ELSEVIER

International Journal of Pharmaceutics 140 (1996) 175–183

international journal of pharmaceutics

Uptake and transport of the ACE-inhibitor ceronapril (SQ 29852) by monolayers of human intestinal absorptive (Caco-2) cells in vitro

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Received 4 April 1996; accepted 30 April 1996

Abstract

The angiotensin-converting enzyme (ACE)-inhibitor ceronapril (SQ 29852) is shown to be a substrate of the intestinal dipeptide transporter. Uptake by Caco-2 cells, grown as confluent monolayers, follows a major saturable pathway (K_m, 0.91 ± 0.11 mM; ~90% at 1 mM) together with a minor passive component (k_d, 32.3 ± 6.6 ng $(10^6$ cells$)^{-1}$ $(20$ min$)^{-1}$. Uptake was inhibited by competition with dipeptides such as L-Ala-L-Pro (K_i, 2.96 mM) and L-Phe-Gly (K_i, 3.84 mM) but not by cephalosporins such as cephalexin. In contrast, transport was non-saturable, flux increased linearly with concentration and data were consistent with a passive transepithelial transport mechanism. Transport profiles showed a biphasic dependence upon time with an initial flux of 0.83 ± 0.02 ng insert^{-1} min^{-1} (k_1) and a terminal value of 1.65 ± 0.08 ng insert^{-1} min^{-1} (k_2) at 100 μM. It is concluded that the basolateral efflux is retarded so that the passive paracellular transport controls the overall transepithelial transport characteristics in the Caco-2 model. Carrier-mediated uptake into intestinal enterocytres, followed by rate-limiting basolateral efflux, may explain the extended t_{max} in vivo following oral administration.

Keywords: ACE-inhibitor; Caco-2 cells; Intestinal dipeptide transporter; Gastrointestinal uptake; Radioimmunoassay

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1. Introduction

Angiotensin-converting enzyme (ACE)-inhibitors are important therapies in the treatment of hypertension and congestive heart failure and act by limiting the conversion of angiotensin I to angiotensin II, a vasoconstrictor, through competition with the natural substrate. Active entities

PII S0378-5173(96)04582-6

are stable, dipeptide-like structures and there has been much interest in the mechanisms through which absorption from the gastrointestinal tract is mediated. A peptide transporter, which serves di- and tri-peptides, but not larger units (Addison et al., 1972; Matthews, 1991), has been identified and the existence of more than one transporter has been suggested (Daniel et al., 1991; Skopicki et al., 1991; Iseki et al., 1989). To facilitate such studies, Caco-2 cell monolayers, a line derived from a human colon carcinoma which demonstrates enterocyte morphology and absorptive behaviour, is now used extensively as a model for the study of intestinal drug transport (Artursson, 1991; Nicklin et al., 1995). The expression of a dipeptide-transporter in culture was demonstrated (Dantzig and Bergin, 1988, 1990) with D-cephalexin, the uptake of which could be described by both saturable and non-saturable pathways. In sodium-free conditions, uptake was inhibited by a range of dipeptides but not by amino (and imino) acids and stereoselectivity, present as L-cephalexin, was a more potent inhibitor than the D-enantiomer. Uptake is pH-sensitive with steady-state accumulation at pH 6.0 being some ten-fold higher than at pH 7.2. Furthermore, the rate of transport from apical (pH 5.0–6.5) to basolateral (pH 7.4) compartments was double that seen when the pH values were similar (7.0–7.4 → 7.4) (Gochoco et al., 1994). In contrast, cephalexin transport from the basolateral to apical surface was not pH-dependent; similar observations on other substrates have been made (Hori et al., 1993; Saito and Inui, 1993) confirming the presence of distinguishable transporters on apical and basolateral surfaces. These observations suggest the cephalexin transporter in Caco-2 cells is located at the apical surface and poor transport characteristics probably reflect poor basolateral efflux although the passive paracellular component may also be important, particularly in vivo. Cefaclor uptake into Caco-2 monolayers is mediated by the same proton-dependent dipeptide-carrier as cephalexin (Dantzig et al., 1992) and carrier-mediated uptake and transport of cephradine via a peptide-transporter by Caco-2 monolayers has been reported (Inui et al., 1992). Cephradine uptake occurs preferentially at the apical surface, it is maximal at pH 6.0 and it is reduced significantly at 4°C. Moreover, uptake is saturable and is inhibited by a range of peptides. Similarly, cephradine transport is vectorial (apical-to-basolateral transport > basolateral-to-apical), is pH-dependent (maximal at pH 6.0–6.5) and is selectively inhibited by a range of dipeptides. Efflux from pre-loaded Caco-2 cells occurs at a greater rate from the basolateral surface and, again, this is markedly reduced at 4°C. Transport is usually driven by an inward proton gradient (Matthews, 1991), although peptide flux in its absence is known (Rajendran et al., 1987). The instability of simple dipeptides has limited the extension of these studies but the effect of the linked proton-gradient has been used successfully to monitor uptake by intracellular pH measurement (Thwaites et al., 1993a,b,c). The presence of a polarised efflux system for peptides in the apical membrane of Caco-2 cells, consistent with a P-glycoprotein-mediated efflux mechanism for peptides in Caco-2 cells, has also been demonstrated (Burton et al., 1993) using methylated D-phenylalanine oligomers.

