International Journal of Pharmaceutics, 38 (1987) 33–38
Elsevier

IJP 01271

Crystal forms and bioavailability of erythromycin

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(Received 26 January 1987)
(Accepted 12 February 1987)

Key words: Crystal forms of erythromycin; Bioavailability; Erythromycin anhydrate; Erythromycin dihydrate

Summary

The effect of four physical forms on the rate and extent of bioavailability of erythromycin has been studied in healthy volunteers. The different forms – amorphous form, anhydrate, dihydrate, and commercially available "base" – are characterized by X-ray, TG, and DSC measurements. Erythromycin anhydrate and dihydrate are obviously faster and more completely absorbed than the amorphous form or commercially available "base".

Introduction

In the discussion about the oral usefulness of erythromycin the main questions have been the influence of chemical entities, dosage forms, and dosage regimen on the rate and extent of absorption of this acid-labile antibiotic. In this debate no one has reported the possible effect of the crystal forms in which erythromycin exists. Erythromycin base appears at least in 4 different structural forms representing an anhydrate, a dihydrate, an amorphous, and a partially crystalline, commercially available form. Studies concerning the crystal forms of erythromycin have been published earlier (Shtolts et al., 1966; Pelizza et al., 1976; Allen et al., 1978; Fukumori et al., 1983; Bauer et al., 1985; Murthy et al., 1986), but the water in erythromycin structure has caused some inconsistency of the nomenclature of these forms. Although the amount of water in hydrous erythromycin corresponds well to the stoichiometric value calculated for a dihydrate, the question is not strictly of bound hydrated water but rather of unbound, entrapped or clathrated water, as also reported by Bauer et al. (1985). Water in hydrous erythromycin is easily removed, but not at any exact transition temperature. The process is rather time dependent than energy dependent. Differences between in vitro dissolution rates of various structural forms of erythromycin have also been reported (Allen et al., 1978; Fukumori et al., 1983; Murthy et al., 1986) but those results have been inconsistent to some extent, too, and the most recent study (Murthy et al., 1986) warns against the use of amorphous erythromycin because of its poor wettability. However, no one has considered the possible effect of the crystal forms of this drug on the blood serum levels to give the desired

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pharmacologic response. An absorption study was therefore planned to establish whether the differences in crystal forms of erythromycin affect its bioavailability. The question of biopharmaceutical importance of physical forms of pharmaceuticals is also of general interest.

Materials and Methods

The forms of erythromycin studied were: partially crystalline "base", amorphous form, anhydrate, and dihydrate. The partially crystalline "base" and the dihydrate were of commercial origin. The anhydrous sample was made from the dihydrate by boiling an aqueous solution of the powdered drug. The amorphous form was ob-

