Bacampicillin Uptake Is Shared with Thiamine in Caco-2 Cells

Masako ODA, Kaori FUJIMOTO, Michiya KOBAYASHI, and Hiroshi SAITOH*

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido; 1757 Kanazawa, Ishikari-Tobetsu, Hokkaido 061–0293, Japan. Received September 7, 2006; accepted April 13, 2007

Bacampicillin was developed as a prodrug to improve the intestinal absorption of its metabolite ampicillin. This study was undertaken to characterize bacampicillin transport in Caco-2 cells. The uptake of bacampicillin in Caco-2 cells was significantly greater than those of ampicillin and pivampicillin. An Eadie–Hofstee plot obtained from 5-min uptake of 0.2—5 mM bacampicillin was linear, indicating the presence of a saturable transport system for bacampicillin with \( K_m \) and \( V_{max} \) of 3.6 mM and 23.9 nmol/mg protein/min, respectively. Hydrophilic organic cations such as choline, cimetidine, guanidine, nicotinamide, 1-methylnicotiamide, and tetraethylammonium failed to modulate bacampicillin uptake in Caco-2 cells whereas diphenhydramine, procainamide, and thiamine significantly depressed it. Moreover, when thiamine was preloaded in Caco-2 cells, bacampicillin uptake was significantly increased, indicating that this cationic vitamin was capable of trans-stimulating bacampicillin transport across the apical membrane of Caco-2 cells. However, trans-stimulated bacampicillin uptake was not observed in the presence of diphenhydramine. Bacampicillin uptake increased with elevation of the medium pH, and the known modulators of thiamine transport such as amiloride and oxythiamine significantly inhibited bacampicillin uptake. Thiamine also significantly decreased the apical-to-basolateral transport of bacampicillin across Caco-2 cell monolayers. However, thiamine did not exert any modulating effect on pivampicillin uptake and its apical-to-basolateral permeation in Caco-2 cells. These results suggest that bacampicillin is transported in Caco-2 cells, sharing a carrier-mediated system with thiamine.

Key words bacampicillin; pivampicillin; prodrug; carrier-mediated transport; thiamine; Caco-2 cell

Bacampicillin is a semisynthetic ester of ampicillin, an amino \( \beta \)-lactam antibiotic. As with other ampicillin prodrugs such as pivampicillin and talampicillin, bacampicillin was developed almost 30 years ago under the hypothesis that the addition of a lipophilic moiety to the hydrophilic ampicillin molecule would improve intestinal absorption of the parent drug.\(^1\) It has been reported that ampicillin exhibits 33—54% bioavailability after oral administration\(^2,3\) and that bacampicillin increases the oral bioavailability of ampicillin up to approximately 90%.\(^4\) Over the past two decades, a large number of papers have provided evidence that several \( \beta \)-lactam antibiotics are absorbed as potent substrates of an \( \text{H}^+\)-coupled peptide transporter (PEPT1) present on the apical membranes of enterocytes.\(^5\) Since ampicillin interacts with PEPT1 to a much lesser extent than other orally active \( \beta \)-lactam antibiotics such as ciclacillin and cefitubten,\(^6\) the contribution of PEPT1 to ampicillin absorption is thought limited. Although PEPT1 exhibits considerably broad substrate specificities to various peptide-like drugs, it has not been clarified whether it is capable of transporting bacampicillin as a substrate.

By introducing a 1’-ethoxycarbonyloxyethyl group into the ampicillin molecule, bacampicillin also acquired the characteristics of a lipophilic organic cation. It is well known that a variety of organic cations are absorbed via specialized transport systems in the small intestine.\(^7,8\) It is therefore possible that a kind of organic cation transporter is unexpectedly involved in the intestinal absorption of bacampicillin. Caco-2 cells express a large number of transporters with which to handle various endogenous and exogenous organic cations such as azasetron,\(^9\) carnitine,\(^10,11\) choline,\(^12\) diphenhydramine,\(^13\) guanidine,\(^14\) 1-methyl-4-phenyl-pyridinium,\(^15\) nicotine,\(^16\) and thiamine.\(^17\) This study was undertaken to characterize bacampicillin uptake in Caco-2 cells with special focus on its interaction with various transporters for organic cations.

