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The Predominant Contribution of Oligopeptide Transporter PepT1 to Intestinal Absorption of β-Lactam Antibiotics in the Rat Small Intestine

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Abstract

Although recent evidence suggests that certain β-lactam antibiotics are absorbed via a specific transport mechanism, its nature is unclear. To confirm whether peptide transport in the rat can be largely ascribed to the intestinal oligopeptide transporter PepT1, the transporter has been functionally characterized and its significance in the intestinal absorption of β-lactam antibiotics was evaluated.

For evaluation of transport activity complementary RNA (cRNA) of rat PepT1 was synthesized in-vitro and expressed in *Xenopus laevis* oocytes. cRNA induced uptake of several β-lactam antibiotics and the dipeptide [^{14}C]glycylsarcosine; this was specifically inhibited by various dipeptides and tripeptides but not by their constituent amino acids or by tetra- or pentapeptides. The transport activity of PepT1 for β-lactam antibiotics correlated well with their in-vivo intestinal transport and absorption. Furthermore, mutual inhibitory effects on uptake were observed between glycylsarcosine and β-lactam antibiotics. Hybrid depletion of the functional expression of rat PepT1 in oocytes injected with rat intestinal epithelial total mRNA was studied using an antisense oligonucleotide corresponding to the 5′-coding region of PepT1. In oocytes injected with rat mRNA pre-hybridized with the antisense oligonucleotide against rat PepT1, the uptake of [^{14}C]glycylsarcosine was almost completely abolished, whereas its uptake was not influenced by a sense oligonucleotide for the same region of PepT1. Similarly, the uptake of β-lactam antibiotics was also reduced by the antisense oligonucleotide against rat PepT1.

These results demonstrate that the intestinal proton-coupled oligopeptide transporter PepT1 plays a predominant role in the carrier-mediated intestinal absorption of β-lactam antibiotics and native oligopeptides in the rat.

Evidence has been obtained by use of intestinal tissue preparations (Addison et al 1975; Tsuji et al 1979; Nakashima et al 1984; Sinko & Amidon 1989), isolated intestinal brush-border membrane vesicles from animals (Okano et al 1986; Tsuji et al 1987a, b; Kramer et al 1988; Muranushi et al 1989) and the cultured adenocarcinoma cell line Caco-2 from man (Inui et al 1992; Dantzig et al 1994a; Gochoco et al 1994) that certain hydrophilic β-lactam antibiotics are transported by oligopeptide transporters in the small intestine. We have also reported the expression of a transporter for both zwitterionic (cefadroxil) and dianionic β-lactam antibiotics (ceftibuten), both of which are absorbed via carrier-mediated mechanisms in isolated membrane vesicles (Kimura et al 1985; Yoshikawa et al 1989) and in *Xenopus laevis* oocytes injected with mRNA obtained from the small intestinal epithelial cells of rat, rabbit or man (Tamai et al 1994, 1995). The transporter expressed in oocytes was considered to be an oligopeptide transporter, as judged from the pH-dependence of the transport activity and from the substrate-specificity evaluated on the basis of inhibitory and counter-transport effects. Furthermore, an intestinal oligopeptide transporter PepT1 cloned recently is present at the brush-border membrane and has transport activity for β-lactam antibiotics and for the dipeptide glycylsarcosine (Boll et al 1994; Fei et al 1994; Miyamoto et al 1996; Sai et al 1996; Saito et al 1996). All these lines of evidence suggest that certain β-lactam antibiotics are absorbed via a specific carrier-mediated transport mechanism (or mechanisms), probably a proton-coupled oligopeptide transporter.

To understand fully the intestinal absorption of β-lactam antibiotics by a carrier-mediated transport mechanism, the multiplicity of the transporters involved and the species difference in the intestinal absorption of the antibiotics must be clarified. Using isolated intestinal brush-border membrane vesicles and Caco-2 cells, multiple transporters which have distinct substrate specificity or transport activity, or both, have been suggested (Inui et al 1988; Kramer et al 1993; Hu et al 1994; Muranushi et al 1994). In addition, species differences in the intestinal absorption mechanisms for β-lactam antibiotics among rat, rabbit and man have been also demonstrated (Sugawara et al 1990, 1992), implying the involvement of multiple transporters for the antibiotics. Furthermore, the presence of HPT1 cloned from Caco-2 cells, which is partially related to the intestinal transport of the β-lactam antibiotics, also suggests a multiplicity (Dantzig et al 1994b). In contrast, differential transport characteristics among β-lactam antibiotics were ascribed to the variable ionization properties of derivatives, resulting in different affinities to PepT1 in rabbits and man (Wenzel et al 1996). Fei et al (1994) and Sai et al

