Mechanisms of Transport of Quinapril in Caco-2 Cell Monolayers: Comparison with Cephalexin

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Purpose. To determine the transport mechanisms of quinapril and cephalexin in Caco-2 cell monolayers, a cell culture model of the human small intestinal epithelium. *Methods.* Uptake, transepithelial transport and intracellular accumulations of these two drugs were measured using Caco-2 cell monolayers grown onto Millicells® and magnetically stirred diffusion chambers. *Results.* Transepithelial transport, apical (AP)[4] uptake and intracellular accumulation of both drugs depended on the maintenance of a transepithelial proton gradient and temperature of the medium. However, quinapril transport and accumulation, which did not display a maximum at approximately pH 6, was more sensitive to proton gradient change, whereas cephalexin transport was more sensitive to concentration change (range 0.5-5 mM). In addition, quinapril (1 mM) transport was decreased significantly ($p<0.05$) by 10 mM cephalexin, loracarbef, Gly-Pro and Phe-Pro, but not by enalapril; whereas cephalexin (0.1 mM) transport was decreased significantly ($p<0.05$) by all four compounds. Similarly, AP quinapril (1 mM) uptake was also decreased by 10 mM loracarbef, Gly-Pro, cephalexin, and enalapril, but these inhibitory effects (20-50%) were quantitatively less than their inhibitory effects on cephalexin uptake (50-90%). Finally, the AP uptake of quinapril was also significantly ($p<0.05$) inhibited by FCCP (10 μg/ml), amiloride (0.5 mM), DEP (0.5 mM), and staurosporine (5 nM). *Conclusions.* The transport of quinapril in the Caco-2 cells is via a combination of the carrier-mediated proton gradient-dependent peptide transporter and passive diffusion.

KEY WORDS: oral β-lactam antibiotic; cephalexin; ACE inhibitor; quinapril; peptide carrier system; proton-gradient; intestinal absorption; Caco-2.

INTRODUCTION

The study of transport of a class of important peptide-like drugs, the angiotensin-converting enzyme (ACE) inhibitors, has not been reported in the Caco-2 cell culture model, although the transport mechanisms of peptides (e.g., Gly-Sar) and peptide like drugs (e.g., β-lactam antibiotics) in this model system have been well characterized (1-8). Because two of the main purposes of developing the Caco-2 cell monolayer as an intestinal models is to study the drug transport mechanism and to screen for oral drug candidates, it would be important to know whether the ACE inhibitors are transported by the peptide transporter, as described previously in the rat small intestine (9-12).

Evidence generated from the rat intestinal studies support the hypothesis that the ACE inhibitors are transported, at least in part, by a carrier-mediated pathway via the peptide carrier in the rat small intestine (9-12). However, it was unclear from the previous studies, which used disappearance rates, whether the observed kinetic characteristics were due to uptake or transcellular transport (or transport) (9-12). Therefore, the purpose of the present study is to determine the transport and AP uptake mechanisms of quinapril. Furthermore, the transport characteristics of quinapril were compared to that of cephalexin, an oral β-lactam antibiotic, whose transport is well characterized and known to be transported via a carrier-mediated proton-gradient peptide transporter. In addition, the present study employs a relatively new diffusion apparatus (13), which was not used in previous publications characterizing cephalexin transport.

MATERIALS AND METHODS

Materials. [^{14}C]-Cephalexin was provided by Lilly Research Laboratories (Indianapolis, IN). Quinapril was provided by Parke-Davis/Warner-Lambert (Ann Arbor, MI). [^{3}H]-Mannitol was purchased from DuPont-NEN (Boston, MA). Cell culture supplies and chemicals used were the same as previously described (7). Additional chemicals such as DEP, FCCP and staurosporine were purchased from Sigma. Cephalexin · HCl and loracarbef were supplied by Lilly Research Laboratories (Indianapolis, IN). Enalapril was kindly provided by Merck and Co. (West Point, PA).

Cell Culture. Caco-2 cell monolayers were grown as described previously (7, 14). The quality of the cell monolayers was determined by measuring the transepithelial electrical resistance (TEER) (normally 400-800 ohm · cm^2) and the leakage of [^{3}H]-mannitol (normally < 0.23%/hr/cm^2). These quality control measures are similar to those reported previously (2,4-7).