MATERIALS AND METHODS

Materials Ampicillin anhydrous, L-carnosine, diphenhydramine hydrochloride, glycyglycine, nicotinamide, tetraethylammonium bromide, and thiamine hydrochloride were obtained from Wako Pure Chem. Ind. (Osaka, Japan). Bacampicillin hydrochloride, guanidine hydrochloride, \( N \)-methylnicotinamide chloride, procainamide hydrochloride, and Dulbecco’s modified Eagle’s medium (DMEM) were purchased from Sigma Chem. Co. (St. Louis, MO, U.S.A.). Choline chloride and cimetidine were obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Oxythiamine hydrochloride and fetal calf serum (FCS) were obtained from ICN Biomedicals Inc. (Aurora, OH, U.S.A.). Non-essential amino acid solution, penicillin, and streptomycin were purchased from Invitrogen Corporation (Grand Island, NY, U.S.A.). All other chemicals and reagents were of the highest grade available.

Cell Culture Caco-2 cells (passage #40) were obtained from Riken Cell Bank (Tsukuba, Japan). They were kept frozen in aliquots in liquid nitrogen until use. The cells were maintained in DMEM (culture medium) containing 5% FCS, 1% nonessential amino acids, 100 U/ml benzylpenicillin, and 100 \( \mu \)g/ml streptomycin. The cells were seeded on 60-mm tissue culture plastic dishes (Becton Dickinson Labware, Bedford, MA, U.S.A.), which were coated with rat tail collagen type I, and grown in an atmosphere of 5% CO\(_2\), in air and 90% relative humidity at 37°C. They were routinely passaged every 6—7 d using 0.02% EDTA and 0.25% trypsin.

Uptake Study in Caco-2 Cells Caco-2 cells at passages between 54 and 70 were seeded at a density of \( 1.8 \times 10^5 \) cells/cm\(^2\) on 35-mm tissue culture plastic dishes. The culture medium was replaced every 3—4 d and Caco-2 cells were used between 20 and 22 d (mostly on day 21) after
seeding. Dulbecco’s phosphate-buffered saline (DPBS) containing 137 mM NaCl, 3 mM KCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 1 mM CaCl₂, 0.5 mM MgCl₂, and 5 mM D-glucose was used as an experimental medium. After removing the culture medium, Caco-2 cells were washed three times with drug-free DPBS (pH 7.4) and preincubated with 2 ml of drug-free DPBS (pH 7.4) at 37 °C for 10 min. The medium was then replaced with 1 ml of drug solution, and the cells were incubated at 37 °C. Drug-free DPBS and drug solution were warmed to 37 °C in advance. At a designated time point, the drug solution was gently and quickly removed from each dish. The cells were washed three times with 2 ml of ice-cold drug-free DPBS, scraped with a cell scraper into 2 ml of ice-cold DPBS, and homogenized with a polytron (Kinematica, Kriens-Luzern, Switzerland) in iced water. An aliquot of the homogenate was then mixed with an equal volume of methanol. The mixture was let stand in iced water for 10 min and centrifugated at 1500×g at room temperature for 5 min. The resulting supernatant was applied to HPLC assay. In most cases, the homogenate was immediately used for drug assay. It was kept frozen at −30 °C until assay, where necessary.

In the case of trans-stimulation experiments, Caco-2 cells were seeded at a density of 4×10⁵ cells/cm² on 60-mm tissue culture plastic dishes. The cells were then treated as described above. The Caco-2 cells were incubated with a medium containing the test compound at 37 °C. The solution was removed after 30 min and the cells were quickly washed twice with 2 ml of drug-free ice-cold DPBS. Then, 2 ml of drug solution was added to each dish, and the cells were further incubated for 1.5 min.

**Permeation Study across Caco-2 Cell Monolayers**

For permeation experiments, Caco-2 cells at passages between 54 and 70 were seeded at a density of 4×10⁵ cells/cm² on polycarbonate filter inserts with a surface area of 4.2 cm² in Transwell chambers (Becton Dickinson Labware, Bedford, MA, U.S.A.). The culture medium was replaced every 3—4 d and Caco-2 cells were used between 20 and 22 d (mostly on day 21) after seeding. After the culture medium was removed, Caco-2 cells were washed three times with DPBS (pH 7.4) and preincubated at 37 °C for 10 min. The apical-to-basolateral permeation was initiated by adding drug solution, which was warmed to 37 °C in advance, to the apical side of the Caco-2 cell monolayers. The medium volume of the apical and basolateral side was 1.5 and 2.2 ml, respectively. After 60 min, 0.2 ml of the basolateral medium was taken and centrifuged at 1500×g at room temperature, and the supernatant was applied to HPLC assay.