Such carrier-mediated processes have been implicated in determining the absorption profile of ACE-inhibitors after oral medication in vivo. For example, although the bioavailability of captopril (60–75% (Kubo and Cody, 1985)) is dose-independent (Duchin et al., 1982), its absorption is reduced markedly in the presence of food (Singhvi et al., 1982). A carrier-mediated absorption pathway has been implicated in an in situ rat intestine luminal perfusion model (Hu and Amidon, 1988) where the rate of luminal disappearance was concentration-, energy-, pH- and sodium-dependent. Absorption was also inhibited by a range of dipeptides and the β-lactam antibiotic, cephradine. Active transport has been similarly implicated in the absorption of other ACE inhibitors. Enalapril, a prodrug (bioavailability 60–70% (Kubo and Cody 1985)) has a saturable uptake component (K_m, 0.07 mM) which is inhibited by the dipeptide L-tyrosylglycine and cephradine, but not by amino acids (Friedman and Amidon, 1989a) but, in the same study, the uptake of a heavily substituted ACE inhibitor, fosinopril, was shown to occur via a non-satu-

rable (passive) mechanism. Additionally, two ACE inhibitors of the lysyl-proline class, lisinopril and ceronapril (SQ 29852), undergo carrier-mediated absorption from the rat intestine (Friedman and Amidon, 1989b).

Ceronapril (Fig. 1. (*S*)-1-(6-amino-2-([hydroxy-(4-phenylbutyl)-phosphinyl]oxy)-1-oxo-hexyl)--L-proline (SQ 29852)) is a potent ACE inhibitor in vitro (IC_{50}, 36 nM) and in vivo following intravenous (ED_{50}, 0.063 μmol kg^{-1}) or oral (ED_{50}, 0.53 μmol kg^{-1}) administration to rats (Karenewsky et al., 1988). After oral administration to human volunteers, its plasma appearance occurs slowly (t_{max}, ~ 4 h) and, is dose-dependent (Moore et al., 1988). Moreover, its luminal disappearance from the in situ rat intestine (Friedman and Amidon, 1989b) and intestinal ring (Stewart et al., 1990) model has both passive and carrier-mediated components; disappearance is also inhibited by L-tyrosylglycine and cephradine, but not by amino acids. Although it has been suggested that a carrier-mediated uptake mechanism may account for the non-linear pharmacokinetics in humans, no direct interaction between ceronapril and the peptide transporter has been established. The present study was designed to investigate the possibility of carrier-mediated ceronapril uptake into monolayers of human intestinal absorptive (Caco-2) cells and to assess the importance of this mechanism in the transepithelial transport of this drug (Nicklin et al., 1992c).

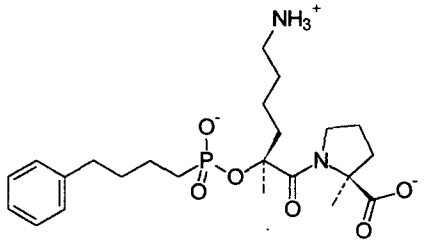

Fig. 1. Ceronapril (SQ29852).

