Metabolism, Uptake, and Transepithelial Transport of the Diastereomers of Val-Val in the Human Intestinal Cell Line, Caco-2

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Purpose. The purpose of this study was to determine whether the binding of the diastereomers of Val-Val to the apical oligopeptide transporter(s) could be correlated with their cellular uptake and transepithelial transport.

Methods. The Caco-2 cell culture system was used for all experiments. The binding of the diastereomers of Val-Val was evaluated by determining their ability to inhibit [^{3}H]cephalexin uptake. The stability of the diastereomers was determined in a homogenate of Caco-2 cells and in the apical bathing solution over Caco-2 cell monolayers. The cellular uptake and transepithelial transport properties of the individual diastereomers were studied using Caco-2 cell monolayers.

Results. 10 mM concentrations of L-Val-L-Val, L-Val-D-Val, D-Val-L-Val and D-Val-D-Val inhibited cellular uptake of [^{3}H]cephalexin (0.1 mM) by 92%, 37%, 70%, and 18%, respectively. When the cellular uptake of Val-Val diastereomers (1 mM) were evaluated, the intracellular concentrations of L-Val-D-Val and D-Val-L-Val were 15 and 50 times higher, respectively, than that of D-Val-D-Val. The cellular uptake of L-Val-D-Val and D-Val-L-Val was inhibited by Gly-Pro (10 mM) (>95%), whereas Gly-Pro had no effect on the cellular uptake of D-Val-D-Val. L-Val-L-Val was not detected in the Caco-2 cells, probably due to its metabolic lability. When the transepithelial transport of the Val-Val diastereomers (1 mM) was determined, L-Val-D-Val, D-Val-L-Val and D-Val-D-Val transport rates were similar. The transepithelial transport of L-Val-D-Val and D-Val-L-Val was inhibited by Gly-Pro (10 mM) 36% and 30%, respectively, while Gly-Pro inhibited carnosine (1 mM) transepithelial transport by 65%. Gly-Pro had no effect on the transepithelial transport of D-Val-D-Val.

Conclusions. These results suggest that the major transepithelial transport route of L-Val-D-Val, D-Val-L-Val and D-Val-D-Val is passive diffusion via the paracellular route. The binding of Val-Val diastereomers to the oligopeptide transporter(s) is a good predictor of their cellular uptake, however, the binding is not a good predictor of their transepithelial transport. It appears that the stereochemical requirements for the transporter that mediates efflux of the peptide across the basolateral membrane may be different from the requirements for the apical transporter that mediates cellular uptake.

KEY WORDS: oligopeptide transporter; structure-transport; Caco-2; dipeptides; carnosine; cephalexin.

INTRODUCTION

The intestinal peptide transporter(s) is involved in the absorption of natural di-/tripeptides and peptidomimetic drugs (1–5). Recently, molecules have been rationally designed to target the intestinal oligopeptide transporter in attempts to enhance their oral absorption (6–8). To take advantage of the oligopeptide transporter(s) for drug delivery, a greater understanding of what structural features of a peptide optimize its binding to the transporter(s), its cellular uptake, and its transepithelial transport is needed. Some information regarding the substrate specificity of this transporter(s) has been generated through competition studies of apical uptake between peptides and/or peptidomimetics (9). From these types of experiments, one can determine whether a molecule binds to the apical transporter(s), but binding does not necessarily mean that the molecule will be taken up into the cell or whether it will undergo transepithelial transport (1, 5, 10–12). Moreover, recent studies using enterocyte basolateral membrane vesicles or Caco-2 cells point to the existence of an oligopeptide transporter(s) on the basolateral membrane (13–17).

In this study, we have used the diastereomers of Val-Val as model peptides to determine their metabolism, binding to the apical oligopeptide transporter(s), cellular uptake, and transepithelial transport using the Caco-2 cell culture system as a model of the intestinal epithelium. We have also determined whether the binding of the Val-Val diastereomers to the apical transporter(s) could be correlated with their cellular uptake and transepithelial transport. Furthermore, based on the observation seen in the uptake and transepithelial transport studies, conclusions were made about the substrate specificity of the basolateral transporter(s).

