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# Role of the monocarboxylic acid transport system in the intestinal absorption of an orally active β-lactam prodrug: carindacillin as a model

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**Abstract**

Transport of carbenicillin (CBPC) and its orally active prodrug (carindacillin, CIPC) was studied with rat intestinal brush border membrane vesicles (BBMV). CIPC was transported uphill into BBMV in the presence of a $H^+$ gradient, indicating that CIPC absorption is carrier-mediated. Indeed, CIPC was predominantly transported by the monocarboxylic acid transport system, although it might be possible that CIPC possesses some affinity to the oligopeptide transporter. In contrast, CBPC exhibited no affinity to either the oligopeptide or the monocarboxylic acid transport system. Apparent uptake clearance of CIPC was approximately 70-fold greater than that of CBPC. It was clarified that the modification of the chemical structure of CBPC (a dicarboxylic acid) to CIPC (a monocarboxylic acid) by ester formation may have resulted in the increased affinity to the monocarboxylic acid transport system, which, in turn, led to improved absorption of the prodrug. 

*Keywords:* Carindacillin; Prodrug; Intestinal absorption; Brush border membrane vesicles; Monocarboxylic acid transport system

## 1. Introduction

It has been demonstrated that not only di/tripeptides but also some β-lactam antibiotics are transported by the $H^+$-coupled oligopeptide transporter which exists at the brush border membrane (BBM) of the small intestine (Oh et al., 1993; Tsuji and Tamai, 1996). β-Lactams without an α-amino group in the side chain, e.g. ceftibuten (CETB) and cefixime, as well as those with an α-amino group in the side chain, e.g. cephalexin and cyclacillin, are transported by the oligopeptide transporter, which makes these β-lactams orally active (Tsuji et al., 1987; Muranushi et al., 1989; Yoshikawa et al., 1989). It is also reported that not only various anionic drugs but also some β-lactams are transported by the monocarboxylic acid transport system which also exists at BBM of the small intestine (Tsuji and Tamai, 1996). The β-lactams transported by the monocarboxylic acid transport system include cefdinir and phenethi-

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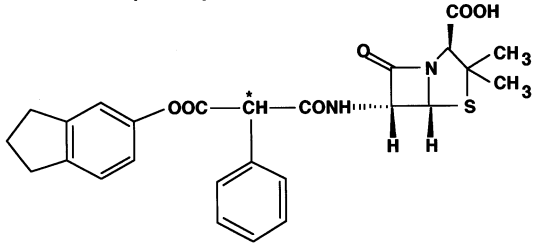
cillin, which are mono-anionic at physiological pH, lack an α-amino group in the side chain, and are orally active (Tsuji et al., 1993; Itoh et al., 1998). Therefore, orally active β-lactams appear to be absorbed via either the oligopeptide transporter or the monocarboxylic acid transport system.

Carbenicillin (CBPC) is a semi-synthetic β-lactam antibiotic which has been used as an injection because of poor stability and absorption in the gastro-intestinal tract (Fig. 1). CBPC is clinically used as a mixture of two epimers due to the chirality of the side chain (Bird and Steele, 1982; Itoh and Yamada, 1995). On the other hand, carindacillin (CIPC, carbenicillin indanyl sodium) is a prodrug of CBPC and is orally administered (Fig. 1). Although improved absorption of CIPC was accounted for mainly by the improved stability under acidic conditions, it is also reported that the rate of CIPC absorption from the rat small intestine is greater than that of CBPC and that the absorption rate is comparable or greater than those of other orally active penicillins (Tsuji et al., 1978, 1979, 1982). Absorption of CIPC was also studied in humans and the result indicated that the CIPC absorption follows Michaelis-Menten kinetics (Tanigawara et al., 1982). However, no information is available with regard to the chirality of the side chain of CIPC.

**Carindacillin (CIPC)**




**Carbenicillin (CBPC)**

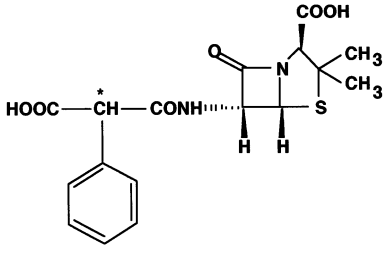



Fig. 1. Chemical structures of carindacillin (CIPC) and carbenicillin (CBPC). *, Chiral carbon in the side chain.

