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Experiences with the Rectal use of Trimethoprim

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Abstract

The possibility of rectal use of trimethoprim was studied. The in-vitro liberation of the drug from 24 different suppository bases was examined and the results used to select bases for in-vivo examination.

The in-vitro liberation from the suppositories containing 50–200 mg trimethoprim was studied by the method of dynamic diffusion, and the released drug content was measured spectrophotometrically. The in-vivo examinations were performed in anaesthetized rats. The concentration of trimethoprim in blood was determined by bioassay. The absorption of the drug in the form of oral suspension, rectal solution and suppository was also studied. The pharmacokinetic parameters obtained after blood-level curve fitting were compared by use of the MedUSA 1.6 program. The best in-vivo results were achieved with the lipohydrophilic Witepsol W 35 vehicle containing 10% polysorbate 20 and 10% polysorbate 61 (bioavailability = 63·8%) and with Witepsol W 35 containing 10% polysorbate 60 (bioavailability = 63·8%). The results for hydrophilic Macrogol 1540 vehicle containing 5% of Macrogol 400 were only slightly worse (bioavailability = 52·9%). In the case of the lipohydrophilic Witepsol W 35 vehicle with 10% polysorbate 20 and 10% polysorbate 61 content a significant negative exponential relationship was found between the administered doses and their respective bioavailability values; this tendency was also observed during in-vitro examinations.

When incorporated in the appropriate vehicle trimethoprim was absorbed well. With three vehicles the extent of absorption exceeded that for oral administration on the same model (bioavailability = 38·8%). Trimethoprim rectal suppositories, which are formulated with the vehicles having the best in-vitro and in-vivo results, are suitable for clinical pharmacological investigation.

The world-wide success of the trimethoprim–sulphamethoxazol combination (cotrimoxazol) has also revealed that approximately 90% of strains sensitive to the combination are also sensitive to trimethoprim alone (Brumfitt & Hamilton-Miller 1982). It has also become evident that resistance to trimethoprim is independent of whether it is used alone or with sulphonamides (Anon 1986).

Most of the side-effects of cotrimoxazol (allergy, renal and hepatic toxicity) are caused by the sulphonamide component. Because of its lipophilic character, trimethoprim has good tissue penetration, which thus leads to high concentrations in most tissues and body fluids (Robb et al 1971; Welling et al 1973; Brogden et al 1982; Cockerill & Edison 1987). Pure trimethoprim has been available in tablet form since 1972. If the appropriate vehicle is found experimentally, its administration in the form of a suppository will also be possible because its spectrum of action excludes the anaerobic bacteria mainly responsible for the protection of the intestinal wall, and hypersensitivity against the drug is rare. It is known that the absorption of the rectally administered drug is influenced considerably by the composition of the given suppository base (Thoma 1980; Müller 1986); this is confirmed by our own results (Regdon et al 1978; Regdon & Selmeczi 1988).

According to the studies of Liedtke & Haase (1979) on the rectal administration of cotrimoxazol in man, therapeutic blood levels can be achieved with both components. The aim of our experiments was to determine, with in-vitro and in-vivo methods, the near-optimum vehicle composition for subsequent clinical pharmacological trials. For this purpose the in-vitro liberation of drug from 24 different suppository bases was measured by dynamic diffusion. The validity of the conclusions drawn from the in-vitro results was studied in anaesthetized rats. The bioavailability values obtained with five mixed vehicles, and other pharmacokinetic parameters, were compared with each other and also with the results obtained by administration of trimethoprim intravenously, orally, or rectally in the form of a solution.

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Materials and Methods

Chemicals

Trimethoprim was purchased from EGIS Pharmaceuticals Ltd, Budapest, Hungary. Theobroma Oil, Witepsol W 35, Witepsol H 15, Massa Estarinum, Macrogol 1540 and Lipoxol 1550 bases were used as vehicles. Macrogol 400, Macrogol 4000, Lipoxol 400, Lipoxol 4000, Macrogol Stearate, Miglyol 812, Span 20, Polysorbate 20, Polysorbate 60 and Polysorbate 61 served as additives. All were obtained from Hüls AG or Witten and Goldschmidt AG, Essen, Germany.

In-vitro dynamic diffusion examination

The liberation and membrane diffusion of trimethoprim as a function of time was measured for five samples of each suppository containing different vehicles at a temperature of $37 \pm 0{\cdot}5°C$ by dynamic diffusion through a kidney-dialysing membrane (Union Carbide, Chicago, USA). The membrane had a pore diameter of 2–8 nm and a diffusion surface of approximately 18 cm^2. The suppositories were placed into a membrane sack and were examined individually with a Vibrotherm shaking water bath (Hungarian Academy of Sciences Kutesz, Budapest, Hungary) in such a way that the

20 mL of distilled water serving as the acceptor phase was replaced with an equivalent amount of water of 37°C. Samples were taken after 30, 60, 120 and 240 min of diffusion and their trimethoprim content was determined spectrophotometrically at $\lambda = 278$ nm either directly or after dilution (the various suppository bases did not interfere with measurement). For each experiment the mean values were calculated from results from five parallel examinations.