MATERIALS AND METHODS

Materials

[^{3}H]Cephalexin (3.7 μCi/mmol) was synthesized by the Department of Synthetic Chemistry, SmithKline Beecham Pharmaceuticals (King of Prussia, PA). [^{14}C]Mannitol (55 mCi/mmol) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). The dipeptides L-Val-L-Val, L-Val-D-Val, D-Val-L-Val and D-Val-D-Val were synthesized as described previously (17). Carnosine, Gly-Pro, L-Valine, 2-(N-morpholino)ethanesulfonic acid (MES) and Dulbecco's phosphate buffer solution (D-PBS; powder form) were purchased from Sigma Chemical Co. (St. Louis, MO). N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonate] (Hepes), Hanks' balanced salt solution (HBSS), Dulbecco's modified Eagle's medium (DMEM), and non-essential amino acids (NEAAs) were obtained from JRH Biosciences (Lenexa, KS). Fetal bovine serum (FBS) was from Intergen Company (Cambridge, MA). Rat tail collagen (Type I) was from Collaborative Research (Lexington, MA). Penicillin and streptomycin were obtained as a mixture from Irvine Scientific (Santa Ana, CA). Transwell® clusters, PVP free, 24.5 mm

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ABBREVIATIONS: D-PBS, Dulbecco's phosphate buffer solution; DMEM, Dulbecco's modified Eagle medium; EBSS, Earle's balanced salt solution; FBS, Fetal bovine serum; HBSS, Hanks' balanced salt solution; Hepes, (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonate]); MES, 2-(N-morpholino) ethanesulfonic acid; NEAA, non-essential amino acids; Val, Valine.

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in diameter (4.71 cm^2 surface area), and 3.0 μm pore size were purchased from Costar Corporation (Bedford, MA). Acetonitrile was of HPLC grade. Other chemicals were used as received.

Caco-2 Cell Culture

Caco-2 cells were plated and grown according to previously published procedures (19). Briefly, cells were grown in 165 cm^2 culture flasks (Costar Corp.) in culture medium consisting of DMEM with 100 U/ml penicillin and 100 μg/ml streptomycin, 1% NEAA and 10% FBS. Before reaching confluence, cells were trypsinized with 0.25% trypsin and 0.02% EDTA and plated at a density of 63,000 cells/cm^2 in a culture medium on Transwell® polycarbonate membranes (3.0 μm pore size) that had previously been coated with collagen. The culture medium was replaced (1.5 ml apical side and 2.6 ml basolateral side) every other day for the first week and daily thereafter. Cells were maintained at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity. All cells used in this study were between passages 34 and 70. The monolayers used in this study were 19–25 days postseeding. The quality of the monolayer was controlled by checking the paracellular transport of [^{14}C]mannitol. The [^{14}C]mannitol leakage is normally less than 1% per 1 hr per well.

Uptake Studies

The uptake of [^{3}H]cephalexin (0.1 mM), carnosine (1 mM) and Val-Val diastereomers (1 mM) was determined in Caco-2 cells in the presence or absence (controls) of competitors (10 mM of the Val-Val diastereomers or Gly-Pro). On the day of the experiment, cells were rinsed with HBSS containing 25 mM glucose and 10 mM Hepes (pH 7.4 buffer) at 37°C. Plates were then preincubated at 37°C for 10 min with Earle's balanced salt solution (EBSS) containing 25 mM glucose and 10 mM MES (pH 6.0 buffer) on the apical side and pH 7.4 buffer on the basolateral side. After the preincubation period, the control monolayers were incubated for 15 min or otherwise specified time at 37°C with pH 6.0 buffer containing a peptide of interest on the apical side. The monolayers treated with inhibitors were incubated with a peptide of interest plus the inhibitor on the apical side at 37°C. The basolateral sides of monolayers in both control and inhibitor-treated groups were maintained in the pH 7.4 buffer. Subsequently, the incubation medium was removed and the cells were washed three times with ice-cold pH 7.4 buffer to stop further uptake and to remove unbound peptide.

