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The effect of proteolytic enzymes on the disposition of tetracycline

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The acute administration of a mixture of trypsin and chymotrypsin did not significantly affect the rate or extent of disappearance of tetracycline from the rat gut in situ, nor did chronic administration of enteric coated tablets containing trypsin and chymotrypsin to rats significantly influence the rate or extent of absorption of tetracycline compared with rats receiving enteric coated placebo tablets. Similarly, chronic enzyme administration did not effect either the systemic clearance, half life or apparent volume of distribution of intravenously administered tetracycline compared with rats receiving placebo tablets.

Oral administration of the proteolytic enzymes trypsin and chymotrypsin has been reported to enhance the absorption (Seneca & Peer 1963, 1965; Thevenot et al 1966) and tissue penetration (Wohlman et al 1968, 1969) of several drugs. Combinations of proteolytic enzymes with tetracycline have been claimed to improve therapy (Stankler 1976) but MacDonald et al (1964) found serum concentrations of tetracycline after its oral administration were not significantly altered by concomitant administration of chymotrypsin to animals or man and that no added degree of protection was conferred by the enzyme on mice infected with a lethal strain of *Escherichia coli* over that produced by tetracycline alone.

We have investigated the influence of acute administration of trypsin and chymotrypsin on the absorption of tetracycline from a rat in situ gut preparation and also the effect of chronic administration of the enteric coated enzymes on the disposition of orally and intravenously administered tetracycline in rats.

MATERIALS AND METHODS

Unlabelled tetracycline (as the anhydrous hydrochloride salt) was from Sigma, London. [7-^{3}H]-tetracycline powder (Radiochemical Centre, Amersham, Bucks), specific activity 1·63 mCi mg^{-1} was dissolved in methanol and stored at −20 °C. Its purity was reported to be 95% according to t.l.c. analysis on silica gel developed in 10% citric acid saturated with butan-1-ol and 98% by paper chromatography in ethyl acetate saturated with McIlwain's buffer pH 4·3. This was found by t.l.c. to be 93% (Willekens 1975). Decomposition on storage (2–3 months) was 4% at −20 °C.

In situ absorption technique

The disappearance of tetracycline (1 mg) + [7-^{3}H]-tetracycline (0·1 mCi) from a buffer solution (10 ml) containing NaH_2PO_4 $7H_2O$ (12·9 g litre^{-1}) and NaCl (4·5 g litre^{-1}) was studied in urethane anaesthetized rats in situ according to Doluisio et al (1969).

Absorption of tetracycline was followed in the presence or absence of a trypsin-chymotrypsin concentrate (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex—300 Armour Units) given into the loop.

In vivo disposition study

Pairs of rats were dosed twice daily with two enteric coated tablets (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex) containing either an inert material, or a mixture comprising trypsin and chymotrypsin in a ratio of 6 : 1 and 300 Armour units per tablet, for 3 days. On the fourth day, two more tablets were given followed 50 min later by tetracycline (orally or intravenously, 5 mg kg^{-1}) containing [7-^{3}H]tetracycline hydrochloride (0·1 mCi). Blood samples were removed at timed intervals from the tail artery. Plasma was assayed for radioactivity by liquid scintillation counting. The animals were housed in glass metabolism cages and urine and faeces collected for 3 days.

Assay of tetracycline in biological media

Samples of luminal fluid were assayed by liquid scintillation counting, either in 'Unisolve' (Koch-

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Light Laboratories Ltd., Colnbrook, Bucks.) or a cocktail comprising *PPO (10 g litre^{-1}) and **POPOP (0·1 g litre^{-1}) in a toluene–Triton X100 mixture (2:1 v/v) in a Packard Tri-Carb liquid scintillation spectrometer. Plasma, urine and luminal fluid samples were counted directly. Faecal material was dried, ground, digested with a mixture of 30% hydrogen peroxide and 60% perchloric acid (2:1 v/v) for 30 min at 60 °C and the resulting mixture olubilized in a cocktail comprising PPO (10 g litre^{-1}) in a toluene-Triton mixture (2:1 v/v).

Samples of luminal fluid (0·1 ml) and suitably diluted urine (1·0 ml) were additionally analysed by the method of Lever (1972) which is specific for the antibiotic.

Plasma protein binding of tetracycline (1·0 mg litre^{-1}) was assessed by ultracentrifugation of plasma from rats dosed orally with either enzyme or placebo tablets and containing [7-^{3}H]tetracycline as a marker.

Statistical analysis

The statistical significance of observed differences was assessed by use of Student's *t*-test for both paired observations (in vivo disposition studies) and unpaired observations (in situ disappearance studies).