In the present study, transport of CIPC was studied using brush border membrane vesicles (BBMV) prepared from rat jejunum. The results were compared to those of CBPC in an attempt to clarify the mechanism(s) of improved oral absorption of CIPC.

## 2. Materials and methods

### 2.1. Materials

Carindacillin (CIPC) and ceftibuten (CETB) were kindly donated by Pfizer (Tokyo, Japan) and Shionogi (Osaka, Japan), respectively. Carbenicillin disodium salt (CBPC), sodium salicylate, sodium benzoate and oligopeptides (Gly-Gly, Gly-Pro, Gly-Sar, Ala-Ala-Ala) were purchased from Sigma (St. Louis, MO, USA). The CBPC used in the present study was a mixture of two epimers with an R-epimer to S-epimer ratio of ~1.1. Acetic acid, L-lactic acid, Hepes (2-[4-(2-hydroxylethyl)-1-piperazinyl]ethanesulfonic acid), Mes (2-morpholinoethanesulfonic acid, monohydrate) and Tris (tris(hydorxymethyl) aminomethane) were purchased from Wako (Osaka, Japan). $^{14}$C-labeled L-lactic acid and glycylsarcosine (Gly-Sar) were purchased from DuPont NEN Products (MA, USA). All other chemicals were of the highest grade available.

### 2.2. Preparation of rat intestinal brush border membrane vesicles

Brush border membrane vesicles (BBMV) were prepared by the calcium precipitation method as previously described (Itoh et al., 1998). Prepared vesicles were stored at −80°C and used for uptake experiments within 7 days.

The purity of BBMV was evaluated by comparing the activities of alkaline phosphatase (a marker enzyme of the brush border membrane)

and $Na^+/K^+$-ATPase (a marker enzyme of the basolateral membrane) to those of the initial homogenate. Protein concentrations of BBMV were determined using a Bio-Rad Protein assay kit (Bio-Rad Laboratories, Richmond, CA, USA).

### 2.3. Uptake experiment

Uptake of CETB, L-lactic acid, CIPC and CBPC into BBMV was measured at 25°C by the rapid filtration method. The uptake was initiated by adding 180 μl of the incubation medium to 20 μl of the BBMV suspension. Composition of the incubation medium is listed in the legend of the figures. At the appropriate time, the reaction was stopped by the addition of 1 ml of ice-cold stop solution consisting of 100 mM mannitol, 100 mM KCl and 10 mM Mes/Tris (pH 5.5). The mixture was immediately filtered (Millipore filter, HAWP, 0.45-μm pore size, 25 mm in diameter) followed by washing with 5 ml of ice-cold stop solution. For the assay of L-lactic acid and Gly-Sar, the filter was transferred into a scintillation vial and 10 ml of scintillation cocktail (Aquasol®-2, Packard Instrument, Meriden, CT, USA) was added. Radioactivity was measured with a liquid scintillation counter (Beckman LS5000TD). For the assay of CETB, CIPC and CBPC, the filter was transferred into a glass tube and the drug trapped in the vesicles was extracted with 400 μl of distilled water using a vortex mixer. A 200-μl aliquot was injected onto HPLC.

Adsorption of the drug to the vesicles was measured by adding the drug after the stop solution was added to the vesicle suspension. Adsorbed drug was assayed in the same manner as described above. Adsorbed amount was subtracted and the corrected value was considered as the uptake amount.

### 2.4. HPLC conditions

A high-performance liquid chromatograph (HPLC) was used to determine the concentrations of CETB, CIPC and CBPC. For the determination of CIPC, PEGASIL®-ODS (2.1 mm (i.d.) × 150 mm; Senshu Kagaku, Tokyo, Japan) was used as an analytical column. The mobile phase was 50 mM $CH_3COONH_4$-$CH_3OH$ (4:6, v/v) with a flow rate of 0.3 ml/min. CIPC was detected at 220 nm. For determination of CBPC and CETB, Cosmosil® ($5C_{18}$-AR, 4.6 mm (i.d.) × 250 mm; Nakalai Tesque, Kyoto, Japan) was used as an analytical column. The mobile phase compositions were 0.07 M $CH_3COONH_4$-$CH_3OH$ (8:1, v/v) and 50 mM phosphate buffer (pH 3.0)-$CH_3OH$ (7:1, v/v) for CBPC and CETB, respectively, with a flow rate of 0.8 ml/min for both drugs. CBPC and CETB were detected at 235 and 262 nm, respectively.