Study Protocol. The uptake and transport experiments were performed the same way as described previously, using a diffusion chamber (7). Transport buffers at pH7.4 and 6.0 were the same as previously described (7). The pH 6 Na^+-free buffer contained 250 mM mannitol, 25 mM glucose and 25 mM MES plus 0.9 mM calcium chloride and magnesium chloride. Samples were taken from trans side of the loading chamber every 30 or 40 min for a total of four samples. The amount of radioactivity taken up was assayed using liquid scintillation counting as described (7). Alternatively, the cells are homogenized with ultrasonic probe, filtered through a 0.22 μm filter, and injected into an HPLC. Total cellular protein was assayed according to Bradford's method (15).

Inhibition Studies. Transport or uptake in the presence of potential inhibitors was determined by loading a solution

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[4] **Abbreviations:** **ACE**, angiotensin-converting enzyme; **AP**, apical; **BL**, basolateral; **DEP**, diethyl purocarbonate; **FCCP**, carbonyl cyanide p-trifluoromethoxy-phenylhydrazone; **HBSS**, Hank's balanced salt solution; **HEPES**, N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid; **MES**, 2-[N-morpholino]-ethanesulfonic acid; **NaCN**, sodium cyanide; **TEER**, transepithelial electrical resistance.

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containing both the test inhibitor and substrate in the same side of the diffusion chamber. When the compound was a noncompetitive inhibitor (e.g., NaCN), a 60 min preincubation was used followed by the actual experiment. When a high concentration of a potential inhibitor (e.g., cephalexin · HCl) was used in the transport solution, the pH of the solution was always adjusted to the desired value after the compound had dissolved.

Temperature Effects. The protocol for performing these experiments was similar to ordinary transport experiments except the transport medium and waterbath were kept at 4°C prior to and during the sampling period.

Sample Analysis. The [^{14}C]-cephalexin and [^{3}H]-mannitol was determined using liquid scintillation spectrophotometry (Model 2500 TR, Packard Ins. Co., Meriden, CT) with quench correction. A program stored in the computer was used to obtain the DPM under dual labeled conditions.

Cephalexin and quinapril were also analyzed with HPLC after internal standards were added (if applicable) and samples were filtered. The conditions for cephalexin were: column, Beckman Ultrasphere (5 μm) C-18; mobile phase, 80% 0.05 M sodium phosphate buffer (pH 5) plus 20% methanol; retention time, 9 min. whereas the conditions for quinapril were: column, Beckman Ultrasphere (5 μm) C-18; mobile phase, 70% methanol plus 30% 50 mM pH7.4 sodium phosphate buffer; internal standard, taurocholic acid; retention time, 5.5 min for quinapril, 7.5 min for taurocholic acid; wavelength, 220 nm.

Using the above HPLC procedures, both cephalexin and quinapril were determined to be stable in freshly prepared Caco-2 cell homogenates within the 3 hr time period.

Data Analysis. Results of mannitol leakage were expressed as % transported versus time, results of transport were expressed as amount transported versus time, and results of intracellular accumulation of cephalexin were expressed as cumulative amount per cell monolayer, which contains approximately 1.6 mg of cellular protein. The lag time is calculated for each amount transported versus time curve because it is inappropriate to calculate the average lag time from the average amount transported versus time curve. The permeabilities may be calculated by dividing the rate of transport (uptake) with concentration and surface area. In a saturable transport, permeability decreased as concentration increases to a level approaching and exceeding K_m.

Since it was not always possible to perform all the inhibition experiments using the same batch of cells, the results of inhibition experiments were always normalized to this control value as described previously (7).

Statistical analyses of the data presented in the "Results" section were performed by a one way ANOVA or a Student's T-test. A prior level of significance was set at 5% or $p<0.05$. The software used was Systat®.

RESULTS

Transcellular Transport

Transcellular transport (or transport) represents the overall process of absorption (secretion). It describes an apparent kinetic process of transport that include uptake at one side and subsequent efflux at the other.

Effect of Time and pH on Transport

Vectorial transport of 1 mM quinapril was measured in the AP to BL direction or vice versa (Fig.1). In the presence of a proton gradient (pH6-7.4), AP to BL transport rate (0.683±0.026 nmol/min/cm^2) was approximately 72% faster than the BL to AP transport rate (0.401±0.016 nmol/min/cm^2), and 5 times faster than transport against a proton gradient (Fig.1A). In addition, there was a 12-15 min (average) lag time for transport in the presence of a proton gradient, compared to 3-5 min (average) lag time in the absence of a proton gradient.