For the [^{3}H]cephalexin experiments, cells and filters were dissolved in a Ready-Safe scintillation cocktail and radioactivity was determined in a Beckman LS6000IC liquid scintillation counter. For carnosine and Val-Val diastereomers, cells were scraped from the membrane into 1 ml of ice-cold pH 7.4 buffer. After briefly being spun down in a microcentrifuge (Marathon 21K/BR, Fisher Scientific, 13,000 rpm), the cells were resuspended in 250 μl of pH 7.4 buffer, vortexed and sonicated for approximately 10 min. The peptide was extracted from the cells by a modification of the method of Wessel and Flugge (20). Briefly, dichloromethane (250 μl) was added to the samples, and they were vortexed and centrifuged for 15 min (13,000 rpm). The aqueous layer containing the peptide was removed, acidified with an equal volume of 0.04 N HCl and analyzed by HPLC.

Uptake was expressed as nmol/mg protein. Total protein content of cells cultured on polycarbonate filters for various days after seeding was previously determined (21). The percent inhibition of cellular uptake was calculated by comparing the amount of uptake in the presence and absence of inhibitors.

Transepithelial Transport Studies

The transepithelial transport of Val-Val diastereomers (1 mM) and carnosine (1 mM) was determined in Caco-2 cells at 37°C in the presence or absence (controls) of Gly-Pro (10 mM). Cell monolayers were rinsed and preincubated as described above for the uptake experiment. After removal of the preincubation medium, 2.6 ml of the pH 7.4 buffer was placed in the basolateral side and 0.75 ml of the pH 6.0 buffer containing a compound of interest with or without competitors was placed in the apical side. Samples (200 μl) were removed at designated times from the receiver chamber and replaced with fresh pH 7.4 buffer. The samples were then acidified by addition of 0.08 N HCl (100 μl) and analyzed by HPLC.

The permeability coefficient (P_{app}) was calculated according to the following equation:

$$P_{app} = \frac{V \cdot dC}{A \cdot C_0 \cdot dt}$$

where $V \cdot (dC/dt)$ is the steady-state rate of appearance of the apically applied peptide in the receiver chamber after initial lag time; C_0 is the initial peptide concentration in the donor chamber; and A is the area of the Transwell®. Percent inhibition of transepithelial transport was calculated by comparing the amount of peptide transported in the receiver chamber during a 2 hr incubation in the presence and absence of inhibitors.

Metabolism Studies

The metabolism of the Val-Val diastereomers was determined in the Caco-2 cell homogenate at 37°C. Caco-2 cells were washed twice with the same pH 7.4 buffer that was described in the uptake experiment. The cells were scraped into ice-cold pH 7.4 buffer and homogenized in a Dounce homogenizer (~1 mg protein/ml). After mixing the peptide (1 mM) with the cell homogenate, aliquots (200 μl) were removed at various times, and the reaction was quenched by the addition of 200 μl of ice-cold 0.08 N HCl. Samples were analyzed by HPLC.

The metabolism of the Val-Val diastereomers (1 mM) in the apical bathing solution over the Caco-2 cell monolayers was also determined. The Val-Val diastereomers (1 mM, pH 6.0) were added on the apical side and 2.6 ml of pH 7.4 buffer was placed on the basolateral side. The samples (200 μl) were removed from the apical side after a 2-hr incubation. The reaction was quenched by the method described above. The amount of the Val-Val diastereomers in the samples was determined by HPLC.

HPLC Analysis

The analysis conditions of the Val-Val diastereomers and carnosine were as follows: column, C18 (Vydac, 4.6 × 250 mm, Hesperia, CA); mobile phase, 70 mM phosphate buffer (pH 3.5) containing 10 mM heptane sulfonic acid and 5–15% acetonitrile; detection, 210 nm; flow rate, 1 ml/min. The reten-

tion times of L-Val-L-Val, L-Val-D-Val, D-Val-L-Val, D-Val-D-Val and carnosine were approximately 9, 12, 12, 9 and 8 min, respectively.

Statistical Analysis

Statistical analysis of the data was performed by one way ANOVA. A prior level of significance was set at 5% or $P < 0.05$. The software used was Minitab™.

RESULTS

Inhibition of [^{3}H]Cephalexin Uptake by the Val-Val Diastereomers

Figure 1 shows the cumulative uptake of [^{3}H]cephalexin (0.1 mM) in the presence or absence of the Val-Val diastereomers (10 mM) during a 15 min incubation at 37°C. L-Val-L-Val, L-Val-D-Val, D-Val-L-Val and D-Val-D-Val inhibited cellular uptake of [^{3}H]cephalexin by 92%, 37%, 70%, and 18%, respectively.