RESULTS

In situ absorption of tetracycline

The data for the disappearance of radioactivity from the rat small intestine (Fig. 1) were fitted to a simple biexponential function

$$R = Ae^{-\alpha t} Be^{-\beta t}$$

using the least squares regression program *NONLIN* (Metzler et al 1973) where R is the ratio of radioactivity present at time t to that initially introduced. α and β are the rate constants (min^{-1}) characterizing the rapid and more slowly declining phases respectively. A and B are constant coefficients the sum of which represents the value of R at t = 0.

The results from the fluorimetric method of Lever (1972) were identical to those in Fig. 1 indicating that the species measured by the radiochemical method was tetracycline.

The derived kinetic parameters are in Table 1. There were no statistically significant differences in the kinetic parameters following enzyme administration, nor was there a significant difference between the values of R at t = 30 or t = 90 min following enzyme treatment.

* PPO 2,5-Diphenyloxazole.
** POPOP 1,4-Di-2(5-phenyloxazolyl)-benzene.

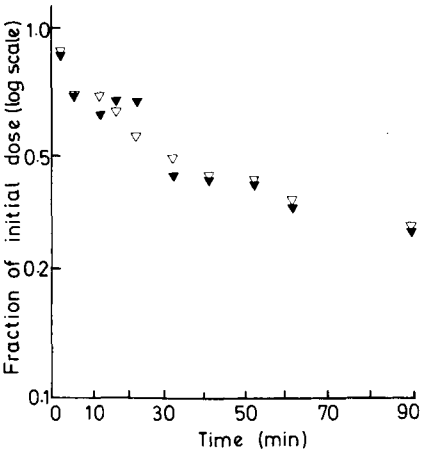

FIG. 1. Disappearance of tetracycline from the rat gut in situ. Samples were assayed radiochemically and results expressed as fractions of the initial amount. Values are mean of five experiments. (▽) control (▼) enzyme treated.

In vivo disposition study

The values for the parameters of in vivo disposition after oral and intravenous administration of tetracycline are given in Table 2. The plasma concentration vs time profile of total radioactivity (fraction of dose ml^{-1} × 10^4) after oral [7^3-H]tetracycline and constructed from mean plasma concentrations within each group is shown in Fig. 2. T.l.c. of urine samples indicated that radioactivity was associated only with tetracycline. Additionally Kelly & Buyske (1960) reported that no metabolism of tetracycline occurred in the rat. Therefore, total radioactivity concentrations reflect those of tetracycline. There was no significant difference between enzyme-treated animals

Table 1. Kinetic parameters for the disappearance of tetracycline from the rat intestine. Values are estimates ± standard deviations obtained from a non-linear least squares regression fit of the function $R = Ae^{-\alpha t} + Be^{-\beta t}$. R is the ratio of the amount of tetracycline present at time t to that initially introduced. α and β are the rate constants (min^{-1}) characterizing the rapid and more slowly declining phases respectively. A and B are constant coefficients, such that A + B = R at t = 0.

	A	B	α	β
Control	0·38 ± 0·04	0·65 ± 0·02	0·97 ± 0·35	0·0072 ± 0·0011
Enzyme treated	0·34 ± 0·06	0·66 ± 0·04	0·58 ± 0·25	0·0078 ± 0·0015
Significance	$P > 0·5$	$P > 0·5$	$P < 0·5 > 0·4$	$P > 0·5$

Table 2. Parameters of tetracycline disposition after oral (a) and intravenous (b) administration to pairs of rats dosed orally with either proteolytic enzymes (Enz) or placebo tablets (Pla)

(a) Oral administration

Parameter	Pla Mean (n = 10)	s.d.	Enz Mean (n = 10)	s.d.	Difference Mean	s.d.	Significance
$AUC_{0-\infty}$ (fraction of dose $ml^{-1} \times 10^4 \times h$)	200·3	79·8	220·6	80·3	20·3	36·7	$P < 0{\cdot}2 > 0{\cdot}1$
$t_{\frac{1}{2}}$ (h)	4·2	1·2	4·2	1·6	0·0	0·8	$P > 0{\cdot}5$
XU (% dose)	48·9	4·2	52·7	7·0	3·8	7·2	$P < 0{\cdot}2 > 0{\cdot}1$