The intracellular accumulations of quinapril were also determined under identical conditions after 160 min experiments (Table 1). In the presence of a proton gradient, the

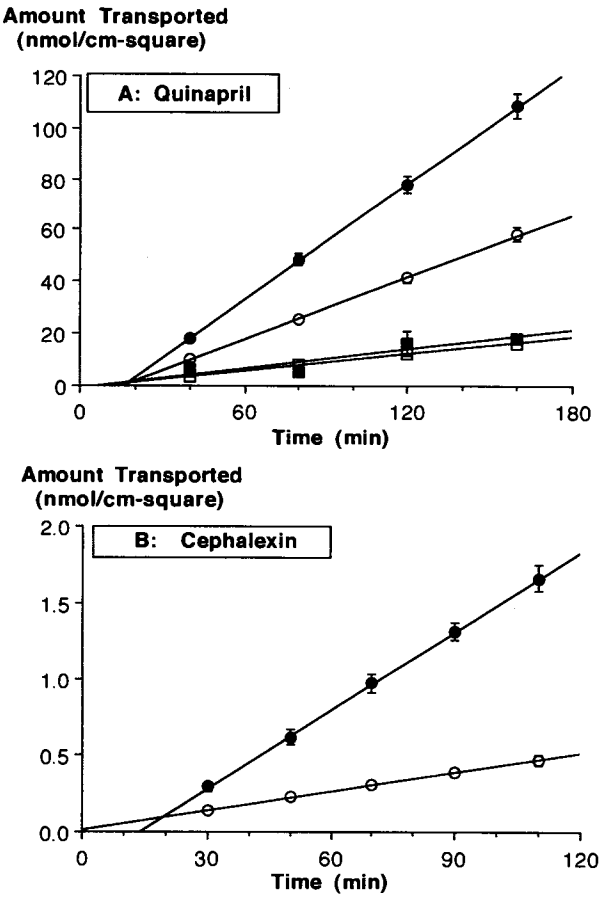

Fig. 1. Transcellular transport of quinapril (Panel A) and [^{14}C]-cephalexin (Panel B) in the AP to BL direction (solid symbols) versus the BL to AP direction (hollow symbols). Panel A shows 1 mM quinapril transport following a transepithelial proton gradient (pH6 to pH7.4, circles) versus against a proton gradient (pH7.4 to pH6, squares). Panel B shows transport of 0.2 mM cephalexin following (solid circles) or against (hollow circles) the same gradients as those used in quinapril transport studies. Each line represents the best fit to the data using linear regression. Each point is the average of three determinations with three different monolayers. The error bar represents standard deviation of the mean.

Table 1. Intracellular Accumulation of Quinapril and Cephalexin Under Various Conditions. The Accumulations Were Measured After Transport Experiments Were Completed[a]

Compound	Transport direction	AP pH	BL pH	Accumulation ± SD (nmol/monolayer)
Quinapril (1 mM)	AP → BL	6.0	7.4	30.5 ± 3.4
	AP → BL	7.4	6.0	1.3 ± 0.5
	BL → AP	6.0	7.4	20.8 ± 2.1
	BL → AP	7.4	6.0	0.99 ± 0.12
Cephalexin (0.2 mM)	AP → BL	6.0	7.4	3.17 ± 0.20
	BL → AP	7.4	6.0	0.30 ± 0.05

[a] See Fig. 1 legend and text for further details.

accumulations were approximately 20 to 25 times higher than the accumulations without a proton gradient. The accumulation was also favored when loaded apically (30%, $p<0.05$). Using an intracellular volume of 3.66 μl/mg protein (1), the distribution ratio of quinapril was 14:1 in favor of the intracellular domain.

Cephalexin transport and accumulation were also measured to determine how the transport of cephalexin compares to that of quinapril. The results indicated that AP to BL transport in the presence of a proton gradient (0.017 ± 0.001 nmol/min/cm^2) was approximately 4 times higher than that in the BL to AP transport in the absence of a proton gradient (0.0041 ± 0.0003 nmol/min/cm^2) (Fig.1B). On the other hand, the cephalexin accumulation differed by approximately 10 times (Table 1) under the same conditions.

Because transport of quinapril was particularly sensitive to the proton gradient change, its transport was also determined at different AP pH with a constant basolateral pH (pH7.4). The results indicated that transport at AP 8.0 were approximately 90 folds smaller than transport at AP pH5.5 (Fig. 2), whereas the accumulation was 30 folds smaller. The transport was also much higher with acidic pH at both sides of the epithelium compared with neutral pH at both sides (Fig.2). Compared to transport, quinapril accumulations were less sensitive to the change in AP pH (Fig.2).