### 2.5. Kinetic analysis

In order to calculate kinetic parameters for the uptake of CIPC, the following equation (Eq. (1)) was used and the parameter values were obtained by a non-linear least squares method (MULTI) (Yamaoka et al., 1981):

$$v = \frac{V_{max}[S]}{K_m + [S]} + K_d[S] \quad (1)$$

where $v$ is the initial uptake rate, $V_{max}$ is the maximum uptake rate, $K_m$ is the Michaelis constant, $K_d$ is the coefficient of passive diffusion and $[S]$ is the initial concentration of the drug.

Inhibition constant ($K_i$) of CIPC was calculated from the Lineweaver-Burk plots. Assuming competitive inhibition, the ratio of the slopes for L-lactic acid uptake in the presence of CIPC to that in the absence of CIPC is equal to $(1 + [I]/K_i)$, where $[I]$ is the concentration of the inhibitor. Knowing the inhibitor concentration, the $K_i$ value can be obtained from the Lineweaver-Burk plots.

Apparent uptake clearance ($CL_{uptake,\ app.}$) was calculated according to the following equation (Eq. (2)) in order to compare the transport of CIPC with that of CBPC:

$$CL_{uptake,app.} = \frac{\text{Initial uptake rate (nmol/mg protein per s)}}{\text{Concentration in the medium (nmol/}\mu\text{l)}} \quad (2)$$

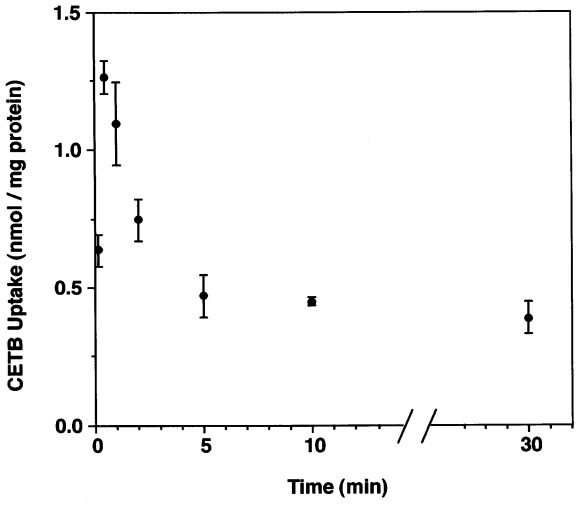



Fig. 2. Time course of CETB uptake into rat intestinal BBMV. Vesicles (20 μl, 152 mg protein) suspended in 100 mM mannitol, 100 mM KCl and 10 mM Hepes/Tris (pH 7.5) were incubated in the incubation medium (180 μl) containing 100 mM mannitol, 100 mM KCl, 10 mM Mes/Tris (pH 5.5) and 0.5 mM CETB. Each point represents the mean ± S.D. of three determinations.

Uptake of CIPC in the presence of an inhibitor was calculated according to Eq. (3) with an assumption of competitive inhibition:

$$v^* = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_i}\right)+[S]} + K_d[S] \quad (3)$$

where $v^*$ is the initial uptake rate in the presence of an inhibitor. The extent of inhibition of the uptake was calculated as the ratio of $v^*/v$.

### 2.6. Statistical analysis

Statistical analysis was conducted with a Student's $t$-test.

## 3. Results

According to the present HPLC method, no peaks interfering with CIPC, CBPC or CETB were observed on the chromatograms of blank samples. The detection limit was approximately 0.01, 0.03 and 0.02 nmol for CIPC, CBPC and CETB, respectively. Moreover, no conversion of CIPC to CBPC was observed during the uptake experiment or the HPLC analysis. On the other hand, for the present membrane preparations, the enrichment factors of alkaline phosphatase and $Na^+/K^+$-ATPase were $12.4 \pm 1.5$ (mean ± S.D., $n = 5$) and $0.87 \pm 0.41$ (mean ± S.D., $n = 5$), respectively.

