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In vitro and in vivo evaluation of four co-trimoxazole oral suspensions

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Summary

The in vitro dissolution, in vitro absorption and in vivo absorption characteristics of sulfamethoxazole (SMZ) and trimethoprim (TMP) from four commercially available oral suspensions have been studied. In vitro dissolution and absorption profiles for both drugs were similar for all four products. SMZ and TMP both followed first-order dissolution kinetics at pH 2.0 and 7.4, although rates were faster at pH 7.4. In vitro absorption was dissolution-rate limited with lag-times of about 2 h. Two-to three-fold variations in observed and estimated C_{max} and T_{max} values, respectively, were found using the rabbit model. However, there were no common criteria found to correlate dissolution, absorption and/or serum data to differentiate between the four suspension products.

Introduction

Co-trimoxazole contains sulfamethoxazole (SMZ) and trimethoprim (TMP) in a 5 to 1 ratio and is widely used in the treatment of a variety of gram-negative and gram-positive infections. The synergistic bactericidal effect of this combination is due to sequential blockade of bacterial enzyme systems associated with tetrahydrofolate synthesis, a metabolically active form of folic acid (Bushby, 1977). SMZ and TMP have similar pharmacokinetic characteristics in humans and obey first-order absorption, distribution, and elimination kinetics (Welling, 1980). Both drugs are well absorbed from the gastrointestinal tract, are rapidly and widely distributed to various tissues and fluids, and undergo mainly hepatic metabolism with subsequent renal excretion (Welling, 1980).

Both SMZ and TMP are only very slightly water soluble with aqueous solubilities of 0.5 μg/ml and 0.4 μg/ml, respectively, at 25 °C (Rudy and Senkowski, 1973; Manius, 1978). While SMZ is a weakly acidic sulfonamide ($pK_a = 5.6$), TMP is a weak base ($pK_a = 6.6$) which is structurally related to pyrimethamine (Rudy and Senkowski, 1973; Manius, 1978). Usually cotrimoxazole is administered orally either in tablet or liquid suspension form. When oral therapy is not feasible or

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if infections are severe, the drug combination may be administered by intravenous injection. The pH of the oral suspension is between 5.0 and 6.5, while the injectable form is adjusted to pH 10 with sodium hydroxide to ensure that both drugs are in solution (McEvoy, 1987).

Numerous studies have reported the bioavailability characteristics of co-trimoxazole when it is administered orally (Welling et al., 1973), by intravenous infusion (Grose et al., 1971), or rectally (Liedtke and Haase, 1979). However, no reports describing the bioavailability of co-trimoxazole in liquid oral suspensions were found in the literature. For some drugs correlations have been established between in vitro and in vivo data. For other drugs this has not been possible. Maeda et al. (1979) found that the rabbit is a useful animal model for examining comparative bioavailability properties of oral dosage forms.

The purpose of this investigation was to study the dissolution and absorption characteristics of TMP and SMZ from four commercially available oral suspension formulations, and to determine if correlations were observed between the rate and/or extent of in vitro dissolution, in vitro absorption, and in vivo absorption. The four suspensions that were studied were selected on the basis of their availability.

Materials and Methods

Materials

SMZ, TMP and glycine were obtained from S.D.I., Iraq. Other reagents were obtained from the following commercial sources and used as received: KH_2PO_4 (Merck, F.R.G.); $Na_2HPO_4 \cdot 12H_2O$ (Prolabo, France); and NaCl and HCl (Fluka, Switzerland). Four oral suspensions, each containing 8 mg TMP and 40 mg SMZ ml, were procured commercially and are subsequently referred to as products A, B, C and D. Viscosity of the products was determined using a Ferranti viscometer model VM (Manchester, U.K.), while the sedimentation volume was defined as the ratio of the final volume of the sediment at room temperature after 24 h to the original volume of the suspension.