2. Materials and methods

2.1. Materials

Ceronapril radioimmunoassay (RIA) kits were provided by Bristol-Myers Squibb, USA and ceronapril and cephradine were supplied by Bristol-Myers Squibb, UK. The culture medium comprised Hanks' balanced salt solution containing bovine serum albumin (0.1% w/v), phenol red (0.01%) and D-glucose (5 mM). Solutions were buffered with *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulphonic acid] (HEPES; 14 mM; pH, 7.4) or with 2-(*N*-morpholino)ethane sulphonic acid (MES, pH 6.0).

2.2. Methods

2.2.1. Uptake experiments

Ceronapril uptake experiments were performed using Caco-2 monolayers cultured on impermeable plastic supports (Nicklin et al., 1992a,b). Briefly, 18 d Caco-2 monolayers were initially washed (2×5 ml $\times$ 5 min) with phosphate-buffered saline (PBS) and finally (1×2 ml $\times$ 15 min) with the medium (pH 6.0) before the ceronapril in the medium (pH 6.0) was applied to their surface. After a 15 min incubation at 37°C, the donor solution was aspirated and the monolayers carefully rinsed (3×5 ml $\times$ 5 min) with ice-cold PBS- sodium azide stop-solution. Each Caco-2 monolayer was solubilised by shaking (2 min) with 1 ml of 0.1% v/v Triton X100 (TX100) in double distilled water followed by 10 μl of 4 M sodium hydroxide. The solubilised monolayers were neutralised by 10 μl of 4 M hydrochloric acid. The ceronapril concentration in the solubilised Caco-2 monolayers was determined by radioimmunoassay.

2.2.2. Transport experiments

Ceronapril transport studies were performed using Caco-2 monolayers cultured for 21 d on nitrocellulose permeable-supports (Nicklin et al., 1992a,b). Briefly, 21 d Caco-2 monolayers were washed ($1 \times$ (*apical*: 2 ml medium, pH 6.0 + *basolateral*: 2 ml medium, pH 7.4) $\times$ 15 min) before ceronapril in the medium (pH 6.0) was applied to

Table 1
Protocol for the radioimmunoassay of ceronapril

Assay	Tube	1 Assay medium (μl)	2 Sample (10 μl, ng ml^{-1})	3 [^{125}I]ceronapril (μl)	4 Ceronapril antiserum (μl)
Totals	1, 2	None	None	200	None
Standards	3, 4	10	0	200	100
	5, 6	10	1	200	100
	7, 8	10	5	200	100
	9, 10	10	10	200	100
	11, 12	10	25	200	100
	13, 14	10	50	200	100
	15, 16	10	100	200	100
	17, 18	10	250	200	100
	19, 20	10	500	200	100
Controls	21, 22	10	Control 1	200	100
	23, 24	10	Control 2	200	100
Samples	25, 26	10[a]	Sample 1	200	100
	..N, $N+1$	10[a]	Sample N	200	100

[a] Standard diluent, potassium phosphate buffer.

the apical surface. Apical to basolateral transport (pH 6.0–7.4) was determined at 30 min intervals for kinetic studies or after a fixed 1 h time-point. Ceronapril concentrations in the receiver solutions were determined by RIA.

2.2.3. Ceronapril radioimmunoassay protocol

For uptake studies, monolayer-associated ceronapril was assayed following a 15 min incubation. The assay-medium used for the calibration standards and control samples (tubes 3 through 24) comprised Caco-2 monolayers solubilised by 0.1% v/v TX100 in double distilled water. This was prepared from Caco-2 monolayers cultured for 18 d in an identical manner to those used for uptake experiments. The monolayers were solubilised by shaking (2 min) with 1 ml of 0.1% v/v TX100 in double distilled water followed by 10 μl of 4 M sodium hydroxide. The solubilised monolayers were neutralised by the addition of 10 μl of 4 M hydrochloric acid and used as the assay-medium for the ceronapril RIA. For transport studies, the receiver-solution was assayed for ceronapril content. The medium (pH 7.4) was used as the assay-medium for the calibration standards and control samples (tubes 3 through 24).

