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BIOLOGICAL AVAILABILITY AND *IN VITRO* DISSOLUTION OF OXYTETRACYCLINE DIHYDRATE TABLETS

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1 The concentration of oxytetracycline in plasma was studied by microbiological assay after oral administration of four different preparations of oxytetracycline dihydrate tablets.

2 There were statistically significant differences in biological availability between the four preparations, as assessed by the peak plasma level, the area under the plasma concentration-time curve, or the cumulative fraction of the dose excreted in urine at 405 minutes. In contrast, differences between the subjects were not statistically significant.

3 The differences in biological availability were not predictably related to the *in vitro* dissolution of the tablets.

Introduction

Differences in the biological or physiological availability of chemically equivalent preparations have been reported for many drugs, including several antibiotics. The relevant literature has been recently reviewed by Wagner (1971). Thus, when different commercial preparations of oxytetracycline hydrochloride are administered orally, there is considerable variation in the concentration of the antibiotic detected in blood. In the U.S.A., Brice & Hammer (1969) studied the serum levels achieved by 16 different samples of oxytetracycline hydrochloride capsules, including the original commercial product; seven preparations resulted in serum concentrations which were considered to be less than the minimum acceptable therapeutic level, and all were inferior to the original product. These results were confirmed in subsequent studies involving the comparison of 10 different preparations with the reference drug (Blair, Barnes, Wildner & Murray, 1971).

The explanation for these differences in the bioavailability of oxytetracycline hydrochloride is at present obscure. In the case of some drugs, for instance acetylsalicylic acid (Wood, 1967), griseofulvin (Katchen & Symchowicz, 1967), and digoxin (Shaw, Raymond, Howard & Hamer, 1973), there is a direct correlation between the plasma concentration of the drug after oral administration and the dissolution rate *in vitro*. With different preparations of oxytetracycline hydrochloride capsules, the relation between the plasma level *in vivo* and drug solubility *in vitro* appears to be more complex. Although low serum concentrations were generally associated with a poor dissolution rate, reliable estimations of biological availability could not be made from the results of *in vitro* tests (Brice & Hammer, 1969). These studies did not include comparable data with oxytetracycline dihydrate tablets, and this preparation is not included in the current United States Pharmacopoeia (1970). In contrast, the British Pharmacopoeia (1973) contains a monograph for oxytetracycline dihydrate tablets, and in addition to the official generic preparation, there are 13 different proprietary preparations included in the current Monthly Index of Medical Specialities. Since data on these preparations has not been reported, we have compared the plasma level and urinary excretion of four different varieties of oxytetracycline dihydrate tablets, and tried to correlate the results with their dissolution characteristics.

Methods

In vivo *studies*

Four samples of oxytetracycline dihydrate tablets (250 mg) of different origins were obtained from the manufacturers or from the Pharmacy Department at Liverpool Royal Infirmary. Any

identifying trademarks on commercial preparations were removed from the tablets by gentle treatment with ethanol. All the samples were sugar-coated, and were similar in appearance to the original commercial product (Terramycin, Pfizer Ltd, Sandwich, Kent).

Sixteen experiments were performed on four student volunteers (mean body weight, 68 kg; range = 63-73 kg). Alternative concurrent drug therapy was excluded, and the informed consent of each subject was obtained. The sequence of the 16 experiments was determined by a 4 x 4 Graeco-Latin square, so that each subject received all four tablets on different occasions. The key to the square was not revealed until the study was completed. In all subjects, there was an interval of at least two weeks between successive experiments.

After overnight fasting, a tablet of oxytetracycline dihydrate (250 mg) was administered orally with approximately 250 ml water. Fasting conditions were maintained for at least 3 hours. Samples of blood (approximately 1.0 ml) were removed by venepuncture at 30, 60, 90, 120, 180, 270 and 360 min, and added to tubes containing lithium heparin. In some experiments, an initial control blood sample was also removed. Plasma was promptly obtained by centrifugation. Measured volumes of urine were collected at 0, 15, 45, 75, 105, 135, 225, 315 and 405 min; the urine flow rate was maintained above 100 ml/h by water diuresis. Oxytetracycline in specimens of plasma and urine or diluted urine was assayed microbiologically (Grove & Randall, 1955), using the test organism *Bacillus cereus* (var. mycoides), and the results were expressed as μg oxytetracycline/ml. There was a linear relation between the logarithm of the oxytetracycline concentration and the diameter of the zone of inhibition. The limit of sensitivity of the assay was 0.25 μg/ml, and the inter-assay coefficient of variation of single observations about the mean was 8%.