As shown in Fig. 2, uptake of CETB into BBMV showed an overshoot phenomenon in the presence of an inwardly directed $H^+$ gradient ($pH_{in} = 7.5$ and $pH_{out} = 5.5$). Initial uptake of CETB (uptake in 10 s) was significantly reduced in the presence of a dipeptide (Gly-Sar), suggesting that CETB was transported by the oligopeptide transporter (Table 1). Transport of CETB was inhibited by CIPC, not by CBPC. The results indicated that CIPC has affinity to the oligopeptide transporter, whereas CBPC does not.

Table 1
Effects of various compounds on the initial uptake of CETB, Gly-Sar, L-lactic acid or CIPC[a]

| Substrate | Inhibitor | Uptake (% of control) |
|---|---|---|
| CETB (0.5 mM) | 10 mM Gly-Sar | 55.1 ± 13.0** |
| | 0.1 mM CIPC | 73.3 ± 5.3* |
| | 25 mM CBPC | 99.7 ± 6.2 |
| [$^{14}$C]-Gly-Sar (0.2 μM) | 3.5 mM CETB | 34.6 ± 13.1** |
| | 0.8 mM CIPC | 115 ± 12 |
| | 20 mM CBPC | 107 ± 22 |
| L-[$^{14}$C]Lactic acid (6 μM) | 20 mM benzoic acid | 50.3 ± 15.2** |
| | 20 mM salicylic acid | 55.9 ± 7.3** |
| | 0.6 mM CIPC | 58.9 ± 10.6* |
| | 20 mM CBPC | 115 ± 16 |
| CIPC (0.3 mM) | 20 mM Gly-Pro | 72.9 ± 15.3 |
| | 20 mM Gly-Gly | 72.7 ± 18.1 |
| | 20 mM Ala-Ala-Ala | 75.0 ± 23.4 |
| | 40 mM L-lactic acid | 63.4 ± 11.6* |
| CIPC (0.2 mM) | 10 mM CETB | 89.2 ± 17.6 |
| | 20 mM CBPC | 84.3 ± 38.1 |

[a] Each value represents the mean ± S.D. of three to four determinations.

* $P < 0.05$, significantly different from control.

** $P < 0.01$, significantly different from control.

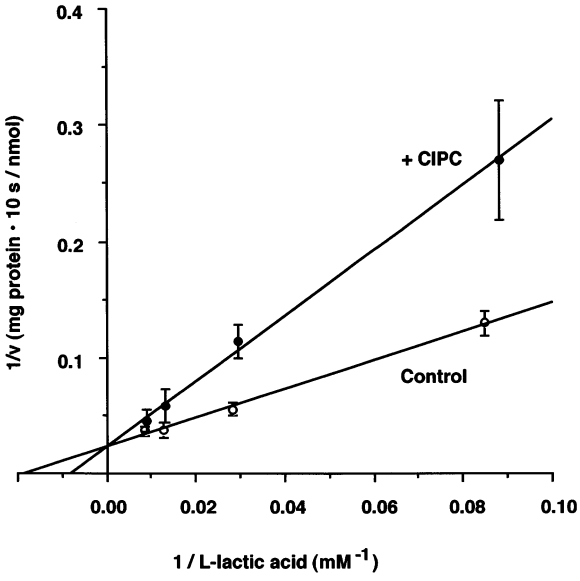



Fig. 3. Lineweaver-Burk plot of the initial uptake of L-lactic acid in the absence (○) or presence (●) of 0.74 mM CIPC. Vesicles (20 μl, 84.5 mg protein) suspended in 100 mM mannitol, 100 mM KCl and 10 mM Hepes/Tris (pH 7.5) were incubated in the incubation medium (180 μl) containing 100 mM mannitol, 100 mM KCl, 10 mM Mes/Tris (pH 5.5) and 12–100 mM of L-lactic acid. Each point represents the mean ± S.D. of four determinations. Solid lines were obtained by linear regression analyses.

