J. Pharm. Pharmacol. 1989, 41: 795–796
Communicated March 14, 1989

Transport of cephalosporin antibiotics in rat renal basolateral membranes

MIKIHISA TAKANO, TOMONOBU OKANO, KEN-ICHI INUI, RYOHEI HORI, *Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan*

Abstract—Transport mechanisms of cephalosporin antibiotics in rat renal basolateral membranes have been studied in isolated membrane vesicles. Uptake of [^{14}C]*p*-aminohippurate, a typical organic anion, by basolateral membrane vesicles was enhanced when membrane vesicles were preloaded with unlabelled *p*-aminohippurate (counter-transport). Cephalosporins such as cephradine, cephalexin, and cefazolin inhibited the counter-transport of [^{14}C]*p*-aminohippurate, as did unlabelled *p*-aminohippurate and probenecid. In contrast, cephalexin did not affect the uptake of [^{3}H]tetraethylammonium, an organic cation, by basolateral membrane vesicles. These results suggest that most cephalosporins are transported via organic anion transport systems in rat renal basolateral membranes.

How cephalosporins are dealt with by the kidney has been extensively studied by methods such as the in-vivo clearance technique and in-vitro cortical slice experiments. From such studies, most cephalosporins have been shown to be actively secreted by an organic anion transport system in the proximal tubules (Nightingale et al 1975; Hori et al 1982a; Kamiya et al 1983). However, epithelial cells in the proximal tubules are composed of two functionally different membranes, i.e. luminal brush-border and antiluminal basolateral membranes. Therefore, it is difficult to estimate the actual membrane events during transepithelial transport of drugs by the techniques described above.

To resolve this problem, we have been studying the transport of drugs in isolated brush-border and basolateral membrane vesicles, which should provide an effective system for analysing the transport mechanisms in both membranes separately. In fact, by the use of brush-border membrane vesicles isolated from rat kidney cortex, it has recently been found that aminocephalosporins could be actively secreted via an H^+/organic cation antiport system (Inui et al 1985), in addition to the organic anion transport system (Inui et al 1983). Furthermore, aminocephalosporins are reabsorbed via dipeptide transport systems across renal brush-border membranes (Inui et al 1984), as well as in the intestinal brush-border membranes (Okano et al 1986a, b; Hori et al 1988). However, until now there has been little information concerning the transport of cephalosporins in renal basolateral membranes.

In this study, the transport mechanisms of cephalosporins have been studied, using isolated renal basolateral membrane vesicles, and it was discovered that most are transported by an organic anion transport system in renal basolateral membranes.

Materials and methods

Basolateral membrane vesicles were isolated from the renal cortex of male Wistar albino rats (200–230 g) by Percoll density gradient centrifugation (Inui et al 1981). The purified membranes were suspended in a buffer comprising (mM) mannitol 100, Hepes-Tris (pH 7·5) 20 and KCl 100. The uptake of [^{14}C]*p*-aminohippurate (Du Pont-New England Nuclear, 45·8 mCi mmol^{-1}) by the freshly isolated membrane vesicles was measured at 25°C by a rapid filtration technique (Inui et al 1986). The reaction was initiated by adding 180 μL of buffer, containing [^{14}C]*p*-aminohippurate with or without cephalosporins, to 20 μL of membrane vesicles preloaded with unlabelled *p*-aminohippurate (counter-transport condition). Uptake of [^{3}H]tetraethylammonium (Du Pont-New England Nuclear, 109 mCi mmol^{-1}) was measured as described previously (Takano et al 1985). Protein was determined, after precipitation with ice-cold 10% (w/v) trichloroacetic acid, by the method of Lowry et al (1951) with bovine serum albumin as a standard. Cephalexin and cephaloridine (Shionogi & Co., Osaka, Japan), cephradine (Sankyo Co., Tokyo, Japan), cefazolin and cefixime (Fujisawa Pharmaceutical Co., Osaka, Japan), and cefotiam (Takeda Chemical Industries, Osaka, Japan) were gifts. Data were analysed statistically using one-way analysis of variance followed by Dunnett's t-test.

Correspondence to: R. Hori, Department of Pharmacy, Kyoto University Hospital, Sakyo-ku, Kyoto 606, Japan.

