Pharmaceutical Research, Vol. 6, No. 9, 1989

Research Article

Evidence for Site-Specific Absorption of a Novel ACE Inhibitor

George M. Grass[1,2] and William T. Morehead[1]

Received December 1, 1988; accepted March 29, 1989

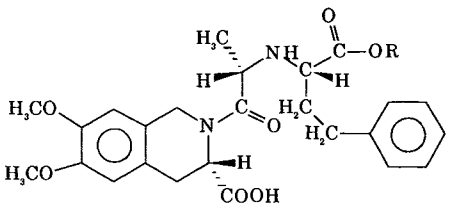
Moexipril {2-[(1-ethoxycarbonyl)-3-phenylpropyl]amino-1-oxopropyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (S,S,S)}, an ester prodrug of an ACE inhibitor, was formulated in controlled-release preparations with a range of *in vitro* release rates, to provide a prolonged input of drug *in vivo*. However, pharmacokinetic studies with the controlled-release dosage forms in humans produced plasma profiles with the same characteristics and time to peak as an immediate-release capsule. *In vitro* dissolution data from the controlled-release dosage form, as well as the known characteristics of the polymer used to control drug release from the dosage form, suggest no reason to suspect an abrupt halt to the *in vivo* release of the drug after 1–2 hr. The lack of sustained blood levels is, therefore, most likely due to failure of the GI tract to absorb the drug beyond some location in the upper small intestine, i.e., site-specific absorption. This theory is supported by a series of computer simulations involving moexipril and the active moiety, moexipril diacid. Possible mechanisms include poor drug permeability, a pH effect whereby the zwitterionic form of the drug is more rapidly absorbed, and esterase cleavage of moexipril to the poorly absorbed moexipril diacid.

KEY WORDS: site-specific absorption; moexipril; Stella; pharmacokinetic modeling; ACE inhibitor.

INTRODUCTION

Moexipril (Scheme I) is an ACE inhibitor having potential as a therapeutic agent for the treatment of hypertension and congestive heart failure. Similar to enalapril (Scheme II), moexipril is an ester prodrug of the therapeutically active diacid form, moexipril diacid. Because of its unknown duration of action, development of a controlled-release formulation for this compound was investigated.

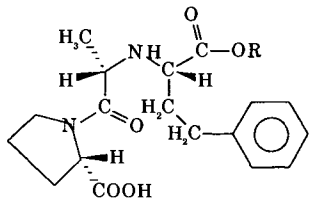
MOEXIPRIL DIACID, R=H
MOEXIPRIL, R= C_2H_5



Scheme I

The goal in designing sustained- or controlled-release delivery systems is to reduce the frequency of dosing and/or to increase effectiveness of the drug by localization at the site of action. For oral delivery, targeting to the site of action is generally possible only for compounds with therapeutic activity in the GI tract. Effective controlled-release dosage forms for systemic drug delivery are usually designed to localize drug at the site of absorption. In some cases, this may reduce the dose required and minimize extremes in plasma concentrations, thus reducing side effects. Overall, this can increase patient compliance and is particularly beneficial during long-term therapy (1).

ENALAPRILAT, R=H
ENALAPRIL, R= C_2H_5



• MALEATE

Scheme II

For most orally administered compounds, the site of absorption is assumed to be throughout the gastrointestinal tract, with passive processes controlling the rate and extent of absorption. For some compounds, however, absorption may occur over a particular portion of the GI tract, i.e., "site-specific absorption." When this involves a specific transport receptor or active/facilitated process, a "carrier-mediated" process is described. Transport phenomena of this type have been described for riboflavin and chlorothiazide among others (2).

[1] Institute of Pharmaceutical Sciences, Syntex Research, 3401 Hillview Avenue, Palo Alto, California 94303.
[2] To whom correspondence should be addressed.

0724-8741/89/0900-0759$06.00/0

Formulation of these compounds into sustained- or controlled-release (CR) forms can have a significant impact. If drug release is not complete before the dosage form passes beyond the region of absorption, bioavailability can be severely decreased (3).

