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Report

Passive and Carrier-Mediated Intestinal Absorption Components of Two Angiotensin Converting Enzyme (ACE) Inhibitor Prodrugs in Rats: Enalapril and Fosinopril

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The intestinal absorption mechanism of two ACE inhibitor prodrugs, enalapril and fosinopril, was investigated in rats using a single-pass perfusion method. A modified boundary layer solution was applied to determine the apparent intestinal wall permeability. The prodrug enalapril is well absorbed from rat jejunum, whereas the parent drug, enalaprilat, is poorly absorbed. The permeability of enalapril is concentration dependent and is decreased by the dipeptide Tyr-Gly and by cephradine but not by the amino acids L-leucine or L-phenylalanine, indicating a nonpassive absorption mechanism via the small peptide carrier-mediated transport system. In contrast, fosinopril is readily absorbed by a concentration-independent mechanism without the involvement of the peptide carrier.

KEY WORDS: angiotensin converting enzyme (ACE) inhibitors; cephradine interactions; drug absorption mechanism; enalapril; enalaprilat; fosinopril; prodrugs; Tyr-Gly.

INTRODUCTION

Recently it has been shown that captopril, the first orally active angiotensin converting enzyme (ACE) inhibitor to be widely used clinically, exhibits a nonpassive absorption pattern in rats (1). Like captopril, other ACE inhibitors are amino acid or dipeptide derivatives (Fig. 1), which suggests that they may also share the same carrier-mediated transport system of the amino acids or dipeptides. Enalapril is an Ala-Pro derivative containing two amino groups, one in the proline ring and a second in an α position to the peptide bond. Fosinopril contains only one amino group in the proline ring and a phosphinic group in the position corresponding to the α-amino group.

Enalapril and fosinopril are converted via deesterification to the active drugs by hepatic biotransformation. The active drugs are enalaprilat and SQ 27,519 (Fig. 1). It has been demonstrated that high doses of enalapril do not saturate hepatic biotransformation of the prodrug to the active diacid enalaprilat (2–5).

Enalaprilat, the diacid, binds slowly and tightly to ACE, producing well-defined clinical effects, but it is poorly absorbed from the gastrointestinal tract (6). SQ 27,519 (parent drug of fosinopril) is a potent ACE inhibitor, but like enalaprilat it exhibits a low bioavailability. Both prodrugs, enalapril and fosinopril, were developed for their superior oral absorption over their deesterified parent drugs. It is of interest to note that there are more than seven prodrugs of diacid type ACE inhibitors currently in clinical phase II or III, which are esterified in a similar manner to enalapril (7).

Enalapril oral absorption has been studied previously. Gardner *et al.* (8) studied the influence of different buffers and solutions on enalapril absorption in *in situ* rat perfusion in the pH range of 2.9 to 7.4 and postulated a narrow absorption window for enalapril due to its limited passive site-specific absorption in the duodenum. However, the hydrolysis of the prodrug to enalaprilat is not reported, nor is the concentration used reported in that study. While passive absorption may predominate in the duodenum, the fact that oral absorption occurs over several hours and that the extent of absorption is only 39–64% suggests that absorption may be occurring from the small intestine as well. In another study Pang *et al.* (9) used intestine-liver *in situ* rat vein perfusion in an experiment designed to investigate enalapril liver and jejunal metabolism. The liver was found to be the major transformation site of enalapril to enalaprilat, with apparently little contribution from the jejunum. In one case enalapril was dosed in to the duodenum and a very small fraction of enalapril and enalaprilat was subsequently collected from the GI tract. Levels of enalaprilat in the jejunum were higher than in the hepatic perfusion reservoir, which was interpreted as intestinal lumen hydrolysis of enalapril. However, no concentration study was done and only a small fraction was recovered.