Results and discussion

Basolateral membranes isolated from rat kidney cortex have a carrier-mediated transport system for organic anions like *p*-aminohippurate. We have previously reported that labelled *p*-aminohippurate transport is markedly stimulated when basolateral membrane vesicles were preloaded with high concentrations of unlabelled *p*-aminohippurate (counter-transport), and an apparent overshoot uptake was observed (Inui et al 1986). By using this experimental condition, the effect of cephalosporins on [^{14}C]*p*-aminohippurate uptake was estimated, to test whether the drugs can interact with an organic anion transport system in the basolateral membranes (Table 1). [^{14}C]*p*-aminohippurate

Table 1. Effect of various drugs on [^{14}C]*p*-aminohippurate (PAH) uptake by basolateral membrane vesicles. Basolateral membrane vesicles were preloaded with (With PAH Preload) or without (Without PAH Preload) 5 mM unlabelled PAH. The concentrations of [^{14}C]PAH and drugs in the uptake medium were 0·1 and 10 mM, respectively. Each value represents the mean ± s.e. of four determinations.

	[^{14}C]PAH Uptake (pmol (mg protein)$^{-1}$/15 s)	% of Control
A. Experiment I		
Without PAH preload	31·5 ± 2·0*	31
With PAH preload		
Control	100·9 ± 1·2	100
Probenecid	16·4 ± 1·2*	16
Cefazolin	29·4 ± 1·1*	29
PAH	35·1 ± 2·8*	35
Cephradine	60·1 ± 1·4*	60
Cephalexin	81·4 ± 1·2*	81
Tetraethylammonium	103·2 ± 3·5	102
B. Experiment II		
Without PAH preload	29·1 ± 1·8*	31
With PAH preload		
Control	94·4 ± 1·2	100
Cefotiam	59·4 ± 3·2*	63
Cephaloridine	63·1 ± 3·3*	67
Cefixime	89·4 ± 3·6	95

* $P < 0.01$, significant difference from control using one-way analysis of variance followed by Dunnett's t-test.

transport was inhibited by unlabelled *p*-aminohippurate, probenecid (a potent inhibitor of organic anion transport) and various cephalosporins, though it was not affected by tetraethylammonium (an organic cation). The potency of inhibition by the antibiotics on *p*-aminohippurate transport could be ranked as follows: cefazolin > cephradine = cefotiam = cephaloridine > cephalexin.

Among cephalosporins tested, cefixime had a marginal inhibitory effect on [^{14}C]*p*-aminohippurate uptake. In-vivo study showed that cefixime did not undergo the tubular secretion in dog kidney, though the secretion was observed in rabbits (Sakamoto et al 1985). Tamai et al (1988) reported that cefixime was transported via an organic anion transport system in rat renal brush-border membranes.

Aminocephalosporins such as cephalexin and cephradine are amphoteric molecules, having both amino and carboxyl groups, and are ionized as mostly zwitterion and anion at the physiological pH. Aminocephalosporins can be transported, in part, via an organic cation transport system in renal brush-border membranes (Inui et al 1985). Therefore, the effect of cephalexin (2 and 5 mM) on the transport of [^{3}H]tetraethylammonium in basolateral membranes was also examined and found to be without effect: the uptake of TEA not differing significantly from the control value of 214 ± 9 pmol(mg protein)$^{-1}$/15 s (n = 4). These results indicate that most cephalosporin antibiotics are transported via an organic anion transport system in rat renal basolateral membranes. Our results are in accordance with those of Kasher et al (1983) who reported the inhibitory effect of cephaloridine and cefazolin on *p*-aminohippurate transport in dog basolateral membranes.

Based on the present results and our previous findings, transport of cephalosporins in renal epithelial cells may be characterized as follows. i) Cephalosporins are secreted across renal epithelial cells via organic anion transport systems located in basolateral (this study; Hori et al 1982a) and brush-border membranes (Inui et al 1983), though the transport systems in both membranes have different properties (Hori et al 1982b). ii) In brush-border membranes, aminocephalosporins (oral cephalosporins) are secreted via organic cation transport systems (Inui et al 1985) and are reabsorbed via dipeptide transport systems (Inui et al 1984). In contrast to the organic anion transport system, an organic cation transport system in brush-border membranes is an active process, which is driven by an H^+ gradient (H^+/organic cation antiport system). Therefore, the H^+ gradient across renal brush-border membranes should serve as a driving force for the secretion of aminocephalosporins (Inui et al 1985). However, aminocephalosporins do not interact with organic cation transport systems in basolateral membranes.

Unfortunately, the driving force for organic anion transport in basolateral membranes is still controversial. At this stage, anion-anion exchange is a likely candidate to explain the active transport of organic anions in basolateral membranes (Inui et al 1986; Shimada et al 1987; Pritchard 1988). Therefore, the counter-transport condition we used may be a suitable system to mimic the in-vivo situation, and to enhance the sensitivity for evaluating the drug interaction with the organic anion transport system in basolateral membranes.