Tubes (polyethylene LP3, Luckman, UK) 1 and 2 represent the total counts, tubes 3 through 20 constitute a standard curve (0–500 ng ml^{-1}) and tubes 21, 22 and 23, 24 are sample controls I and II, respectively. Two additional tubes were required for a duplicate assay of each ceronapril sample. The RIA protocol is summarised in Table 1. Briefly, 10 μl of receiver-medium (solubilised Caco-2 monolayer or the medium, pH 7.4, for uptake or transport studies, respectively) was added to tubes 3 through 24 and an equal volume of standard diluent to each sample tube. [^{125}I]ceronapril (200 μl) was added to each tube followed by 100 μl of diluted ceronapril-antiserum to tubes 3 onwards. The tubes were vortexed gently and incubated overnight at 4°C. The ceronapril bound to the primary antibody was separated from unbound ceronapril by specifically precipitating the ceronapril-primary antibody complex with 1 ml of separant (1% v/v goat anti-rabbit IgG in 7.5% w/v polyethylene glycol in potassium phosphate buffer; 4°C). Each tube was gently vortexed and incubated at 4°C for 10 min. Tubes 3 onwards were centrifuged at 3000 rpm for 10 min at 4°C (Chilspin, Fisons, UK) to pellet the precipitate. The supernatant (containing the unbound ceronapril fraction) was decanted and the tube inverted for 10 min on absorbent paper. Excess supernatant on the walls of each tube was removed with a wick of absorbent paper. The

[^{125}I]-content of each pellet (which represented the bound [^{125}I]ceronapril) was determined by gamma counting for 1 min using an LKB Multigamma II gamma counter.

Standard calibration-curves were prepared using an assay-medium comprising Caco-2 monolayers solubilised with 0.1% v/v TX100 in double distilled water (uptake studies) or the medium pH 7.4 (transport studies), as appropriate. In both cases, a cubic function (i.e. $y = a_0 + a_1x + a_2x^2 + a_3x^3$) could be fitted to the standard curve of pellet-associated CPM versus log (ceronapril) profile. Typical results were: (i) in the presence of 0.1% TX100. $a_0 = 17361$, $a_1 = 316.22$, $a_2 = -6430.9$, $a_3 = 1563.6$, $r^2 = 0.998$; (ii) without added TX100, $a_0 = 17661$, $a_1 = 271.08$, $a_2 = -6420.0$, $a_3 = 1556.8$, $r^2 = 0.997$. Sample concentrations were calculated using the equation which described the best fit through the standard curve data.

The apparent permeability coefficient (P_{app} cm s^{-1}) of ceronapril was calculated from Eq. 1 (Artursson and Magnusson, 1990):

$$P_{app} = \frac{dM/dt}{A \cdot [S_0]} \quad (1)$$

where dM/dt is the rate of appearance of substrate in the receiver phase (mmol s^{-1}), A is the area of the permeable support (cm^2) and $[S_0]$ is the initial concentration of substrate in the donor compartment (mmol ml^{-1}).

The transport inhibition constant (K_i mM) of competitors was estimated from the observed inhibition of carrier-mediated ceronapril uptake using the ratio (r) of the uptake rate in the absence (v_0) of an inhibitor to the value in the presence (v_i) of an inhibitor ($r = v_0/v_i$) according to Eq. 2 (Nicklin et al., 1995):

$$K_i = \frac{K_m \cdot [I]}{(r-1) \cdot (K_m + [S])} \quad (2)$$

where K_m is the Michaelis constant (mM) and [S] and [I] are the concentrations of substrate and inhibitor (mM), assuming that the compounds inhibit transport competitively. The approximate solution to this equation (Takada et al., 1991; Walker et al., 1994), where $K_i \approx [I]/(r-1)$ for cases where $K_m \gg [S]$, is in error by $\sim 10\%$ for the conditions used here.