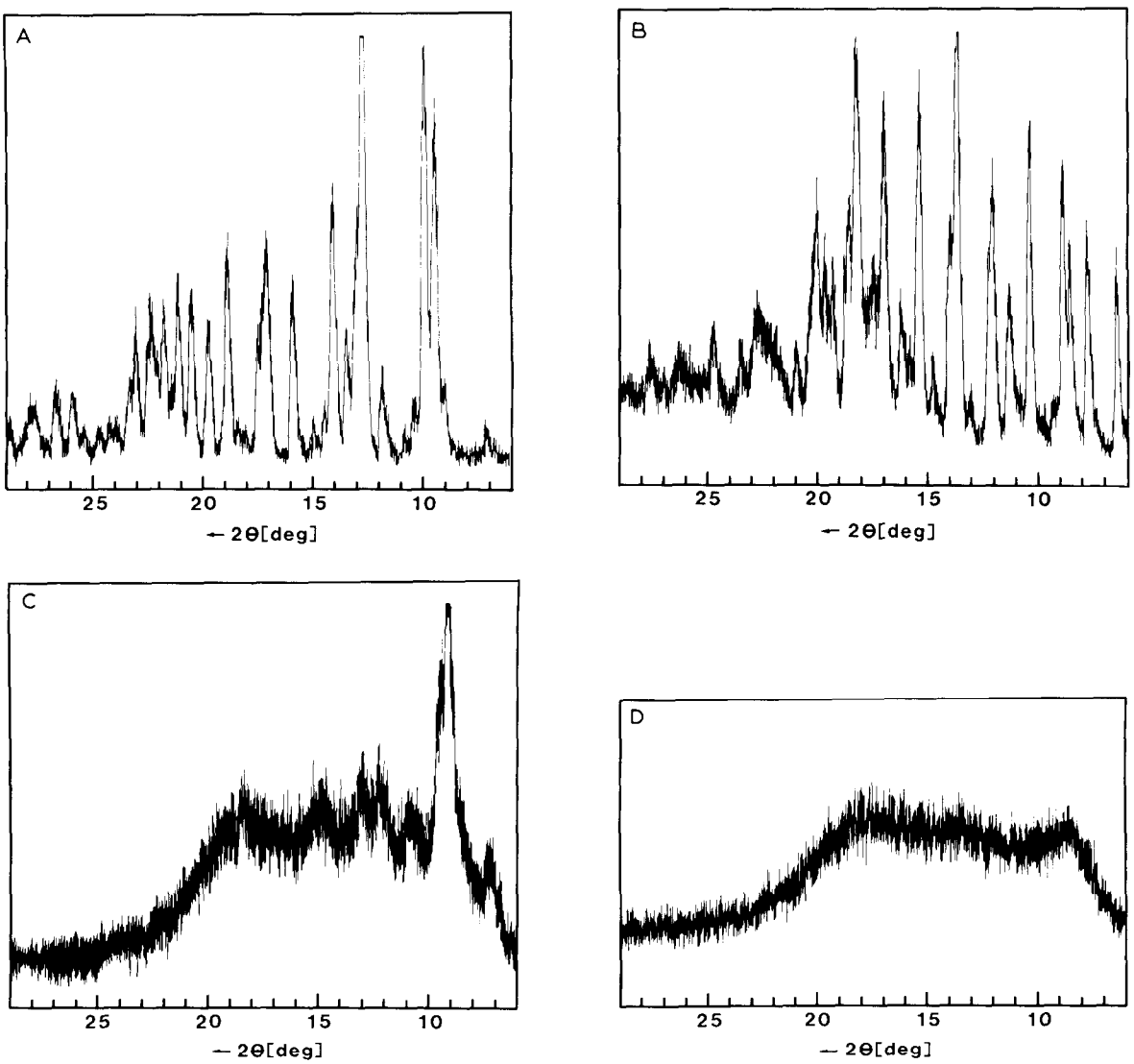

Fig. 1. X-Ray spectrum of: (A) erythromycin dihydrate; (B) erythromycin anhydrate; (C) partially crystalline erythromycin ("base"); and (D) amorphous erythromycin.

tained by heating the dihydrate at 135° C, cooling the melt to room temperature and grinding the transparent amorphous product in a mortar.

Physical studies

X-Ray diffraction measurements were carried out using a Philips diffractometer equipment with a sample-holder that can be heated above room temperature. Nickel-filtered copper radiation was used in the measurements. The reflected radiation was investigated by means of a scintillation detector. The pulses from the detector were integrated in a ratemeter circuit. The resulting analogue output was displayed on the recorder.

Thermogravimetric (TG) analyses were performed with Mettler TA 3000/TG 50 system, and differential scanning calorimetric (DSC) analyses with Mettler TA 3000/DSC 20 system.

Absorption studies

The absorption studies were carried out in 24, according to a physiological examination healthy, ambulatory volunteers – 19 females and 5 males with medical histories devoid of evidence of any gastrointestinal or hematological problems. Their ages varied betwen 23 and 44 years and they weighed between 53 and 83 kg. The subjects gave their consent after the objectives and the proce-

