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Intestinal absorption of cephalosporin antibiotics: correlation between intestinal absorption and brush-border membrane transport

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Abstract—The absorption of seven cephalosporin antibiotics from the in-situ intestinal loop at pH 7·4 and their transport by brush-border membrane vesicles in the presence of an inward H^+ gradient ($[pH]_i = 7·5$, $[pH]_o = 6·0$) were examined. A good correlation was found between the intestinal absorption rate and the initial uptake rate by brush-border membrane vesicles. The data suggest that the transport study using intestinal brush-border membrane vesicles is useful as a model system for the intestinal absorption of β-lactam antibiotics.

It is well known that amino-β-lactam antibiotics are efficiently absorbed from the small intestine even though they are ionized at physiological pH and have very low lipid solubilities. Several groups have reported that amino-β-lactam antibiotics are absorbed by a carrier-mediated system in the rat small intestine (Kimura et al 1978, 1983; Tsuji et al 1981; Miyazaki et al 1982; Nakashima et al 1984; Nakashima & Tsuji 1985). Transcellular transport across the enterocyte involves (a) uptake from the gut across the brush-border membranes, (b) diffusion through the cytoplasm, and (c) exit to the blood across the basolateral membranes. In general, the transport across the brush-border membranes is regarded as the most important step for the intestinal absorption of drugs. To elucidate the intestinal absorption mechanisms of amino-β-lactam antibiotics, we have studied the transport characteristics of those drugs by the intestinal brush-border membrane vesicles, and found that amino-β-lactam antibiotics are transported via common carrier system with dipeptides and that their transport is driven actively by an inward H^+ gradient (Okano et al 1986a, b). Furthermore, to evaluate the brush-border membrane vesicles as a model system for the intestinal absorption of various β-lactam antibiotics, we examined the correlation between in-situ intestinal absorption and brush-border membrane transport of those drugs.

Materials and methods

Cephradine (Sankyo Co., Tokyo, Japan), cephalexin and cefaclor (Shionogi and Co., Osaka, Japan), cefadroxil (Bristol Myers Co., Tokyo, Japan), cefixime and cefazolin (Fujisawa Pharmaceutical Co., Osaka, Japan), and cefotiam (Takeda Chemical Industries, Osaka, Japan) were gifts. *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (Hepes) and 2-(*N*-morpholino)-ethanesulphonic acid (Mes) were obtained from Nakarai Chemicals Ltd, (Kyoto, Japan).

Male Wistar albino rats (180–220 g), fasted for 16–20 h, were anaesthetized with pentobarbitone (40 mg kg^{-1} i.p.). Whole small intestine were used for the in-situ loop absorption experiment. The drugs (1 mM) were dissolved in the phosphate buffered saline, consisting of (mM) NaCl 145, KCl 4·56, $CaCl_2$ 1·25, Na_2HPO_4 1·33 and NaH_2PO_4 0·33 (pH 7·4) (Schanker et al 1958). After washing the loop with the phosphate buffered saline, 5 mL of the drug solution was injected into the loop using a syringe. After 20 min, the contents of the loop were withdrawn as completely as possible, and the lumen washed with the phosphate buffered saline to give a volume of 50 mL. The decrease in the amount of drug from the luminal solution was determined and the amount absorbed was calculated.

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Brush-border membrane vesicles were isolated from the upper half part of the small intestine of male rabbits (2·1–2·3 kg) as described previously (Okano et al 1986a), according to the calcium precipitation method of Kessler et al (1978). Brush-border membranes were suspended in the buffer, consisting of (mM) mannitol 100, KCl 100 and Hepes 10 (pH 7·5). The uptake of cephalosporins (1 mM) was measured by a rapid filtration technique (Okano et al 1986a). In brief, membrane vesicles (20 μL) were incubated at 25°C for 30 s with the substrate mixture (200 μL) comprising (mM) mannitol 100, KCl 100, Mes 10 (pH 6·0) and cephalosporins 1. The cephalosporin trapped on the Millipore filter (HAWP, 0·45 μm, 2·5 cm diameter) was extracted with 300 μL of distilled water and was used for the determination by high performance liquid chromatography.

Cephalosporins were analysed with a high performance liquid chromatograph (LC-3A, Shimadzu Co., Kyoto, Japan) (Okano et al 1986a). Protein was estimated by the method of Bradford (1976), using the Bio-Rad Protein Assay Kit with bovine γ-globulin as a standard.