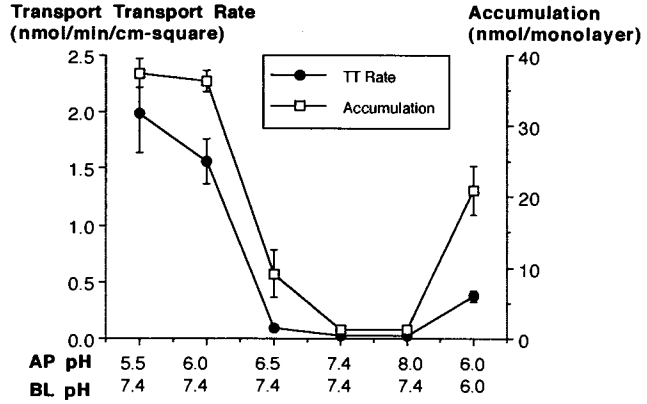

Fig. 2. Effect of proton gradient on the transcellular transport (Panel A) and accumulation (Panel B) of quinapril. The transport rates were calculated after plotting amount transported versus time curves as shown in Figure 1. Each horizontal column represents the average of three determinations and the error bar represents standard deviation of the mean.

Effect of Temperature on Transport

The effect of temperature on the transport of 1 mM quinapril and cephalexin was determined by measuring the rates of transport at 37°C and 4°C. At 37°C, the transport rate of quinapril was approximately 17 times higher than transport rate of cephalexin (Table 2), whereas the intracellular accumulation of quinapril was only 4 times as high (Table 2). At 4°C the difference was about 7 times (Table 2). In addition, as the concentration increased, the transport of cephalexin became less sensitive to temperature change. For example, cephalexin transport decreased 90% at 0.1 mM but only 75% at 5 mM when media temperature dropped from 37°C to 4°C (not shown).

Effect of Concentration on Transport

The rates of transported were measured over the concentration range of 0.5 to 5 mM at 37°C (Table 3). Higher concentration of quinapril was not used due to a solubility limit (approximately 5.5 mM in the current buffer system). The results indicated that the permeabilities of quinapril stayed relatively the same from 0.5 to 3 mM and became somewhat higher at 5 mM ($p<0.05$) (Fig.5A). On the other hand, the permeability of cephalexin gradually decreased as the concentration increased ($p<0.05$) (Table 3).

Effects of Peptides and Peptide Analogs on Transport

Transport of cephalexin at a concentration of 0.1 mM was measured in the absence and in the presence of an excess amount of Phe-Pro, Gly-Pro, loracarbef, and enalapril. The results indicated that the transport of cephalexin was inhibited significantly ($p<0.05$) by the natural peptides, Gly-Pro and Phe-Pro, as well as by the peptide analogs, loracarbef (a new synthetic β-lactam antibiotic) and enalapril (an ACE inhibitor reported to be transported by the peptide carrier, ref.9) (Fig.3).

The same set of inhibitors were also used to challenge the transport of 1 mM quinapril. The results indicated quinapril transport was much less affected by these inhibitors (ranging from 26 to 50%) whereas enalapril had no effect (Fig.3).

Apical Uptake

The study of the apical uptake process alone using initial uptake rate can help determine the drug influx mechanisms into the cells, since transcellular transport is a process that starts with apical uptake followed by basolateral efflux.

Table 2. Effect of Temperature on the Transport and Accumulation of 1 mM Cephalexin and Quinapril. The Experiments Were Performed at 37°C and 4°C, and the Rate of Transport and Accumulation Were Measured as Described in Fig. 1 and Table 1

Compound	Temperature (°C)	Transport ± SD (nmol/min/cm^2)	Accumulation ± SD (nmol/monolayer)
Quinapril	37	1.57 ± 0.19	36.3 ± 1.5
	4	0.094 ± 0.008	4.61 ± 1.08
Cephalexin	37	0.087 ± 0.003	7.41 ± 0.81
	4	0.0133 ± 0.0003	—

Table 3. Effect of Concentration on the AP to BL Transport of Cephalexin and Quinapril Following a Proton Gradient (pH 6.0 to 7.4). In Carrier-Mediated Transport, Permeability Decreases as Concentration Approaches K_m Value