Uptake of the Val-Val Diastereomers and the Effect of Gly-Pro

Figure 2 shows the apical uptake of the Val-Val diastereomers (1 mM) and carnosine (1 mM) during a 2-hr incubation at 37°C in the presence or absence of Gly-Pro (10 mM). Cellular concentrations of L-Val-D-Val, D-Val-L-Val, D-Val-D-Val and carnosine were 0.79 ± 0.22, 2.65 ± 0.54, 0.05 ± 0.06 and 4.56 ± 0.32 nmol/mg protein, respectively. The cellular uptake of L-Val-D-Val, D-Val-L-Val and carnosine was significantly inhibited by inclusion of Gly-Pro in the incubation mixture. Gly-Pro had no effect on the cellular uptake of D-Val-D-Val. L-Val-L-Val was not detected in the Caco-2 cell monolayers. Based on the detection limit of the HPLC analysis, the concentration of L-Val-L-Val in the Caco-2 cell monolayers was less than 0.1 nmol/mg protein.

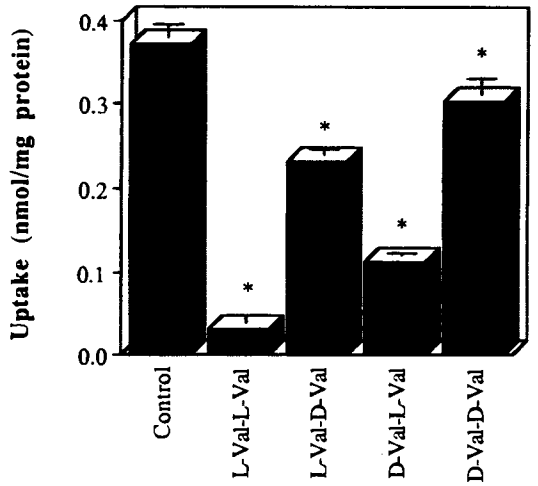

Fig. 1. Uptake of [^{3}H]cephalexin (0.1 mM) in the presence or absence of the Val-Val diastereomers (10 mM) for 15 min at 37°C. Caco-2 cell monolayers were incubated with pH 6.0 buffer on the apical side and pH 7.4 buffer on the basolateral side. Results are the means ± SD for three separate filters. The asterisks (*) indicate that the differences from the control level were statistically significant ($P < 0.05$) according to a one-way ANOVA test.

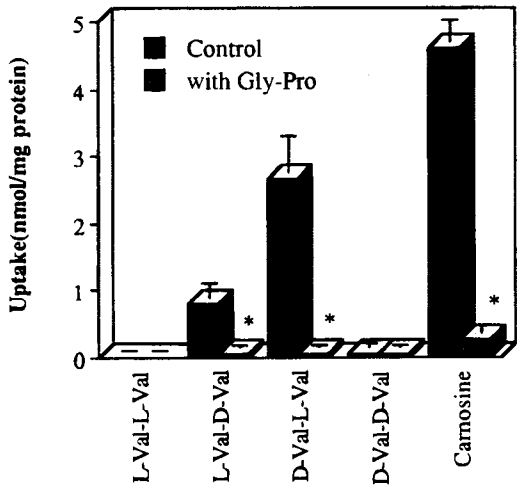

Fig. 2. Uptake of the Val-Val diastereomers (1 mM) and carnosine in the presence or absence of Gly-Pro (10 mM) during a 2 hr incubation at 37°C. Caco-2 cell monolayers were incubated with pH 6.0 buffer on the apical side and pH 7.4 buffer on the basolateral side. Results are the means ± SD for three separate filters. The asterisks (*) indicate that the differences from the control levels were statistically significant ($P < 0.05$) according to a one-way ANOVA test.

Metabolism of the Val-Val Diastereomers

Figure 3A shows the time course of metabolism of the Val-Val diastereomers in the Caco-2 cell homogenate at 37°C. L-Val-L-Val was degraded completely within 15 min. In contrast, L-Val-D-Val, D-Val-L-Val and D-Val-D-Val were stable for up to 2 hr. Figure 3B shows the extent of metabolism of the Val-Val diastereomers in the apical bathing solution (pH 6.0) over Caco-2 cell monolayers after a 2-hr incubation at 37°C. When 10 mM concentrations of the dipeptides were used for the experiments, little or no degradation of the peptides was observed. However, a 1 mM concentration of L-Val-L-Val showed significant degradation (approx. 80%) during a 2-hr incubation. The Val-Val diastereomers were all stable for at least 12 hr at 37°C in the pH 7.4 buffer (data not shown).