In vitro *studies*

Dissolution tests on batches of tablets were carried out at pH 2.0, using the apparatus described by Poole (1969). The dissolution medium used (0.37% KCl in 0.011 N HCl; 500 ml) was placed in a glass vessel at 37° C. The contents of the vessel were stirred at 120 rev/min, and samples were removed at 5, 10, 15, 20, 25, 30, 45 and 60 minutes. The optical density of the samples was measured spectrophotometrically at 353 nm (British Pharmacopoeia, 1958), using an authentic sample of oxytetracycline dihydrate B.P. as a standard. The inter-assay coefficient of variation of single observations about the mean was 4%. Because of the linear relationships involved, the results of spectrophotometric and microbiological assay are correlated.

Results

In vivo *studies*

The mean concentration of oxytetracycline in plasma after oral administration of four different preparations of the dihydrate is shown in Figure 1. Although the antibiotic was usually identified in blood after 1 h, there was considerable variation in the oxytetracycline levels produced by the four preparations. The mean maximum plasma level varied from 0.19-1.01 μg/ml, which was attained approximately 3 h after administration of the tablets. Mean levels which were lower than the limit of sensitivity of the assay were due to the failure to detect the antibiotic in plasma in some

Table 1 Mean excretion rate and cumulative fraction of oxytetracycline eliminated in urine after oral administration of four different preparations of the dihydrate (tablets A, B, C and D) to four subjects

Time (min)	*Mean excretion rate (μg/min)*				*Cumulative fraction excreted (x 10^3)*			
	A	B	C	D	A	B	C	D
45	13.8	5.7	2.1	2.6	2.1	0.7	0.5	0.5
75	41.1	25.3	14.6	7.6	5.5	3.8	3.2	1.4
105	53.2	48.5	33.3	7.9	9.7	9.6	7.5	2.3
135	91.3	65.5	36.3	9.0	18.1	19.3	12.1	3.4
225	87.3	61.8	26.7	12.7	40.5	38.8	25.3	7.9
315	47.5	48.1	14.1	10.5	53.8	57.0	36.9	11.6
405	44.2	37.0	16.6	10.2	63.0	70.3	44.6	15.7
24 h					138.2	149.6	82.8	36.6

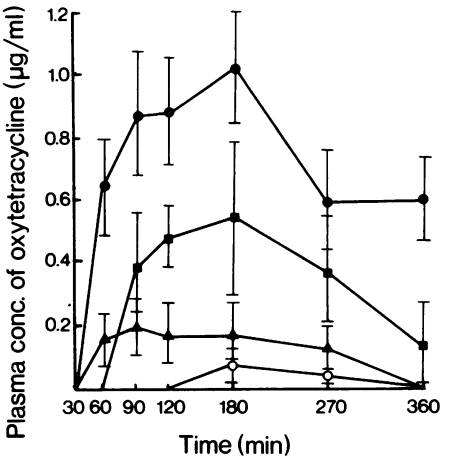

Fig. 1 Plasma concentration of oxytetracycline after oral administration of the four different preparations of oxytetracycline dihydrate. Tablet A (●); tablet B (■); tablet C (▲); tablet D (○). Each point and vertical bar represents the mean and s.e. mean of four experiments.

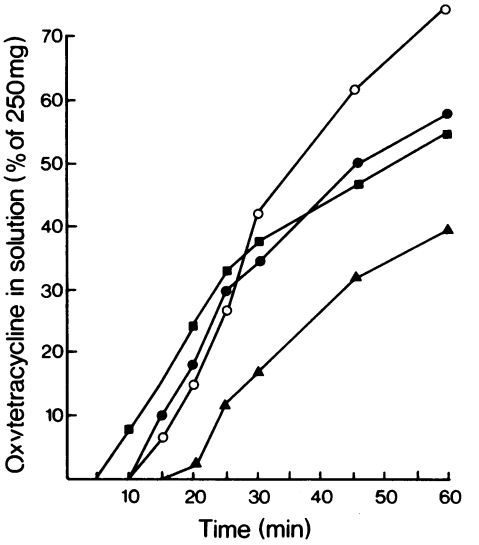

Fig. 2 Dissolution profiles of the four preparations of oxytetracycline dihydrate tablets that were used in the *in vivo* studies. Tablet A (●); tablet B (■); tablet C (▲); tablet D (○). Each point represents the mean of at least four experiments.

experiments. In five instances, oxytetracycline was not detected in blood during the 6 h study. With all four preparations, there was a marked individual variation in the plasma levels, as shown by the large standard error (Figure 1). Such variation in the plasma level of oxytetracycline between individuals is apparently independent of tablet dissolution, since it has been observed when the drug is administered orally as an aqueous solution (Scales & Assinder, 1973).