**Protein Assay**

The protein content in the cell homogenate was determined according to the method of Bradford using a protein assay kit (Bio-Rad Laboratories, Richmond, CA, U.S.A.). Bovine serum albumin was used as standard.

**Drug Analyses**

Since it was expected that bacampicillin and pivampicillin would be extensively converted to ampicillin in the Caco-2 cells, the concentrations of ampicillin, bacampicillin, and pivampicillin in each sample solution were separately determined by HPLC, and the sum of each concentration was regarded as the total bacampicillin or pivampicillin in Caco-2 cells. The three β-lactam antibiotics were determined by Shimadzu LC-10A HPLC system (Kyoto, Japan), which was equipped with a Shimadzu SPD-10A UV detector. Chromatographic conditions were as follows: column, Cosmosil 5C18-AR (i.d. 4.6 mm×150 mm, Nacalai Tesque, Kyoto, Japan); column temperature, 50 °C; mobile phases, 0.05 M KH₂PO₄/acetonitrile (6:4) for bacampicillin and pivampicillin and 0.05 M KH₂PO₄/methanol (8:2) for ampicillin; flow rate, 0.5—1.0 ml/min; wavelength, 215 nm; and injection volume, 20 μl. Under these HPLC conditions the retention times of ampicillin, bacampicillin, and pivampicillin were about 5 min, 6 min, and 8 min, respectively, and they were reproducibly determined with assay variances of 5—10%.

**Statistical Analysis**

Statistical analysis was evaluated by Student’s t-test. The level of significance was considered p<0.05.

**RESULTS**

**Uptake of Ampicillin, Bacampicillin, and Pivampicillin in Caco-2 Cells**

Preliminary HPLC analyses indicated the presence of minimal amounts of intact bacampicillin or pivampicillin in all sample solutions obtained from Caco-2 cells to which bacampicillin or pivampicillin was applied. Therefore it was considered that bacampicillin and pivampicillin were promptly converted to ampicillin after they entered the Caco-2 cells. As shown in Fig. 1, the uptake of ampicillin itself was very small (0.6 nmol/mg protein) during a 10-min period at a concentration of 0.2 mM whereas those of bacampicillin and pivampicillin, both of which were detected as ampicillin, were much greater than that of ampicillin (bacampicillin, 17.3 nmol/mg protein; pivampicillin, 11.4 nmol/mg protein). It was also shown that bacampicillin uptake was significantly greater than that of pivampicillin.

**Relationship between Bacampicillin Concentrations and Its Uptake in Caco-2 Cells**

Figure 2 shows an Eadie–Hofstee plot obtained from bacampicillin uptake in Caco-2 cells during a 5-min period over the concentration range from 0.2 to 5 mM. Prior to these experiments, it was confirmed that bacampicillin uptake at 0.2 mM linearly increased with time at least up to 10 min. Here, bacampicillin was again recovered as ampicillin in all sample solutions. A straight line was obtained with a correlation coefficient of 0.988, although the number of data points was not enough, and Kₚ and Vₘₐₓ were roughly determined to be 3.6 mM and 20.
cis-Inhibition of Bacampicillin Uptake by Various Compounds in Caco-2 Cells

Table 1 shows bacampicillin uptake in Caco-2 cells in the presence of various compounds. The medium pH was set at 6 as two dipeptides, L-carnosine and glycylglycine, were included in this experiment so as to assess the likely involvement of a H+/H1001-coupled peptide transporter in bacampicillin uptake in Caco-2 cells. There were no significant changes in bacampicillin uptake in the presence of 25-fold greater concentrations of choline, cimetidine, guanidine, nicotinamide, N-methyl nicotinamide, tetraethylammonium, or the two dipeptides. On the other hand, bacampicillin uptake was significantly reduced in the presence of diphenhydramine, procainamide, thiamine, oxythiamine, and amiloride. These compounds also significantly decreased bacampicillin uptake to 60—75% of the control at pH 7.4.

trans-Stimulation of Bacampicillin Uptake in Caco-2 Cells

Figure 3 shows bacampicillin uptake in Caco-2 cells in which four organic cations were preloaded at a concentration of 0.2, 1, or 5 mM. When Caco-2 cells were preincubated with 5 mM thiamine, bacampicillin uptake was significantly increased both at a medium pH of 6 (134% of the control value) and 7.4 (112% of the control value). When Caco-2 cells were preincubated with a lower concentration (0.2 or 1 mM) of thiamine at pH 7.4, bacampicillin uptake increased to approximately 130% of the control. On the other hand, choline, diphenhydramine, and tetraethylammonium failed to modulate bacampicillin uptake when they were preloaded in Caco-2 cells at concentrations of 0.2 and 5 mM.