Transepithelial Transport of the Val-Val Diastereomers and the Effect of Gly-Pro

Table I shows the permeability coefficients of the Val-Val diastereomers (1 mM) and carnosine (1 mM) in the Caco-2 cell monolayers during a 2 hr incubation. The order of their permeation was: carnosine > L-Val-D-Val = D-Val-L-Val = D-Val-D-Val > L-Val-L-Val. As shown in Figure 4, transepithelial transport of L-Val-D-Val, D-Val-L-Val and carnosine was inhibited by inclusion of Gly-Pro (10 mM) in the incubation mixture. However, Gly-Pro did not show an inhibitory effect on the transepithelial transport of L-Val-L-Val and D-Val-D-Val.

DISCUSSION

Hidalgo *et al.* (18) have demonstrated that incorporation of one D-amino acid in the diastereomers of Val-Val does not abolish their affinities for the apical oligopeptide transporter(s).

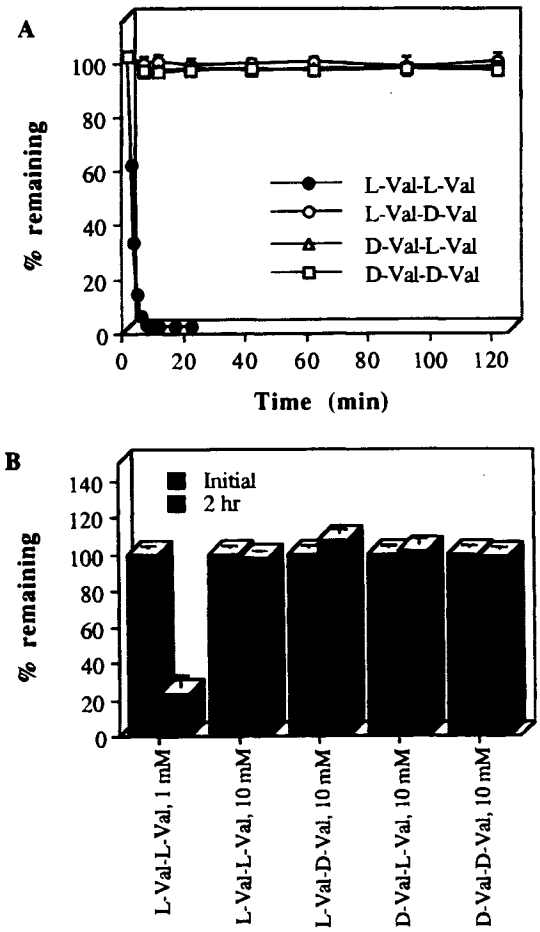

Fig. 3. (A) Time course of metabolism of the Val-Val diastereomers (1 mM) in the Caco-2 cell homogenate (~1 mg protein/ml, pH 7.4) and (B) metabolism of the Val-Val diastereomers in the apical bathing solution (pH 6.0) over Caco-2 cell monolayers at 37°C. Results are the means ± SD for three separate experiments.

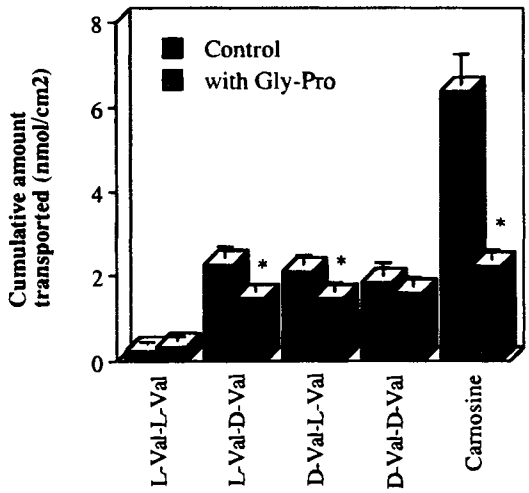

Fig. 4. Effect of Gly-Pro (10 mM) on the transepithelial transport of the Val-Val diastereomers (1 mM) and carnosine (1 mM) at 37°C. Caco-2 cell monolayers were incubated with pH 6.0 buffer on the apical side and pH 7.4 buffer on the basolateral side. The total amount of each peptide transported into the basolateral chamber during a 2 hr incubation is compared. Results are the means ± SD for three separate filters. The asterisks (*) indicate that the differences from the control levels were statistically significant ($P < 0.05$).