3. Results and Discussion

Ceronapril uptake into Caco-2 monolayers was concentration-dependent over the range of 10 μM to 5 mM as shown in Fig. 2A. The shape of this curve suggests that both active and passive processes are involved, as described by Eq. 3 where dM/dt is the initial rate of ceronapril uptake at a given substrate concentration [S], V_{max} is the maximum transport rate, K_m is the Michaelis constant, equal to the substrate concentration at which the reaction rate is half its maximum value, and k_d is the passive diffusional rate constant.

$$\frac{dM}{dt} = \frac{V_{max}[S]}{K_m + [S]} + k_d[S] \quad (3)$$

Total ceronapril uptake was resolved into saturable and non-saturable components using this model by least-squares non-linear regression analysis (Irwin, 1990). The saturable component was characterised by a K_m of 0.91 ± 0.11 mM and a V_{max} of 538 ± 46 ng (10^6 cells)$^{-1}$ (20 min)$^{-1}$ while the non-saturable process had a k_d of 32.3 ± 6.6 ng (10^6 cells)$^{-1}$ (20 min)$^{-1}$ mM^{-1}. At substrate concentrations of 1 mM or less, this equates with over 90% of the uptake being accounted for by an active process. The observed K_m value contrasts with an estimate of 0.08 mM for the K_m of the carrier-mediated uptake of ceronapril in the rat intestine (Friedman and Amidon, 1989b; Stewart et al., 1990) which is an order of magnitude lower than that found here; a discrepancy which may reflect inter-species or model differences. Despite this highly saturable uptake-mechanism, the apical-to-basolateral (pH 6.0 → 7.4) transport of ceronapril was proportional to the concentration in the apical donor solution between 50 μM and 10 mM (Fig. 2B). When transport profiles, dependent upon time, were measured from donor solutions containing 100 μM and 500 μM of ceronapril, across confluent Caco-2 monolayers, transport appeared to be biphasic (Fig. 3). The initial linear phase, which continued for over 60 min, showed no lag period and revealed rate constants (k_1) of 0.83 ± 0.02 ng insert^{-1} min^{-1} (100 μM) and 4.17 ± 1.01 ng insert^{-1} min^{-1} (500 μM). The second, faster, phase corresponded to lag times for transepithe-

lial transfer of 48.7 min (100 μM) and 59.3 min (500 μM) and rate constants (k_2) of 1.65 ± 0.08 ng insert^{-1} min^{-1} (100 μM) and 8.53 ± 1.17 ng insert^{-1} min^{-1} (500 μM). The direct interaction of ceronapril with the monolayer, to compromise integrity by progressively increasing permeability,

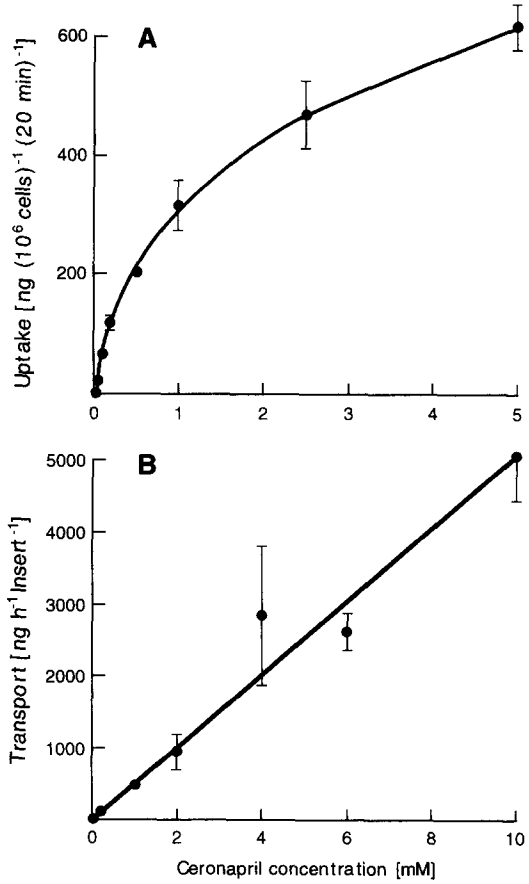

Fig. 2. Concentration-dependence of ceronapril uptake and transport. (A) Uptake: Caco-2 monolayers were washed (*apical*: 1 × 2 ml × 15 min) with medium (pH 6.0). Ceronapril uptake was determined for a range of apical concentrations (10 μM–5 mM). The total uptake was resolved into saturable (K_m, 0.91 ± 0.11 mM; V_{max}, 538 ± 46 ng (10^6 cells)$^{-1}$ (20 min)$^{-1}$) and non-saturable (k_d, 32.3 ± 6.6 ng (10^6 cells)$^{-1}$ (20 min)$^{-1}$ mM^{-1}) components by non-linear regression analysis. (B) Transport: Caco-2 monolayers were washed (*apical*: 1 × 2 ml × 15 min) with medium (pH 6.0) and (*basolateral*: 1 × 2 ml × 15 min) with medium at pH 7.4. Apical (pH 6.0) to basolateral (pH 7.4) Ceronapril transport was determined for a range of concentrations (50 μM–10 mM). Data are presented as mean values ± SD for three monolayers.