EXPERIMENTAL

Preparation of Controlled-Release Formulations

A mixture of 80% (w/w) microcrystalline cellulose (Avicel PH 101 FMC Corp, Newark, Del.) and 20% (w/w) moexipril (Syntex Research, Palo Alto, Calif.) was granulated with water in a rotogranulator (Versa-Glatt, Model GPCG-1, with rotor insert, Glatt Air Techniques Inc., Ramsey, N.J.). The resulting spherically shaped pellets, 0.5 to 1.2 mm in diameter, were coated with an aqueous dispersion of a neutral copolymer based on poly(meth)acrylic acid esters. The rate of drug release was altered by varying the thickness of the polymer coat.

In Vitro Dissolution Testing

Dissolution was measured using the USP dissolution method II (paddle). UV absorbance was measured at a wavelength of 280 nm, and unless otherwise stated, the stirring speed was 100 rpm. The dissolution medium was analyzed after the 24-hr time point by HPLC to verify that moexipril was the only species released from the dosage form and no degradation to moexipril diacid was noted.

In Vivo Human Trials

Both immediate-release (30-mg) and a series of controlled-release (30-mg) moexipril dosage form preparations were administered in single-dose experiments in male human volunteers. The immediate release preparation was evaluated in a randomized, single-dose, two-way crossover study with and without food (a standard high-fat breakfast consisting of orange juice, 2 fried eggs, 3 bacon strips, 1 slice of toast with butter and grape jelly, and 1 cup of coffee with cream and sugar), with a 2-day washout period between doses. For the controlled-release preparations, the study was an open-label, randomized, single-dose, three-way Latin square crossover, with a washout period of 1 week following each dose. Twelve subjects were used for both study designs. Plasma samples from both studies were taken at the time points indicated and the concentration of moexipril diacid was determined by a specific RIA method with a limit of detection of 0.5 ng/ml.

Computer Simulations

Computer simulations were conducted using a modeling program (STELLA, Hyperformance Systems, Inc., Lyme, N.H.) on a Macintosh SE (Apple Computer, Cupertino, Calif). The model chosen and parameters used were as described in the Appendix.

RESULTS AND DISCUSSION

Figure 1 shows the mean plasma concentration profiles of moexipril diacid for three different moexipril CR formulations of varying release rate. Only moexipril diacid (the active metabolite of moexipril) was measured. Peak plasma concentrations compare as expected, with the fast release giving the greatest value and the slow release the lowest. However, the general shape of the curves bear a close resemblance to that generally seen in an immediate-release product (Fig. 2), in that the peak concentrations are not sustained, and the time to peak is identical for all three release rates. When compared with the profiles obtained with the immediate release form, the peak plasma levels and AUC values of the controlled-release formulations are much less (see figure legends). The immediate-release dosage form shows a significant ($P < 0.001$) difference in the T_{max} values, 1.5 (0.52) and 2.83 (0.58) for fasting and nonfasting, respectively. Errors are given as 1 standard deviation.

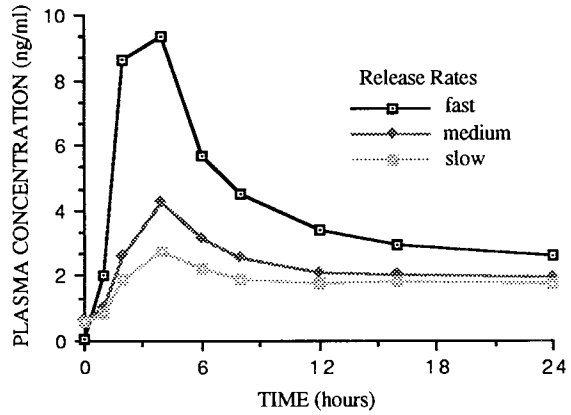


Fig. 1. Mean plasma concentrations of moexipril diacid following oral administration of 30 mg of moexipril CR with various release rates. AUC values (ng · hr/ml) for 0 to 24 hr were 100.38 (27.19), 56.23 (31.86), and 44.61 (25.42) for fast, medium, and slow, respectively. Numbers in parentheses represent 1 SD.