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(1996) suggested after a hybrid-depletion study in rabbits that cloned PepT1 is very important for evaluation of the intestinal absorption of the dipeptide and some β-lactam antibiotics, implying the participation of a single transporter. Because of these conflicting results and the apparent species-difference in the intestinal transport of β-lactam antibiotics, it is essential to confirm whether or not peptide transport in the rat is largely ascribed to PepT1, because rats are often used for the in-vivo evaluation of the intestinal absorbability of drugs.

Although Miyamoto et al (1996) and Saito et al (1995) have recently cloned a rat PepT1, functional characterization of the rat PepT1 and the elucidation of its significance in the intestinal absorption of β-lactam antibiotics are still required. Accordingly, the aim of this study was to evaluate whether or not transporters other than PepT1 function in the intestinal absorption of β-lactam antibiotics, by characterizing the transport activity of PepT1 for β-lactam antibiotics and glycylsarcosine in *Xenopus laevis* oocytes and by the hybrid-depletion of the expressed transport activity by rat intestinal total mRNA using antisense oligonucleotide against rat PepT1.

Materials and Methods

Materials

[Glycine-1-^{14}C]glycylsarcosine ([^{14}C]glycylsarcosine; 60 m Ci mmol^{-1}) was obtained from Amersham International (Buckinghamshire, UK). Cefadroxil (Bristol Banyu, Tokyo, Japan), ceftibuten, *trans*-ceftibuten and cephalexin (Shionogi, Osaka, Japan), cefazolin and cefixime (Fujisawa Pharmaceutical Industry, Osaka) and cephradine (Sankyo, Tokyo) were gifts. mRNA purification kit and mRNA capping kit were from Pharmacia LKB (Piscataway, NJ) and Stratagene (La Jolla, CA), respectively. Di-, tri-, tetra- and pentapeptides were purchased from Sigma (St Louis, MO). Restriction endonuclease Xba I was purchased from New England Biolabs (Beverly, MA). All other chemicals were reagent-grade products, and were used without further purification.

Preparation of poly(A)$^+$RNA from tissues, and in-vitro transcription

Male Sprague-Dawley rats were obtained from Japan SLC (Hamamatsu, Japan). Poly(A)$^+$RNA (mRNA) from rat intestinal epithelial cells was isolated by guanidinium thiocyanate and caesium chloride purification then oligo(dT)-cellulose affinity chromatography as described previously (Terasaki et al 1993).

In-vitro transcription of the cloned rat homologue of rabbit intestinal oligopeptide transporter, PepT1, which was subcloned into pBluescript SK II (+), was performed as follows. Template DNA was linearized by Xba I digestion. Transcription by T7 RNA polymerase and subsequent capping were performed by use of the mCAP mRNA capping kit. After digestion of template DNA, the synthesized complementary RNA (cRNA) was purified by phenol-chloroform extraction, precipitated with ammonium acetate and ethanol, and resuspended in water pre-treated with diethylpyrocarbonate.

Transport experiments in Xenopus laevis *oocytes*

Oocytes from *Xenopus laevis* were manually dissected in medium A (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ and 5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) adjusted to pH 7·6 with NaOH) and defolliculated in modified Barth's solution (88 mM NaCl, 1 mM KCl, 2·4 mM $NaHCO_3$, 0·82 mM $MgSO_4$, 0·33 mM $Ca(NO_3)_2$, 0·41 mM $CaCl_2$, 100 units mL^{-1} penicillin G, 100 mg mL^{-1} streptomycin and 10 mM HEPES adjusted to pH 7·5 with NaOH) as described previously (Tamai et al 1994). mRNA solution (50 nL containing 50 ng mRNA), water or in-vitro-synthesized cRNA (25 ng) was micro-injected directly into each oocyte. The oocytes were cultured for 5 days in modified Barth's solution and used for transport experiments. Before initiation of uptake of [^{14}C]glycylsarcosine or β-lactam antibiotics, oocytes were pre-incubated for 30 min in medium B (100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM HEPES adjusted to pH 7·5 with Tris) at 25°C. Uptake at pH 7·5 was measured in medium B containing [^{14}C]glycylsarcosine or β-lactam antibiotics at 25°C. When uptake was measured at acidic pH the pH of medium B was maintained by use of 10 mM MES (2-(*N*-morpholino)ethanesulphonic acid) instead of HEPES after adjustment to pH 5·5 with Tris. In most experiments, the concentrations of [^{14}C]glycylsarcosine and β-lactam antibiotics were 50 μM and 2 mM, respectively. Uptake was terminated by washing the oocytes three times with ice-cold medium B.

