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COMMUNICATIONS

The effect of the molecular weight of polyethylene glycol on the bioavailability of paracetamol–polyethylene glycol solid dispersions

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A controlled study of the bioavailability of paracetamol in solid dispersion with PEG 6000, 10000 and 20000 has been made. The total amount of paracetamol excreted in urine increased with molecular weight of the PEG, but the rate of absorption of the drug was unaffected.

In Chodos & DiSanto's review (1974) of the bioavailability of drugs, paracetamol is classified among those for which bioinequivalence is well documented, while Ritschel (1972) includes it among drugs for which bioavailability problems do not necessarily involve therapeutic inequivalence. The fact that the drug's solubility in gastric juice is some 1% w/v, together with the wide differences in gastric emptying time between individuals, prompted us to investigate the bioavailability of paracetamol in solid dispersion with PEG 20000 (Vila-Jato et al 1983) and the effect of ageing of the solid dispersion on the bioavailability of the drug (Vila-Jato et al 1984).

Comparisons of the relative effectiveness of PEG of different molecular weights have been inconclusive. Thus while increase of molecular weight of PEG decreased the enhancement of dissolution of spironolactone (Geneidi & Hamacher 1980) and digoxin (Ammar et al 1980) from solid dispersions, increase in molecular weight increased the enhanced dissolution of papaverine (Salib & Ebian 1978), sulfamethoxydiazine (El-Gindy et al 1976) and chlorothiazide (Salama et al 1981).

The object of the present study was to investigate the influence of molecular weight of PEG on the bioavailability of paracetamol in solid dispersions.

Materials and methods

Preparation of solid dispersions. The method consisted in melting the PEG (mol. wt 6000, 10000 and 20000), adding a solution of paracetamol, and evaporating the solvent under vacuum. All the solid dispersions prepared contained 20% of drug and 80% of PEG.

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Determination of paracetamol in urine. To 2 ml of urine were added 8 ml of acetic acetate buffer (pH = 5) and 800 units of β-glucuronidase with sulphatase activity (sigma G-0751). After incubation for 1 h at 37 °C, total drug was extracted according to Möes (1974). The residue was dissolved in methanol and injected into a HPLC (detector at 245 nm) with a Lichrosorb RP-8 column and eluted with water–methanol–formic acid (86:14:0·5) at a flow rate of 2 ml min^{-1} (retention time: 4·2 min).

Bioavailability protocol. Fifteen healthy volunteers aged 20–35 years were randomly distributed in a 3 × 3 latin square design and written informed consent was obtained from each individual before enrolment. Half an hour before a standard breakfast (200 ml coffee + milk and 4 biscuits), each subject took 200 ml of water with a dose of solid dispersion equivalent to 250 mg of drug. Urine samples were taken 1, 2, 3, 4, 5, 6, 8, 10 and 12 h after administration of the drug and the period between assays was 1 week.

Pharmacokinetic analysis. The three statistical moments (Cutler 1978; Yamaoka et al 1978) were used. The first moment is the total quantity of drug excreted in the 12 h following administration (E_{12}) and the second and third moments (MRT and VRT) were calculated using the equations:

$$MRT = [\int(dE/dt).t\,dt]/E_{12}$$

$$VRT = [\int(t - MRT)^2\,(dE/dt)\,dt]/E_{12}$$

where dE/dt is the urinary excretion rate in mg h^{-1} and t is the time in h. The units employed for E_{12}, MRT and VRT were respectively, mg, h and h^2.

Statistical analysis. The data were analysed by a two-way MANOVA, the utility of which in bioavailability studies has been proved (Vila-Jato et al 1980). Before carrying out the MANOVA, Barlett's test was used to check the homogeneity of the variances.

Table 1. Statistical moments of each of the solid dispersions studied.