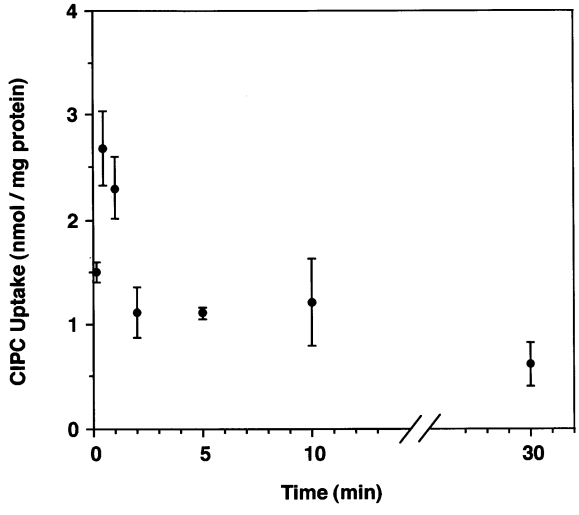



Fig. 4. Time course of CIPC uptake into rat intestinal BBMV. Uptake of CIPC (0.3 mM) was measured in the same manner as described in the legend of Fig. 3. Each point represents the mean ± S.E. of three determinations.

Inhibitory effects of CETB, CIPC and CBPC on the transport of [$^{14}$C]-Gly-Sar, another model substrate of the oligopeptide transporter, were also studied in the presence of a $H^+$ gradient. As summarized in Table 1, CETB inhibited the transport of Gly-Sar, but CIPC and CBPC did not show any inhibitory effect.

According to our previous study, uptake of L-[$^{14}$C]lactic acid in the presence of an inwardly directed $H^+$ gradient exhibited an overshoot phenomenon, suggesting that L-[$^{14}$C]lactic acid was transported uphill into BBMV (Itoh et al., 1998). Initial uptake of L-[$^{14}$C]lactic acid (uptake in 10 s) was significantly reduced in the presence of benzoic and salicylic acids, demonstrating that L-[$^{14}$C]lactic acid was transported by the monocarboxylic acid transport system (Table 1). Uptake of L-[$^{14}$C]lactic acid was also inhibited by CIPC, whereas CBPC did not inhibit L-lactic acid uptake (Table 1). These results suggested that CIPC has affinity to the monocarboxylic acid transport system and that CBPC does not have affinity to it.

Initial uptake (uptake in 10 s) of L-[$^{14}$C]lactic acid was measured at various concentrations of L-lactic acid in the presence or absence of 0.74 mM CIPC. The results are shown as Lineweaver-Burk plots (Fig. 3), which clearly demonstrated that CIPC competitively inhibit the L-[$^{14}$C]lactic acid uptake. The inhibition constant ($K_i$) of CIPC was 0.59 mM.

Uptake of CIPC in the presence of a $H^+$ gradient showed an overshoot, suggesting that CIPC was actively transported into BBMV (Fig. 4). Adsorption of CIPC to the vesicles accounted for ~35% of the total uptake at 30 s, indicating that CIPC was actually transported into the vesicles. Uptake of CIPC was also measured at different pH (Fig. 5). The uptake rate increased with a decrease of the pH of the incubation medium, suggesting that the transport of CIPC is driven by the $H^+$ gradient.

Uptake of CIPC at 0.3 mM was measured in the presence of a $H^+$ gradient at 4°C. The uptake amount linearly increased with time without showing overshoot (data not shown). The $K_d$ value obtained was 0.0494 μl/mg protein per s. Since the initial uptake clearance of CIPC at 25°C

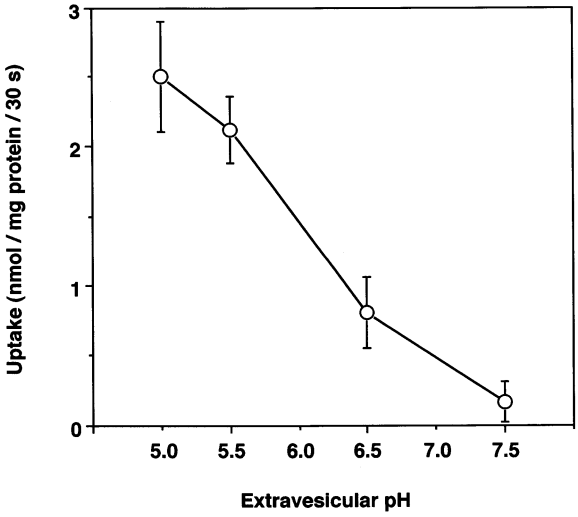



Fig. 5. Effect of the extravesicular pH on the initial uptake of CIPC. Uptake of CIPC in 30 s was measured in the same manner as described in the legend of Fig. 4, except that the pH of the incubation medium was varied between 5.0 and 7.5. Each point represents the mean ± S.D. of three determinations.