In conclusion, most cephalosporins should be transported via an organic anion transport system in renal basolateral membranes. The present results confirm and extend previous findings obtained by other techniques such as in-vivo clearance and in-vitro cortical slice methods.

References

Hori, R., Ishikawa, Y., Takano, M., Okano, T., Kitazawa, S., Inui, K. (1982a) The interaction of cephalosporin antibiotics with renal cortex of rats: Accumulation to cortical slices and binding to purified plasma membranes. Biochem. Pharmacol. 31: 2267–2272

Hori, R., Takano, M., Okano, T., Kitazawa, S., Inui, K. (1982b) Mechanisms of p-aminohippurate transport by brush-border and basolateral membrane vesicles isolated from rat kidney cortex. Biochim. Biophys. Acta 692: 97–100

Hori, R., Okano, T., Kato, M., Maegawa, H., Inui, K. (1988) Intestinal absorption of cephalosporin antibiotics: correlation between intestinal absorption and brush-border membrane transport. J. Pharm. Pharmacol. 40: 646–647

Inui, K., Okano, T., Takano, M., Kitazawa, S., Hori, R. (1981) A simple method for the isolation of basolateral plasma membrane vesicles from rat kidney cortex: Enzyme activities and some properties of glucose transport. Biochim. Biophys. Acta 647: 150–154

Inui, K., Okano, T., Takano, M., Kitazawa, S., Hori, R. (1983) Carrier-mediated transport of amino-cephalosporins by brush border membrane vesicles isolated from rat kidney cortex. Biochem. Pharmacol. 32: 621–626

Inui, K., Okano, T., Takano, M., Saito, H., Hori, R. (1984) Carrier-mediated transport of cephalexin via the dipeptide transport system in rat renal brush-border membrane vesicles. Biochim. Biophys. Acta 769: 449–454

Inui, K., Takano, M., Okano, T., Hori, R. (1985) H^+ Gradient-dependent transport of aminocephalosporins in rat renal brush border membrane vesicles: Role of H^+/organic cation antiport system. J. Pharmacol. Exp. Ther. 233: 181–185

Inui, K., Takano, M., Okano, T., Hori, R. (1986) Role of chloride on carrier-mediated transport of p-aminohippurate in rat renal basolateral membrane vesicles. Biochim. Biophys. Acta 855: 425–428

Kamiya, A., Okumura, K., Hori, R. (1983) Quantitative investigation on renal handling of drugs in rabbits, dogs and humans. J. Pharm. Sci. 72: 440–443

Kasher, J. S., Holohan, P. D., Ross, C. R. (1983) Effect of cephaloridine on the transport of organic ions in dog kidney plasma membrane vesicles. J. Pharmacol. Exp. Ther. 225: 606–610

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Nightingale, C. H., Greene, D. S., Quintiliani, R. (1975) Pharmacokinetics and clinical use of cephalosporin antibiotics. J. Pharm. Sci. 64: 1899–1927

Okano, T., Inui, K., Maegawa, H., Takano, M., Hori, R. (1986a) H^+ Coupled uphill transport of aminocephalosporins via the dipeptide transport system in rabbit intestinal brush border membranes. J. Biol. Chem. 261: 14130–14134

Okano, T., Inui, K., Takano, M., Hori, R. (1986b) H^+ Gradient-dependent transport of aminocephalosporins in rat intestinal brush-border membrane vesicles: Role of dipeptide transport system. Biochem. Pharmacol. 35: 1781–1786

Pritchard, J. B. (1988) Coupled transport of p-aminohippurate by rat kidney basolateral membrane vesicles. Am. J. Physiol. 255: F597–F604

Sakamoto, H., Hirose, T., Nakamoto, S., Mine, Y. (1985) Mechanism of renal excretion of FK027 in dogs and rabbits. J. Antibiotics 38: 1088–1095

Shimada, H., Moewes, B., Burckhardt, G. (1987) Indirect coupling to Na^+ of p-aminohippuric acid uptake into rat renal basolateral membrane vesicles. Am. J. Physiol. 253: F795–F801

Takano, M., Inui, K., Okano, T., Hori, R. (1985) Cimetidine transport in rat renal brush border and basolateral membrane vesicles. Life Sci. 37: 1579–1585

Tamai, I., Tsuji, A., Kin, Y. (1988) Carrier-mediated transport of cefixime, a new cephalosporin antibiotic, via an organic anion transport system in the rat renal brush-border membrane. J. Pharmacol. Exp. Ther. 246: 338–344