Figure 3 shows previously published plasma profiles for enalapril and enalaprilat after a single oral 10-mg dose of enalapril. The general features of the enalaprilat curve with respect to time of peak, initial rate of concentration decay, and presence of an extended terminal half-life compare with

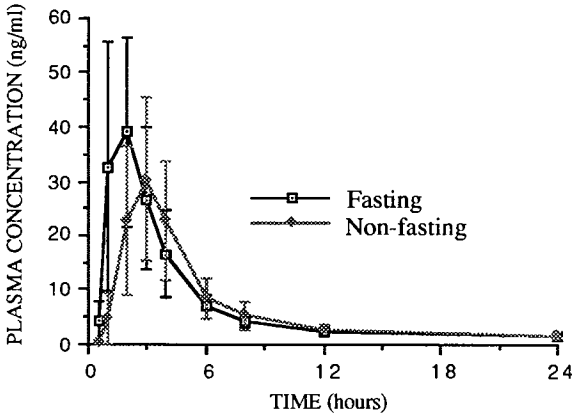


Fig. 2. Mean plasma concentrations of moexipril diacid following oral administration of 30 mg of moexipril as an immediate-release dosage form. AUC values (ng · hr/ml) for 0 to 24 hr were 173.77 (60.20) and 158 (60.76) for fasted and nonfasted, respectively. Numbers in parentheses represent 1 SD.

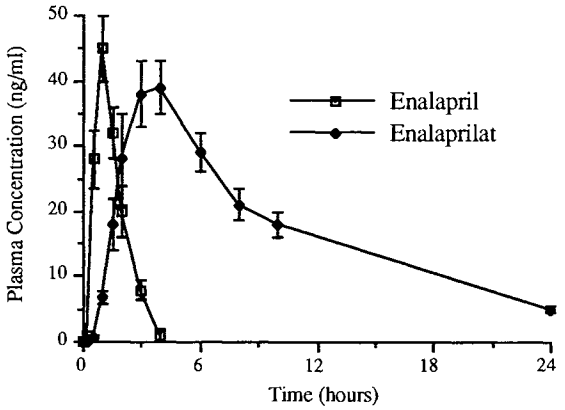


Fig. 3. Mean serum concentrations of enalapril and enalaprilat following oral administration of enalapril 10 mg. Data taken from Ref. 14.

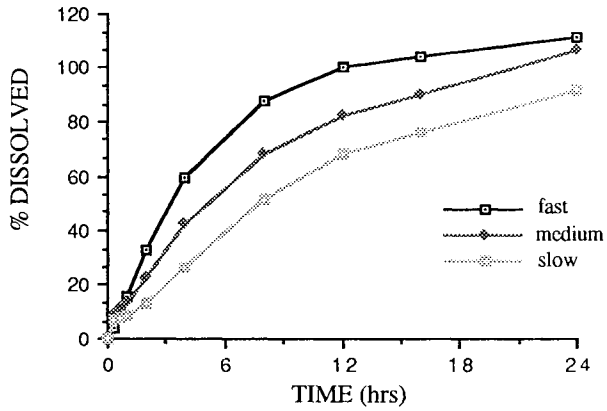


Fig. 5. *In vitro* moexipril controlled-release capsule dissolution in pH 7.4 phosphate buffer at 100 rpm.

the plasma concentrations determined for moexipril diacid. Enalapril is known to be rapidly absorbed but to have an average bioavailability of only 60% (4).

If the *in vivo* rate of conversion of moexipril to moexipril diacid is similar to that of enalapril to enalaprilat, the peak plasma concentrations of moexipril can be inferred to occur about 2 hr before the moexipril diacid peak. This would be 1–2 hr postdosing. This comparison seems reasonable, given the similar chemical structures of enalapril and moexipril. Moexipril also appears to be rapidly yet incompletely absorbed, and this absorption does not occur after 2 hr with either the immediate- or the controlled-release dosage forms.

Moexipril CR Formulation

The moexipril CR dosage form consists of film-coated spherical pellets. The permeability of this film is known to be independent of pH (5,6) and has been shown for a number of drugs to be largely independent of individual fluctuations in the environment of the digestive tract (6). Figures 4 and 5 show *in vitro* dissolution results for the three release rates in deionized water and pH 7.4 phosphate buffer medium (Simulated Intestinal Fluid USP, without pancreatin). Figure 6 shows a comparison of two different release rates of a development batch of the same formulation comparing media of 0.1 *N* HCl and deionized water. While a small effect on release rate is shown, the basic characteristics of the release curves are maintained. Figure 7 shows the results of varying the dissolution paddle speed on the CR formulation from 25 to 100 rpm. Virtually no stirring speed effect is demonstrated, confirming that release is membrane controlled and virtually independent of hydrodynamics. Performance of the dosage form *in vitro* was predictable, such that there is no reason to suspect that it will behave in an unusual manner *in vivo*.