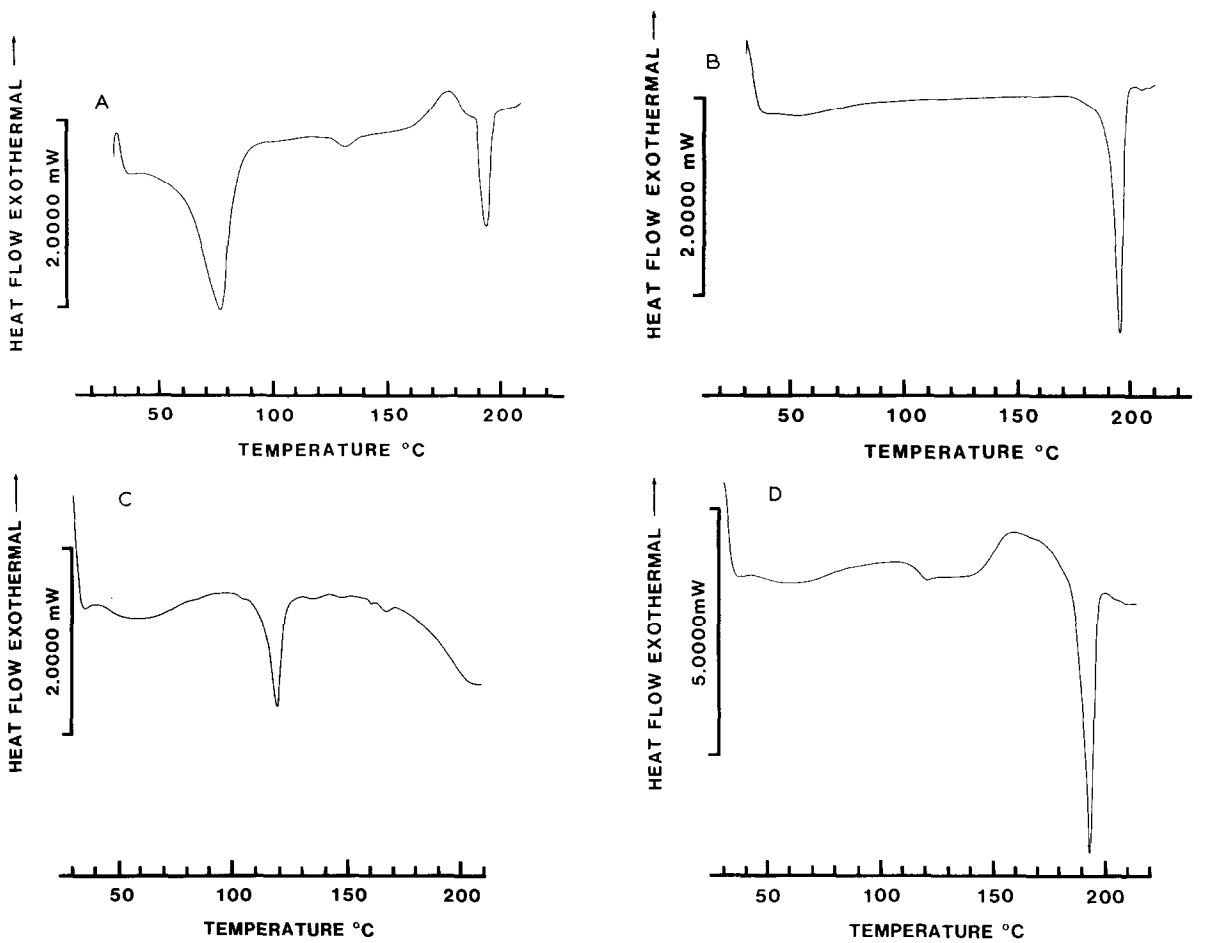

Fig. 2. DSC-curve of: (A) erythromycin dihydrate; (B) erythromycin anhydrate; (C) partially crystalline erythromycin ("base"); (D) amorphous erythromycin. Heating rate 5° C/min.

dure of the trial had been explained to them.

The subjects received 500 mg of erythromycin with 150 ml of water 08.00 h after an overnight fast on an empty stomach. Eating was allowed 3 h after the administration. Other drugs and alcohol intake was not permitted two days before and during the study. A randomized, cross-over experimental design was followed throughout the study.

Blood samples were drawn before the study and at 0.25, 0.5, 1, 1.5, 2, 3, 4 and 6 h postadministration. Serum was separated by centrifugation and stored frozen (−20 °C) until analysis which was performed during 4 days after sampling.

Erythromycin levels in serum were determined microbiologically using the cylinder agar plate method with *Sarcina lutea* ATCC 9341 as test organism. The plates were prepared from medium 1 (Growe and Randall, 1955).

The concentrations of erythromycin in serum were plotted against time and the peak concentration (C_{max}) and the time to peak concentration (t_{max}) were assessed from the individual curves. The area-under-the-curve ($AUC_{0\text{-}6h}$) was calculated by the trapezoidal rule.

The statistical significance of differences was assessed by Student's *t*-test for paired data.

Results and Discussion

Physical studies

X-Ray diffractograms of studied erythromycin forms are presented in Fig. 1, while the *d*-values and the relative intensities I/I_0 expressed as percentages of the strongest line in the pattern are given in Table 1.

Upon heating with DSC (Fig. 2A) the hydrous form dehydrates at 30–110 °C depending on the heating rate used, and transforms to the amorphous liquid at 130–135 °C. At about 160 °C the anhydrous form starts to crystallize from the melt. This anhydrous form has the same X-ray pattern as the form obtained by boiling a dihydrate. The anhydrous form is thermally stable: it only exhibits a melting endotherm at about 195 °C when heated with DSC (Fig. 2B).

When the partially crystalline "base" is heated from room temperature (Fig. 2C), it does not crystallize more completely but the crystal structure starts to collapse until it melts entirely at 115–120 °C. The melt does not recrystallize, when heated more or cooled back to room temperature.

The powdered amorphous sample liquefies at 118 °C as determined on DSC (Fig. 2D). At temperatures below 118 °C the molecules do not have sufficient motional degree of freedom to flow as a liquid. The anhydrous form crystallizes from the amorphous liquid at 157 °C.

Absorption studies

The mean serum erythromycin levels for the crystal forms studied are shown in Fig. 3. The means of calculated biopharmaceutical parameters are collected in Table 2.

According to the results we can find out two fast absorbing forms – the anhydrate and the dihydrate – with great absorption rate constants and short times to peak levels. The amorphous form seems to be the form of the slowest absorption. Its peak concentration is reached at about 2.25 h after the drug administration (Table 2).