In this report the mechanism of absorption of these two nonsulfhydryl ACE inhibitors is investigated in an *in situ* rat perfusion over a wide range of concentrations and with absorption inhibitors, e.g., amino acids, dipeptides, and cephradine which are transported via the amino acid or the peptide carrier system, respectively. In order to determine the

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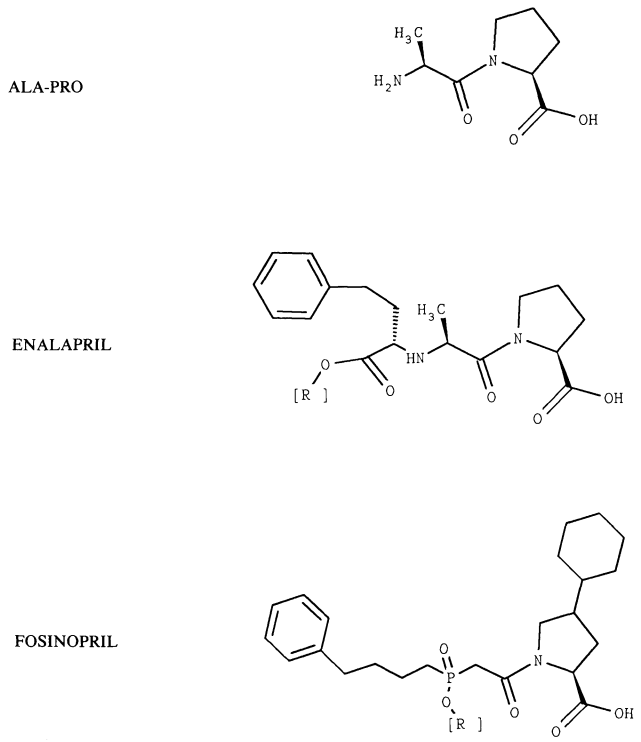

Fig. 1. The chemical structure of Gly-Pro, enalaprilat ([R] = H) and SQ 27,519 ([R] = H) and their respective prodrugs enalapril ([R] = ethyl) and fosinopril (SQ 28,555) ([R] = isobutandiol acetate).

absorption mechanism the results are analyzed according to the modified boundary layer approach published previously (10,11) to describe carrier-mediated transport.

EXPERIMENTAL

Materials

Fosinopril sodium (batch NN037NB) and cephradine (batch 3146412167629) was generously provided by Squibb & Sons (Princeton, NJ). Enalapril maleate (batch L-154,739-001D076) and enalaprilat (batch L-154,628-004Z007) were kindly supplied by Merck Sharp and Dohme Research Laboratories (Rahway, NJ). ^{14}C-Polyethylene-glycol (Du Pont-NEN, Boston, MA), Tyr-Gly, L-leucine, L-phenylalanine, and polyethylene-glycol 4000 (Sigma Chemical Co., St. Louis, MO) were used. All buffer and mobile-phase components were analytical or HPLC grade and used as received.

Perfusate Solution

The perfusion solution consisted of Sorensen phosphate and citrate buffers of pH 6.0 and 5.0, respectively, 0.01% PEG 4000 with a trace amount of its ^{14}C isotope, enalapril maleate or fosinopril sodium, and NaCl to adjust the final solution osmolality to 290 (±10) mOsm/kg.

Rat Perfusion

The method has been described previously (1,10) and was used without modification. Throughout the inhibition experiments, two adjacent segments of the jejunum of the same animal were perfused (8). One segment was perfused with the tested drug and the other with the drug and the inhibitor, so that each animal served as its own control.

Assay methods

Radioactivity. Sample (0.5 mL) was mixed with 10 ml of scintillation cocktail (Bio-Safe, RPI, Mount Prospect, IL) and counted using a Beckman LS-9000 counter (Beckman Instruments Inc., Fullerton, CA). Readings of the nonabsorbed marker ^{14}C-polyethylene-glycol were analyzed to quantify volume changes.