However, the binding of the Val-Val diastereomers to the apical oligopeptide transporter(s) does not necessarily mean that these molecules will be taken up and will undergo transepithelial transport (1,5,10–12). Therefore, the purpose of our study was to determine whether binding of these diastereomers to the apical oligopeptide transporter(s) could be correlated with their cellular uptake and/or transepithelial transport. We used the Caco-2 cell culture system as a model of the intestinal epithelium. The transepithelial transport characteristics of the Val-Val diastereomers were compared to those of carnosine, N-β-alanyl-L-histidine, which is known to be transported actively by the oligopeptide transporter in the intestinal epithelium and is enzymatically stable (22–25).

Table I. Permeability Coefficients of the Val-Val Diastereomers and Carnosine across the Caco-2 Cell Monolayers

Dipeptides	Papp × 10^7 (cm/sec)
L-Val-L-Val	1.86 (0.08)[a]
L-Val-D-Val	5.07 (0.70)
D-Val-L-Val	4.92 (0.33)
D-Val-D-Val	4.42 (0.55)
Carnosine	13.04 (1.53)

[a] Mean (±SD).

To evaluate the binding of the Val-Val diastereomers to the apical oligopeptide transporter(s), inhibition studies of [^{3}H]cephalexin uptake by the Val-Val diastereomers were performed. The result of the inhibition studies (Figure 1) suggests that the diastereomers have different affinities for the apical oligopeptide transporter(s). Incorporation of one D-amino acid significantly reduced the affinity of the dipeptides for the transporter(s). Incorporating the D-amino acid at the C-terminal end had a more dramatic effect than inserting it at the N-terminal end. Inserting two D-amino acids into the peptide further reduced its affinity for the transporter(s). Hidalgo *et al.* (18) did not see significant differences in the inhibitory activities of L-Val-L-Val, L-Val-D-Val and D-Val-L-Val on [^{3}H]cephalexin uptake. The differences in the results seen in our study compared with those reported by Hidalgo *et al.* (18) may be due to the different concentrations of the Val-Val diastereomers used in the experiments (we employed 10 mM, Hidalgo *et al.* (18) employed 20 mM).

To determine whether the binding of these diastereomers can be correlated with their cellular uptake from the apical side, we determined the apical uptake of these diastereomers and carnosine in the presence or absence of Gly-Pro. As seen in Figure 2, the apical uptake of L-Val-D-Val, D-Val-L-Val and carnosine was 15, 50 and 88 times higher, respectively, than that of D-Val-D-Val. Also, Gly-Pro inhibited the apical uptake of L-Val-D-Val, D-Val-L-Val and carnosine by 95%, 98% and 95%, respectively, whereas Gly-Pro had no effect on that of D-Val-D-Val. These results suggest that the apical uptake process of L-Val-D-Val and D-Val-L-Val are mainly carrier mediated by the oligopeptide transporter(s) existing on

the apical membrane, and D-Val-D-Val uptake into the cell monolayers is by passive diffusion. L-Val-L-Val was not detected in the Caco-2 cells. Since L-Val-L-Val is a natural dipeptide and has the highest affinity for the apical oligopeptide transporter(s) among the Val-Val diastereomers based on the [^{3}H]cephalexin studies, we assume that it is taken up actively into the cells but then rapidly metabolized. Therefore, the binding of Val-Val diastereomers to the apical oligopeptide transporter(s) appeared to be a good predictor of their cellular uptake.

