstain from alcoholic and caffeine-containing beverages 48 h prior to each dosing.

After an overnight fast (at least 12 h), all subjects had a light breakfast. Prior to drug administration, a blood sample was taken and subsequently each subject received two tablets of the reference formulation. Blood samples were collected in heparinized glass tubes, with particular care being taken to avoid blood contact with the rubber stopper, at 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0 and 12.0 h post-dose. The plasma was immediately separated by centrifugation and stored at −20°C until analysis.

After a washout period of 15 days, two capsules of the tested formulation were administered. Plasma concentrations of SMZ and TMP were assayed simultaneously by an HPLC method.

5 μl of internal standard solution (500 μg/ml of phenacetin in methanol) and 100 μl of 1.0 M trichloroacetic acid were added in 1 ml of plasma. After vortex mixing for 30 s, the samples were centrifuged for 15 min at 3000 rpm. Aliquots were introduced into a C_{18} column through a Rheodyne 7125 injector with a 50 μl loop valve. The HPLC system used was a Spectra Physics model 8800 pump equipped with a Spectra 100 variable-wavelength detector (set at $\lambda_{max} = 230$ nm), which was connected with a Spectra Physics Chromjet integrator. Standard curves for each analyte were generated daily by analyzing samples containing standard amounts of SMZ and TMP added to aliquots of blank plasma. The daily standard curves were found to remain linear over the range of 8.0–64.0 μg/ml for SMZ and 0.4–2.4 μg/ml for TMP, throughout the entire study period. The correlation coefficient ($\pm$SD) of standard curves was 0.998 ($\pm$0.0010) for SMZ and 0.994 ($\pm$0.0012) for TMP. The method was reproducible with the coefficient of variation (CV) not exceeding 2.0% for TMP and 1.88% for SMZ, as assessed by repeated analysis of plasma samples ($n = 10$) spiked with TMP and SMZ at concentrations of 0.4 and 16.0 μg/ml, respectively.

Pharmacokinetic parameters for SMZ and TMP were determined from plasma concentration-time data. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were obtained directly from the plasma concentration-time data. The area under the plasma concentration-time curve up to the last sampling time (AUC_0^{last}) was determined by using the linear trapezoidal rule. The apparent elimination rate constant (k_{el}) was calculated by the technique of least-squares regression from the data for the last three points of each plasma concentration-time curve. The AUC_0^{∞} values were determined according to the equation:

$$AUC_0^{\infty} = AUC_0^{last} + C_{last}/k_{el}$$

Since one-compartment model kinetics adequately described the plasma profiles of both drugs (Patel and Welling, 1980), the elimination half-life ($t_{1/2}$) was calculated according to the equation:

$$t_{1/2} = \ln 2/k_{el}$$

TABLE 1

Mean plasma concentrations of SMZ and TMP (CV%) following administration of test and reference formulations in human volunteers

Time (h)	[SMZ] (μg/ml) (CV%)		[TMP] (μg/ml) (CV%)	
	Test	Reference	Test	Reference
1.0	26.20 (40.6)	26.26 (28.6)	0.936 (29.9)	0.969 (12.4)
1.5	47.77 (18.6)	43.99 (18.3)	1.564 (29.4)	1.573 (32.4)
2.0	38.27 (29.5)	37.12 (23.8)	1.438 (23.6)	1.434 (18.8)
3.0	28.74 (28.5)	26.17 (25.2)	1.130 (22.1)	1.132 (13.3)
4.0	27.61 (26.3)	24.18 (34.4)	1.122 (17.8)	1.096 (16.4)
6.0	23.49 (33.5)	20.67 (34.4)	0.996 (21.1)	0.983 (20.3)
8.0	18.94 (21.8)	18.87 (13.7)	0.760 (23.7)	0.912 (32.8)
10.0	13.56 (37.5)	10.56 (21.1)	0.608 (23.0)	0.590 (20.9)
12.0	9.29 (20.9)	9.58 (28.4)	0.450 (24.4)	0.440 (15.7)

All statistical tests were carried out at the 95% confidence level. Effects of formulation and subjects on the total variation in pharmacokinetic parameters were examined by two-way analysis of variance (ANOVA). The ANOVAs of AUC parameters and C_{max} values were carried out on the linear data (Bolton, 1984). The power of each ANOVA to detect a difference equivalent to 20% of a reference mean was computed according to the method of Winer (1971). Equality of variances for a given parameter between the two formulations was tested by the variance ratio (F) test (Devore, 1982).

