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Effect of compression force on biopharmaceutical characteristics of Eudragit RS-based cephalexin tablets *

Ramón Martínez-Pacheco, José L. Vila-Jato, Angel Concheiro, Consuelo Souto, Cándida M. Losa and Teresa Ramos

Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Santiago de Compostela (Spain)

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Summary

This paper describes the biopharmaceutical evaluation of a series of cephalexin tablet formulations with a view to their utility for controlled release of antibiotics. Our earlier study of the dissolution rates of double-layer cephalexin tablets with matrices based on the acrylic resins Eudragit E and Eudragit RS had shown that a wide variety of dissolution profiles could be obtained by varying the compression force used to punch tablets containing Eudragit RS alone. The formulations tested in the present study were accordingly punched under 30, 40 or 50 MPa from a granulate containing 10% Eudragit RS. The bioavailabilities of these formulations were compared with each other and with that of a capsule formulation using a latin square design for multivariant analysis of the statistical moments of curves of urinary excretion of cephalexin. The results showed that the proportion of the dose that was absorbed decreased progressively with increasing punch pressure. Further studies in which the same formulations were subjected to dissolution rate and mercury porosimetry tests were carried out to investigate in vivo–in vitro correlations and the influence of total porosity and pore-size on release profiles.

Introduction

Owing to its pharmacokinetics and bactericidal activity (Welling, 1983; Kato et al., 1977), cephalexin is one of the antimicrobial agents which have received most attention in the development of controlled-release formulations. Matrices for such formulations have included cellulose (Hasegawa et al., 1980), acrylic polymers (Higashikawa, 1983) and lipid-based substances (Kato et al., 1981). However, very few data are available concerning the influence of technological variables on the release of cephalexin from such formulations.

In a previous study (Martínez-Pacheco et al., 1986), we determined the rates of dissolution of cephalexin from a series of double-layer tablets prepared using several punch pressures and several matrices based on the acrylic resins Eudragit E and Eudragit RS. We concluded that a great variety of antibiotic dissolution profiles could be obtained using Eudragit RS alone and varying the punch pressure. The main aim of the study now reported was to evaluate the effects of punch

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Correspondence: J.L. Vila-Jato, Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, Laboratorio de Farmacia Galénica, Facultad de Farmacia, Santiago de Compostela, Spain.

pressure on the bioavailability of cephalexin in this kind of tablet so as to establish in vivo–in vitro correlations and to assess the potential of such tablets for controlled absorption of the antibiotic.

Materials and Methods

Formulations

The 3 tablet formulations studied all contained 88.5% cephalexin (from Antibioticos S.A., lot CFX 1358), 10% Eudragit RS, 1.0% talc and 0.5% magnesium stearate. Formulation A was punched using a maximum pressure of 50 MPa, Formulation B under 40 MPa, and Formulation C under 30 MPa. The preparation method basically consisted in punching tablets from a granulate composed of a mixture of the antibiotic and a solution of the polymer in acetone. Technological details of the method and the punch machine used have been published elsewhere (Martínez-Pacheco et al., 1986). The dose of cephalexin was in all cases 500 mg per tablet.

Dissolution rate and porosity

The rate of dissolution of cephalexin from the formulations studied was measured using a flow-through, non-accumulating apparatus developed in our laboratory (Llabrés et al., 1978). This apparatus allows the use of a pH gradient. The dissolution media were artificial gastric and enteric juices without enzymes (U.S.P., XXI edn.). Three tablets of each formulation were tested separately. The concentration of cephalexin in samples taken from the test media was determined by direct U.V. spectrophotometry ($E_{1\%,1\,cm} = 211$ at 262 nm). The corresponding quantities of cephalexin dissolved were calculated by our "dissolved percentage" method (Llabrés et al., 1978).

Total porosity and pore size distribution were determined by mercury intrusion porosimetry between 0 and 2100 atm. Pore size distribution was analysed applying a probit transformation to the logarithmic cumulative curves.

Equally spaced punch pressures were employed to produce the tablets of the different formulations so as to allow analysis of variance to be used to estimate the separate effects of linear and quadratic terms in punch pressure on dissolution rate and total porosity.

Bioavailability

The bioequivalence of Formulations A, B and C with capsules containing an equal dose of cephalexin was studied using the "statistical moments" E_{12} (total amount of cephalexin excreted in the 12 h following drug administration), *MRT* (mean residence time) and *VRT* (variance of residence time) to characterize profiles of the quantity of unmetabolized antibiotic in urine (Vila et al., 1980). The experimental design employed was a 4×4 latin square with 3 replicates. Formulations were administered to fasting volunteers and urine samples were taken 1, 2, 3, 4, 5, 6, 8, 10 and 12 h postadministration. The data were processed statistically by multivariant analysis of variance of E_{12}, *MRT* and *VRT* (Vila et al., 1980).

Cephalexin concentrations in urine were determined by reverse-phase HPLC using a C_{18} column, 70 : 30 : 0.5 water–methanol–acetic acid as the mobile phase and a detection wavelength of 254 nm. Preliminary runs confirmed that the normal components of urine did not interfere with the determination of cephalexin.