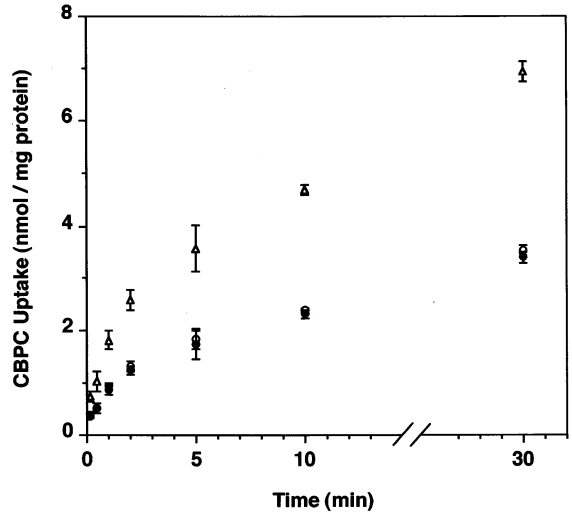



Fig. 7. Time course of CBPC uptake (○, R-CBPC, ●, S-CBPC and △, R-CBPC plus S-CBPC). Uptake of CBPC was measured in the same manner as described in the legend of Fig. 4, except that the incubation medium contained 10 mM RS-CBPC instead of CIPC. Each point represents the mean ± S.D. of three determinations.

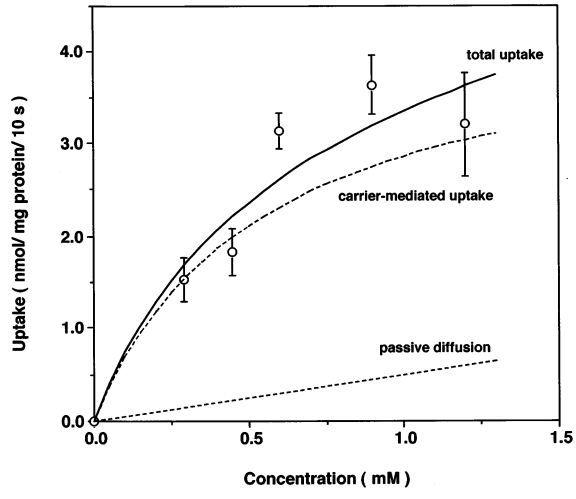



Fig. 6. Concentration dependence of the initial uptake of CIPC. Uptake of CIPC in 10 s was measured in the same manner as described in the legend of Fig. 4, except that the concentration of CIPC was varied between 0 and 1.0 mM. Each point represents the mean ± S.D. of three to four determinations. The solid curve shows the total uptake and the dotted lines show the contribution of carrier-mediated uptake and passive diffusion.

was 0.286 μl/mg protein per s (Fig. 4), the contribution of passive diffusion ($K_d$) to the total uptake was 17%. In other words, the major portion of the uptake ( ~ 83%) was accounted for by the carrier-mediated uptake.

Initial uptake (uptake in 10 s) was measured at various concentrations of CIPC. The results are shown in Fig. 6. The $K_m$ and $V_{max}$ values were calculated according to Eq. (1) assuming the $K_d$ value as 0.0494 μl/mg protein per s as mentioned above. The $K_m$ and $V_{max}$ thus obtained were 0.54 mM and 0.44 nmol/mg protein per s, respectively. The same experiment was conducted with BBMV prepared from other rats, and the obtained $K_m$ value was 0.45 mM (data not shown).

Effects of Gly-Pro, Gly-Gly, Ala-Ala-Ala, L-lactic acid, CETB and CBPC on the initial uptake (uptake in 30 s) of CIPC were studied (Table 1). L-Lactic acid inhibited the CIPC uptake, whereas CETB and CBPC did not show any inhibitory effects on CIPC uptake. Although oligopeptides slightly inhibited CIPC uptake, the uptake amounts were not significantly different from the control.

The time course of CBPC uptake in the presence of a $H^+$ gradient is shown in Fig. 7. Uptake of CBPC was not stereoselective since the R-epimer to S-epimer ratios in the vesicles were similar to that in the incubation medium. Moreover, the uptake did not show an overshoot. Uptake of CBPC was measured at a concentration much greater than that of CIPC (0.3 mM for CIPC as shown in Fig. 4, and 10 mM for CBPC), since the uptake amount was close to the detection limit when the uptake of CBPC was measured at a concentration equal to that of CIPC.