With the exception of a low capacity, tightly bound compartment thought to be responsible for the long terminal half-life of moexipril diacid (7), there is little evidence to suggest that this compound follows nonlinear elimination kinetics. If this is the case, then plasma level peak heights should be proportional to drug input rates. Since absorption appears to occur up to 2 hr, a comparison of peak plasma levels (initial baseline subtracted) was made to *in vitro* dissolution results. If the different formulations release *in vivo* as predicted by *in vitro* results, ratios of plasma peak heights for any two release rates should equal the ratio of the amount of drug released. Ratios of the plasma concentration maximums of the fast to medium (F/M) and medium to slow (M/S) release rates have been compared to the amount released *in vitro* at both 1 and 2 hr (Table I). These ratios are essentially

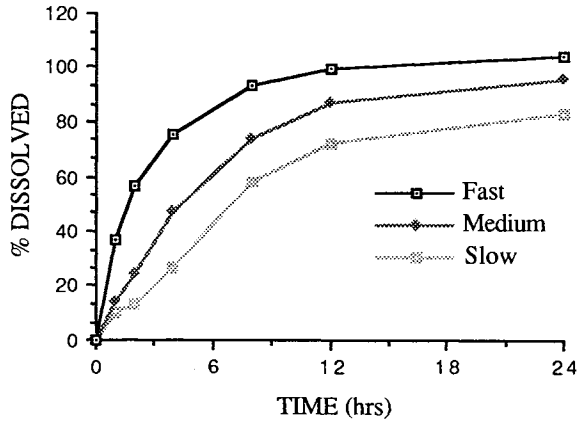


Fig. 4. *In vitro* moexipril controlled-release capsule dissolution in deionized water at 100 rpm.

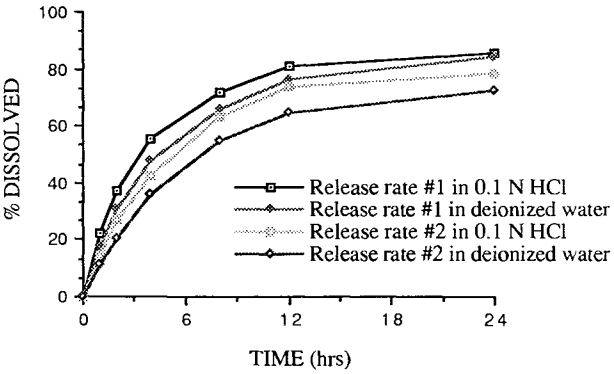


Fig. 6. *In vitro* dissolution of two different release rates of a moexipril CR trial batch in 0.1 *N* HCl and deionized water.

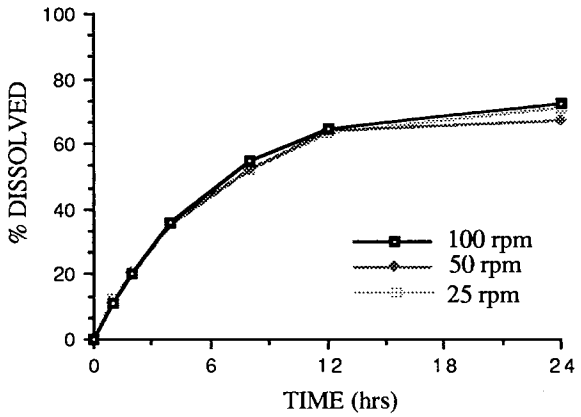


Fig. 7. *In vitro* dissolution of a single release rate of moexipril CR in deionized water at different stirring speeds.

the same; therefore, the dosage forms appear to be performing as expected for the first 2 hr.

Simulations of Controlled-Release Formulations

A simulation model was developed to provide some insight concerning the performance of the controlled-release moexipril dosage form. It should be noted that this model was designed to test the relationships that might exist among the release rate of the dosage form, various absorption parameters, and their potential effects on observed plasma levels. It is not intended to be the definitive pharmacokinetic model for the description of moexipril/moexipril diacid, since much of the required data to construct such a model does not exist. However, every effort was made to choose a model and associated rate constants which would present a reasonable picture using the information currently available. Assumptions and restrictions of the model are described in Fig. 8 (Scheme 1) and the Appendix.