Concentration (mM)	Permeability ± SD ($\times 10^4$ cm/min)	
	Quinapril	Cephalexin
0.5	1.30 ± 0.01	1.6 ± 0.01
0.75	—	1.15 ± 0.12[a]
1	1.60 ± 0.10	0.95 ± 0.05[a]
1.5	1.50 ± 0.10	0.94 ± 0.02[a]
2	1.78 ± 0.18	—
2.5	—	0.82 ± 0.02[a]
3	1.80 ± 0.01	—
5	2.60 ± 0.20[b]	0.78 ± 0.03[a]

[a] Indicates that the difference between permeability at this concentration and that at 0.5 mM were statistically significant according to one way ANOVA plus Post hoc test using statistical software Systat.

[b] Indicates that the permeability at this concentration is significantly higher than those observed at other concentration.

Effect of Time and pH on Uptake

Uptake of quinapril (1 mM) and cephalexin (0.2 mM) was linear with time for approximately 15 min (not shown). Therefore, subsequent uptake experiments were performed for 15 min. In the absence of a proton gradient, the rate of 1 mM quinapril uptake was decreased approximately 10 times, whereas the uptake of 0.2 mM cephalexin was decreased approximately 6 times (not shown).

Effect of Concentration on Apical Uptake

Uptake of quinapril and cephalexin was measured from 0.5 to 5 mM. Quinapril permeability stayed relatively unchanged within the concentration range, whereas cephalexin permeability decreased significantly ($p<0.05$) as the concentration increased (Table 4).

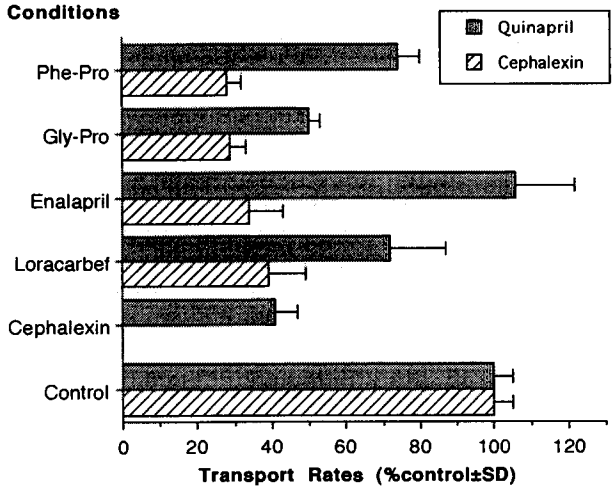

Fig. 3. Effect of various peptides and analogs on the transcellular transport rates of 1 mM quinapril and 0.1 mM cephalexin at 37°C. The transport rates were calculated after plotting amount transported versus time curves as shown in Figure 1. The control values ranged from 10.3-11.7 pmol/min/cm^2 for 0.1 mM cephalexin, and 700-1500 pmol/min/cm for 1 mM quinapril. Each column represents the average of three determinations, and the error bar represents standard deviation of the mean.

Effect of Peptides and Peptide Analogs on Initial Uptake and Accumulation

Uptake of 1 mM quinapril was significantly ($p<0.05$) inhibited by 10 mM Gly-Pro (50%), enalapril (20%), loracarbef (20%), and cephalexin (23%), but not by Phe-Pro and 5 mM quinapril (Fig.4A). However, their inhibitory effects on quinapril uptake were quantitatively smaller than their inhibitory effects on 0.2 mM cephalexin uptake using the same inhibitors (ranged from 60% to 90%). In addition, both 10 mM Phe-Pro and 5 mM quinapril inhibited 0.2 mM cephalexin uptake by approximately 90%.

Effect of Other Compounds on Initial Uptake

Initial uptake of quinapril was significantly ($p<0.05$) inhibited by 10 μg/ml FCCP (56%), 0.5 mM DEP (28%), 0.5 mM amiloride (43%), and 5 nM staurosporine (37%), but not by 10 μM NaCN or by the absence of Na^+ (Fig.5). In contrast, cephalexin transport was significantly ($p<0.05$) inhibited by FCCP (39%), amiloride (18%), and by the absence of Na^+ (37%), but not by 10 μM NaCN (Fig.5).