Hybrid depletion study

Antisense and sense oligonucleotides were synthesized by Biologica (Nagoya, Japan) and purified by HPLC. The sequences of the antisense and sense oligonucleotides (23-mer), corresponding to the 5′ coding region of PepT1 cDNA, were 5′-CCCCGAGACTTGGACATCCCCAT-3′ and 5′-ATGGGGATGTCCAAGTCTCGGGG-3′, respectively. These oligonucleotides were incubated at 42°C for 1 h in the presence of 50 mM NaCl with intestinal total mRNA previously denatured by 5-min incubation at 65°C. Final concentrations of antisense and sense oligonucleotides were 50 ng mL^{-1} and that of rat intestinal mRNA was 1000 ng mL^{-1}. The mRNA hybridized with antisense oligonucleotide were micro-injected into oocytes and the oocytes were cultured.

Analytical procedures

Oocytes were washed with ice-cold medium B, homogenized in phosphate buffer (50 mM, pH 7·4; 300 mL), then deproteinized with 0·5% perchloric acid for HPLC analysis of β-lactam antibiotics. The HPLC system was equipped with a BIP-I (Japan Spectroscopic, Tokyo, Japan) constant-flow pump, Uvidec-100V (Japan Spectroscopic) UV detector and Chromatopac CR4A (Shimadzu, Kyoto, Japan) integrator. The analytical column was a 4·6 mm × 15 cm reversed-phase TSK-gel ODS-80Ts (Tosoh, Tokyo). The mobile phases were water-acetonitrile (96:4) for cefadroxil and (91:9) for cefixime, cephradine, cephalexin and cefazolin, and water-methanol (88:12) for ceftibuten and *trans*-ceftibuten, each containing 10 mM ammonium acetate. The mobile-phase flow-rate was 1·0 mL min^{-1} and the eluate was monitored at 230 nm for cefadroxil, 290 nm for cefixime, 260 nm for cephradine, cephalexin and cefazolin, and 262 nm for ceftibuten and *trans*-ceftibuten. When radiolabelled compounds were used, after termination of uptake oocytes were solubilized in 5% sodium dodecylsulphate solution. Liquid scintillation cocktail (Cleasol I, Nacalai Tesque, Kyoto, Japan) was then added and the radioactivity was measured in a liquid scintillation counter (Aloka, Tokyo, Japan).

Results

Functional characterization of rat PepT1

Table 1 shows the uptake of [^{14}C]glycylsarcosine by cRNA- or water-injected oocytes at a medium pH of 5·5 in the absence and in the presence of various free amino acids and di-, tri-, tetra- and pentapeptides. It is clearly apparent that di- and tripeptides composed of various amino acids, e.g. glycine, alanine, sarcosine, proline and lysine, at concentrations ranging from 0·5 to 20 mM caused significant inhibition whereas their constituent amino acids or the tetra- or pentapeptides at the same concentrations were not inhibitory. For alanine-dipeptide the stereoisomer, D-Ala-D-Ala was not inhibitory even at the higher concentration of 40 mM, whereas L-Ala-L-Ala at 2 mM completely prevented the uptake of [^{14}C]glycylsarcosine. Similarly, D-cephalexin was less potent at reducing uptake than was L-cephalexin. Thus, the oligopeptide transporter is stereoselective. The absence of inhibition by glycine implies that we are measuring uptake of the intact radiolabelled dipeptide, not a radiolabelled amino acid produced by hydrolysis of [^{14}C]glycylsarcosine.