HPLC. The instrumentation consisted of two Model 510 pumps, a WISP Model 712 automatic sampler, and a Model 481 UV detector (Waters, Milford, MA). Data acquisition and integration were performed with a Waters Baseline 810 software package. Enalapril maleate was hydrolyzed with sodium hydroxide to enalaprilat and then separated on a reverse-phase column, Ultrasphere ODS, 5 μm (Beckman, San Ramon, CA). The mobile phase consisted of 12% acetonitrile in 0.05 *M* phosphate buffer at pH 3.2. Samples were eluted at a flow rate of 1 ml/min. Fosinopril sodium was separated on a reverse-phase column, μBondapack C18, 10 μm (Waters Milford, MA). The mobile phase consisted of 65% acetonitrile and 8% methanol in 0.05% phosphate buffer acidified with phosphoric acid to pH 3.5. Samples were eluted at a flow rate of 3.0 ml/min.

Hydrolysis Study

Samples were assayed for enalaprilat twice, as collected and after the conversion to enalaprilat. The first assay served to evaluate enalapril stability or hydrolysis to enalaprilat while perfused through the jejunum, and the second to measure output/input enalapril levels. Direct HPLC detection of enalapril at the very low concentrations studied was hindered by jejunal exudates. The sensitivity of the enalaprilat HPLC assay was also better than that for enalapril.

Data Analysis

Water transport, estimated membrane permeability, and the presence of nonpassive transport were determined as described before (10,11).

Equation (1) was used to describe the Michaelis–Menten saturable carrier transport process.

$$P*\mathrm{w} = J*\mathrm{max}/(K_m + C_{\mathrm{wall}}) + P*\mathrm{m} \qquad (1)$$

where $P*$w is the jejunal wall permeability, $J*$max is the maximal flux, K_m is the Michaelis constant, C_{wall} is the drug concentration at the jejunal wall, and $P*$m is the passive permeability.

RESULTS AND DISCUSSION

A concentration-dependent permeability and competitive inhibition are fundamental properties of carrier-mediated transport. The two prodrugs were studied in this manner in order to elucidate their absorption mechanism in rat jejunum. The *in situ* experimental input/output drug level differences are interpreted as loss due to membrane trans-

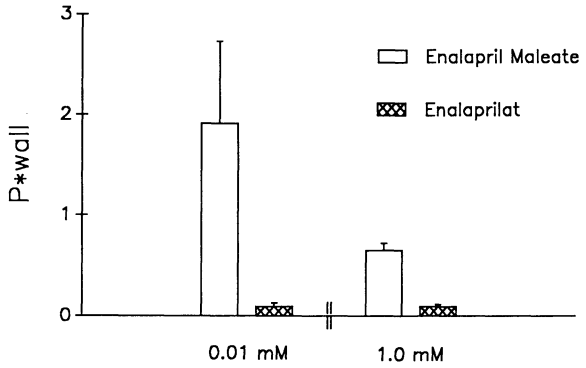

Fig. 2. The dimensionless wall permeability (P*wall) of enalapril and enalaprilat at low (0.01 mM) versus high (1.00 mM) concentrations. Mean of four to eight rats ± SD.

port in the perfused segment. Reduced output levels could not result from instability of the drugs as enalapril and fosinopril were found to be stable for 24 hr in the different buffers and perfused buffers studied (less than 2% hydrolyzed after 24 hr at room temperature).

In addition, no enalaprilat was detected in the perfusate after passing through the jejunal segment. The limit of detection of enalaprilat HPLC assay is 0.05 μM or 0.5% of the lowest enalapril concentration studied. Enalaprilat levels beyond the detection limits of the assay are negligible. This result indicates that enalapril is not hydrolyzed significantly in the jejunum in this type of experimental system. Traces of enalaprilat that were found by Pang *et al.* (9) when enalapril was injected into the duodenum could be attributed to pancreatic enzyme hydrolysis since enalaprilat was measured after 3 hr of intestinal exposure in comparison to a 6- to 8-min average resident time of enalapril in this experimental system.