Results and Discussion

Fig. 1 shows, for each of the 4 formulations tested, the mean distributive curves of urinary excretion of cephalexin, and Table 1 the corresponding mean values of E_{12}, MRT and VRT. Table 2, which lists the results of subjecting the urinary excretion data to multivariant analysis of variance, shows that there were significant differences among the various treatments; and according to Roy's test for multiple contrasts (Roy and Bose, 1953) these differences are attributable solely to the effects of E_{12}, i.e. the fraction of the dose absorbed is affected (decreasing with increasing punch pressure) but not the rate of absorption. This confirms the impression given by the great similarity among the values of MRT and VRT, which suggests that for cephalexin the "anatomi-

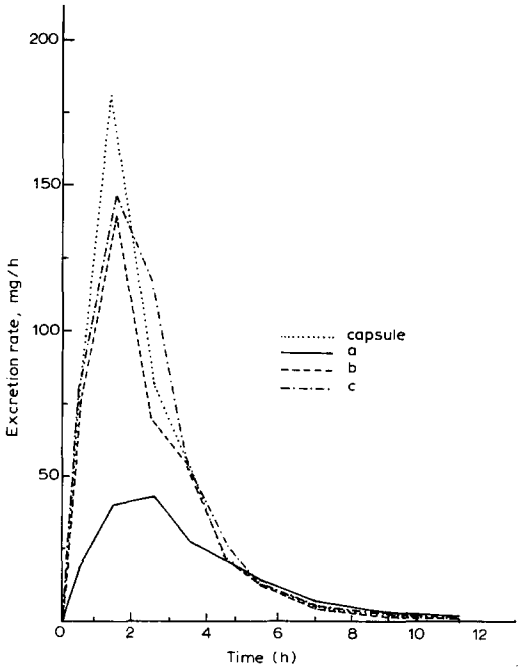

Fig. 1. Mean distributive urinary curves.

cal reserve length for intestinal absorption" (Ho et al., 1983) is quite small. Table 3, which lists the results of applying one-way ANOVA to the E_{12} data obtained for Formulations A, B and C so as to estimate the separate significance of linear and quadratic terms in the response to punch pressure, shows that linear and quadratic terms are significant.

Fig. 2 shows the mean cumulative dissolution curves for the 3 tablet formulations studied. The dissolution parameter best correlating with the in vivo results was the percentage of cephalexin dissolved after 70 min, D_{70}; the corresponding correlation with E_{12} is shown in Fig. 3. The analysis of

TABLE 1

Mean values of the urinary excretion parameters

Formulation	E_{12} (mg)	MRT (h)	VRT (h^2)
A	184.4	2.97	3.17
B	390.6	2.26	2.22
C	420.2	2.38	2.23
Capsule	460.4	2.12	2.10

TABLE 2

Results of MANOVA

Source	Matrix	Sum of squares and products		
Formulations	H	544988.45		
		−1557.60	5.02	
		−2180.43	6.41	8.91
Subjects	S	26875.29		
		143.16	6.72	
		357.99	9.86	32.38
Error	E	108105.17		
		131.75	8.31	
		−23.94	7.94	25.87
Total	T	679968.92		
		−1438.39	20.05	
		−1690.68	24.21	67.16

The greatest eigenvalue of H×E−1 is $Cs = 5.72$ and $Cs/(1+Cs) = 0.85$; the parameters for its distribution are $s = 3$, $m = 0$, and $n = 15$. Null hypothesis rejected at 0.01 level.

TABLE 3

Analysis of variance results for E_{12}

Source	df	M.S.	F
Formulations	2	198064.0	50.9 *
Linear	1	333694.8	85.8 *
Quadratic	1	62424.6	16.0 *
Error	33	3891.0	
Total	35	14986.4	

* Significant at 0.01 level.

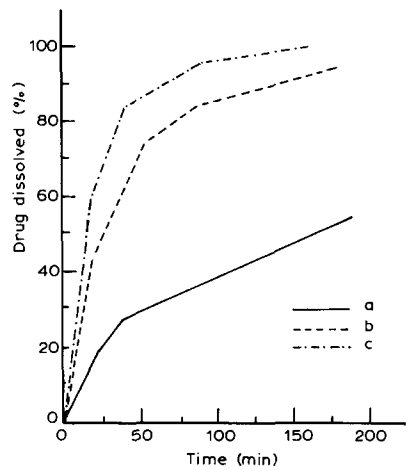

Fig. 2. Mean cumulative dissolution curves.

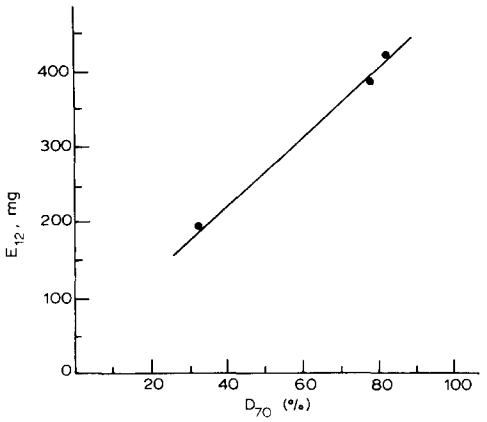

Fig. 3. Correlation between E_{12} and D_{70} ($r^2 = 0.9909$, $F = 100.8$ with 1 and 2 df).