Table 2 shows the uptake of various β-lactam antibiotics by oocytes injected with rat PepT1 cRNA or water. Uptake of zwitterionic derivatives, cephalexin and cephradine, and dianionic derivatives, cefixime and ceftibuten, all of which are orally active, was significantly increased in oocytes injected with rat PepT1 cRNA compared with uptake in water-injected oocytes, whereas uptake by rat PepT1 cRNA- and water-injected oocytes was comparable for parenterally administered cefazolin. The geometric isomer of ceftibuten, *trans*-ceftibuten, was not taken up by oocytes injected with rat PepT1 cRNA, showing that the uptake activity of PepT1 is stereoselective. Relatively higher uptake of some β-lactam antibiotics such as cefadroxil and cefixime observed in water-injected oocytes suggests that they might be substrates for a native transporter expressed in oocytes, as suggested by the uptake of glycylsarcosine (Miyamoto et al 1991). However, the activity is much less than those expressed by rat PepT1 cRNA injection.

Table 2. Uptake of various β-lactam antibiotics by *Xenopus laevis* oocytes injected with rat PepT1 cRNA.

Substrate	Uptake rate (pmol/2 h oocyte^{-1})	
	PepT1 cRNA-injected	Water-injected
Cefadroxil	21·9 ± 2·87*	4·67 ± 1·49
Cefazolin	0·642 ± 0·379	0·101 ± 0·101
Cefixime	13·5 ± 1·01*	2·23 ± 0·924
Ceftibuten	24·1 ± 5·19*	0·797 ± 0·354
trans-Ceftibuten	1·11 ± 0·444	1·71 ± 0·972
Cephalexin	12·0 ± 2·31*	1·51 ± 0·287
Cephradine	7·72 ± 1·23*	1·43 ± 0·909

Uptake of β-lactam antibiotics (2 mM) was measured at 25°C for 2 h at pH 5.5. Each datum represents the mean ± s.e.m. of three to eight determinations. *P<0·05, significantly different compared with uptake by water-injected oocytes.

Table 3 shows the mutual competitive inhibitory effects between glycylsarcosine and β-lactam antibiotics. Uptake of [^{14}C]glycylsarcosine by cRNA-injected oocytes was reduced in the presence of ceftibuten (2 mM) or cefadroxil (20 mM) in the uptake medium. Similarly, uptake of both ceftibuten and cefadroxil was inhibited by 20 mM glycylsarcosine. Accordingly, it was demonstrated that native oligopeptide and β-lactam antibiotics have a common binding site(s) on rat PepT1.

Hybrid depletion of transport activity

The hybrid depletion of the transport activity for [^{14}C]glycylsarcosine and β-lactam antibiotics in *Xenopus laevis* oocytes injected with total mRNA from rat intestinal epithelial cells was examined by using antisense oligonucleotide against rat PepT1. Whereas injection of mRNA alone into oocytes induced an approximately threefold increase in uptake of [^{14}C]glycylsarcosine compared with that by water-injected oocytes (Table 4), when mRNA pre-hybridized with the antisense oligonucleotide was injected the uptake was significantly reduced to a level comparable with that of water-injected oocytes. The uptake obtained by using mRNA pre-incubated

Table 1. Inhibitory effects of amino acids and oligopeptides on PepT1-induced [^{14}C]glycylsarcosine uptake in the rat.

Inhibitor	Uptake rate (% of control)	Inhibitor	Uptake rate (% of control)
Water injected	31·2 ± 3·4		
cRNA injected (Control)	100·0 ± 7·4		
Amino acids		Tripeptides	
+20 mM Gly	118·3 ± 21·1	+20 mM Gly3	20·0 ± 2·92*
+20 mM Sar	95·6 ± 17·5	+0·5 mM Ala3	82·1 ± 4·87*
+20 mM Pro	107·7 ± 10·8	+2 mM Ala3	17·3 ± 2·68*
+10 mM Ala	97·1 ± 4·55	+10 mM Lys3	17·7 ± 4·50*
+10 mM Lys	101·8 ± 11·9	Tetrapeptides	
Dipeptides		+20 mM Gly4	92·1 ± 19·5
+10 mM Gly2	31·6 ± 8·34*	+10 mM Ala4	100 ± 7·46
+20 mM Gly2	24·4 ± 4·24*	Pentapeptides	
+20 mM Gly-Sar	18·2 ± 5·55*	+10 mM Gly5	88·0 ± 14
+20 mM Gly-Pro	31·6 ± 5·83*	+5 mM Ala5	92·2 ± 5·52
+0·5 mM Ala2	50·0 ± 8·77*	+10 mM Lys5	110 ± 36·3
+2 mM Ala2	10·1 ± 1·44*	Cephalosporins	
+2 mM D-Ala2	117·8 ± 27·4	+5 mM L-CEX	55·6 ± 4·13*
+40 mM D-Ala2	90·2 ± 13·8	+5 mM D-CEX	76·2 ± 6·67*