The model chosen was based upon the known pharmacokinetic profiles for enalapril and enalaprilat (Fig. 3) and the limited human data for moexipril diacid and moexipril. Data from the immediate-release moexipril dosage form (Fig. 2) were used to estimate rate constants, and the dissolution phase was predicted from *in vitro* data for both the immediate-release and the controlled-release dosage forms. For all dosage forms, absorption could not be modeled as a continuous function and required that no absorption occur after 1.5 hr. These characteristics are consistent with a rapid yet incomplete absorption phase. Figures 9–11 suggest that this interpretation fits the existing data very well. Only the *in vitro* dissolution rates have been changed among each of these simulations.

Table IA. Percentage Moexipril Dissolved *in Vitro* at 1 and 2 hr for Three Different Release Rates

	Formulation Release Rate		
Time	Fast	Medium	Slow
1 hr	37	15	10
2 hr	57	24	14

Table IB. Ratio of Plasma Concentration Maximums and Ratio of the Amount of Moexipril Released *in Vitro* at 1 and 2 hr for Different Release Rate Formulations

	Maximal Plasma Concentration Ratio (baseline subtracted)	*in Vitro* Dissolution Ratio	
		1 Hr	2 Hr
Fast/medium	2.5	2.5	2.4
Medium/slow	1.7	1.5	1.7

The following scenarios can be constructed for interpretation of this moexipril/moexipril diacid absorption data.

Site Specificity

Site specificity infers preferential drug absorption at a specific location, usually due to a physiological membrane phenomena or active transport site. If the present data suggest that absorption occurs only over the initial 1.5- to 2-hr period, then two potential sites seem possible. In one case, the stomach could be invoked as a primary site. One hypothesis is that the pH of the gastric region or upper duodenum would be in the range from 3 to 5, and moexipril (pK_{a_1} = 3.0, pK_{a_2} = 5.4) would exist as the zwitterionic form. This form, containing both positive and negative regions could self-associate or pair with other molecules of the same type, mutually shielding the existing charges of the molecule, and allow increased transport of the now neutral molecules. Supporting this premise is the fact that all absorption appears to occur at very early time points, presumably when the dose resides very high in the gut. However, several points are contradictory. First, absorption for enalapril (and the simulations for moexipril) appears to occur over a period of approximately 1.5 hr. In the fasted state, dissolution of the

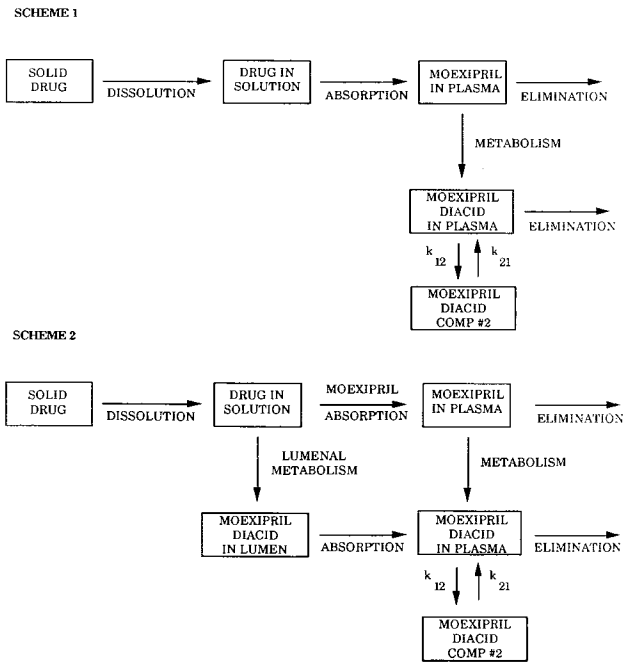


Fig. 8. Pharmacokinetic schemes used to model oral moexipril.

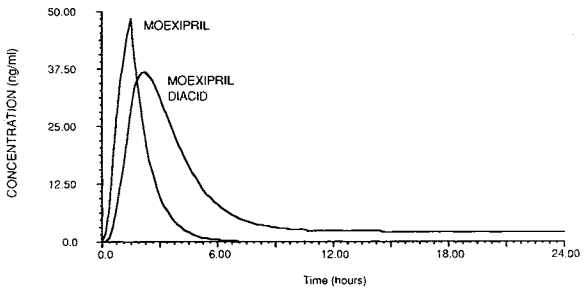


Fig. 9. Computer simulation of moexipril immediate-release formulation.