Experimental animals and their preparation

Female SPRD rats, 200–250 g, previously fasted for 8–10 h, were used for the experiments. Water was freely available during fasting. The animals were anaesthetized with 1 g kg^{-1} urethane administered intraperitoneally, then one lateral carotid artery was cannulated for the purpose of blood sampling. After the administration of various doses of trimethoprim, blood was taken through the carotid artery at the times given below. Whole blood (0·05 mL) was required for each measurement.

Trimethoprim blood level determination

A microbiological method (bioassay) was used on Müller-Hinton culture medium of pH 7·2, with *Bacillus pumilus* serving as the test organism. The calibration line was determined in each case with drug-free blood and trimethoprim serial dilutions. After incubation at 37°C for 16 h, the diameters of the inhibition zones were measured to within ±0·1 mm. The trimethoprim concentrations of our samples were calculated according to the regression equation of the calibration line. The correlation coefficient (r) thereof exceeded 0·99 in each case. The number of parallel experiments ranged from 5 to 10.

Determination of the pharmacokinetic parameters

The pharmacokinetic parameters were determined by analysing the blood level curves obtained with an IBM compatible computer with a one- or two-compartment open model, using the MedUSA 1·6 pharmacokinetic program. As the areas under the curves (AUC) were known, the absolute biological availability was calculated by taking into consideration the AUC values obtained after intravenous administration.

Results and Discussion

The membrane diffusion of 100 mg trimethoprim powder without vehicle was taken as the basis for calculating in-vitro relative availability (100%). Because trimethoprim is poorly water-soluble, it is not surprising that only part of the drug could diffuse through the membrane in a dissolved form.

Table 1 shows the composition and the relative availability values of the suppositories studied. Samples 1–11 are suppositories prepared with lipophilic bases, whereas suppositories 12–24 are hydrophilic. Additives with various HLB values added to the Witepsol bases form a lipohydrophilic system, which had a positive influence on liberation and diffusion in several cases. These include the Witepsol W 35 suppository base containing 10% polysorbate 20 and 10% polysorbate 61, which is known officially in Hungary under the name Adeps solidus compositus. This vehicle has also given favourable results with other drugs (Regdon et al 1994, 1996). Five- or sixfold better liberation and in-vitro diffusion were observed with the use of hydrophilic bases (numbers 12–24).

The results from the in-vitro diffusion experiments were analysed mathematically in an effort to characterize the process of liberation of trimethoprim from the suppository bases.

Table 1. Experimental suppository compositions and their in-vitro relative availabilities and s.e.m. values (calculated according to the entire quantity of trimethoprim diffused in 240 min).

No.	Suppository base	Additive	Amount trimethoprim (mg per 2 g suppository)	In-vitro relative availability	
				%	s.e.m.
1	Massa Estarinum	–	100	46·5	3·0
2	Witepsol W 35	–	100	93·0	6·5
3	Theobroma Oil	–	100	98·1	4·8
4	Witepsol H 15	–	100	90·0	1·9
5	Witepsol W 35	10% Polysorbate 20 10% Polysorbate 61	100	119·3	5·7
6	Witepsol H 15	10% Span 20	100	72·5	4·5
7	Witepsol H 15	10% Polysorbate 60	100	151·9	4·2
8	Witepsol W 35	–	50	170·6	13·8
9	Witepsol W 35	10% Polysorbate 20 10% Polysorbate 61	50	258·3	12·9
10	Witepsol W 35	–	200	55·8	4·0
11	Witepsol W 35	10% Polysorbate 20 10% Polysorbate 61	200	83·4	1·5
12	Macrogol 1540	–	100	622·5	24·6
13	Macrogol 1540	5% Macrogol 400	100	538·4	16·5
14	Macrogol 1540	10% Macrogol 400	100	553·8	54·7
15	Macrogol 1540	10% Macrogol 4000	100	611·1	41·4
16	Macrogol 1540	5% Polysorbate 20	100	499·9	19·5
17	Macrogol 1540	5% Macrogol stearate	100	608·8	13·3
18	Macrogol 1540	5% Span 20	100	527·6	21·2
19	Macrogol 1540	5% Span 20	50	668·1	48·4
20	Macrogol 1540	5% Span 20	200	200·3	14·6
21	Lipoxol 1550	–	100	522·9	53·0
22	Lipoxol 1550	5% Lipoxol 400	100	536·9	13·6
23	Lipoxol 1550	10% Lipoxol 400	100	601·1	28·7
24	Lipoxol 1550	10% Lipoxol 4000	100	643·0	47·6

It was found that the diffusion process could be plotted as lines in a double logarithmic system. The closeness of the relationship is indicated by the values of the correlation coefficients, which approximate to 1·000 (between 0·990 and 0·999).

