Saturable absorption of amino-cephalosporins by the rat intestine

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We have previously presented kinetic evidence indicating the existence of an interrelationship between concentration and in situ absorption of amoxicillin and cyclacillin by rat intestine (Tsuji et al 1977, 1978) and proposed that some types of carrier-mediated kinetics underlie the absorption mechanisms. In the present communication we report new findings indicating that in vivo absorption of amino-cephalosporins by rat intestine is governed by saturable kinetics in a manner similar to that of amino-penicillins.

Cephalexin monohydrate and cephradine monohydrate were kindly supplied by Shionogi & Co., Osaka, Japan and Sankyo Co., Tokyo, Japan, respectively. Experimental conditions and in situ loop and recirculating perfusion methods were described previously (Tsuji et al 1977). Disappearance of antibiotics from the intestine was calculated from the amount of residual amino-cephalosporin assayed by high-pressure liquid chromatography (h.p.l.c.) as follows: A Model FLC A-700 equipped with a Model UVIDEC 100 ultraviolet detector set at 254 nm (Japan Spectroscopic Co., Tokyo, Japan) and a reversed phase column (SC-02, Japan Spectroscopic Co.) were used. The carrier was 7% acetonitrile-93% 0·01 M ammonium acetate. 50 μl of an appropriately diluted solution was injected through a variable loop-injector on flow, and peak heights were measured. The calibration curves of the peak heights against concentration of the antibiotics were satisfactorily linear.

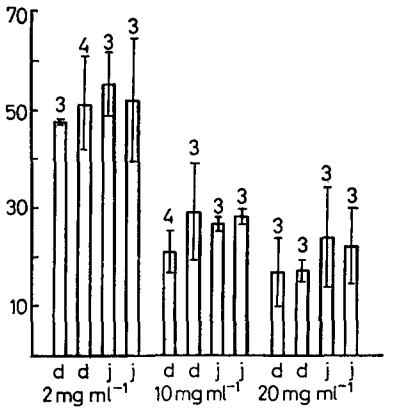

FIG. 1. Percentage disappearance of cephradine (ordinate) obtained from the rat intestinal loop 1 h after administration. Each dose was dissolved in isotonic phosphate buffer (pH 7·4) and injected in a 1 ml volume into a 5 cm intestinal loop. In each experiment, two loops were prepared. The loop was made 2 cm from the pylorus, with 1 cm of intestine separating the duodenal loop. For jejunal study, the loops were made 15 cm from the pylorus. They were consecutive loops in each area of the intestine. Mean absorption and standard deviation (bars) are shown. The number of rats used is indicated d = duodenum; j = jejunum.

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The in vitro first-order degradation of the two antibiotics (Yamana & Tsuji 1976) during the absorption experiments was determined in an isotonic phosphate buffer of pH 7·0 at 37 °C, and yielded 4·0 and 3·6% after 1 h and 7·8 and 7·2% after 2 h respectively for cephalexin and cephradine, being consistent with that in the rat intestinal washing solution with pH 7·0 isotonic phosphate buffer. The degradation of the two antibiotics in the in situ intestinal lumen solution, therefore, is likely to proceed to a similar extent and to follow apparent first-order kinetics.

Figs 1 and 2 show the percentage disappearance 1 h after administration. The total loss of cephradine by all portions of the rat intestine (Fig. 1) was greater at a concentration of 2000 μg ml^{-1} than at 10 000 μg ml^{-1} ($P < 0{\cdot}05$) and that at a dose concentration of 10 000 μg ml^{-1} was not significantly greater than that at 20 000 μg ml^{-1}. In the lower part of the jejunum, the disappearance of cephalexin (Fig. 2) was significantly higher at 2000 μg ml^{-1} than at 20 000 μg ml^{-1} ($P < 0{\cdot}05$). These results suggest that intestinal absorption of cephradine and cephalexin, like that of amoxicillin and cyclacillin, is governed by dose-dependent and capacity-limited transport kinetics. Quay (1972) and Quay & Foster (1970) suggested that the transport of cephalexin across the rat isolated intestine includes simultaneous passive diffusion and some active entrance step, such as carrier-mediated transport.

Further detailed kinetic studies using the in situ recirculating perfusion technique were carried out at pH 7·0 with concentrations of cephradine ranging from 181–34 800 μg ml^{-1} and concentrations of cephalexin ranging from 826–33 000 μg ml^{-1}. Fig. 3 shows the total percentage disappearance 2 h after administration. Like the results of static loop experiments, the disappearance of cephradine decreased with increase in initial antibiotic concentration. Values obtained from at least 3 animals were (mean with s.d.) 52·2 (12·4), 45·6 (7·6), 34·7 (3·1), 39·9 (2·9), 29·3 (2·4), 22·6 (4·4), and 21·9 (2·5%) from initial cephradine concentrations of 181, 345, 875, 2250, 5450, 18 300, and 34 800 μg ml^{-1}, respectively, indicating that disappearance from the lumen solution follows Michaelis-Menten and simultaneous first-order kinetics (eqn 1).

$$\frac{dC}{dt} = -\frac{V_{max}C}{K_m + C} - (k_1 + k_2)C \quad .. \quad .. \quad (1)$$

where C is the concentration of antibiotics remaining at time t, V_{max} is the maximum rate, K_m is the Michaelis-Menten constant and k_1 and k_2 are the first-order absorption and degradation rate constants, respectively.