DISCUSSION

We first studied the vectorial transport of quinapril as one of the standard tests. Apical to basolateral transport of quinapril in the presence of a proton gradient was much higher than transport in the opposite direction in the absence of a proton gradient, as observed previously (2,3,5-7). Also, quinapril transport was more sensitive to change in proton gradient than reported for transport of cephradine, cephalexin, loracarbef, Gly-Sar and bestatin (1,2,4-7). For example, when apical pH was increased from 6 to 7.4, quinapril transport was decreased 15 times; whereas, the transport of other compounds only decreased approximately 2-3 times. In addition, when apical pH was increased from 6 to 6.5, the transport rate decreased 8 fold as compared to less than 1 fold with the transport of other peptides substrates (2,5,6). However, quinapril transport did not display a maximal

Table 4. Effect of Concentration on the AP Uptake of Cephalexin and Quinapril Following a Proton Gradient (pH 6.0 to 7.4). In Carrier-Mediated Transport, Permeability Decreases as Concentration Approaches K_m Value

Concentration (mM)	Permeability ± SD ($\times 10^4$ cm/min)	
	Quinapril	Cephalexin
0.5	4.54 ± 0.48	1.76 ± 0.05
1	4.39 ± 0.18	1.69 ± 0.18
2.5	4.74 ± 0.54	1.32 ± 0.08[a]
5	4.35 ± 0.11	1.13 ± 0.04[a]

[a] Indicates that the difference between permeability at this concentration and that at 0.5 mM were statistically significant according to one way ANOVA plus Post hoc test using statistical software Systat.

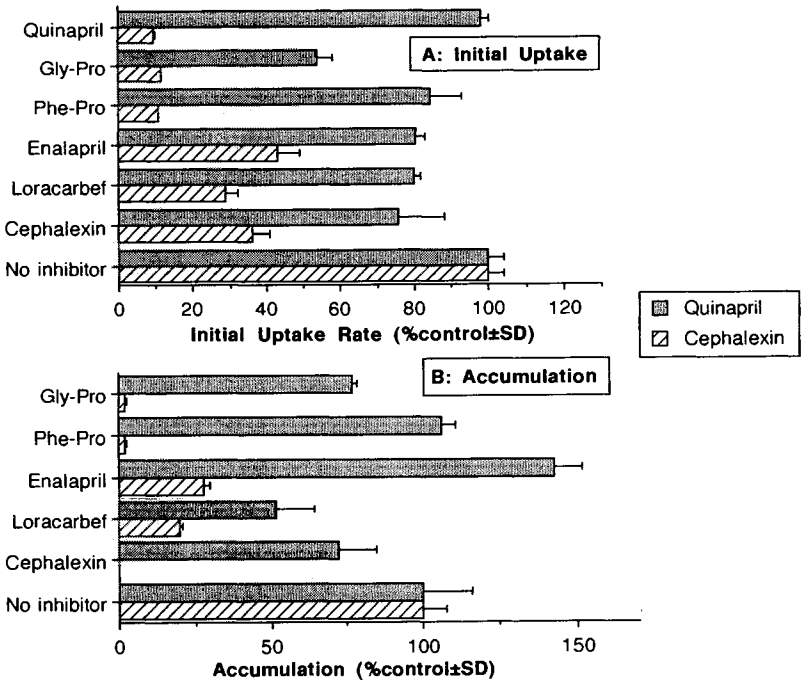

Fig. 4. The effect of peptides and peptide analogs on the initial uptake and accumulation of 1 mM quinapril and 0.2 mM cephalexin. Initial uptake rates were calculated by dividing amount taken up with uptake time. The accumulations were measured after a 120 or 160 min incubation period for cephalexin or quinapril, respectively. The control values for uptake were 38-42 pmol/min/cm^2 for 0.2 mM cephalexin, and 397-444 nmol/min/cm^2 1 mM for quinapril. The control values for accumulation were 30-36 nmol/monolayer for quinapril and 12-15 nmol/monolayer for 0.2 mM cephalexin. Each horizontal column represents the average of three determinations and the error bar represents standard deviation of the mean.

transport at approximately pH 6 as shown previously (1,2,5,6). Because transepithelial transport was much slower when pH was 6 at both sides, the results suggest that the transepithelial proton gradient provides a driving force for quinapril transport.