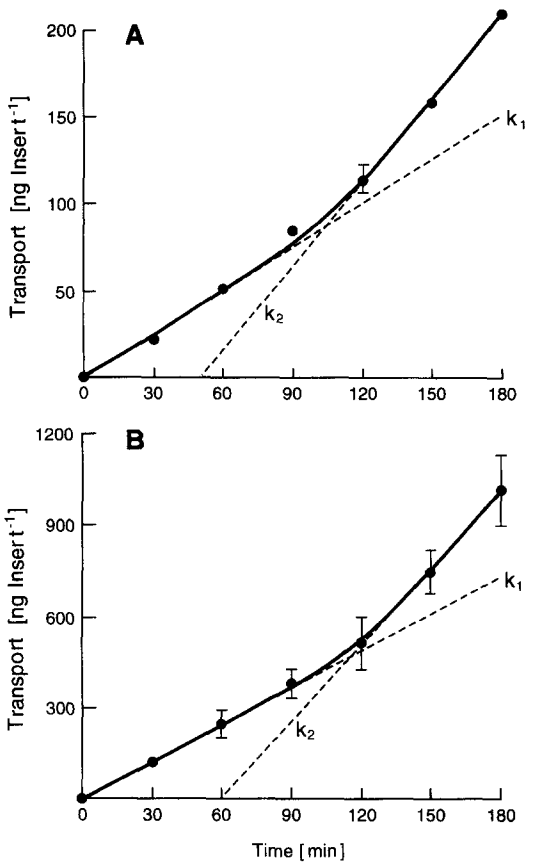

Fig. 3. Ceronapril transport kinetics from (A) 100 μM and (B) 500 μM donor solutions. Caco-2 monolayers were washed (*apical*: 1 × 2 ml × 15 min) with the medium (pH 6.0) and (*basolateral*: 1 × 2 ml × 15 min) with medium at pH 7.4. Ceronapril, 100 μM (A) or 500 μM (B) in medium at pH 6.0, was applied to their apical surface and its appearance in the basolateral receiver-solution was determined at 30 min intervals. Slopes are: (A) k_1, 0.83 ± 0.02 ng insert^{-1} min^{-1}; k_2, 1.65 ± 0.08 ng insert^{-1} min^{-1}; (B) k_1, 4.17 ± 1.01 ng insert^{-1} min^{-1}; k_2, 8.53 ± 1.17 ng insert^{-1} min^{-1}. Data are presented as mean values ± SD for three monolayers.

does not appear likely as the increase in each rate constant (5.02- and 5.17-fold) directly reflects the five-fold increase in donor concentration and parallels the linearity seen in Fig. 2B.

To explore the nature of the active, saturable process, inhibition of ceronapril uptake into, and transport across, Caco-2 monolayers by a range of small peptides or β-lactam antibiotics, as competitive substrates for the intestinal dipeptide

Table 2
Cross-inhibition of ceronapril (100 μM) uptake and transport in the presence competitors (10 mM)

Competitor	Inhibition (%) (mean ± S.D.)		Uptake inhibition constant (K_i mM)
	Uptake	Transport	
L-Ala-L-Pro	75.3 (9.4)[a]	3.6 (38.1)	2.96
Cephalexin	6.3 (1.3)	14.9 (10.9)	134
Cephadrine	14.3 (5.2)	29.1 (10.5)	54
L-Phe-Gly	70.1 (5.5)[a]	30.2 (55.6)	3.84
L-Phe-Gly-Gly	NT	30.9 (10.9)	
TRH[b]	NT	−6.7 (17.8)	
L-Try-Gly	55.6 (4.5)[a]	39.1 (33.4)	7.19
L-Try-Gly-Gly	NT	13.3 (14.8)	
L-Tyr-Gly (2 mM)	57.3 (9.4)[a]	NT	1.34