The permeability of enalapril in fasted rat jejunum was found to be concentration dependent, whereas enalaprilat permeability was much lower and not influenced by a change of two orders of magnitude in concentration (Fig. 2). The wall permeability (P*wall) of enalapril at 1.000 mM was significantly lower than at 0.010 mM ($P < 0.001$). The permeability of enalapril over a wide range of concentrations was determined (Fig. 3) and fitted to a Michaelis–Menten model [Eq. (1)]. The carrier absorption parameters were calculated to be as follows: J*max, 0.13; K_m, 0.07 mM; P*c, 1.9; and P*m 0.35. These results indicate that carrier-mediated transport dominates enalapril absorption in the rat jejunum. Furthermore, enalapril absorption at low concentrations (0.01 to 0.02 mM) is decreased when coperfused with 10 mM Tyr-Gly ($P < 0.001$) or 1 mM cephradine ($P < 0.001$), which has been shown to be absorbed by the peptide carrier in rat jejunum (11) (Fig. 4).

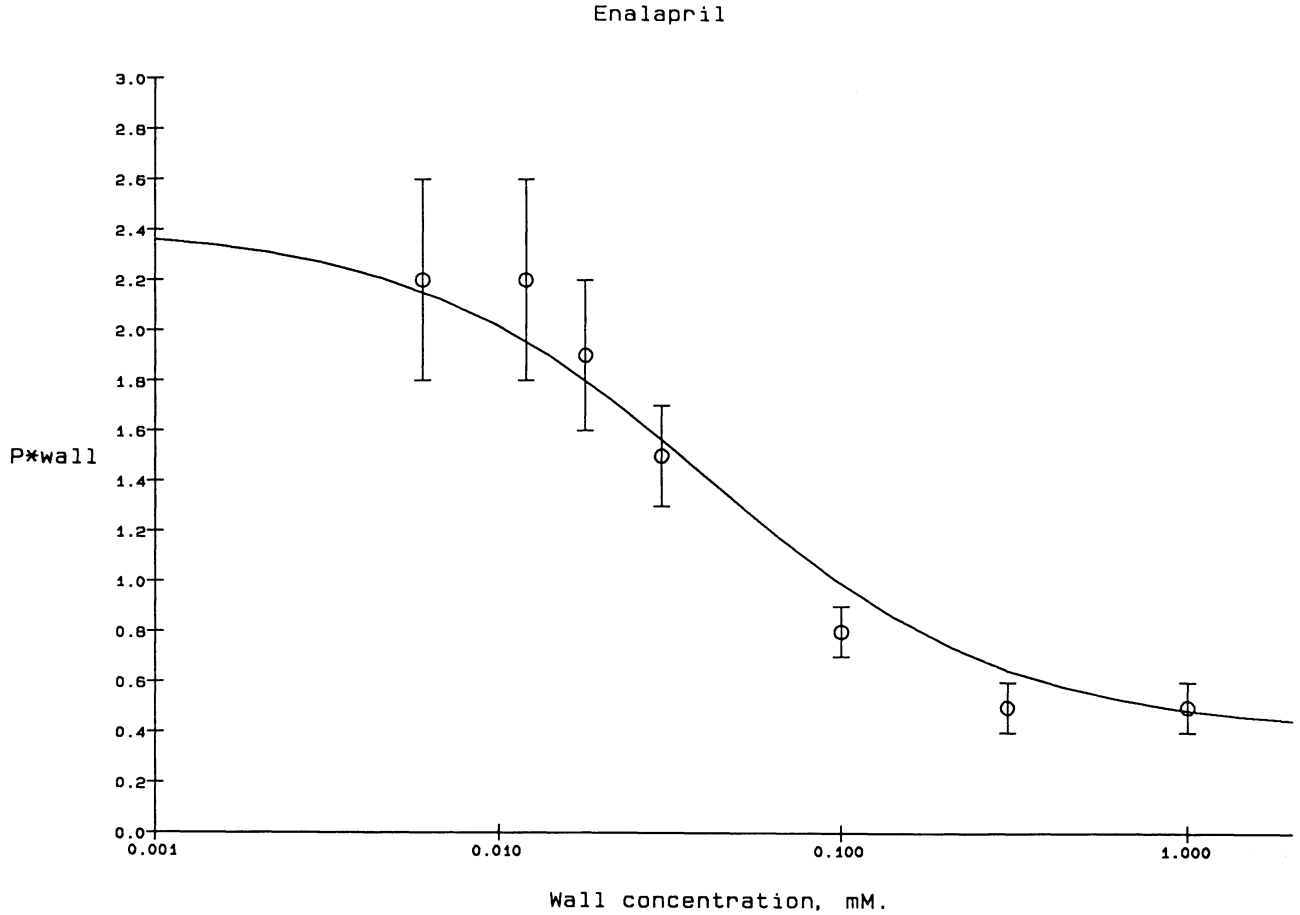

Fig. 3. Comparison of the experimentally determined (open circles) and the simulated (solid line) of the dimensionless wall permeability (P*wall) as a function of wall concentration (C_{wall}). Mean of four to eight rats ± SD at each concentration.

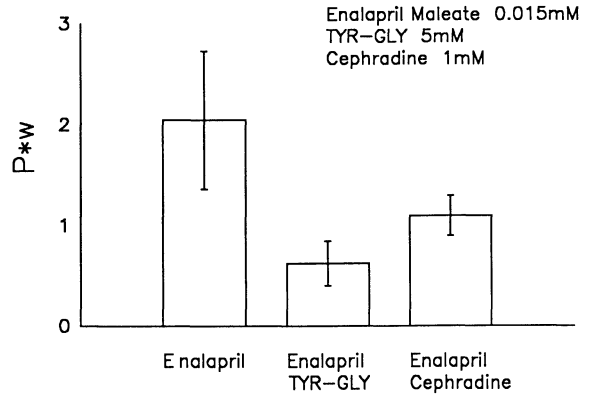

Fig. 4. Influence of Tyr-Gly and cephradine on the dimensionless wall permeability (P*wall) of 0.015 mM enalapril. Mean of four to six rats ± SD.

The cephradine concentration in the inhibition experiment is close to its K_m value of 1.5 mM (11). Higher cephradine concentrations interfered with the HPLC assay and therefore were not studied. This concentration reduced the enalapril wall permeability (P*wall) to 1.10, which is in agreement with the predicted P*wall of 1.31 calculated from Eq. (2) (12).

$$<P*w> = 1 - \frac{[I]}{[I] + K_i(1 + C_w/K_m)} P*c + P*m \quad (2)$$