In vitro dialysis

A 12 cm length of ileum obtained from a freshly killed mature rabbit was washed with normal saline and ligated 1 cm from one end. After 1 ml of phosphate buffer (pH 7.4) was introduced into the ileum, a 2 ml sample of a SMZ/TMP suspension was added and thoroughly shaken to ensure homogeneity. A further ml of buffer was then added and the open end of the ileum ligated. Each ileum sac was suspended 2 cm below the surface of 500 ml of phosphate buffer (pH 7.4) contained in a 1000 ml beaker. The contents of the beaker were maintained at $37.0 \pm 1.0°C$ and stirred at 50 rpm. Aliquots (2 ml) were withdrawn at 0, 30, 60, 90, 120, 180, 240 and 300 min intervals, and assayed as described below. Each dialysis experiment was performed in triplicate for each product.

In vitro dissolution

Dissolution studies were performed in a 6 channel in vitro dissolution assembly (Erweka, F.R.G, Model DT6), using a modified official rotating paddle method (U.S.P., 1983) Two ml of each product was carefully introduced into 500 ml of buffered dissolution media contained in a 1000 ml dissolution vessel maintained at $37.0 \pm 0.1°C$. Triplicate studies on all products were performed in both glycine buffer (pH 2.0) and phosphate buffer (pH 7.4) to simulate gastric and intestinal fluid, respectively. Two ml aliquots were withdrawn using a pipette with filter attachment at regular intervals during a period of 2 h. Each aliquot was extracted and assayed for both SMZ and TMP content using the procedure described below.

Extraction and analysis of SMZ and TMP

The spectrofluorometric method of Lichtenwalner et al. (1979) was modified for the extraction and spectrophotometric analysis of SMZ and TMP in buffered aqueous solutions. Each 2 ml sample was transferred to a 15 ml glass-stoppered centrifuge tube and 0.3 ml of a 0.1 M glycine-sodium hydroxide buffer (pH 9.5) was added. Each solution was vortexed for several seconds followed by the addition of 4 ml of chloroform to each tube, and the tubes were shaken for 5 min on a horizontal mechanical shaker (S.M., Labsco,

TABLE 1

Regression data for the calibration of TMP and SMZ in buffers pH 2.0 and 7.4 [a]

Drug	Buffer/serum	Range (μg/ml)	Slope $\times 10^{-2}$	Intercept	R^2
TMP	buffer pH 2.0	2.5– 12.5	6.1	0.053	0.996
TMP	buffer pH 7.4	1.0– 25.0	2.0	–0.015	1.000
TMP	serum	2.5– 40.0	0.7	–0.004	0.993
SMZ	buffer pH 2.0	10.0–200.0	0.9	0.013	0.999
SMZ	buffer pH 7.4	5.0–125.0	4.9	0.003	0.999
SMZ	serum	10.0–200.0	0.2	0.009	1.000

[a] Wavelengths of detection were 255 and 275 nm for SMZ and TMP, respectively.

F.R.G.) at approximately 100 oscillations per min. The tubes were then centrifuged at $2000 \times g$ for 10 min. The concentrations of SMZ and TMP in the upper aqueous and lower organic layers, respectively, were determined spectrophotometrically at their corresponding absorption wavelength maxima of 255 and 275 nm. Ethanolic stock solutions containing 1 mg/ml TMP or SMZ were used to prepare a series of standard solutions for each drug in buffers (pH 2.0 and 7.4) and serum. The concentration ranges used to prepare each calibration graph are shown in Table 1. Standard curves were prepared from combinations of known concentrations of the two drugs, using the same separation and analysis methods described above. Concentrations of the drugs in the samples were determined from these working standard curves. Extraction efficiencies for both drugs were greater than 80%.

In vivo rabbit study

A random, parallel design was used to determine serum levels of TMP and SMZ attained in adult rabbits (2.0–2.5 kg) after an oral dose of 2 ml/kg was administered with a syringe and oral feeding needle. Each product was tested in three rabbits. Blood samples (2 ml) were collected from the marginal ear vein at 0, 10, 20, 30, 45, 60, 75, 90, 120 and 150 min. All blood samples were allowed to coagulate at room temperature and then centrifuged at $2000 \times g$ for 15 min. One ml of serum was extracted as described by Lichtenwalner et al. (1979) and the concentrations of SMZ and TMP were then determined as described above.