The observed pH-dependent transport cannot be explained by the pH-partition theory. Quinapril (pKa = 2.8 and 5.4) is predominantly negatively charged at all the pH studied ($pK_I = 4.1$) (16). In addition, uptake was also inhibited by other inhibitors which acted on various processes associated with proton-driven protein transporter, including 10 μg/ml FCCP (a protonophore), 0.5 mM DEP (a protein modifying agent known to inhibit peptide transport, ref 17), and 5 nM protein kinase C inhibitor staurosporine (see the proposed PKC modification site in peptide transporter in ref 18). The last result was different from that observed by Brandsch et al using Gly-Sar as substrate (19). The exact reason for this discrepancy is unknown, but it has been proposed that the ACE inhibitors may be bound by a site different from that that binds ordinary peptides (20). Overall, these results indicate that the quinapril uptake is driven by a proton gradient, and mediated by a peptide carrier.

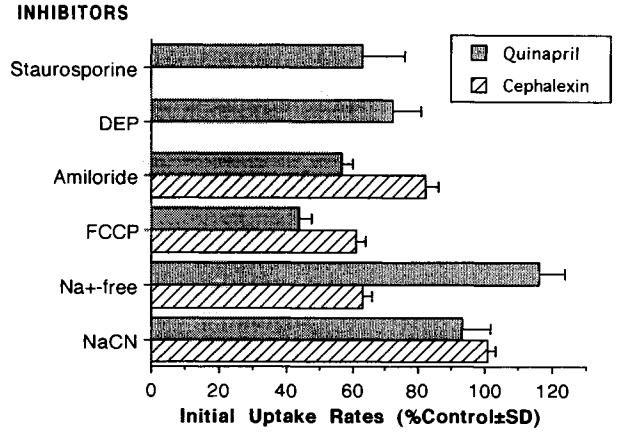

Fig. 5. Effect of various compounds on the uptake of 1 mM quinapril and 0.1 mM cephalexin. Initial uptake rates were calculated by dividing amount taken up with uptake time. The control values for the initial uptake were similar to those shown in Fig. 4. Each horizontal column represents the average of three determinations and the error bar represents standard deviation of the mean.

Moreover, the efflux of quinapril was also likely to be pH-dependent, as shown previously for other peptide substrates (4,7), since transport following a proton gradient was faster than transport with pH 6 at both sides. Taken together, the results suggest that the transepithelial proton gradient is the driving force for the transport of quinapril, although other nonsaturated pathways may also play a significant role (see below).

To further determine the absorption mechanisms of quinapril, effects of temperature, concentration and inhibitors were measured. The results indicated that the quinapril transport was more sensitive to temperature change than

cephalexin, but was less sensitive to concentration change than cephalexin as concentration increased. These results suggest that quinapril probably has weak affinity to the carrier as represented by higher K_m value than cephalexin, which is probably the reason why raising media pH completely abolished its affinity to the transporter. However, because 1 mM quinapril transport was only approximately 50-55% inhibitable in the presence of various inhibitors, nonsaturable component probably made significant contribution to the transport. On the other hand, the less than 90% inhibition could also be partially attributed to a high quinapril concentration (1 mM versus 0.1-0.2 mM for cephalexin). A lower quinapril concentration was not used to avoid inadequate detection by HPLC.

Finally, the kinetic characteristics of uptake and transcellular transport were compared. The results indicated that the quinapril transport was quantitatively more sensitive to inhibition by peptides and peptide analogs than uptake and accumulation. Therefore, it is possible that the inhibitors mainly acted on the basolateral efflux of quinapril. In contrast, cephalexin transcellular transport was quantitatively less sensitive to inhibition by peptides and analogs than uptake and accumulation. Therefore, it is possible that the inhibitors mainly acted on the apical uptake of cephalexin, as observed previously using loracarbef as the substrate (7). Taken together, these results suggest that the same inhibitor may affect the transcellular transport differently, depending on whether the uptake or the efflux is more sensitive to its effect.

In summary, quinapril transport is driven by a transepithelial proton gradient via a carrier-mediated mechanism with a significant contribution from a nonsaturable component, which is not necessary the passive diffusion. The transport characteristics, while qualitatively similar to that of cephalexin, have significant quantitative difference, suggesting different combinations of transport mechanisms for the transport of quinapril and cephalexin.