When the transepithelial transport characteristics of the Val-Val diastereomers were determined, the permeability coefficient (P_{app}) values for L-Val-D-Val, D-Val-L-Val and D-Val-D-Val transport were similar (Table I), even though L-Val-D-Val and D-Val-L-Val are taken up more actively by the apical oligopeptide transporter(s) and accumulated to much higher intracellular concentrations than that of D-Val-D-Val (Figure 2). The transepithelial transport of L-Val-D-Val and D-Val L-Val was inhibited by Gly-Pro by 36% and 30%, respectively. Gly-Pro had no effect on the transepithelial transport of D-Val-D-Val. In contrast, the P_{app} value for transepithelial transport of carnosine was approximately 2.5 times greater than the P_{app} values for L-Val-D-Val, D-Val-L-Val and D-Val-D-Val. In addition, Gly-Pro produced a more significant inhibition of carnosine's transepithelial transport (approx. 65%). These data suggest that the major transepithelial transport route of L-Val-D-Val, D-Val-L-Val and D-Val-D-Val is passive diffusion either via the transcellular or paracellular route. If the major transepithelial transport route of these molecules is transcellular, D-Val-L-Val would have shown a much higher P_{app} value than those of L-Val-D-Val and D-Val-D-Val because of greater accumulation in the cell. These observations suggest that the major route of transepithelial transport of these dipeptides is probably passive diffusion through the paracellular route.

Moreover, the observations above suggest that there is little or no contribution of the oligopeptide transporter to the efflux of L-Val-D-Val, D-Val-L-Val and D-Val-D-Val across the basolateral membrane. In contrast, the oligopeptide transporter on the apical membrane facilitates the apical uptake of L-Val-D-Val and D-Val-L-Val. Therefore, the basolateral transporter may have different and much strict structural requirements for peptides to bind and be transported than does the apical transporter(s).

Therefore, since the active transepithelial transport process of peptides or peptidomimetics involves the oligopeptide transporters on both the apical and basolateral membrane, the binding of the Val-Val diastereomers to the apical oligopeptide transporter(s) is not sufficient to predict their transepithelial transport. It appears that the stereochemical requirements for the transporter that mediate efflux of the peptide across the basolateral membrane may be different from the requirements for the apical transporter that mediates cellular uptake.

The characteristics of the basolateral oligopeptide transporter are controversial. Inui *et al.* (14–15) suggested that the basolateral oligopeptide transporter is involved in the transport of cephalosporins and bestatin across the basolateral membranes. Furthermore, they also demonstrated that the transport of cephalsporins across the basolateral membrane are mediated by the oligopeptide transporter in an H^+ gradient-independent manner (16). In contrast, Thwaites *et al.* (17) have compared the properties of the apical and basolateral oligopeptide transporters and shown both to be H^+-coupled but they exhibit different kinetic properties. These published observations and the observations described in this manuscript regarding the different substrate specificities of the apical and basolateral transporters would suggest that the proteins mediating the apical and basolateral flux of dipeptides in Caco-2 cells might be structurally different.