Data analysis

Linear regression analyses were performed on all in vitro dissolution and absorption mean data for both TMP and SMZ from each product. First-order dissolution and absorption rate constants and corresponding T50% values (i.e., the time taken for 50% of the available drug to dissolve or be absorbed) were calculated for both drugs. In vitro absorption lag-times were obtained from the abscissa intercept after extrapolation of the linear segment of the cumulative amount absorbed verses time graphs. The peak serum drug concentration (C_{max}) and the time required to reach this level (T_{max}) for both drugs were obtained from rabbit serum data using a PC NONLIN program for a one-compartment model showing first-order absorption and elimination. These estimated values were compared with observed values obtained from serum drug concentration versus time graphs.

Results and Discussion

Microbiological, colorimetric, spectrofluorometric and chromatographic methods have been reported for the analysis of SMZ and TMP in biological fluids (Patel and Welling, 1980). However, the spectrophotometric procedure which was employed was developed for its relative simplicity (Lichtenwalner et al., 1979). Regression analysis of the calibration data for both SMZ and TMP in buffers (pH 2.0 and 7.4) and serum confirmed the linearity between absorption and drug concentrations over the concentration range tested with

TABLE 2

Physical properties of co-trimoxazole suspensions

Product	Viscosity (P)	Sedimentation volume after 24 h	Color
A	1.12	0.91	light pink
B	0.88	0.92	pink
C	1.65	0.90	milky white
D	1.43	0.92	pink

coefficients of variation between 0.993 and 1.000 (Table 1).

Particle size distribution is an important consideration in assessing dissolution and absorption characteristics of drugs in suspension. Therefore, the viscosity and sedimentation volumes of the four co-trimoxazole products were examined (Table 2). Colors of the suspensions were also noted and varied from white to pink. No differences existed in the sedimentation volumes of the four products. However, large differences were observed in the viscosities of the products with product C having a 1.9-fold higher viscosity than product B.

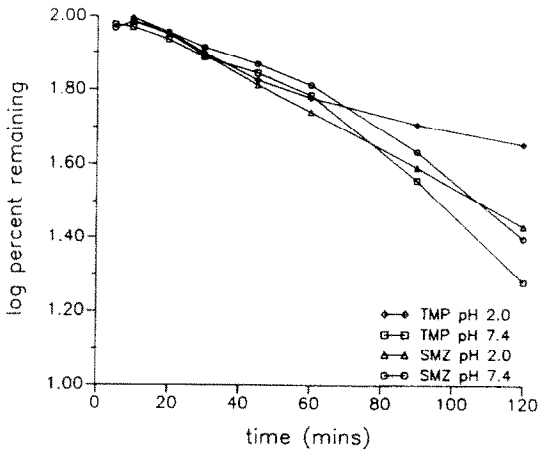

Fig. 1. Typical first-order in vitro dissolution profile for sulfamethoxazole (SMZ) and trimethoprim (TMP) from an oral suspension (product A) at pH 2.0 and 7.4.

The results obtained in this study indicate that the in vitro dissolution and absorption characteristis for SMZ and TMP were similar for all four

TABLE 3

Dissolution and absorption data for sulfamethoxazole (SMZ) from four commercial co-trimoxazole oral suspensions

Parameter	Product			
	A	B	C	D
In vitro dissolution, pH 2.0				
Percent dissolved	71.4 ± 2.8	51.8 ± 3.2	54.7 ± 2.0	64.8 ± 3.1
$k \times 10^{-3}$ (min^{-1})	11.70	6.52	7.05	8.97
R^2	0.999	0.682	0.975	0.980
T50% (min)	59.2	106.3	98.3	77.3
In vitro dissolution, pH 7.4				
Percent dissolved	74.7 ± 5.7	90.1 ± 3.0	60.5 ± 1.4	97.1 ± 0.9
$k \times 10^{-3}$ (min^{-1})	11.31	18.73	7.41	18.87
R^2	0.954	0.944	0.974	0.948
T50% (min)	61.3	37.0	93.5	36.7
In vitro absorption				
Percent absorbed	22.4 ± 2.1	19.5 ± 1.3	19.4 ± 1.9	22.4 ± 1.9
$k \times 10^{-4}$ (min^{-1})	8.88	7.11	7.64	8.78
R^2	0.888	0.898	0.928	0.937
T50% (h)	13.0	16.3	15.1	13.2
lag time (min)	142	160	148	126
In vivo rabbit serum				
T_{max} observed (min)	50	75	70	75
T_{max} estimated (min)	48 ± 2	117 ± 39	77 ± 6	85 ± 9
C_{max} observed (μg/ml)	90	63	74	130
C_{max} estimated (μg/ml)	88 ± 2	59 ± 7	71 ± 3	125 ± 2

