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COMMUNICATIONS

Kinetics of Michaelis-Menten absorption of amino-penicillins in rats

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Much interest has been focused on the absorption mechanism of amino-penicillins such as ampicillin, amoxicillin, and cyclacillin and amino-cephalosporins such as cephalexin and cephradine. Recent findings suggested that the intestinal absorption of amoxicillin in rats is governed by simple diffusion following first-order kinetics at high dose and is favoured by a specialized transport process following Michaelis-Menten kinetics at low doses (Tsuji, Nakashima & others, 1977).

The present communication describes the kinetic evidence for the Michaelis–Menten absorption of cyclacillin which has been recognized to be completely and rapidly absorbed after oral administration both in man and in animals (Warren, 1976).

Cyclacillin anhydrate was kindly supplied from Takeda Chemical & Ind. Co. Ltd., Japan. The experimental conditions and the procedures for the *in situ* loop and recirculating perfusion methods were as described previously (Tsuji & others, 1977). Cyclacillin was assayed by the imidazole method (Bundgaard & Ilver, 1972) after the acylation of the amino group with acetic anhydride at pH 9 for the loop samples. The aliquots, 10–50 μl, of the sample withdrawn periodically from the recirculating perfusion solutions were analysed both by high-pressure liquid chromatography (h.p.l.c.) using a cation-exchange chromatomode and by microbiological assay (paper disk diffusion method) employing *Sarcina lutea*. The conditions used for h.p.l.c. were as follows: instrument, JASCO FLC A-700 equipped with a variable wavelength ultraviolet detector setting at 210 nm, Model UVIDEC-100; column, Zipax SCX, Dupont; carrier, 0·1 M phosphate buffer of pH 4. The values from these three different analytical methods were confirmed to be in good agreement within the experimental errors.

The % absorption of cyclacillin over 1 h from the *in situ* intestinal loop is presented in Fig. 1. The results indicate the marked difference in the extent of absorption depending upon the initial dose. At a low concentration (2 mg ml^{-1}), cyclacillin was well absorbed (*ca* 80%) from every segment of the rat intestine, while at high concentration (20 mg ml^{-1}), it was poorly absorbed (*ca* 18%), the difference being statistically highly significant ($P < 0.001$). The intermediate absorption (42–53 %) was observed at 5 mg ml^{-1}. There

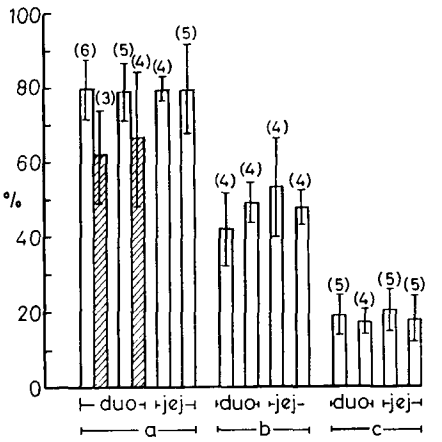

FIG. 1. The % of absorption (ordinate) obtained from the rat intestinal loop of cyclacillin with (hatched columns) or without (open columns) 2,4-dinitrophenol (abscissa). All studies were over 1 h. Each dose was dissolved in pH 7·4 isotonic phosphate buffer and injected in a volume of 1 ml into a 5 cm intestinal loop. In each experiment, two loops were prepared. The first loop was made at 2 cm from the pylorus, with 1 cm of intestine separating the consecutive loop in duodenum. For the study in jejunum, the loops were prepared at 15 cm from the pyrlorus. The points represent the mean absorption with standard deviation shown as a bar. Experimental number is shown in parentheses; duo-duodenum, jej-jejunum. (a) 2; (b) 5; (c) 20 mg ml^{-1}.

was no detectable, and negligible, degradation to its penicilloic acid at 2 and above 5 mg ml^{-1} (*ca* 2–3%) respectively, that could be determined by h.p.l.c. Addition of 5 mM of 2,4-dinitrophenol, a metabolic inhibitor, to the *in situ* intestinal loops caused a slight decrease in the absorption. At cyclacillin 2 mg ml^{-1}, the % absorption was changed from 79·5 ± 8·0 (n = 6) to 61·6 ± 12·4 (n = 3) and 78·9 ± 7·5 (n = 5) to 66·2 ± 18·0 (n = 4) from the proximal and distal duodenum, respectively.

Our results from the *in situ* loop experiments strongly suggest that there is some form of saturable process in cyclacillin transport across the intestinal mucosa and they show some similarities to those of Dixon & Mizen (1977) who found active transport of cyclacillin by the *in vitro* everted intestinal sac method.