Since the uptake of CBPC was measured at a concentration greater than that of CIPC, $CL_{uptake,app.}$ was calculated according to Eq. (2) in order to compare the transport efficiency. For CBPC, total uptake of the two epimers was used for the calculation of $CL_{uptake,app.}$ because the uptake was non-stereoselective as mentioned above. $CL_{uptake,app.}$ values of CIPC and CBPC thus obtained were $499 \pm 57 \times 10^{-3}$ and $7.29 \pm 0.98 \times 10^{-3}$ μl/mg protein per s (mean ± S.D., $n = 3$), respectively, indicating that the transport efficiency of CIPC was ~70-fold greater than that of CBPC.

## 4. Discussion

For the present BBMV preparations, enrichment factors of the marker enzymes were comparable to those reported by other groups (Yoshikawa et al., 1989; Simanjuntak et al., 1990; Tamai et al., 1995). Also, in the present study, the uptake of CETB, a model substrate of the oligopeptide transporter, into BBMV exhibited an overshoot in the presence of an inwardly directed $H^+$ gradient. Transport characteristics of CETB observed in the present study were similar to those reported previously (Yoshikawa et al., 1989).

According to our previous study, the uptake of L-[$^{14}$C]lactic acid, a model substrate of the monocarboxylic acid transport system, showed an overshoot in the presence of an inwardly directed $H^+$ gradient (Itoh et al., 1998). Transport characteristics of L-lactic acid were similar to those reported by other groups (Storelli et al., 1980; Tiruppathi et al., 1988). These results indicated that the BBMV was well prepared in the present study and that both oligopeptide and monocarboxylic acid transport systems were present in our BBMV preparations.

Since CETB and Gly-Sar mutually inhibited the uptake (Table 1), it was confirmed that these compounds are transported by the common transport system, i.e. the oligopeptide transporter. Although CIPC inhibited CETB uptake, CIPC did not inhibit the uptake of Gly-Sar. Moreover, neither CETB nor oligopeptides significantly inhibited CIPC uptake (Table 1). Since the reported $K_m$ value of CETB is 0.17 mM (Yoshikawa et al., 1989), the concentration of CETB used in the present inhibition study was much greater than its $K_m$ value. Also, the concentrations of oligopeptides used in our inhibition studies are sufficient to inhibit CETB uptake (Muranushi et al., 1989). All these observations strongly indicate that CIPC may not be transported by the oligopeptide transporter although it might have some affinity to it. In contrast, CBPC did not inhibit the uptake of CETB or Gly-Sar, demonstrating that the affinity of CBPC to the oligopeptide transporter is very low.

Uptake of L-[$^{14}$C]lactic acid was inhibited by benzoic and salicylic acids (Table 1), which are the substrate of the monocarboxylic acid transport system (Tsuji et al., 1994; Ogihara et al., 1996; Tamai et al., 1997). L-Lactic acid and CIPC mutually inhibited the uptake (Table 1), and CIPC inhibited L-[$^{14}$C]lactic acid uptake in a competitive manner (Fig. 3). From the Lineweaver-Burk plots, the $K_m$ and $K_i$ values of L-lactic acid and CIPC were 55 mM and 0.59 mM, respectively. The $K_i$ value of CIPC was very similar to the $K_m$ value of 0.54 mM obtained from Fig. 6, supporting the hypothesis that L-lactic acid and CIPC share the common transport system. On the other hand, the $K_m$ value of L-lactic acid in the present study was greater than the reported values. The reported $K_m$ values are 12.7, 39.2 and 3.75 mM for rabbit brush border membrane vesicles (Tiruppathi et al., 1988), rat basolateral membrane vesicles (Orsenigo et al., 1997) and Caco-2 cells (Ogihara et al., 1996), respectively. Since the $K_m$ value of L-lactic acid for rat intestinal brush border membrane has not been reported, we are

unable to compare our value with the literature value. It is possible that the affinity of L-lactic acid to the monocarboxylic acid transporter(s) of rat intestinal BBM may be smaller than those of other animal species.