TABLE 4

Dissolution and absorption data for trimethoprim (TMP) from four commercial co-trimoxazole oral suspensions

Parameter	Product			
	A	B	C	D
In vitro dissolution, pH 2.0				
Percent dissolved	47.4 ± 1.8	40.7 ± 4.3	44.7 ± 5.8	40.5 ± 1.8
$k \times 10^{-3}$ (min^{-1})	7.20	5.04	5.99	4.64
R^2	0.956	0.999	0.997	0.998
T50% (min)	96.3	137.4	115.7	149.3
In vitro dissolution, pH 7.4				
Percent dissolved	80.9 ± 2.4	85.6 ± 1.4	70.0 ± 0.2	100.4 ± 1.4
$k \times 10^{-3}$ (min^{-1})	13.52	16.27	9.83	18.77
R^2	0.955	0.960	0.985	0.909
T50% (min)	51.3	42.6	70.5	36.9
In vitro absorption				
Percent absorbed	19.3 ± 5.0	16.2 ± 3.0	22.0 ± 2.4	15.2 ± 1.9
$k \times 10^{-4}$ (min^{-1})	6.83	5.01	7.87	5.12
R^2	0.880	0.935	0.980	0.945
T50% (h)	16.9	23.1	14.7	22.6
lag time (min)	111	115	59	128
In vivo rabbit serum				
T_{max} observed (min)	53	61	60	90
T_{max} estimated (min)	38 ± 5	74 ± 13	56 ± 3	103 ± 23
C_{max} observed ($\mu g/ml$)	3.80	2.65	3.05	4.75
C_{max} estimated ($\mu g/ml$)	3.30	1.88	2.95	4.35

suspension products. Typical in vitro dissolution profiles obtained from product A for both drugs at pH 2.0 and 7.4 are shown in Fig. 1. Mean in vitro dissolution data for both SMZ and TMP from each oral suspension product are shown in Tables 3 and 4, respectively. As expected for these sparingly soluble drugs under non-accumulating conditions, both SMZ and TMP followed first-order dissolution kinetics with coefficients of variation between 0.938 to 0.999. First-order dissolution rate constants and dissolution half-lives are also presented in Tables 3 and 4 for the two drugs in the four products. Approximately 50% of the available SMZ and TMP had dissolved after 2.0 h at pH 2.0, suggesting that both drugs were near their saturation solubilities under these conditions. Both drugs dissolved more rapidly and completely at higher pH although greater variations were found between products (Tables 3 and 4).

The data indicate that no significant variations in the rate or extent of in vitro absorption of SMZ or TMP were found between the four products (Tables 3 and 4). However, using this experimental approach, the rate and extent of absorption were low for both drugs when compared with in vivo rabbit serum data. Figure 2 shows the mean cumulative amounts of SMZ and TMP absorbed for all four products using the in vitro ileum dialysis procedure. The standard deviations about the mean data points indicate that variations between products were minor. However, only approximately 20% of the available SMZ and TMP was absorbed in vitro after 5 h. These observations are consistent with results obtained for the absorption of phenethicillin from oral preparations using a similar in vitro dialysis procedure (Marty and Hersey, 1975). Like SMZ and TMP, phenethicillin also has very poor aqueous solubility.

The prolonged absorption lag-times of about 2 h can be attributed to the low aqueous solubility of both drugs and the small volume (4 ml) of buffer medium inside the rabbit ileum sacs. Similar absorption lag times of 1.4 to 2.4 and 0.5 to 2.7 for TMP and SMZ, respectively, have been reported in humans (Welling et al., 1973). The ab-