Where, $<P*w>$ is the predicted wall permeability, [I] is the inhibitor concentration, and K_i is taken to be the K_m for cephradine determined previously (11).

It has been reported (9) that the n-octanol/aqueous buffer system partition coefficient of enalapril increases 10-fold between pH 7.0 and pH 4.5 and the permeability reaches a maximum between pH 4.0 and pH 5.0. The absorption of enalapril from phosphate and citrate buffers solutions in the pH range of 4.5 to 6.5 was also measured in this study. The permeability of enalapril was concentration dependent and was not influenced by the nature of the buffer solution or the pH in the range tested (Fig. 5).

It has been shown (13) that enalaprilat is poorly absorbed in rats and dogs, with 11% oral absorption compared to i.v. administration. This is in agreement with the previously published (14) correlation of wall permeability (P*wall) measured in rats, with the fraction of drug absorbed (F) in man. In contrast to this poorly absorbed drug, its prodrug enalapril is well absorbed, with a P*wall of 2.1 at concentrations below 0.03 mM (Fig. 3).

Enalapril wall permeability at low concentrations was not reduced by 25 mM concentrations of the amino acids L-Leu and L-Phe, which together with the inhibitory effect of Tyr-Gly and cephradine, indicates that the small peptide carrier-mediated transport system is involved in the nonpassive enalapril absorption rather then the amino acid permeases.

Esterification of enalaprilat to enalapril apparently facilitates nonpassive absorption through the peptide carrier-mediated transport system. A less significant increase in the small passive component of enalapril absorption is also observed, probably due to reduced hydrophilicity, as can be seen from the difference in the wall permeability of enalaprilat and the passive component of enalapril absorption, P*wall 0.1 and 0.3, respectively.