Data are presented as mean values ± standard deviation (S.D.) for three monolayers
NT, not tested.
[a] Significant inhibition, $P<0.01$.
[b] Thyrotrophin-releasing hormone.

transporter, was investigated (Table 2). Ceronapril uptake was significantly inhibited ($P < 0.05$) by the dipeptides: L-alanyl-L-proline (K_i 2.96 mM), L-phenylalanylglycine (K_i 3.84 mM), L-tryptophanylglycine (K_i 7.19 mM) and L-tyrosylglycine (K_i 1.34 mM). This confirms that ceronapril uptake into Caco-2 monolayers is a carrier-mediated, saturable process and parallels observations in the rat intestine, which suggest that ceronapril is a substrate for the intestinal peptide transporter (Friedman and Amidon, 1989b; Stewart et al., 1990). Neither cephalexin nor cephradine exerted a significant reduction on intracellular ceronapril levels. This contrasts with uptake from the rat intestine (Friedman and Amidon, 1989a) and is surprising as most of the work identifying the intestinal dipeptide transporter in culture has used cephalosporins as relatively stable dipeptide analogues. This observation may result from a relatively high K_i value for cephalosporins or it may indicate the existence of competing transporter subgroups with distinct structural specificities which interact differently with substrates and competitors.

Paradoxically, the rate of ceronapril transport, from apical to basolateral chambers, across Caco-2 monolayers is directly proportional to its concentration between 50 μM and 10 mM and is not significantly inhibited by the peptides which suppress uptake. This implies that, despite the observed carrier-mediated uptake into Caco-2 cells, the overall transepithelial transport of ceronapril are consistent with a passive mechanism. This implies that the major localisation of the dipeptide transporter is at the apical surface with little, if any, expression basolaterally. Ceronapril is hydrophilic (Log $D < -3.0$ at pH 7.0; Ranadrive et al., 1992) with three ionisation sites and, in the absence of a carrier-mediated basolateral export mechanism, the passive efflux of ceronapril across the basolateral lipid membrane would be expected to occur very slowly. Although ceronapril has an oral bioavailability of 67% in humans (Foley et al., 1988), it is poorly transported across Caco-2 monolayers. Indeed, its apparent permeability coefficient, calculated from the initial (0–60 min) rate (P_{app} $1.3 \pm 0.4 \times 10^{-8}$ cm s^{-1}; $P_{app} = v/A/[S]$) is less than that of PEG_{4000} (3.6 ± 0.1 cm s^{-1}) and predicts a low oral bioavailability ($< 1\%$) in man according to the correlation for purely passively absorbed compounds (Artursson and Karlsson, 1991). Such discrepancies may reflect the importance of carrier-mediation, the expression of fewer peptide-transporters in Caco-2 cells compared with normal absorptive cells in vivo or that more efficient carrier-mediated basolateral efflux mechanisms are present in normal absorptive cells.

These data highlight the possibility that ceronapril enters Caco-2 cells via an apical peptide-transporter but that its basolateral efflux may be retarded such that the passive and paracellular transport dictates the overall transepithelial transport characteristics. In support is the observation that the maximal plasma-concentration following the oral administration of ceronapril to humans is achieved only after 4 h (Moore et al., 1988). Its absorption from the GI tract, thus, occurs slowly, while the oral bioavailability is dose-dependent; an observation which may suggest either the importance of a saturable pathway or the existence of a narrow gastrointestinal absorption window. Later work, using SQ 29852 as a probe for the dipeptide transporter, suggests, however, that absorption occurs uniformly throughout the gastrointestinal tract in rats (Marino et al., 1996). The biphasic transport profile (Fig. 3) may reflect this difference showing distinct paracellular (k_1) and transcellular (k_2) transport pathways with the passive paracellular transport dictating the overall transepithelial transport characteristics. This work also illustrates a general concern that, although the Caco-2 monolayer model may be an excellent mechanistic tool which also provides a useful screen to identify drug candidates that recognise carrier-mediated transport pathways, it may be quantitatively unreliable for predicting the extent of absorption of molecules that are absorbed primarily by the dipeptide transporter and of those for which passive transport predominates (Chong et al., 1996).

Acknowledgements

The authors thank the Biotechnology and Biological Sciences Research Council (formerly SERC) for the award of a post-graduate research studentship (to PLN).

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