Subject	PEG	E_{12}*	MRT	VRT	PEG	E_{12}*	MRT	VRT	PEG	E_{12}*	MRT	VRT
1		166·00	4·53	8·93		144·59	4·93	8·57		169·45	4·37	8·97
2		181·67	4·57	8·70		135·93	5·01	10·20		161·10	4·19	7·27
3	20000	213·62	3 80	7·42	10000	166·44	4·90	10·00	6000	128·22	4·19	11·41
4		190·95	4·72	8·84		167·74	3·59	7·68		145·15	5·32	10·13
5		181·95	5·32	9·64		180·06	4·13	7·83		130·07	5·39	10·86
6		141·17	5·08	10·14		122·95	5·26	9·88		188·04	4·18	8·08
7		182·40	4·43	9·11		154·35	4·64	8·91		175·70	4·88	9·29
8	10000	181·49	4·45	10·13	6000	160·01	4·56	8·75	20000	208·55	3·70	7·67
9		162·82	3·70	6·88		132·75	4·58	9·92		221·29	3·75	6·26
10		158·74	4·57	7·72		129·80	4·73	8·76		189·90	4·84	9·21
11		136·90	4·89	9·73		128·92	4·00	6·54		161·25	5·02	10·34
12		142·72	4·00	7·37		1478·37	4·62	9·96		158·29	4·66	10·20
13	6000	134·62	4·90	9·74	20000	185·22	4·16	8·30	10000	171·28	3·98	7·62
14		138·85	4·22	8·35		209·57	4·02	8·60		195·62	5·17	10·35
15		118·12	4·95	9·50		194·82	4·05	8·56		156·96	4·89	9·39

* Total amount of paracetamol excreted in the 12 h following administration.

Table 2. Results of MANOVA. The greatest eigenvalue of $H.E^{-1}$ is 1·538 $c_s/1 + c_s = 0·606$. The parameters for its distribution are: s = 2; m = 0 and n = 12. Null hypothesis rejected at $\alpha = 0·05$ level.

Source of variation	Matrix	Sum of squares and products		
Subjects	I	5 370.00		
		−48·008	2·3201	
		−32·2078	3·3507	8·8984
Formulations	H	15 400·6286		
		−141·3437	1·3045	
		−303·9815	2·8582	6·6406
Error	E	10 050·5867		
		−102·7886	7·4432	
		−205·5667	16·1820	46·5611
Total	T	30 821·5047		
		−292·1408	11·0680	
		−541·7561	22·3910	62·0500

Table 3. Roy's test for multiple contrast.

Parameter	Least significant difference = 0·05	6000 vs 10000	20000 vs 6000	10000 vs 20000
E_{12}	31·47	23·98	41·05*	21·03
MRT	0·86	0·19	0·41	0·22
VRT	2·14	0·22	0·90	0·68

* Significant.

Table 4. Mean values and coefficient of variation of the parameters, for each solid dispersion.

Solid dispersion	E_{12}	CV	MRT	CV	VRT	CV
6000	141·00	10·00	4·76	9·54	9·30	12·20
10000	164·72	10·30	4·57	11·19	9·07	13·60
20000	185·67	13·20	4·34	11·05	8·40	12·60

Results and discussion

Table 1 shows the statistical moments calculated from the urinary excretion data of each subject and Table 2 shows the matrices of the sums of squares and products corresponding to the various sources of variance. The largest eigenvalue or greatest characteristic roots (Morrison 1976, p. 189) of the matrix $H.E^{-1}$ is $c_s = 1·538$ and therefore the statistic $(c_s/1 + c_s) = 0·606$ which has distribution parameters: s = 2; m = 0 and n = 12. Since the critical value of this statistic for $\alpha = 0·05$ as given by Hecks charts (Morrison 1976) is 0·425, the null hypothesis was rejected.

The next step was to find out which parameters were affected by the factor under study, as well as how they were affected. This was done by means of Roy's test (Roy & Bose 1953). Table 3 shows the results obtained and, as can be observed, the only parameter affected was the total quantity of drug excreted (E_{12}) between the PEG 20000 solid dispersion and that of PEG 6000. The least significant difference of 31·47 mg for this parameter (which amounts to 15·6%) is remarkably low for a bioavailability study, reflecting the sensitivity of the experimental design employed. The coefficients of variation of E_{12}, which ranges from 10 to 13·3% (Table 4), are much lower than those found by McGilveray et al (1971).