Effect of Medium pH on Bacampicillin Uptake in Caco-2 Cells

Figure 4 shows bacampicillin uptake in Caco-2 cells during a 10-min period at a concentration of 0.2 mM over a pH range from 5 to 8. The uptake of bacampicillin, which was mostly detected as ampicillin, was markedly pH-dependent and increased markedly when the medium pH was increased from 5 to 7. However, the pH-sensitive increase in bacampicillin uptake was not so marked beyond pH 7.

Effect of Various Compounds on Pivampicillin Uptake in Caco-2 Cells

Table 2 shows the effects of diphenhydramine, tetraethylammonium, thiamine, and L-carnosine on pivampicillin uptake in Caco-2 cells. Whereas diphenhy-
The concentrations of pivampicillin and compounds tested were 0.2 and 5 mM, respectively. Caco-2 cells were incubated at medium pH of 7.4 for 10 min. Each value represents the mean±S.E. of three determinations. *p<0.01, significantly different from pivampicillin alone.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Uptake (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pivampicillin alone</td>
<td>100.0±2.5</td>
</tr>
<tr>
<td>+ Diphenhydramine</td>
<td>50.0±1.7*</td>
</tr>
<tr>
<td>+ Tetracyclammonium</td>
<td>107.4±4.1</td>
</tr>
<tr>
<td>+ Thiamine</td>
<td>93.0±3.6</td>
</tr>
<tr>
<td>+ L-Carnosine</td>
<td>104.3±5.2</td>
</tr>
</tbody>
</table>

The mechanism of ampicillin transport across Caco-2 cell monolayers was investigated in the presence of these two compounds. Thiamine but not in the presence of diphenhydramine. Therefore, the absorption mechanisms of these ampicillin prodrugs have not been fully investigated until now. Recently, Chanteux et al. reported that pivampicillin, which was added to the apical side of Caco-2 cells, was efficiently hydrolyzed to ampicillin inside the cells and that the ampicillin was preferentially released to the basolateral medium via an MRP-like transporter. The aim of this study was to assess how Caco-2 cells deal with bacampicillin as compared with pivampicillin.

First, we observed that when bacampicillin and pivampicillin were applied to Caco-2 cells, the compound recovered from the cells was primarily ampicillin and the amount of intact bacampicillin and pivampicillin was small. Since bacampicillin and pivampicillin are less stable in an aqueous medium, it was expected that a not small part of the bacampicillin and pivampicillin would be hydrolyzed to ampicillin in the apical medium and the ampicillin would be translocated into the Caco-2 cells. However, the uptake of ampicillin itself was very small (Fig. 1), indicating that the ampicillin recovered from Caco-2 cells resulted exclusively from the bacampicillin and pivampicillin taken up by the cells. Thus, it was considered that bacampicillin was transported into the Caco-2 cells to a much greater extent than either ampicillin (ca. 32 fold) or pivampicillin (ca. 1.6 fold) and that bacampicillin was efficiently converted to ampicillin inside the cells. It was also thought that pivampicillin was easily converted to ampicillin in the Caco-2 cells, which is consistent with results presented in recent papers.

Because an Eadie–Hofstee plot (Fig. 2) implied possible involvement of a specialized transport system for bacampicillin, the changes in its uptake were investigated in the presence of various organic cations. As shown in Table 1, bacampicillin uptake was significantly suppressed in the presence of diphenhydramine, procainamide, thiamine, oxythiamine, and amiloride. The presence of specialized transport systems for diphenhydramine and thiamine were previously reported in Caco-2 cells. It is also known that procaainamide permeation across the rabbit intestinal brush-border membrane is mediated by an H^+-tertiary amine antiport system. It has also been suggested that diphenhydramine and procainamide share a common organic cation transporter.