The means and CV of the plasma concentrations at each sampling time following administration of test and reference are given in Table 1. At all time points, the treatment means were not significantly different from each other (ANOVA test: $p < 0.05$). The ratio of unchanged SMZ/TMP plasma concentrations ranged from 20.7 to 28.0 for the reference and from 20.6 to 30.5 for the test formulation during a period of 12 h following administration.

Fig. 1 depicts the mean (±SD) plasma concentration of SMZ and TMP observed in the five subjects studied after administration of reference and test formulations. The plasma concentrations of SMZ and TMP showed large intersubject vari-

TABLE 2

Mean pharmacokinetic parameters of SMZ and TMP (CV%) for the reference and test formulations and relative bioavailability of the test formulation

Formulation	AUC_0^{last} (μg h ml^{-1})	AUC_0^{∞} (μg h ml^{-1})	C_{max} (μg ml^{-1})	t_{max} (h)	k_{el} (h^{-1})	$t_{1/2}$ (h)
SMZ						
Test	276.82	333.34	43.99	1.70	0.116	5.98
(CV%)	(18.73)	(22.56)	(18.3)	(15.8)	(18.9)	(6.5)
Reference	304.33	357.17	47.77	1.76	0.127	5.95
(CV%)	(30.35)	(26.44)	(18.6)	(15.0)	(14.9)	(10.6)
Bioavailability of the test (%)	90.96	93.33	–	–	–	–
TMP						
Test	11.71	15.07	1.573	1.66	0.109	7.3
(CV%)	(19.59)	(15.86)	(32.4)	(15.6)	(13.7)	(11.8)
Reference	11.98	15.70	1.564	1.52	0.107	7.5
(CV%)	(26.00)	(26.88)	(29.4)	(15.7)	(24.3)	(19.5)
Bioavailability of the test (%)	96.66	95.98	–	–	–	–

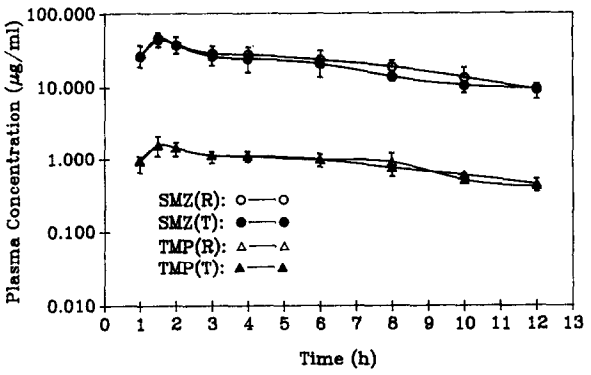

Fig. 1. Plasma SMZ and TMP concentration-time profiles (mean of five determinations ±SD) after administration of reference (R) and test (T) formulation.

ation for both formulations at all sampling times (CV ranged between 12.4 and 40.6%), which was also reflected in the calculated pharmacokinetic parameters.

Table 2 lists the mean values and CVs of the pharmacokinetic parameters for SMZ and TMP obtained from the plasma concentration data after separate administrations of reference and test formulations. The mean (±SD) k_{el} values and range of correlation coefficients associated with SMZ were 0.127 (±0.019) and 0.964–0.999 for the reference and 0.116 (±0.022) and 0.964–0.996 for the test formulation, respectively. The corresponding values for TMP were 0.107 (±0.026) and 0.987–0.998 for the reference and 0.109 (±0.015) and 0.998–0.999 for the test formulation.

Results of the ANOVA of parameters indicate that there was no significant effect of the formulation on any of the above AUC or C_{max} parameters. However, significant subject effects ($p < 0.005$) were noted for all the parameters, as would be expected in view of the wide intersubject variations. The statistical power to detect a 20% difference in the mean AUC and C_{max} was greater than 80% for both parameters. In the case of t_{max}, comparison between the two formulations was carried out by t-test. No significant difference was observed in this parameter between the two formulations.

Although the CVs of parameters of the test formulation were lower than the respective CVs of the reference formulation, the hypothesis that $\sigma_T^2 < \sigma_R^2$, of the pharmacokinetic parameters, was rejected at the 0.05 level (Devore, 1982).

The two formulations did not show any statistically significant difference on the basis of AUC parameters, C_{max} and t_{max}.

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