The serum levels of erythromycin reflect also the rapidity of absorption of the anhydrous and dihydrous forms (Fig. 3). The concentrations at the beginning of the experiments are higher for them than for "base" or amorphous form.

The concentration maxima also indicate differences in absorption between the physical forms

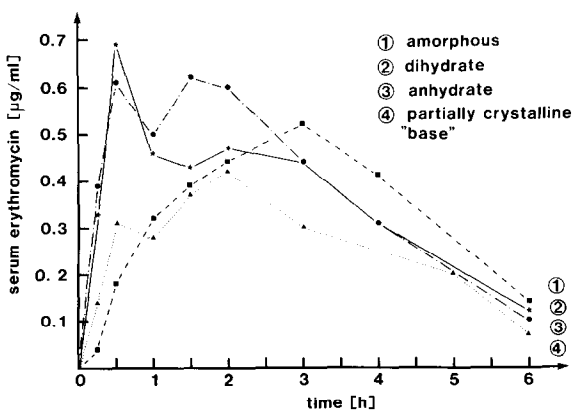

Fig. 3. Mean serum levels of different crystal forms of erythromycin.

TABLE 1

Powder diffraction data of anhydrous (A), hydrous (B) and partially crystalline (C) forms of erythromycin

Form A			Form B			Form C		
2θ (°)	I/I_0	d (nm)	2θ (°)	I/I_0	d (nm)	2θ (°)	I/I_0	d (nm)
6.48	44	1.364	7.10	10	1.245	6.30	24	1.403
7.40	15	1.195	9.00	16	0.983	6.80	32	1.300
7.83	49	1.129	9.45	56	0.940	7.25	39	1.219
8.58	45	1.031	9.95	77	0.889	9.10	100	0.972
8.90	59	0.994	10.40	13	0.851	9.45	74	0.936
9.30	17	0.951	10.85	10	0.816	10.88	48	0.813
9.70	15	0.912	11.80	18	0.750	12.25	56	0.723
10.40	67	0.851	12.75	100	0.694	13.00	55	0.681
10.81	19	0.819	13.45	23	0.658	14.80	49	0.599
11.30	37	0.783	14.10	43	0.628	18.33	51	0.484
12.08	60	0.733	14.45	13	0.613			
13.03	19	0.680	14.95	10	0.593			
13.68	100	0.647	15.90	31	0.558			
14.00	50	0.633	17.12	37	0.518			
14.75	24	0.601	17.48	24	0.507			
15.39	76	0.576	18.03	09	0.492			
15.82	28	0.560	18.40	10	0.482			
16.22	36	0.547	18.85	36	0.471			
17.00	72	0.522	19.75	24	0.450			
17.46	42	0.508	20.50	29	0.433			
18.25	81	0.486	21.13	31	0.421			
18.58	53	0.478	21.45	17	0.414			
19.31	42	0.460	21.78	27	0.408			
19.68	46	0.451	22.08	20	0.403			
20.03	56	0.443	22.38	28	0.397			
21.00	27	0.423	23.05	26	0.386			
21.70	25	0.410	23.35	16	0.381			
21.95	29	0.405	23.95	11	0.372			
22.22	30	0.400	24.25	11	0.367			
22.77	35	0.391	24.75	09	0.360			
23.50	27	0.379	25.40	10	0.351			
24.76	30	0.360	25.95	14	0.343			
26.30	27	0.339	26.70	16	0.334			
27.00	22	0.330	27.65	13	0.323			
27.65	27	0.323	27.87	14	0.320			

2θ = twice the angle of incidence or reflection; I/I_0 = percent relative intensity (based on maximum intensity of 100); d = interplanar spacing.

TABLE 2

Calculated biopharmaceutical parameters for erythromycin forms studied. S.D. in parentheses

Physical form	AUC (μg·h/ml)	C_{max} (μg/ml)	t_{max} (h)
Amorphous	2.03 (1.19)	0.67 (0.44)	2.25 (0.86)
"Base"	1.46 (1.12)	0.49 (0.38)	1.49 (0.62)
Anhydrous	2.36 (2.07)	0.82 (0.73)	1.08 (0.65)
Dihydrous	2.16 (2.20)	0.76 (0.68)	0.91 (0.66)

of erythromycin. The "base" gives significantly lower C_{max}-values than the amorphous and anhydrous forms. The difference between the "base" and the dihydrate is not statistically significant but obvious. The same is also valid when comparing AUC-values. The extent of bioavailability is obviously lower for the "base" than for the other forms though the only statistically significant difference at the 5% level exists between the "base" and the anhydrate.

The difficulties to show statistically significant differences between the different physical forms are due to the fluctuation of the individual results which is – according to current knowledge – dependent on the erratic absorption of erythromycin. The variation is clearly to be seen from maximum and minimum values collected in Table 2.

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