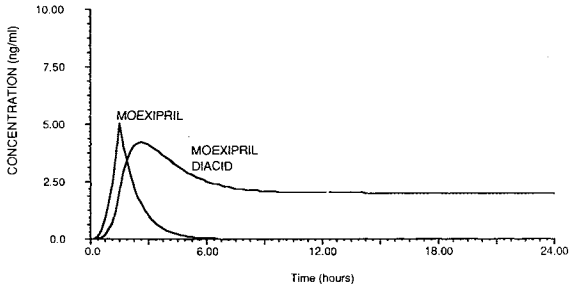


Fig. 11. Computer simulation of moexipril CR medium release rate.

immediate release capsule is expected to occur very promptly (within 15 min). Drug in solution is expected to leave the stomach by a fairly rapid first-order process (8) to the duodenum which is likely to have a resident pH of 5.5 to 6.0 (9). The transit time of the upper GI tract to reach the ileocecal junction is known to be approximately 2–3 hr (8). Thus, during the 1.5-hr time period, much of the drug should reside in the duodenum and jejunum, at a pH of 6.0 or greater. Finally, one would expect a potential increase in total absorption when coadministered with food, since depending upon the quantity and type of food given, this should delay gastric emptying (10). Figure 2, however, suggests that this does not occur.

More plausible is the possibility of site-specific absorption in the duodenum or jejunum and may be a carrier-mediated process. This is partially consistent with the data presented for interaction with food, since a longer lag time would be expected as a result of the decreased rate of stomach emptying for the drug. However, decreased bioavailability was observed, and this is inconsistent with a delay in the total time for passage of drug past the absorption site.

Esterase Degradation of Moexipril

The plasma profile of enalapril suggests a relatively large absorption rate constant despite the fact that incomplete absorption is known to occur. Kinetically, it is possible to have a very large apparent rate constant when competing parallel elimination also occurs (11). Such a model is shown in Fig. 8 (Scheme 2) for moexipril. Competing with the absorption of moexipril in the gut is brush border or lumenal metabolism of moexipril to a nonabsorbable state, presumably conversion to a very poorly absorbed moexipril diacid. If this conversion rate constant is great enough, it can appear as a large absorption rate constant and would result in decreased bioavailability when compared to a true absorption rate constant of the same magnitude.

Bioavailability

Because the simulations are a series of calculations at each individual time interval (*dt*), it is possible to estimate the absolute percentage absorbed for the moexipril immediate-release dosage form by noting the amount of drug remaining in the solid drug compartment at the end of the simulation. This value is approximately 10%, however, its precision is obviously questionable given the high degree of speculation concerning the rate constants used. It does, however, seem reasonable in light of the following: Enalapril generates similar plasma levels of drug from a 10-mg dose when compared to the plasma levels generated from the 30-mg moexipril immediate release. This would predict an average absorption of 20% for moexipril, given the known value for enalapril absorption, i.e. 60%.

Possible Sustaining Effects from the Fast CR Dosage Form

Although the plasma profiles from controlled-release dosage forms are nearly identical in their time to reach peak plasma levels, the fast-releasing CR formulation demonstrates a consistently higher plasma level of moexipril diacid than the slow- or medium-release rate forms which continues to 24 hr. Because of the rapid elimination rates used to model the initial declining phases for both moexipril and moexipril diacid in the simulations, the only way that this could be modeled was to have a very slow but continuous input of moexipril over the 24-hr time period. Figure 12 shows this simulation, which uses the fast CR dissolution rate and allows absorption to occur as with the immediate release for 1.5 hr. However, the simulation differs in that over the time period from 1.5 to 24 hr, an extremely slow (0.004 hr^{-1}) rate

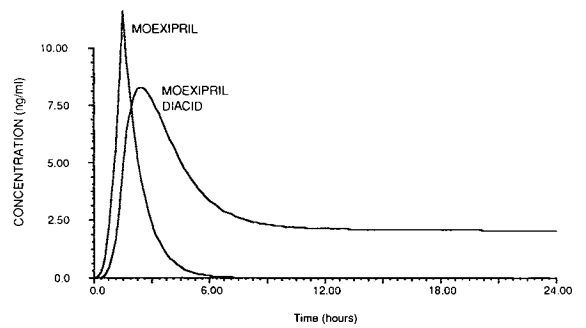


Fig. 10. Computer simulation of moexipril CR fast release rate.