The experimental results for fosinopril indicate that the effective permeability is constant over two orders of magnitude of concentration (Fig. 6). The high effective permeability value indicates that the prodrug is well absorbed and to the same extent over a wide range of concentrations. This absorption was not reduced by L-amino acids, Tyr-Gly, or cephradine. Although carrier-mediated transport has not been demonstrated, it cannot be ruled out since it may be masked by the high passive component. However, nonpassive transport of fosinopril, if it exists, is relatively low and its contribution to the total absorption is not likely to be significant.

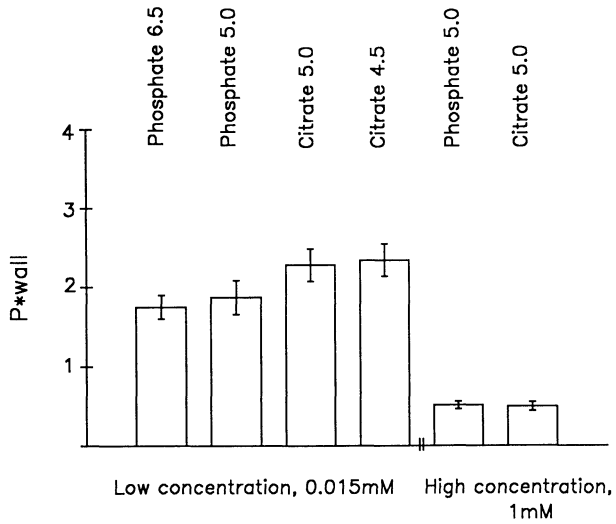

Fig. 5. Enalapril dimensionless wall permeability (P*wall) at pH 4.5, 5, and 6.5, phosphate or citrate buffers. P*wall values at low concentrations are statistically not different ($P > 0.2$). Mean of four rats ± SD.

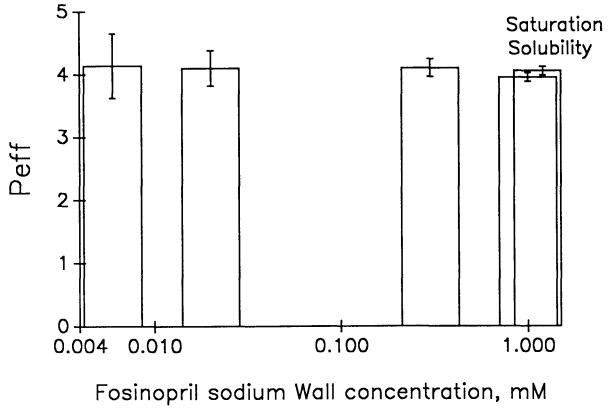

Fig. 6. Effective dimensionless wall permeability (P*eff) of fosinopril at different wall concentrations (C_{wall}). The highest concentration studied is the solubility saturation in the phosphate buffer. Mean of four rats ± SD.

Fosinopril has been found to be stable in the buffers and perfusate for 24 hr. While SQ 27,519, the active parent compound, has not been monitored after fosinopril perfusions, the large difference in the input/output concentration even at relatively high perfusion rates together with the insensitivity to coperfusion with dipeptides and the lack of dependence on concentration indicates that nonlinear phenomena such as nonpassive absorption and enzymatic hydrolysis are not likely to occur.

A comparison of the oral efficacy and intrinsic activity profiles of enalapril and enalaprilat is noteworthy. Enalaprilat binds tightly to ACE yet has a low efficacy for the peptide carrier. The prodrug enalapril is an ester and has a higher apparent affinity for the peptide carrier. This indicates that the reason for good oral absorption of enalapril is that it makes enalaprilat more peptide-like rather than more nonpolar. The fact that all of the dicarboxylic acid ACE inhibitors under clinical development (7) are esterified on the same carboxyl is consistent with the progroup serving a more structurally specific binding site requirement, rather than altering the polarity/passive transport requirement.

Enalapril intestinal absorption by the peptide carrier transport system extends the previous observation that captopril is transported by the peptide carrier-mediated system. Moreover, the data presented here add to the existing knowledge of the nonpassive absorption mechanism of other peptide-like drugs, the β-lactam antibiotics, and will aid in the design of other ACE inhibitors and small peptide drugs and prodrugs with an improved oral efficacy.

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