Using the $K_m$ values of L-lactic acid and CIPC, the extent of inhibition was calculated as $v^*/v$ (Eqs. (1) and (3)). With the concentrations of L-lactic acid and CIPC being 6 μM and 0.6 mM, respectively, the uptake of L-[$^{14}$C]lactic acid was calculated to be 47% of the control. This value was similar to the observed uptake, i.e. 58.9% as listed in Table 1. With the concentrations of L-lactic acid and CIPC being 40 and 0.3 mM, respectively, the uptake of CIPC was calculated to be 70% of the control, which was again similar to the observed uptake, i.e. 63.4% as listed in Table 1. These observations also support that CIPC shares the common transport system with L-lactic acid. In contrast, CBPC did not inhibit the uptake of L-[$^{14}$C]lactic acid or CIPC, indicating that the affinity of CBPC to the monocarboxylic acid transport system is much lower than that of CIPC or L-lactic acid.

Uptake of CIPC into BBMV was uphill with a $H^+$ gradient as the driving force (Figs. 4 and 5), demonstrating that CIPC is actively transported into BBMV by the monocarboxylic acid transport system. Although CIPC might possess affinity to the oligopeptide transporter as mentioned above, it is predominantly transported by the monocarboxylic acid transport system since the transport of CIPC was not inhibited by either CETB or oligopeptides.

The $K_m$ value of CIPC was similar to those of the β-lactams to the oligopeptide transporter; the reported $K_m$ are 0.17, 0.83 and 0.79 mM for CETB, cefixime and loracarbef, respectively (Tsuji et al., 1987; Yoshikawa et al., 1989; Hu et al., 1994). The $K_m$ values of L-lactic acid and phenethicillin, which are the substrates of the monocarboxylic acid transport system, are 20–50 mM (Itoh et al., 1998). On the other hand, the $K_d$ value of CIPC (0.0494 μl/mg protein per s) was greater than those reported for CETB and phenethicillin; the reported $K_d$ values are 0.005 and 0.008 μl/mg protein per s for CETB and phenethicillin, respectively (Yoshikawa et al., 1989; Itoh et al., 1998). The greater $K_d$ value of CIPC may reflect the greater lipophilicity.

Although the $CL_{uptake,\ app.}$ of CIPC was 0.499 μl/mg protein per s as mentioned above, the uptake clearance calculated as the $V_{max}/K_m$ was 0.815 μl/mg protein per s. Since the CIPC concentration in the time course study was 0.3 mM (Fig. 4), it was close to the $K_m$ value of 0.59 mM. The uptake observed in Fig. 4 may be partially saturated, which may have resulted in the underestimation of the $CL_{uptake,\ app.}$. In any case, the fact that the uptake clearance of CIPC is at least 70-fold greater than that of CBPC demonstrates that CIPC is much more efficiently transported into BBMV, which may, in turn, result in the increased absorption from the intestine. The uptake clearance of CIPC is comparable to those of other orally active β-lactams. For example, the uptake clearance of CETB and phenethicillin is 0.46 and 0.14 μl/mg protein per s, respectively (Yoshikawa et al., 1989; Itoh et al., 1998).

Takagi et al. (1998) reported that the pH-dependent uphill transport of a monocarboxylic acid, such as salicylic acid, across the lipid bilayer was observed in the absence of a carrier protein. However, there are differences between their results and our results. In their study, the uptake of salicylic acid was not inhibited by L-lactic acid, which is a typical substrate of the monocarboxylic acid transport system. In contrast, the uptake of CIPC was significantly inhibited in the presence of L-lactic acid in our study (Table 1). Also, the temperature dependency appears to be greater in our study. The uptake of salicylic acid at 25°C was only two-fold greater than that at 4°C in their study, whereas the transport of CIPC at 25°C was more than five-fold greater than that at 4°C in our study. Further studies are necessary to clarify involvement of carrier proteins in the transport of monocarboxylates.

Since CIPC is an indanyl ester of CBPC, the dicarboxylic acid (CBPC) is altered to a monocarboxylic acid (CIPC) in the synthesis of the prodrug. It was demonstrated in the present study that the structural modification resulted in increased affinity to the monocarboxylic acid transport system, which, in turn, led to improved absorption of the prodrug. The present results

should provide valuable information on the design of orally active prodrugs.

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