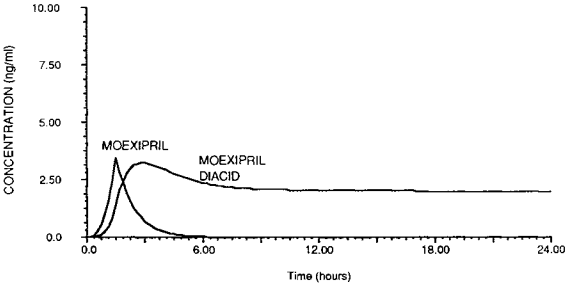


Fig. 12. Computer simulation of moexipril CR slow release rate.

constant for absorption was used to simulate continuing input of moexipril. One possible mechanism for this effect could be release of drug from the CR dosage form, which is fast enough to allow some drug absorption before it is completely converted to moexipril diacid in the intestine. Since the drug is protected from enzymatic decay in the CR dosage form, this can continue over a prolonged period of time. Since the slower CR forms do not produce this effect, it is assumed that the drug release is slower than the rate of enzymatic degradation, and all drug is converted before it can be absorbed to any measurable extent. This result implies that an optimal release rate may be able to be achieved where the rate of moexipril availability to the intestine is greater than the rate of conversion to moexipril diacid, however, unlike the immediate release form, some drug remains protected within the dosage form. An important relationship between the release rate and the enzymatic activity in the intestine is suggested.

CONCLUSIONS

In general, absorption of moexipril appears to occur only over a time period of approximately 1.5 hr, and it is independent of the release rate of the dosage form. These findings are consistent with available information concerning enalapril and more recent studies with captopril (12) where sustaining formulations resulted in decreased bioavailability. The data indicate that moexipril is absorbed in a site-specific manner. The nature of this absorption phenomenon has not been identified, although specific transport locations in the duodenum or jejunum are possible. Absorption controlled by competing enzymatic degradation of the prodrug has also been suggested. Studies using time- or pH-dependent dosage forms, or site-directed intubations, are methods which could be used to clarify these possibilities. The use of the simulation program STELLA has proved helpful in developing mechanistic interpretations for plasma profiles from bioavailability studies.

APPENDIX

The use of STELLA for simulation of plasma profiles from *in vitro* dissolution data has been previously described (13). The program uses the input values and calculates the resultant drug concentrations in each compartment for each time interval. The following were considered in the design of the model and assignment of rate constants:

(1) Peak plasma levels for moexipril diacid occur at 3 to 4 hr after dosing of moexipril. Since peak levels of enalaprilat occur 3 to 4 hr after oral dosing of enalapril, and the peak time for enalapril is about 1 hr, it was assumed that the peak time for moexipril after oral dosing from an immediate-release dosage form was also 1 hr. That is, since there exists few data to the contrary, it was assumed that the pharmacokinetic relationship which exists between enalapril and enalaprilat would also exist for moexipril/moexipril diacid. Hence, the initial rate constants were chosen to fit the known plasma data for the appearance of moexipril diacid from the immediate-release form (Fig. 2) and it was assumed that moexipril should have a peak time of 1 to 1.5 hr at a concentration slightly greater than that of the moexipril diacid peak, with no detectable moexipril levels beyond 6 hr.

(2) Enalaprilat demonstrates a very prolonged terminal half-life at a very low plasma concentration. It has been suggested that this is the result of binding to circulating ACE and, for enalapril, represents 1 mg of enalaprilat, regardless of dose. The residual plasma level of moexipril diacid appears to be less than that for enalaprilat. The long terminal half-life is suggestive of a second compartment for moexipril diacid and this was included in the model. However, it was not described in the classical manner, since circulating ACE is part of the sampled plasma pool and is saturable (limited capacity). The contents of this compartment were part of the calculation for plasma concentration along with the central compartment. The result of this is that the elimination rate constant for moexipril diacid alone does not define the terminal phase but, rather, the initial plasma decline.

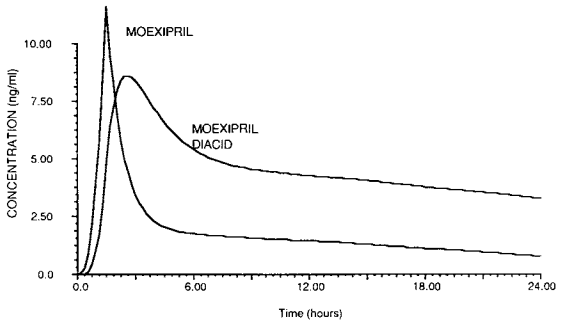


Fig. 13. Computer simulation of moexipril CR fast release with slow absorption continuing for 24 hr.