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REFERENCES

1. A. Dantzig and L. Bergin. Uptake of the cephalosporin, cephalexin, by a dipeptide transport carrier in the human intestinal cell line, Caco-2. Biochim. Biophys. Acta. 1027:211-217 (1990).
2. K. Inui, M. Yamamoto, and H. Saito. Transepithelial transport of oral cephalosporins by monolayers of intestinal epithelial cell line Caco-2: Specific transport systems in apical and basolateral membranes. J. Pharmacol. Expt. Ther. 261:195-201 (1992).
3. A. H. Dantzig, L.B. Tabas, and L. Bergin. Cefaclor uptake by the proton-dependent dipeptide transport carrier of human intestinal Caco-2 cells and comparison to cephalexin uptake. Biochim. Biophys. Acta, 1112:167-173, 1992.
4. D.T. Thwaites, C.D.A. Brown, B.H. Hirst, and N.L. Simmons. H+-Coupled dipeptide (glycylsarcosine) transport across apical and basal borders of human intestinal Caco-2 cell monolayers display distinctive characteristics. Biochim. Biophys. Acta., 1151:237-245 (1993)
5. 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)
6. C. H. Gochoco, F.M. Ryan, J. Miller, P.L. Smith, I.J. Hidalgo. Uptake and transcellular transport of orally absorbed cephalosporin cephalexin, in the human intestinal cell line, Caco-2. Int. J. Pharm., 104:187-202, 1994
7. M. Hu, J. Chen, Y. Zhu, A.H. Dantzig, R.E. Stratford, Jr, M.T. Kuefeld. Mechanism and kinetics of transcellular transport of a new b-lactam antibiotic loracarbef across an intestinal epithelial membrane model system (Caco-2). Pharm. Res., 11:1405-1413, 1994
8. A. H. Dantzig, D.C. Duckworth, and L.B. Tabas. Transport mechanism responsible for the absorption of loracarbef, cefixime, and cefuoroxime axetil into human intestinal Caco-2 cells. Biochim. Biophys. Acta, 1911:7-13 (1994)
9. D. I. Friedman and G. L. Amidon. Passive and carrier-mediated intestinal absorption components of two angiotensin-converting enzyme (ACE) inhibitor prodrugs in rats: enalapril and fosinopril. Pharm. Res. 6:1043-1047 (1989).
10. D. I. Friedman and G. L. Amidon. Intestinal absorption mechanism of dipeptide angiotensin converting enzyme inhibitors of the lysyl-proline type: lisinopril and SQ 29852. J. Pharm. Sci., 78, 995 (1989)
11. M. Hu and G. L. Amidon. Passive and carrier-mediated intestinal absorption components of captopril. J. Pharm. Sci., 77, 1007-1011 (1988).
12. Yee, Shiyin. Oral absorption of ACE inhibitors. Ph.D. Thesis, University of Michigan, Ann Arbor, MI. (1992)
13. M.T. Kuhfeld, J. Chen, M. Hu, and R.E. Stratford, Jr. Design of a novel diffusion apparatus compatible with Millicell permeable support and its characterization using Caco-2 cells. AAPS 7th National Meeting, Orlando, FL.
14. 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)
15. M. Bradford. A rapid and sensitive method for the determination of microgram quantities of protein utilizing the principles of protein-dye binding. Anal. Biochem. 72:248-254 (1976)
16. Alan R. Kugler. "Determinants of renal drug elimination: effect of renal metabolism on the renal disposition of quinapril and quinaprilat." A Ph.D. thesis, The University of Michigan, Ann Arbor, MI, 1993.
17. W. Kramer, C. Dechent, F. Girbig, U. Gutjahr, and H. Neubauer. Intestinal uptake of dipeptides and β-lactam antibiotics I. The intestinal uptake system for dipeptides and β-lactam antibiotics is not part of a brush border membrane peptidase. Biochim. Biophys. Acta., 1030, 41-49 (1990)
18. Y.J. Fei, Y. Kanal, S. Nussberger, V. Ganapathy, F.H. Leibach, M. F. Romero, S.K. Singh, W. F. Boron, M.A. Hediger. Expression cloning of a mammalian proton-coupled oligopeptide transporter. Nature, 368, 563-566 (1994)
19. M. Brandsch, Y. Miyamoto, V. Ganapathy, and F. H. Leibach. Expression and protein kinase C-dependent regulation of peptide/H^+ co-transport system in the Caco-2 human colon carcinoma cell line. Biochem. J., 299, 253-260 (1994)
20. H. Yuasa, D. Fleisher, and G. L. Amidon. Noncompetitive inhibition of cephradine uptake by enalapril in rabbit intestinal brush-border membrane vesicles: an enalapril specific inhibitory binding site on the peptide carrier. J. Pharmacol. Expt. Ther., 269, 1107-1111, 1994