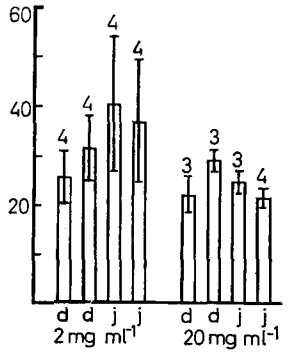

FIG. 2. Percentage disappearance of cephalexin (ordinate obtained from the rat intestinal loop. (Key see Fig. 1).

Our previous communications (Tsuji et al 1977, 1978) confirmed that disappearance of amoxicillin and cyclacillin from the rat intestine occurs according to equation 1. The non-linear least squares analysis of the cephradine data by NONLIN program (Metzler 1969) provided the parameters (mean with s.d.) V_{max} = 297 (36) μg ml^{-1} h^{-1} or 0·808 (0·097) mM h^{-1}, K_m = 1230 (51) μg ml^{-1} or 3·34 (0·14) mM, and $k_1 + k_2$ = 0·119 (0·015) h^{-1}. The percentage disappearance curves against the initial cephradine dose concentration calculated using these parameters fit reasonably well to the experimental data in Fig. 3 (r = 0·938 where r is the correlation coefficient). The application of the simple Michaelis-Menten equation, ignoring the first-order rate process, to the same data yielded too high an apparent Michaelis-Menten constant of 18·7 (0·6) mM (and V_{max} = 6·06 (0·24) mM h^{-1}) and resulted in a poor correlation (r = 0·894) between the predicted and observed percentage disappearances. The present result shows that the disappearance of cephradine from a perfusate does not follow simple saturable kinetics, but can be satisfactory fitted to equation 1 involving both a major saturable and a minor non-saturable rate process. The subtraction of the first-order degradation rate constant of 0·037 h^{-1}, which was obtained from a separate in vitro experiment, from the best fitting rate constant of 0·119 h^{-1} yielded 0·082 h^{-1} as the net first-order absorption rate constant similar to that (0·062 h^{-1}) reported for amoxicillin absorption (Tsuji et al 1978).

Yasuhara et al (1977), using a recirculating perfusion technique, reported that after 1 h there was no significant difference in the disappearance from the whole small intestine of cephalexin in concentrations ranging from 0·01 mM (3·65 μg ml^{-1}) to 10 mM (3650 μg ml^{-1}) (see Fig. 3). Using the same technique, we also observed that the percentage disappearance of cephalexin was almost constant at 826 and 5082 μg ml^{-1} (Fig. 3). The percentage disappearances were 33·5 (3·3)% (n = 3), 33·0 (3·9)% (n = 3) and 25·1 (6·8)% (n = 5) at concentrations of 826, 5082, and 33 000 μg ml^{-1}, respectively. The disappearance from 33 000 μg ml^{-1} decreased slightly, but this was not significant ($P < 0.05$), contrasting with earlier loop experiments which revealed a significant dose-dependence of cephalexin disappearance. In the recirculating perfusion experiments, the disappearance of cephalexin is thought to follow equation 1 but results in an unclear dose-dependent disappearance curve in Fig. 3. However, experiments to discriminate each component of this mixed rate process by using higher concentrations of cephalexin are difficult to carry out because of the limited antibiotic aqueous solubility (33 mg ml^{-1} at 37 °C, pH 7·0) (Tsuji et al 1979). Non-linear least squares analysis of the mean concentration of cephalexin remaining in the perfusion solution after 2 h by equation 1 assuming k_1 = 0·082 h^{-1} (the same as that of cephradine) and k_2 = 0·040 h^{-1} obtained from the in vitro experiment yielded the Michaelis-Menten parameters of V_{max} = 665 (34) μg ml^{-1} h^{-1} or 1·820 (0·092) mM h^{-1}, K_m = 6540 (833) μg ml^{-1}, or 17·90 (2·28) mM. The Michaelis-Menten constants of amino-cephalosporins are greater than those of amino-penicillins, 0·013 mM for amoxicillin and 1·91 mM for cyclacillin, reported previously in the same perfusion experiments in the rat (Tsuji et al 1978).