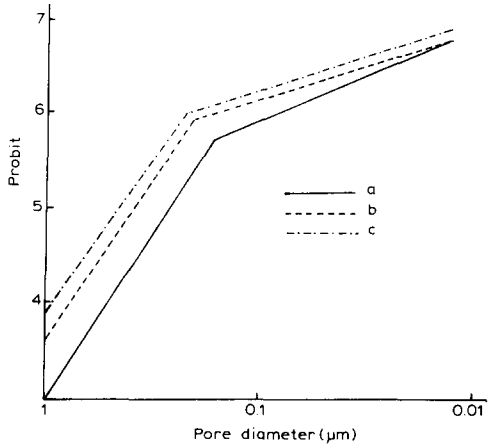

Fig. 4. Pore size distributions for the 3 formulations studied after probit transformation (means of two determinations).

TABLE 4

Analysis of variance for D_{70}

Source	df	M.S.	F
Formulations	2	1950.4	106.0 *
Linear	1	3543.8	192.9 *
Quadratic	1	357.1	19.4 **
Error	4	18.4	
Total	8	791.2	

* Significant at 0.01 level. ** Significant at 0.05 level.

variance results for the D_{70} data (Table 4) are in keeping with this correlation in that, like the E_{12} data, they depend upon the square of the punch pressure. It is perhaps significant that in in vivo–in vitro studies of other drugs, some of them very similar to cephalexin (Llabrés et al., 1982), close correlation was obtained between the in vivo parameters and the in vitro parameter D_{180} which was measured using the same dissolution apparatus and experimental conditions as in the present study; this perhaps lends indirect support to the hypothesis that for cephalexin the intestinal reserve length is small.

Using the methodology developed by Carli et al. (1984) for investigating the mechanisms of drug release from tablet matrices, we estimated the goodness of fit between the experimental cephalexin dissolution profiles and the mathemetical models proposed by Higuchi (1963), Bamba et al. (1979), Gurny et al. (1982) and Hopfenberg et al. (1981). For all 3 formulations, the best fit was with Higuchi's model (Table 5), which suggests that cephalexin release from these tablets is controlled by its diffusion through the pores of the matrix. This is confirmed by the results of the porosimetry experiments: Fig. 4 shows the pore size distributions of the tablets (after probit trans-

TABLE 5

Results of fitting each of the theoretical models tried to the experimental data for the 3 formulations studied

M, % of undissolved drug; t, time, min.; df for the error, 61.

Model	Formulation A			Formulation B			Formulation C		
	r^2	F	slope	r^2	F	slope	r^2	F	slope
$100 - M = K_1 \cdot t^{1/2}$	0.9978	9245.7	3.884	0.9894	1397.2	8.816	0.9812	469.8	12.203
$\ln M = -K_1 \cdot t$	0.6470	36.6	0.034	0.4754	13.6	0.033	0.4866	8.5	0.061
$100^{1/3} - M^{1/3} = K_3 \cdot t$	0.9697	639.9	0.006	0.9716	514.1	0.023	0.9674	266.9	0.045
$100 - M = K_4 \cdot t$	0.9534	409.0	0.346	0.9214	175.9	0.914	0.9129	94.3	1.740

TABLE 6

Total porosities of the formulations studied (means of two determinations)

Formulation	Porosity (%)
A	25.73
B	31.94
C	32.35

formation), and Table 6 the mean values of total porosity. According to Zoglio and Carstensen (1983), the two superposed log-normal distributions which are observed in all cases may be attributed to the presence of inter- and intragranular pores, respectively. Increasing punch pressure progressively reduces total porosity without affecting the mean and S.D. of the pore size; and Table 7 shows that, like D_{70} and E_{12}, total porosity depends also on the square of the punch pressure, with very close correlation between total porosity and the dissolution and excretion parameters ($r^2 = 1.0000$ and $F = 7175.1$ with 1 and 2 df for E_{12} vs. porosity, and $r^2 = 0.9924$ and $F = 125.3$ with 1 and 2 df for D_{70} vs. porosity). These correlations and the invariance of the pore size distribution imply that the chief variable governing the release of cephalexin from the matrices studied is total porosity.

In conclusion it may be pointed out that if the intestinal reserve length for cephalexin is indeed small, then the usefulness of this kind of formulation for controlling the rate of absorption of antibiotic must depend on the rate of transit through the intestine. This will be studied in forthcoming experiments to determine the difference between the bioavailability of cephalexin when the tablets are ingested during meals and on an empty stomach.

TABLE 7

Analysis of variance results for total porosity

Source	df	M.S.	*F*
Formulations	2	25.75	40.2 *
Linear	1	43.82	68.5 *
Quadratic	1	7.68	12.0 **
Error	3	0.64	
Total	5	10.68	

* Significant at 0.01 level. ** Significant at 0.05 level.

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