As shown in Fig. 3, the finding that thiamine, but not diphenhydramine, exerted a significant trans-stimulatory effect on bacampicillin uptake in Caco-2 cells suggests that bacampicillin and thiamine share a common transport system on the Caco-2 cell membranes. Moreover, the results showing that amiloride and oxythiamine, both known modulators of thiamine transporter, significantly decreased bacampicillin uptake in Caco-2 cells (Table 1) further support the idea that bacampicillin uptake occurred in combination with thiamine uptake in Caco-2 cells. According to Dutta et al., the human thiamine transporter, which is different from a multi-vitamin transporter, was not affected by choline, cimetidine, or guanidine. Moreover, thiamine was not a substrate of organic cation transporter 1 (OCT1) or OCT2. Our present results showing that choline, cimetidine, guanidine, NMN, and TEA lack the capability to interfere with bacampicillin, lenampicillin, pivampicillin, and talampicillin pass through the mucosal membranes via simple diffusion because of their high lipophilicities. Therefore the absorption mechanisms of these ampicillin prodrugs have not been fully investigated until now. Recently, Chanteux et al. reported that pivampicillin, which was added to the apical side of Caco-2 cells, was efficiently hydrolyzed to ampicillin inside the cells and that the ampicillin was preferentially released to the basolateral medium via an MRP-like transporter. The aim of this study was to assess how Caco-2 cells deal with bacampicillin as compared with pivampicillin.

First, we observed that when bacampicillin and pivampicillin were applied to Caco-2 cells, the compound recovered from the cells was primarily ampicillin and the amount of intact bacampicillin and pivampicillin was small. Since bacampicillin and pivampicillin are less stable in an aqueous medium, it was expected that a not small part of the bacampicillin and pivampicillin would be hydrolyzed to ampicillin in the apical medium and the ampicillin would be translocated into the Caco-2 cells. However, the uptake of ampicillin itself was very small (Fig. 1), indicating that the ampicillin recovered from Caco-2 cells resulted exclusively from the bacampicillin and pivampicillin taken up by the cells. Thus, it was considered that bacampicillin was transported into the Caco-2 cells to a much greater extent than either ampicillin (ca. 32 fold) or pivampicillin (ca. 1.6 fold) and that bacampicillin was efficiently converted to ampicillin inside the cells. It was also thought that pivampicillin was easily converted to ampicillin in the Caco-2 cells, which is consistent with results presented in recent papers.

Because an Eadie–Hofstee plot (Fig. 2) implied possible involvement of a specialized transport system for bacampicillin, the changes in its uptake were investigated in the presence of various organic cations. As shown in Table 1, bacampicillin uptake was significantly suppressed in the presence of diphenhydramine, procainamide, thiamine, oxythiamine, and amiloride. The presence of specialized transport systems for diphenhydramine and thiamine were previously reported in Caco-2 cells. It is also known that procaainamide permeation across the rabbit intestinal brush-border membrane is mediated by an H^+-tertiary amine antiport system. It has also been suggested that diphenhydramine and procainamide share a common organic cation transporter.

As shown in Fig. 3, the finding that thiamine, but not diphenhydramine, exerted a significant trans-stimulatory effect on bacampicillin uptake in Caco-2 cells suggests that bacampicillin and thiamine share a common transport system on the Caco-2 cell membranes. Moreover, the results showing that amiloride and oxythiamine, both known modulators of thiamine transporter, significantly decreased bacampicillin uptake in Caco-2 cells (Table 1) further support the idea that bacampicillin uptake occurred in combination with thiamine uptake in Caco-2 cells. According to Dutta et al., the human thiamine transporter, which is different from a multi-vitamin transporter, was not affected by choline, cimetidine, or guanidine. Moreover, thiamine was not a substrate of organic cation transporter 1 (OCT1) or OCT2. Our present results showing that choline, cimetidine, guanidine, NMN, and TEA lack the capability to interfere with bacampicillin, lenampicillin, pivampicillin, and talampicillin pass through the mucosal membranes via simple diffusion because of their high lipophilicities. Therefore the absorption mechanisms of these ampicillin prodrugs have not been fully investigated until now. Recently, Chanteux et al. reported that pivampicillin, which was added to the apical side of Caco-2 cells, was efficiently hydrolyzed to ampicillin inside the cells and that the ampicillin was preferentially released to the basolateral medium via an MRP-like transporter. The aim of this study was to assess how Caco-2 cells deal with bacampicillin as compared with pivampicillin.
bacampicillin uptake in Caco-2 cells (Table 1) are in agreement with previous findings. Interestingly, the enhancement of bacampicillin uptake in Caco-2 cells was greater when the cells were preincubated with 0.2 or 1 mM thiamine than when preincubated with 5 mM thiamine (Fig. 3). Although the exact reason for this phenomenon is currently unclear, it might be possible that when Caco-2 cells were preincubated with 5 mM thiamine, back-diffusion of thiamine into the apical medium potently and competitively disturbed bacampicillin transport. It is likely that the affinity of thiamine to the transporter is much greater than that of bacampicillin.