Results and discussion

To examine the absorption characteristics of various cephalosporins, we chose cephalexin, cephradine, cefaclor, cefadroxil (aminocephalosporins for oral use), cefixime (new oral cephalosporin), cefazolin and cefotiam (cephalosporins for parenteral use). The chemical structures of these antibiotics are shown in Fig. 1. Our previous reports demonstrated that the transport of

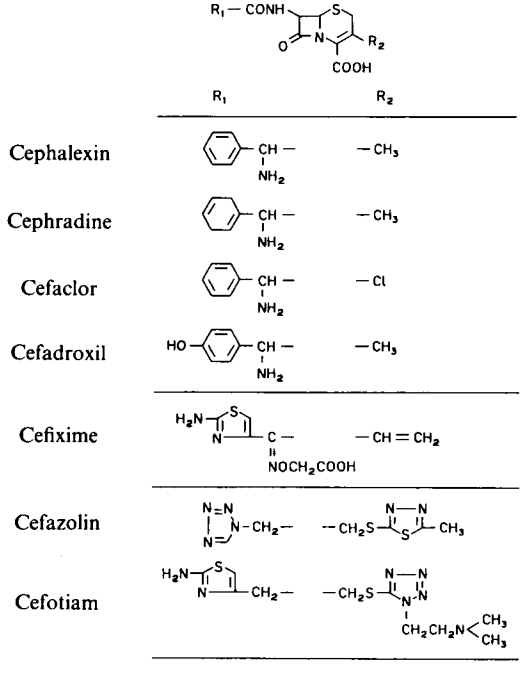

FIG. 1. Structures of cephalosporin antibiotics.

aminocephalosporins can be driven actively by an inward H^+ gradient via the dipeptide transport system in the intestinal brush-border membranes isolated from rats and rabbits (Okano et al 1986a, b). In the present study, therefore, the initial uptake rate of various cephalosporins by brush-border membrane vesicles was examined in the presence of an inward H^+ gradient ($[pH]_i = 7{\cdot}5$, $[pH]_o = 6{\cdot}0$). Apparent absorption rate was estimated by the disappearance of cephalosporins from the intestinal loop 20 min after administration. As shown in Fig. 2, a good correlation was found between in-situ intestinal absorption and the brush-border membrane transport of cephalosporins ($r = 0{\cdot}954$, $P < 0{\cdot}001$). The present data suggest that the transport of oral cephalosporins across the brush-border membranes is the rate-limiting step for the intestinal absorption of these antibiotics. Thus, the transport study using intestinal brush-border membrane vesicles is useful as a model system for the intestinal absorption of β-lactam antibiotics, and this could be used as a primary screening system for the intestinal absorption of new drugs.

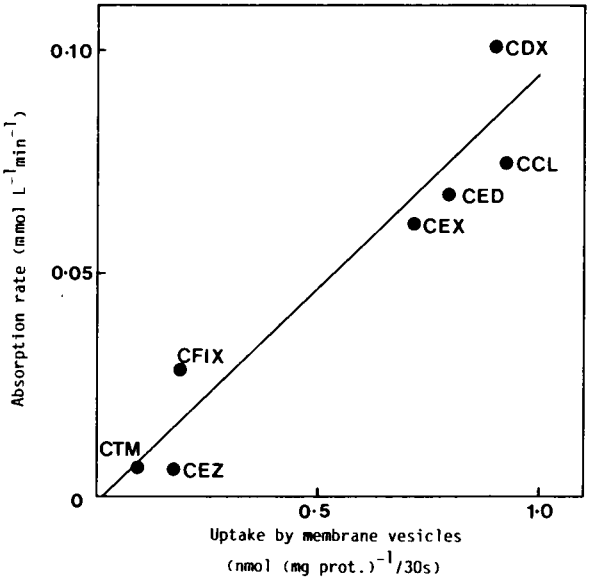

FIG. 2. Correlation between intestinal absorption (A) and brush-border membrane transport (B) of cephalosporin antibiotics. (A) Intestinal absorption. Cephalosporins were dissolved in the phosphate buffered saline (pH 7·4), and 5 mL of drug solution was injected into the intestinal loop. Absorption rate was estimated by the disappearance of cephalosporins from the loop 20 min after administration. Each point represents the mean of five rats. (B) Brush-border membrane transport. The uptake of cephalosporins by brush-border membrane vesicles was measured as described in the text. Each point represents the mean of four determinations. The line was fitted to the data by means of linear regression analysis ($r = 0{\cdot}954$, $P < 0{\cdot}001$). CDX, cefadroxil; CCL, cefaclor; CED, cephradine; CEX, cephalexin; CFIX, cefixime; CEZ, cefazolin; CTM, cefotiam.

Hogben et al (1959) proposed the idea that the pH in the close vicinity of the intestinal brush-border membranes may be acidic compared with the bulk of the luminal solution (virtual pH). By using pH-sensitive microelectrodes, Lucas et al (1976) and Lucas (1983) demonstrated the existence of an acidic microclimate pH (pH 5·5–6·0) at the surface of the small intestine. The Na^+/H^+ exchanger in the brush-border membranes of small intestine is most likely responsible for the secretion of H^+ into the intestinal lumen in-vivo (Murer et al 1976). Thus, the present data suggest that an inward H^+ gradient due to the acidic microclimate pH in the small intestine plays a role as the energy source for the active transport of oral cephalosporins across the brush-border membranes.

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