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REFERENCES

1. C. H. Gochoco, F. M. Ryan, J. Miller, P. L. Smith, and I. J. Hidalgo. Uptake and transepitherial transport of orally absorbed cephalosporine cephalexin in the human intestinal cell line, Caco-2. *Int. J. Pharm.* **104**:187–202 (1994).
2. D. M. Matthews and S. A. Adibi. Peptide absorption. *Gastroenterology* **71**:151–161 (1976).
3. V. Ganapathy and F. H. Leibach. Is intestinal peptide transport energized by a proton gradient? *Am. J. Physiol.* **249**:G153–G160 (1985).
4. A. H. Dantzig and L. Bergin. Carrier-mediated uptake of cephalexin in human intestinal cells. *Biochem. Biophys. Res. Commun.* **155**:1082–1087 (1988).
5. I. J. Hidalgo, F. M. Ryan, G. J. Marks, and P. L. Smith. pH-dependent transepithelial transport of cephalexin in rabbit intestinal mucosa. *Int. J. Pharm.* **98**:83–92 (1993).
6. P. W. Swaan, M. C. Stehouwer, R. I. C. Blok, and J. J. Tukker. Prodrug approach using the intestinal peptide carrier. *Pharm. Res.* **10**:S-295 (1993).
7. M. Hu, P. Subramanian, H. I. Mosberg, and G. L. Amidon. Use of the peptide carrier system to improve the intestinal absorption of L-α-methyldopa: carrier kinetics, intestinal permeabilities, and *in vitro* hydrolysis of dipeptidyl derivatives of L-α-methyldopa. *Pharm. Res.* **6**:66–70 (1989).
8. J. P. F. Bai. pGlu-L-Dopa-Pro: Tripeptide prodrug targeting the intestinal peptide transporter for absorption and tissue enzymes for conversion. *Pharm. Res.* **12**:1101–1104 (1995).
9. P. L. Smith, E. P. Eddy, C.-P. Lee, and G. Wilson. Exploitation of the intestinal oligopeptide transporter to enhance drug absorption. *Drug Delivery* **1**:103–111 (1993).
10. W. Kramer, F. Girbig, U. Gutjahr, S. Kowalewski, F. Adam, and W. Schiebler. Intestinal absorption of β-lactam antibiotics and oligopeptides. *Eur. J. Biochem.* **204**:923–930 (1992).
11. K. Miyazaki, K. Iseki, and T. Arita. Intestinal absorption of several β-lactam antibiotics. IV. Binding to the various components in the intestinal mucosa of rat and role in absorption process. *J. Pharm. Dyn.* **5**:593–602 (1982).
12. F. M. Ryan and P. L. Smith. Effects of altering fluid transport on the absorption of benzylpenicillin and mannitol across rat intestinal mucosa *in vitro*. *Pharm. Res.* **6**:S88 (1989).
13. J. Dyer, R. B. Beechey, J.-P. Gorvel, R. T. Smith, R. Wootton, and S. P. Shirazi-Beechey. Glycyl-L-proline transport in rabbit enterocyte basolateral-membrane vesicles. *Biochem. J.* **269**:565–571 (1990).
14. K.-I. Inui, M. Yamamoto, and H. Saito. Transepithelial transport of oral cephalosporins by monolayers of intestinal epithelial cell line Caco-2: Specific transport system in apical and basolateral membrane. *J. Pharmacol. Exp. Ther.* **261**:195–201 (1992).
15. H. Saito and K.-I. Inui. Dipeptide transporters in apical and basolateral membranes of the human intestinal cell line Caco-2. *Am.J. Physiol.* **265**:G289–G294 (1993).
16. S.-I. Matumoto, H. Saito, and K.-I. Inui. Transepithelial transport of oral cephalosporins in human intestinal epithelial cells, Caco-2: Interaction with dipeptide transport systems in apical and basolateral membranes. *J. Pharmcol. Exp. Ther.* **270**:498–504 (1994).

17. D. T. Thwaites, C. D. A. Brown, B. H. Hirst, and N. L. Simmons. Transepithelial Glycylsarcosine transport in intestinal Caco-2 cells mediated by expression of H^+-coupled carriers at both apical and basal membranes. *J. Biol. Chem.* **268**:7640–7642 (1993).
18. I. J. Hidalgo, P. Bhatnagar, C.-P. Lee, J. Miller, G. Cucullino, and P. L. Smith. Structural requirments for interaction with the oligopeptide transporter in Caco-2 cells. *Pharm. Res.* **12**:317–319 (1995).
19. I. J. Hidalgo, T. J. Raub, and R. T. Borchardt. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* **96**:736–749 (1989).
20. D. Wessel and U. I. Flugge. A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. *Anal. Biochem.* **138**:141–143 (1984).
21. P. J. Chikhale and R. T. Borchardt. Metabolism of L-α-methyldopa in cultured human intestinal epithelial (Caco-2) cell monolayers. Comparison with metabolism *in vivo*. *Drug Metab. Disp.* **22**:592–600 (1994).
22. D. M. Matthews, J. M. Addison, and D. Burston. Evidence for active transport of the dipeptide carnosine (βalanyl-L-histidine) by human jejunum *in vitro*. *Clin. Sci. Molec. Med.* **46**:693–705 (1974).
23. J. M. Addison, D. M. Matthews, and D. Burston. Competition between carnosine and other peptides for transport by human jejunum *in vitro*. *Clin. Sci. Molec. Med.* **46**:707–714 (1974).
24. M. L. G. Gardner, K. M. Illingworth, J. Kelleher, and D. Wood. Intestinal absorption of the intact peptide carnosine in man, and comparison with intestinal permeability to lactose. *J. Physiol.* **439**:411–422 (1991).
25. K. M. Hillgren, P. F. Augustijns, and R. T. Borchardt. Carnosine as a substrate for the peptide carrier in Caco-2 cells. *Pharm. Res.* **10**:S-181 (1993).