Differences between the four preparations were also found when the urinary elimination of the drug was considered. Table 1 shows the mean rate of excretion and the cumulative fraction of the dose eliminated in urine between 45 and 405 min for the four preparations studied. Both the mean excretion rate and the fraction of the dose of oxytetracycline eliminated in urine (summated at each time interval) were consistently greater with preparations A and B than with C or D; in general, both parameters were ranked in the order A > B > C > D, thus reflecting the differences in the plasma concentrations of the four preparations.

Analysis of variance confirmed that the differences between the four preparations were statistically significant, as assessed by the peak plasma level ($F = 8.2$; d.f. = 3,9; $P < 0.01$), the area under the plasma concentration-time curve ($F = 13.3$; d.f. = 3,9; $P < 0.01$), or the cumulative fraction of the dose excreted at 405 min ($F = 3.9$; d.f. = 3,9; $P < 0.05$). In contrast, differences between the four subjects were not statistically significant ($P > 0.8$).

In vitro *studies*

There were considerable differences in the dissolution rates of the four batches of oxytetracycline tablets used in the present study (Figure 2). In general, Tablet D had the best dissolution characteristics; 75% of the antibiotic was in solution within 1 hour. In contrast, only 40% of the drug in Tablet C dissolved during this period. The dissolution profiles of tablets A and B were almost identical (Figure 2).

Discussion

Although oxytetracycline dihydrate is almost insoluble in water (1 part in 2000), it is readily soluble in dilute acids (Martindale, The Extra Pharmacopoeia, 1972). Thus, in physiological conditions solution of the drug is dependent on gastric acidity, but absorption of dissolved oxytetracycline probably occurs in the small intestine. An identical two-step mechanism appears to be responsible for the absorption of tetracycline (Barr, Letcher & Adir, 1970). Dissolution (but not absorption) occurs at the low

pH of the stomach, while absorption from solution takes place in the more alkaline environment of the small intestine after gastric emptying. In the small intestine, the dissolution of undissolved particles is slow and dependent upon variations in formulation.

Differences in the bioavailability of commercial tetracycline products have been recently reported in Britain (Barnett, Smith, Greenwood & Hetherington, 1974). In the present experiments, there were similar statistically significant differences between the four oxytetracycline dihydrate tablets studied. Although other possibilities cannot be excluded, these differences may well be related to the variable dissolution of the four preparations in the fasting stomach. Nevertheless, the *in vivo* differences appear to be quite unrelated to the variable dissolution of the four tablets in the conditions employed in the current study. Thus, the bioavailability of oxytetracycline from the four tablets was ranked in the order A > B > C > D, while the time for 50% of the drug to dissolve, which is probably the best *in vitro* parameter to consider (Wagner, 1971), was ranked in the order D > A > B > C. Particularly anomalous results were obtained with tablet D. The bioavailability of this tablet was lower than the other three products, although it had the best dissolution profile. In addition, tablets A and B, which had very similar dissolution characteristics (Fig. 2), were associated with marked differences in bioavailability (Figure 1). Modification of the conditions and the apparatus in which dissolution tests were carried out failed to produce a closer correlation with the plasma profile or the peak plasma levels. There are two possible explanations for these results. In the first place, the observed differences in bioavailability may be unassociated with tablet dissolution in *in vivo* conditions. Alternatively, the circumstances in which the present dissolution tests were carried out may not be predictably related to the physiological conditions in the human stomach.

Since dissolution of oxytetracycline *in vivo* is an essential prerequisite to intestinal absorption and is dependent on gastric acidity, the use of the relatively insoluble hydrated base in the official preparation (British Pharmacopoeia, 1973) may not be entirely satisfactory. Oxytetracycline hydrochloride is one thousand times more water-soluble than the dihydrate, and other studies suggest that in spite of differences in bioavailability, the serum levels achieved by capsules of the salt are invariably greater (Brice & Hammer, 1969; Blair *et al.*, 1971). Although the use of the hydrated base in oxytetracycline tablets, B.P. may increase drug stability, it would not be surprising if it reduced the bioavailability of the antibiotic.

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