We attributed the intestinal absorption of amino-β-lactam antibiotics such as amoxicillin, cyclacillin, cephalexin, and cephradine in rats to some form of

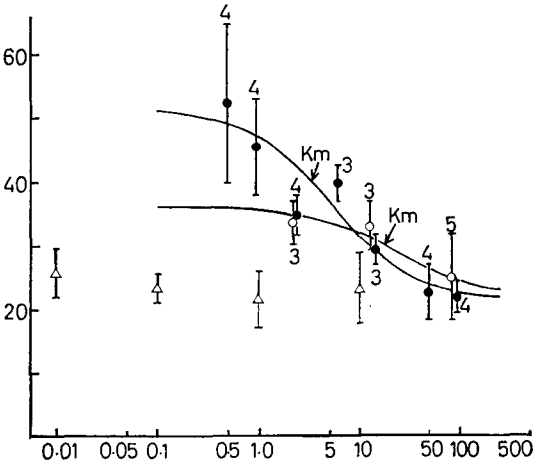

FIG. 3. Experimental values (symbols) and calculated curves for percentage disappearance after 2 h (ordinate) against the initial concentrations of cephradine and cephalexin, C (mM), at pH 7·0 from rat small intestine using the in situ recirculating perfusion technique (abscissa). The perfusion solution (9 ml) was recirculated at a rate of 2 ml min^{-1}. The small intestine used was at a 30 cm distance from the pylorus. Vertical bars represent the standard deviation. Key: ●, cephradine; ○, cephalexin; △, redrawn data by Yasuhara et al (1977) for cephalexin which were determined after 1 h in similar absorption experiments using the whole small intestine, 40 ml perfusion solution and a perfusion rate of 5 ml min^{-1}. The arrows indicated the Michaelis-Menten constant of cephradine and cephalexin. The number of rats used is indicated.

carrier transport and partly to simultaneous first-order diffusion transport. Whether such a carrier-mediated transport system of these antibiotics is present or not in human intestine has not been clarified. It is conceivable that the complete absorption of amoxicillin (Spyker et al 1977), cephalexin and cephradine (Nightingale et al 1975), and an 80% absorption of cyclacillin (Warren 1976) after oral administration in man may be due to the contribution of a saturable carrier system rather than the other possibility of membrane transport of very poorly lipid-soluble and zwitterionized amino derivatives of β-lactam antibiotics.

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Pharmacological data on crinia-angiotensin II

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Crinia-angiotensin II is a natural endecapeptide recently isolated from methanol extracts of the skin of *Crinia georgiana*, an Australian frog (Erspamer et al 1979).

The formulae reported below show that crinia angiotensin differs strikingly from all other known angiotensins II in that it has a tripeptide (Ala-Pro-Gly) attached to the N-terminal Asp residue of the conventional angiotensins, and in that a Ile residue is substituted for the usual Val residue at position 6 from the C-terminus.

Ala-Pro-Gly-Asp-Arg-Ile-Tyr-Val-His-Pro-Phe
Crinia-angiotensin II
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe Ile^5-angiotensin II
Asp-Arg-Val-Tyr-Val-His-Pro-Phe Val^5-angiotensin II

Pure natural crinia-angiotensin II was assayed biologically, in parallel with Val^5-angiotensin-II-Asp^1-amide (Hypertesin Ciba) on a number of test preparations. Rats and rabbits were anaesthetized with urethane (1·5 g kg^{-1}, intraperitoneally or intravenously); rats were pretreated with phenoxybenzamine hydrochloride (1 mg kg^{-1} i.v.). The results are shown in Table 1.

It may be seen that crinia-angiotensin II was approximately equiactive to Val^5-angiotensin-II-Asp^1-β-amide on all tested preparations, with the exception of the isolated guinea-pig gall bladder, on which it was decidedly more potent.

Occasionally, crinia-angiotensin II produced a slightly more sustained elevation of blood pressure, and similarly relaxation of the guinea-pig gall bladder upon washing with fresh nutrient liquid was slightly retarded, always in comparison with Val^5-angiotensin-II-Asp^1-β-amide.

Table 1. The result of parallel bioassay of crinia-angiotensin II and Val^5-angiotensin-II-Asp^1-β-amide on nine test preparations. The activity of Val^5-angiotensin-II-Asp^1-β-amide was always considered equal to 100, that of crinia-angiotensin II was expressed in percent. In parenthesis is the number of experiments.

Test preparation	Crinia-angiotensin II activity (in %)
Guinea-pig ileum	75–100 (5)
Guinea-pig gall bladder (isolated)	200–300 (7)
Rat uterus	70–110 (5)
Rat colon	100–130 (7)
Rat stomach	75–100 (5)
Rabbit urinary bladder	70–80 (3)
Human urinary bladder	70–90 (3)
Rat blood pressure	110–130 (11)
Rabbit blood pressure	115–160 (4)

It will be interesting to investigate in parallel the actions of crinia-angiotensin II and of the mammalian octapeptide angiotensins II on other preparations and biochemical parameters.

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