In linear pharmacokinetic models the mean residence time (MRT) is the sum of two components: mean absorption time and mean disposition time. The use of an experimental design in which all the formulations are tested on all the subjects allows the second component to be filtered out by the analysis of variance. In the present study no significant differences were observed among the MRTs of the three formulations, probably because of the wide differences between individuals and the kinetics of the orally administered drug. In a study carried out using 10 subjects, Albert et al (1974) found the absorption constants corresponding to an open bicompartmental model to range from 0·25 to 4·25 h^{-1}, with a mean of 1·68 h^{-1}.

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Effects of several ergosines on adenylate cyclase activity in synaptosomal membranes of the bovine caudate nucleus

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The effects of several ergosines and different dopamine agonists and antagonists on the activity of dopamine-sensitive adenylate cyclase in synaptosomal membranes of the bovine caudate nucleus were comparatively studied. Among ergot alkaloid derivatives used, ergosinine was the most active in stimulating adenylate cyclase activity. Ergosine, bromoergosine, dihydroergosine, dihydroergocryptine and lisuride also stimulated this enzyme. Dihydroergosinine, bromodihydroergosine and bromoergocryptine did not affect adenylate cyclase activity. Saccharino derivatives of both ergosine and ergosinine were inactive. When used in higher concentrations, ergosine, ergosinine, dihydroergocryptine and lisuride inhibited dopamine-stimulated adenylate cyclase whereas other ergot alkaloid derivatives examined did not. If the extent of dopamine-sensitive adenylate cyclase stimulation is considered as a measure of dopaminergic activity, examination of the structure/dopaminergic activity relationship showed that modifications of ergot alkaloid molecules such as isomerization in position 8, hydrogenation of $\Delta^{9(10)}$-double bond, or introduction of bromine into position 2 of the molecule, lead to a significant decrease of stimulatory effects of adenylate cyclase. Introduction of a saccharino group into position 2 of the molecule caused a total loss of stimulatory activity of both ergosine and ergosinine, probably because of the size of the saccharino residue.

Numerous publications have demonstrated the dopaminergic activity of some ergot alkaloids (Johnson et al 1976; Cannon et al 1981; Euvrard et al 1981; Holohean et al 1982; Lataste 1984) and have led to the introduction of these compounds in the therapy of disorders related to the dysfunction of dopaminergic systems (Berde 1978; Stadler 1980). However, ergot alkaloids from the ergosine series have been neglected. Recent pharmacodynamic studies (Djordjević, personal communication) have suggested that ergot alkaloids from ergosine series have dopaminergic activity. This has prompted us to investigate the effects of these substances on the activity of dopamine-sensitive adenylate cyclase (EC 4.6.1.1) in synaptosomal membranes of the bovine caudate nucleus. Increased synthesis of cAMP is one of the earliest events after dopamine treatment (Seeman 1980) and changes in adenylate cyclase activity may be used to estimate the dopaminergic activity of dopamine agonists (Clement-Cormier et al 1979; Seiler & Markstein 1982). The literature concerning the effects of ergot alkaloids on dopamine-dependent adenylate cyclase, provides an unclear picture of the ergot alkaloid/adenylate cyclase activity relationship (von Hungen et al 1975; Trabucchi et al 1976; Schmidt & Hill 1977; Fuxe et al 1978; Azuma & Oshino 1980) because of different experimental approaches. Our results demonstrate prominent stimulatory effects of several ergot alkaloids from ergosine series on adenylate cyclase activity in synaptosomal membranes of the bovine caudate nucleus.

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Material and methods

Synaptosomal membrane preparation. Nuclei caudata were dissected from bovine brains obtained from a local abattoir 2 h after death. Synaptosomal membranes were prepared by the method of Nishikori et al (1980) for preparation of the M_1 fraction. The final pellet was resuspended in 80 mM Tris HCl, 0·6 mM Na_4EDTA, pH 7·4 to produce a protein concentration of 10 mg ml^{-1} and divided into 4·0 ml aliquots, which were frozen in liquid nitrogen and kept at −20 °C.

The protein concentration was determined according to Lowry et al (1951) using a bovine serum albumin as a standard.