(b) Intravenous administration

Parameter	Pla Mean (n = 5)	s.d.	Enz Mean (n = 5)	s.d.	Difference Mean	s.d.	Significance
CL_s (ml min^{-1})	0·51	0·07	0·55	0·7	0·04	0·10	$P < 0{\cdot}4 > 0{\cdot}3$
Vd (ml)	186·4	45·1	206·8	38·0	20·4	60·9	$P < 0{\cdot}5 > 0{\cdot}4$
$t_{\frac{1}{2}}$ (h)	4·2	0·5	4·5	1·0	0·3	1·0	$P > 0{\cdot}5$
XU (% dose)	74·7	5·0	75·8	5·3	1·1	9·4	$P > 0{\cdot}5$
XF (% dose)	17·2	4·5	17·8	6·2	0·6	4·2	$P < 0{\cdot}5 > 0{\cdot}4$
XU + XF (% dose)	91·9	4·4	93·6	3·4	1·7	7·1	$P < 0{\cdot}4 > 0{\cdot}3$

and controls in values of $t_{\frac{1}{2}}$ or time taken to reach peak plasma concentration, as judged by visual inspection of Fig. 2. Systemic clearance (CL_s) and distribution volume (Vd) were substantially unchanged following enzyme administration. Further, enzymes did not alter either the excretion balance or total recovery of radioactivity between urine and faeces, following the intravenous administration of [7-^{3}H]tetracycline.

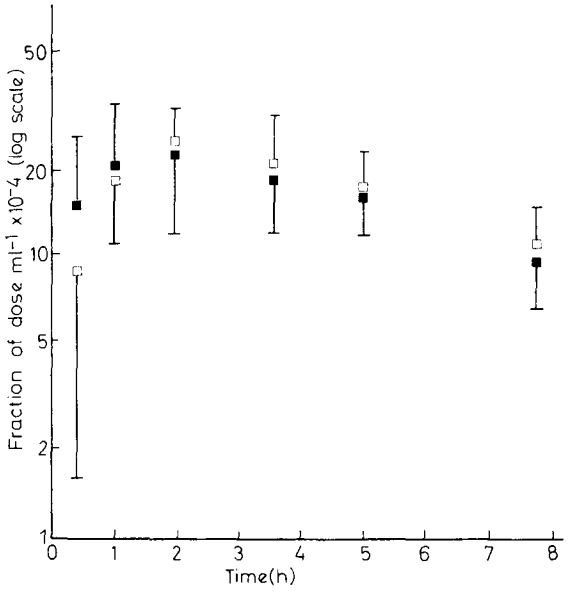

FIG. 2. Mean plasma concentration of total radioactivity within each group of rats (n = 10) following the oral administration of [7-^{3}H]tetracycline to pairs of rats receiving either proteolytic enzyme (□) or placebo (■) tablets. Vertical lines represent standard deviations of the mean.

Plasma protein binding of tetracycline

The percentage of tetracycline bound (at 1 mg $litre^{-1}$) to plasma was 63%, s.d. 2·8 (enzyme-treated) and 66%, s.d. 3·1 (placebo-treated). The binding to the tube in the absence of plasma was 9·6%, s.d. 1·1.

DISCUSSION

The present study was unable to demonstrate that administration of trypsin or chymotrypsin could increase the systemic availability of co-administered drugs, since the enzymes failed to increase the rate of absorption or amount of tetracycline lost from the gut in situ over the time studied.

The mean change in availability due to the administration of proteolytic enzymes was estimated by taking the value of relative availability assessed by measurements of either the area under the plasma/concentration time curve or urinary recovery to infinite time (Table 2). The values of 1·08, s.d. = 0·15 and 1·15, s.d. = 0·25 respectively offer only marginal evidence for an improvement in availability, the results not being statistically significant.

That these observations are in conflict with some existing reports may be attributable, in part, to inadequacies of experimental design inherent in those earlier studies (Seneca & Peer 1963, 1965) and statistical analysis by MacDonald et al (1964) suggested that the 95% confidence limits on the reported % increases were so large as to invalidate any significance that Seneca & Peer had attributed to

an effect of the enzyme. Further MacDonald et al, failed to demonstrate any effect of enzymes on either the availability or therapeutic efficacy of tetracycline, an observation supported by results from Bradbrook et al (1978) who administered orally to volunteers a preparation of tetracycline and bromelain.

The failure of this report to demonstrate changes in distribution volume following enzyme treatment is not in agreement with the results of two animal studies by Wohlman et al (1968, 1969) who suggested that enzyme administration substantially increased the concentration of penicillin in several tissues above that attained when the drug was administered alone. It would be expected that enhanced tissue distribution would result in decreased circulating drug concentrations. Further, the degree of plasma protein binding is such that moderate displacement of drug from binding sites would not significantly alter plasma levels of free antibiotic. Any enhanced distribution into tissues would result in little increase in concentrations due to a relatively large distribution volume.

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