(3) The metabolism of systemic moexipril was assumed to occur rapidly. Since the combination of the unchanged elimination and the metabolism of moexipril defines the declining portion of that curve, the sum for these rate constants was set to mimic the plasma profile of enalapril. The metabolism rate constant for moexipril was chosen to fit properly the ascending portion of the moexipril diacid profile. The elimination rate of moexipril was the remaining difference.

(4) The volume of distribution of both moexipril and moexipril diacid was assumed to be near that of the plasma volume. In reality, this has little effect on the peak time or shape of the plasma profile and affects only the numerical value of the concentrations obtained.

With these assumptions and restrictions, simulations were conducted using a fourth-order Runge–Kutta method with a *dt* value of 0.1 hr. The rate constants used were as follows.

Dissolution, rates obtained from *in vitro* data (Fig. 4)
Moexipril elimination, 0.0693 hr^{-1}
Moexipril diacid elimination, 0.7 hr^{-1}
Moexipril metabolism, 1.0 hr^{-1}
Moexipril volume of distribution, 8500 ml
Moexipril diacid volume of distribution, 8500 ml
k_{12}, 20 hr^{-1}
k_{21}, 0.003 hr^{-1}
Total capacity of moexipril diacid second compartment, 17 μg
Absortion-Rate constant of 0.02 hr^{-1} that discontinues after 1.5 hr (except Fig. 13) [This absorption rate constant could have been modeled as a pair of competing rate constants for absorption of moexipril and lumenal metabolism of moexipril to a very poorly absorbed moexipril diacid (Fig. 8, Scheme 2).]

ACKNOWLEDGMENTS

The authors wish to acknowledge the following: Vince Corvari and Michael Ramsay for technical assistance, Joseph Vallner for helpful discussions, and Department of Bioanalytics and Mass Spectroscopy, Syntex Research, for plasma sample analysis and human pharmacokinetic data.

REFERENCES

1. J. C. Clinite and H. F. Kabat. *J. Am. Pharm. Assoc.* **16**:74–76 (1976).
2. A. B. Morrison, C. B. Perusse, and J. A. Campbell. *N. Engl. J. Med.* **263**:115–117 (1960).
3. E. J. Middleton, E. Nagy, and A. B. Morrison. *N. Engl. J. Med.* **274**:136–139 (1966).
4. B. N. Swanson, P. A. Veasses, R. K. Ferguson, P. A. Bergquist, and A. E. Till. *J. Pharm. Sci.* **73**:1655–1659 (1984).
5. K. Lehmann and D. Dreher. *Int. J. Pharm. Tech. Prod. Mfr.* **2**(4):31–43 (1981).
6. K. Lehmann. *Acta Pharm. Fenn.* **91**:225–238 (1982).
7. W. B. Abrams, R. O. Davies, and H. J. Gomez. *J. Hypertens.* **2**(Suppl 2):31–36 (1984).
8. J. B. Dressman. *Pharm. Res.* **3**:123–131 (1986).
9. W. G. Crouthamel, C. R. Abolin, J. Hsieh, and J. K. Lim. *J. Pharm. Sci.* **64**:1726–1727 (1975).
10. M. Gibaldi. In *Biopharmaceutics and Clinical Pharmacokinetics*, Lea and Febiger, Philadelphia, 1977, pp. 15–67.
11. M. C. Makoid and J. R. Robinson. *J. Pharm. Sci.* **68**:435–443 (1979).
12. Y. Seta, F. Higuchi, Y. Kawahara, K. Nishimura, and R. Okada. *Int. J. Pharm.* **41**:245–254 (1988).
13. M. W. Ramsay, G. M. Grass, and J. J. Vallner. Abstract from AAPS Regional Meeting, Reno, Nevada (Feb. 1988).
14. J. Biollaz, J. L. Schelling, B. Jacot des Combes, D. B. Brunner, G. Desponds, H. R. Brunner, E. H. Ulm, M. Hichens, and H. J. Gomez. Br. J. Clin. Pharm. **14**:363–368 (1982).