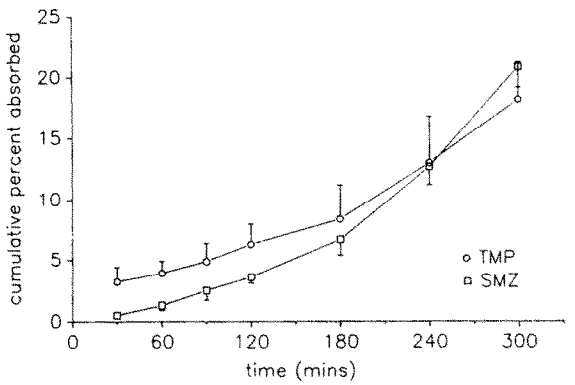

Fig. 2. Mean data from all four products showing the cumulative amount of sulfamethoxazole (SMZ) and trimethoprim (TMP) absorbed as a function of time.

sorption rate constants reported in Tables 3 and 4 are low, for although the studies were performed for 5 h, insufficient data points were obtained in the steady-state region for the lag-time interval to be omitted before calculating rate constants by regression analysis. Similarly, absorption half-lives are correspondingly prolonged. These results, and those obtained by Marty and Hersey (1975), suggest that this dialysis model may be inappropriate for evaluating the in vitro absorption characteristics of insoluble drugs. The slopes of the graph post lag phase in Fig. 2 indicate that the ileum has relatively high intrinsic permeability to both drugs. Hence, the rate of absorption in vitro can be considered dissolution rate-limited.

Large variations between products were observed in vivo as indicated by the T_{max} and C_{max} values for both SMZ and TMP (Tables 3 and 4, respectively). Two- and three-fold variations in both mean observed and estimated values were found. However, it is possible that these large variations reflect intersubject variation rather than variations in product performance. Human studies have shown considerable intersubject variation in absorption rates of SMZ and TMP (Patel and Welling, 1980). The relative bioavailabilities of each product are not reported because insufficient data points were collected during the terminal phase to enable area under the curves to be calculated accurately. The C_{max} and T_{max} values found in this rabbit study are similar to those reported for humans (Welling et al., 1973; Kaplan et al., 1973; Bach et al., 1973; Schwartz and Rieder, 1970).

For SMZ the following rank order was found between products for the dissolution rate at pH 2.0, absorption rate and observed C_{max} values: A > D > C > B (Table 4). For TMP the rank order for the dissolution rate at pH 7.4, and absorption lag-time (D > B > A > C), was similar to the order of T_{max} values (D > B > C > A) (Table 5). However, none of the trends in the dissolution, absorption or serum data criteria were common to both drugs and, therefore, it was not possible to establish a rank-order correlation with the performance of these four co-trimoxazole oral suspension products. In addition, no correlations existed between any of the rank orders and the viscosities of the products. Furthermore, the mean and standard deviation data ($N = 12$) for both drugs from all four products (Table 4) indicates that, with the expected exception of C_{max}, SMZ and TMP demonstrate very similar dissolution and absorption characteristics when formulated as an oral suspension. Based on the in vivo rabbit absorption studies, product D exhibited the highest observed C_{max} and T_{max} for both SMZ and TMP.

TABLE 5

Comparison of mean dissolution and absorption data for sulfamethoxazole (SMZ) and trimethoprim (TMP) from four oral suspensions

	SMZ	TMP
In vitro dissolution, pH 2.0		
Percent dissolved	60.8 ±10.1	45.3 ± 6.9
$k \times 10^{-3}$ (min^{-1})	8.56± 2.34	5.72± 1.14
T50% (min)	85.3 ±21.3	126.7 ±23.5
In vitro dissolution, pH 7.4		
Percent dissolved	80.8 ±16.1	84.3 ±12.5
$k \times 10^{-3}$ (min^{-1})	14.08± 5.68	14.60± 3.83
T50% (min)	57.1 ±26.9	50.3 ±14.7
In vitro absorption		
Percent absorbed	20.9 ± 1.7	18.2 ± 3.1
$k \times 10^{-4}$ (min^{-1})	8.10± 0.87	6.20± 1.30
T50% (h)	14.4 ± 1.6	19.3 ± 4.2
lag time (min)	144.0 ±14.1	103.3 ±30.4
In vivo rabbit serum		
T_{max} observed (min)	68 ±12	66 ±16
T_{max} estimated (min)	82 ±28	68 ±28
C_{max} observed ($\mu g/ml$)	89 ±29	3.56± 0.92
C_{max} estimated ($\mu g/ml$)	86 ±29	3.12± 1.02

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