The vehicle composition of the suppositories studied in-vivo, their lipophilic and hydrophilic properties and the doses administered are shown in Table 2. The doses were chosen according to the extent of absorption and the sensitivity of the bioassay method.

The pharmacokinetic properties of trimethoprim were also examined with intravenous, oral and rectal administration. The blood level curves obtained after an intravenous bolus injection were analysed with a two-compartment open model (Table 3).

A one-compartment open model was used for the analysis of the blood level curves after the oral administration of trimethoprim suspension prepared with mucus containing 1% methylcellulose, and after rectal administration as a solution in dimethylsulphoxide. Curve fitting was also performed according to the one-compartment open model for suppositories prepared with the five different vehicles under study (Table 4). The blood level curves after rectal administration can be seen in Fig. 1.

As our aim was to evaluate the expected therapeutic effect of the suppositories of different composition, biological availability was considered to be the most important kinetic parameter and the basis of comparison. Obviously, the other parameters, e.g. peak concentration (c_{max}) or the time needed to reach the peak concentration (t_{max}) also gave useful additional information. It is easy to see that slight variations in the absorption constant (k_a) or absorption half-life ($t_{\frac{1}{2}a}$) values will not have a significant influence on the therapeutic effect.

Data from the dose of 3·12 mg kg^{-1} were taken as the intravenous basis for comparison, as this is closer to the dose in man (2·5–3·0 mg kg^{-1}). Witepsol W 35 containing 10% polysorbate 60 gave the best results for the rate of absorption ($t_{\frac{1}{2}a}$ = 22·8 min). The fastest elimination was also seen both with this base and with Witepsol W 35, containing 10% polysorbate 20 and 10% polysorbate 61 ($t_{\frac{1}{2}e}$ = 199·18 and 137·61 min, respectively). These two suppository bases had the best properties in respect of peak concentration (c_{max}) and the time needed to reach them (t_{max}), or when the values of absolute bioavailability were compared. Thus absorption is largely influenced by the hydro-, lipo- or lipohydrophilic nature of the vehicle and by its HLB value. This is probably

Table 2. Vehicle composition, properties and administered doses of the studied suppositories.

Base	Additive	Property
Witepsol W 35*	–	Lipophilic
Witepsol W 35	Miglyol 812 (10%)*	Lipophilic
Witepsol W 35	Polysorbate 60 (10%)**	Lipohydrophilic
Witepsol W 35	Polysorbate 20 (10%) Polysorbate 61 (10%)***	Lipohydrophilic
Macrogol 1540	Macrogol 400**	Hydrophilic

*25·0 mg kg^{-1}. **6·25 mg kg^{-1}. ***6·25 mg kg^{-1}, 12·5 mg kg^{-1}, 25·0 mg kg^{-1} and 50·0 mg kg^{-1}.

Table 3. Pharmacokinetic parameters obtained after intravenous administration (analysed with a two-compartment open model).

Dose (mg kg^{-1})	12·5	3·12
Distribution hybrid constant (min^{-1})	0·089	0·064
Distribution half-life (min)	7·79	10·79
Elimination hybrid constant (min^{-1})	0·011	0·004
Elimination half-life (min)	63·03	170·29
Area under the concentration-time curve (mg L^{-1} min)	384·79	199·91

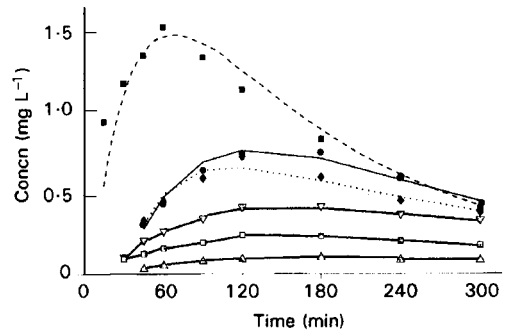

FIG. 1. Blood level curves of rectally administered trimethoprim: ■ rectal solution; ◆ suppository 5; ● suppository 6; ▽ suppository 7; □ suppository 4; △ suppository 3 (numbering as in Table 4).

Table 4. Pharmacokinetic parameters obtained after the administration of oral suspension, rectal solution and suppositories (analysed with a one-compartment open model).