Uptake of [^{14}C]glycylsarcosine (50 μM) was measured at 25°C for 1 h at pH 5·5 in the presence or absence of each inhibitor. Each datum represents the mean ± s.e.m. of the percentage of the control uptake in five to nine determinations. *P<0·05, significantly different compared with control.

Table 3. Mutual competition between glycylsarcosine and cephalosporins for uptake by *Xenopus laevis* oocytes injected with rat PepT1 cRNA.

Substrate	Inhibitor	Uptake rate
[^{14}C]Glycylsarcosine (pmol h^{-1} oocyte^{-1})	Control	4·32 ± 0·49
	+ ceftibuten	0·82 ± 0·17*
	+ cefadroxil	2·28 ± 0·57*
Cefadroxil (pmol/2h oocyte^{-1})	Control	21·9 ± 2·87
	+ glycylsarcosine	3·09 ± 0·34*
Ceftibuten (pmol/2h oocyte^{-1})	Control	23·5 ± 2·13
	+ glycylsarcosine	10·8 ± 1·89*

Uptake of [^{14}C]glycylsarcosine (50 μM) and cephalosporins (2 mM) was measured in the presence or absence of each inhibitor (20 mM for cefadroxil and glycylsarcosine and 2 mM for ceftibuten) at 25°C for 1 h and 2 h at pH 5·5, respectively. Each datum represents the mean ± s.e.m. of four to nine determinations. *$P < 0·05$, significantly different compared with control.

Table 4. Hybrid depletion of rat intestinal mRNA-induced uptake of [^{14}C]glycylsarcosine, ceftibuten and cefadroxil by *Xenopus laevis* oocytes with a rat PepT1 antisense oligonucleotide.

Substrate	Uptake (pmol h^{-1} oocyte^{-1})			
	Water-injected	mRNA-injected control	+ Sense	+ Antisense
[^{14}C]Gly-Sar	0·448 ± 0·057	1·33 ± 0·29*	1·55 ± 0·06*	0·539 ± 0·058
Ceftibuten	0·808 ± 0·064	2·84 ± 0·03*	2·37 ± 0·75*	1·10 ± 0·06
Cefadroxil	0·608 ± 0·009	1·92 ± 0·15*	2·12 ± 0·58*	0·740 ± 0·128

Oocytes were injected with rat intestinal total mRNA (50 nL containing 50 ng) or water. Before injection total mRNA was incubated with rat PepT1 sense or antisense oligonucleotides (2·5 ng). Uptake of [^{14}C]glycylsarcosine (50 μM) and β-lactam antibiotics (2 mM) was measured at 25°C and pH 5·5 for 1 and 2 h, respectively. Each datum represents the mean ± s.e.m. of three to six determinations. *$P<0·05$, significantly different compared with water-injected.

with a sense oligonucleotide was similar to that in control oocytes injected with mRNA alone.

Hybrid depletion of uptake of ceftibuten and cefadroxil was also examined; the results are shown in Table 4. The enhanced uptake of both antibiotics by oocytes injected with rat intestinal mRNA was almost completely abolished by pre-hybridization of mRNA with the antisense oligonucleotide against rat PepT1 to the level of that by water-injected oocytes, whereas the sense oligonucleotide did not have any inhibitory effect.

Discussion

Very recent studies have demonstrated the presence of a family of peptide transporter PepT2 in several tissues (Liu et al 1995; Boll et al 1996; Saito et al 1996), in addition to previously cloned intestinal HPT1 (Dantzig et al 1994b). Both have the capacity to transport β-lactam antibiotics, as was observed for PepT1. Furthermore, functionally different intestinal transport characteristics have been observed among β-lactam antibiotic derivatives (Inui et al 1988; Kramer et al 1993; Hu et al 1994; Muranushi et al 1994) and among animal species (Sugawara et al 1990, 1992). These observations suggest it would be important to assess the multiplicity of peptide/peptide-mimetic β-lactam antibiotic transport in the intestine to obtain further understanding of the mechanism of absorption of these compounds. Accordingly, this study was performed to clarify the functional significance of rat oligopeptide transporter PepT1 in the intestinal absorption both of native peptides and of β-lactam antibiotics.