A well-known feature of thiamine uptake in Caco-2 cells is the pH-dependent manner in which it increased with increases in the pH of the apical medium. In this study, when medium pH was increased to various values between 5 and 8 on the basis of physiological pH of the small intestine, bacampicillin uptake also increased (Fig. 4). In particular, there was a marked increase when the medium pH was shifted from 5 to 7. The partition coefficients of bacampicillin with a value of 6.8 between octanol and DPBS were 0.98 at pH 6 and 1.86 at pH 7.4, suggesting that the lipophilicity of bacampicillin greatly increases with increases in the medium pH. Thus although the pH sensitivity of bacampicillin uptake was similar to that of thiamine, it seemed likely that the sharp increase in bacampicillin uptake between pH 5 and pH 7 was, in part, due to increased passive diffusion of bacampicillin.

With special focus on whether pivampicillin uptake in Caco-2 cells is shared with thiamine, the effect of l-carnosine, diphenhydramine, tetraethylammonium, and thiamine on pivampicillin uptake was evaluated (Table 2). Diphenhydramine significantly lowered pivampicillin uptake, but the other three compounds failed to inhibit it, implying that pivampicillin transport in Caco-2 cells was unrelated to the thiamine transporter. To access the possibility that other carrier-mediated systems are involved in the pivampicillin transport in Caco-2 cells, we tried to evaluate the concentration dependency of pivampicillin uptake. However, the prodrug was very insoluble above 1 mM, making the trial difficult.

Further to characterize the transport of bacampicillin and pivampicillin in Caco-2 cells, we compared their apical-to-basolateral permeation across the cell monolayers in the presence of thiamine and diphenhydramine. As shown in Fig. 5, thiamine significantly decreased the apical-to-basolateral transport of bacampicillin, again suggesting that these two drugs share a common transport system. However, diphenhydramine failed to decrease the transport, indicating that it does not interfere with bacampicillin transport in Caco-2 cells. On the other hand, neither thiamine nor diphenhydramine modulated pivampicillin uptake significantly. The reason why diphenhydramine exerted inhibitory effects on uptake of bacampicillin and pivampicillin in Caco-2 cells (Tables 1, 2) is not well understood at present. It was previously reported that lipophilic organic cations including diphenhydramine efficiently bound to the rat intestinal membrane and that there was competitive inhibition between them. Thus there is a possibility that diphenhydramine simply interfered with the binding of these lipophilic organic prodrugs to the apical membranes of Caco-2 cells.

Previously, using Caco-2 cells, Bretschneider et al. reported that cefoxime-axetil, a prodrug of orally inactive cefoxime, interacted with PEPT1. Thus the question still remains as to whether bacampicillin is a substrate of PEPT1. To assess this, a preliminary study was performed to clarify whether l-carnosine and glycyglycine were capable of interfering with bacampicillin uptake in Caco-2 cells. However, neither of these two dipeptides had any influence on bacampicillin uptake (Table 1), implying that PEPT1 was less involved in the transport of bacampicillin in Caco-2 cells.

In conclusion, the results obtained in this study suggest that bacampicillin, a prodrug of ampicillin, is transported by a carrier-mediated system that shares with thiamine on Caco-2 cell membranes. According to recent papers, two human thiamine transporters, hTHTR-1 and hTHTR-2, are widely expressed in the gastrointestinal tract and play a significant role in carrier-mediated thiamine uptake in the intestine. Accordingly, orally administered bacampicillin is, in part, absorbed via hTHTR-1 or hTHTR-2, challenging the notion that bacampicillin is effectively absorbed by simple diffusion because of its high lipophilicity.

Acknowledgments The authors are deeply grateful to the late Prof. Katsumi Miyazaki, Department of Pharmacy, Hokkaido University Hospital, for the support to this study.

REFERENCES

9) Tamai I., Sahake A., Saitoh R., Sai Y., Yamada I., Tsuji A., J. Pharma-
13) Mizuuchi H., Katsura T., Saito H., Hashimoto Y., Inui K., J. Pharma-
15) Marx E., Grundemann D., Calhau C., Schomig E., Naunyn-Schmiede-