Number	1	2	3	4	5	6	7
Vehicle	Mucilago methylcellulose (oral suspension)	Dimethylsulphoxide (rectal solution)	Witepsol W 35	10% Miglyol 812 90% Witepsol W 35	10% Polysorbate 60 90% Witepsol W 35	10% Polysorbate 20 10% Polysorbate 61 80% Witepsol W 35	5% Macrogol 400 95% Macrogol 1540
Dose (mg kg^{-1})	25·0	6·25	25·0	25·0	6·25	6·25	6·25
Elimination constant (min^{-1})	0·0043	0·0064	0·0019	0·0055	0·0034	0·0050	0·0028
Elimination half-life (min)	161·22	108·08	363·02	126·02	199·18	137·61	242·63
Absorption constant (min^{-1})	0·035	0·032	0·017	0·0079	0·030	0·0017	0·015
Absorption half-life (min)	19·88	21·49	40·95	89·00	22·80	40·49	46·37
Peak concentration (mg L^{-1})	2·046	1·516	0·107	0·238	0·675	0·787	0·416
Time to reach peak concentration (min)	73·76	68·48	170·42	156·83	110·51	131·26	154·62
Area under the concentration-time curve (mg L^{-1}min)	622·05	339·26	72·38	88·36	258·46	255·19	211·78
Bioavailability (%)	38·89	84·85	4·52	5·52	64·89	63·82	52·96

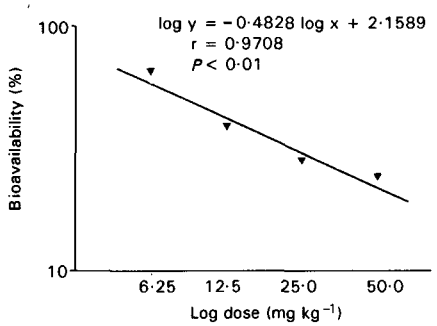

FIG. 2. Relationship between administered dose and bioavailability. Vehicle: 10% polysorbate 20 + 10% polysorbate 61 + 80% Witepsol W 35.

because the solubility of trimethoprim in a lipophilic vehicle results in a reduced rate of absorption. A similar retaining effect was observed in earlier studies (Regdon et al 1978). This might also explain the poor absorption observed by Dorr et al (1981) in the course of their trials with man, as they used lipophilic theobroma oil as a vehicle.

If the solubility of the drug in the vehicle decreases, so does the retaining effect (Fig. 1). This is the reason why, compared with lipophilic substances, much better results are obtained with hydrophilic and lipohydrophilic vehicles. Our data reveal that in our experimental model three of the five different vehicles studied proved better than oral administration (Table 3). This phenomenon was also observed with other drugs (van Hoogdalem et al 1991).

An interesting observation was made for the Witepsol W 35 vehicle containing 10% polysorbate 20 and 10% polysorbate 61, namely that a significant negative exponential relationship existed between the administered doses and their respective bioavailability values. This tendency had also been observed during the in-vitro experiments, but not to a significant degree. Accordingly, if the log of bioavailability was plotted against the log of individual dose, the data give a line (Fig. 2). The relationship proved to be significant ($P < 0.01$). Other authors have referred to a similar phenomenon without confirming the nature of the relationship (Diller & Bünger 1965). Our experiences so far show, however, that this relationship does not apply to all the vehicles studied in our experiments; no such relationship could be found for lipophilic Witepsol W 35 containing 10% miglyol 812. The solubility of the drug in the vehicle might also be important in this instance. Further experiments are needed to find the proper explanation. This phenomenon might have therapeutic consequences, because in such cases increasing the dose does not bring about a proportional increase in efficiency. Our experiments have not exhausted the technological possibilities of increasing the extent of absorption, as an even greater bioavailability value was achieved by administering the drug rectally in solution (Fig. 1). When the drug is administered in solution it is in a state ready for absorption and so the retaining effect of dissolution is absent and the extent of absorption is determined primarily by the physicochemical properties of the drug. The indication is, therefore, that for trimethoprim there is room for improvement in finding the optimum vehicle. The use of dimethylsulphoxide, the solvent chosen as a model, was justified by the solubility properties of trimethoprim, because the administration of the required dose was not possible in aqueous solution. The literature makes many references to the rectal absorption of paracetamol and indomethacin, which have solubility properties similar to those of trimethoprim but belong to a different pharmacodynamic group. These point out that absorption is influenced by the lipo- hydro- or lipohydrophilic properties of the vehicles in a similar way (Jonkman et al 1984; Jensen & Grenabo 1985; Lauroba et al 1990). In our experimental model the bioavailability values of three suppository bases were higher than the oral values, which is very promising in respect of therapy in man. Our experimental results clearly show that the best of our formulated trimethoprim suppositories are ready for clinical pharmacological investigation.

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