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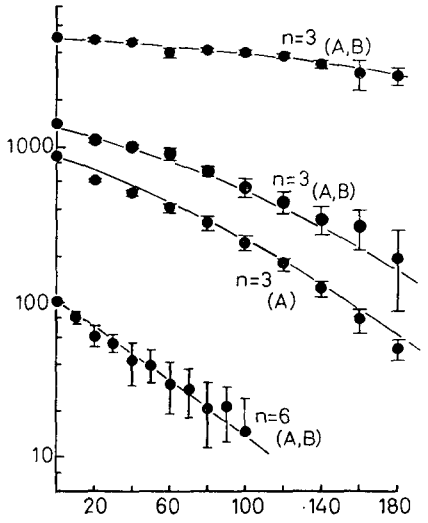

FIG. 2. Time-courses of the disappearance of cyclacillin as a function of dose from the isotonic phosphate buffer perfused through rat small intestine. The perfusion solution (9 ml) was recirculated at pH 7·0 and at a rate of 2 ml min^{-1}. The small intestine was a 30 cm length from the pylorus. The total of the sampling volume was within 0·6 ml. The remaining antibiotic was assayed by h.p.l.c. (A) and/or microbiological assay (B). The points represent the mean concentration and vertical bars represent the standard deviations. The curves are model-predicted concentrations based on the non-linear least squares fitting data to the integral equation of eqn 1. Ordinate: Log concn (μg ml^{-1}). Abscissa: Time (min).

To confirm kinetically the saturable absorption, the experiments using the *in situ* recirculating perfusion technique were conducted at pH 7·0 with various concentrations ranging from 100–5000 μg ml^{-1}. The pH of the perfusion solution was maintained at 37° by means of a pH-stat (Radiometer, Copenhagen) during the absorption experiments. The time-courses for the disappearance of cyclacillin from the rat intestine are illustrated in Fig. 2, indicating that the disappearance follows the Michaelis–Menten kinetics of equation 1

$$\frac{dC}{dt} = -\frac{VmaxC}{Km + C} \quad \ldots \quad \ldots \quad (1)$$

where C is the concentration of cyclacillin remaining at time, t, and Vmax and Km are Michaelis–Menten parameters. The non-linear least squares analysis of the data according to the integrated equation of equation 1 provided the parameters (mean with s.d.); Vmax = 842 s.d. 28 μg ml^{-1} h^{-1} or 2·47 s.d. 0·08 mM h^{-1}, and Km = 654 s.d. 40 μg ml^{-1} or 1·91 s.d. 0·12 mM. On the assumption that the absorption by simple diffusion makes a negligible contribution to the total absorption process and the negligible first-order degradation, the lines calculated by equation 1 fit reasonably to the data within the experimental dose concentrations below 5 mg ml^{-1}.

The computer analysis of the absorption kinetics of amoxicillin obtained under the same conditions (Tsuji & others, 1977) gave the Michaelis–Menten parameters of Vmax = 4·9 s.d. 0·6 μg ml^{-1} h^{-1} or 0·012 s.d. 0·002 mM h^{-1} and Km = 5·3 s.d. 2·3 μg ml^{-1} or 0·013 s.d. 0·006 mM and the simultaneous first-order rate constants for the simple diffusion (k_1) and the degradation (k_2) of k_1 = 0·062 h^{-1} and k_2 = 0·059 h^{-1}.

The present kinetic evidence and the previous observations (Tsuji & others, 1977) suggest that the absorption of amino-penicillins having low lipid-solubility and high polarity is due to carrier-mediated transport which depends upon the nature of the 6-side-chain structure of the α-amino-penicillins.

Such a saturable absorption process, in our opinion, probably also proceeds for amino-cephalosporins such as cephalexin and cephradine. The preliminary observation of the absorption of highly water-soluble cephradine from the *in situ* rat intestinal loop showed the similar dose-dependent absorption behaviour under the same experimental conditions. For example, the absorption over 1 h at 2 mg ml^{-1} and at the duodenum was 51·4 s.d. 9·4% (n = 4), while that at 20 mg ml^{-1} was 17·3 s.d. 2·3% (n = 3), the difference was significant ($P < 0.01$).

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REFERENCES

BUNDGAARD, H. & ILVER, K. (1972). *J. Pharm. Pharmac.*, **24**, 790–794.
DIXON, C. & MIZEN, L. W. (1977). *J. Physiol. Lond.*, **269**, 549–559.
TSUJI, A., NAKASHIMA, E., KAGAMI, I., HONJO, N. & YAMANA, T. (1977). *J. Pharm. Pharmac.*, **29**, 707–708.
WARREN, G. H. (1976). *Chemotherapy*, **22**, 154–182.