The results, which show the specific inhibitory effect of various di- and tripeptides such as Gly-Gly, Gly-Sar, Gly-Pro, Ala-Ala, Gly-Gly-Gly, Ala-Ala-Ala and Lys-Lys-Lys, but not of their constituent amino acids or of tetra- or pentapeptides, is in good agreement with the substrate specificity of peptide transport found in intestinal tissues (Addison et al 1975; Matthews 1991). The stereospecificity of rat PepT1, observed as differences between the inhibitory potencies of L-Ala-L-Ala and D-Ala-D-Ala and between those of D-cephalexin and L-cephalexin (Table 1), is also in accord with previously reported characteristics of oligopeptide transport in the intestine (Tamai et al 1988; Matthews 1991). Furthermore, uptake of [^{14}C]glycylsarcosine, ceftibuten and cefadroxil by oocytes injected with rat PepT1 cRNA was pH-dependent; activity was approximately two to twelve times greater at pH 5·5 than at pH 7·5 (data not shown). These results show that PepT1 apparently explains the intestinal brush-border transport features obtained in studies using membrane vesicles and intestinal tissue preparations.

Rat PepT1 was also shown to have transport activity for several orally active β-lactam antibiotics, including the zwitterionic compounds cefadroxil, cephalexin and cephradine and the di-anionic derivatives cefixime and ceftibuten, whereas cefazolin and *trans*-ceftibuten were not efficiently transported. Because cefazolin and *trans*-ceftibuten were poorly absorbed from intestine, these results correlate well with the feasibility of their intestinal transport and absorption. Furthermore, the mutual inhibitory effects on PepT1-induced uptake for dipeptide and β-lactam antibiotics (Table 3) indicate that dipeptides and β-lactam antibiotics share a common binding site (or sites) on PepT1. All these observations could be additional evidence that intestinal absorption of the antibiotics is mainly caused by PepT1.

By suppressing the expression of PepT1 in oocytes by hybridization of total mRNA with an antisense oligonucleotide against rat PepT1, we also investigated whether transporters other than PepT1 transport oligopeptide and β-lactam antibiotics. In oocytes injected with rat intestinal mRNA hybridized with the antisense oligonucleotide, the increase in transport activity for [^{14}C]glycylsarcosine was virtually abolished, whereas such abolition was not caused by the sense oligonucleotide against rat PepT1 (Table 4). Similar reduction of [^{14}C]glycylsarcosine transport by an antisense oligonucleotide was also observed in rabbits by Fei et al (1994) and by Sai et al (1996). The uptake of ceftibuten and cefadroxil by oocytes injected with rat intestinal total mRNA was also reduced by pre-hybridization with antisense oligonucleotide against rat PepT1 (Table 4). The almost total abolition of transport of ceftibuten and cefadroxil by the antisense oligonucleotide suggests that these antibiotics are transported exclusively by PepT1, not by other transporters. However, we cannot rule out the participation of another transporter (or transporters) with a 5′-terminal sequence very similar (in terms of nucleotides) to that of PepT1 or a low level of expression of other transporters, or both. Furthermore, the possibility that other di- and tripeptides are transported via transporters other than PepT1 cannot be ruled out. Such a multiplicity is also possible for the intestinal absorption of β-lactam antibiotics. Indeed, it has been suggested that the mono-anionic β-lactam antibiotic cefdinir is absorbed by two different transporters, oligopeptide and monocarboxylic acid transporters (Tsuji et al 1993).

In conclusion, this study has demonstrated that in rats the native peptides and β-lactam antibiotics tested were mainly transported by PepT1; this suggests that PepT1 has a predominant role in their intestinal absorption. However, further studies are needed to prove the multiplicity of the intestinal absorption of β-lactam antibiotics, because transporters which are not related to peptide transport might be involved in the transport of some β-lactam antibiotic derivatives.

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