Optimizing Dose Selection with Modeling and Simulation: Application to the Vasopeptidase Inhibitor M100240

Marc Pfister, MD, FCP, Nancy E. Martin, PharmD, Lloyd P. Haskell, MD, MBA, and Jeffrey S. Barrett, PhD, FCP

Dual inhibition of neutral endopeptidase 24.11 (NEP) and angiotensin-converting enzyme (ACE) has gained increased interest in the treatment of hypertension, heart failure, and renoprotection. Specifically, M100240, the thioester of the dual ACE/NEP inhibitor MDL100,173, has been evaluated in the management of hypertension. A model-based analysis, including simulations, was employed to characterize the relationship between individual M100240 drug exposure and neurohormonal response and to optimize the dose selection for future clinical studies. Sixty-two healthy subjects and 189 hypertensive patients were studied after oral once-daily administration of 2.5, 5, 10, 25, or 50 mg M100240. Pharmacokinetic-biomarker and blood pressure response models were fitted to the data with the computer program NONMEM. A direct inhibitory $E_{\text{max}}$ model adequately described the relationship between MDL100,173 concentration and ACE activity. No clear concentration or dose-dependent NEP or blood pressure responses were evident. Given a target 90% ACE inhibition, simulation reveals that (1) 50 mg M100240 once daily produces adequate ACE inhibition 24 hours postdose in only 20% of subjects, and (2) higher and/or more frequent doses on the order of 25 mg three times daily or 50 mg twice daily are required to achieve the target ACE inhibition in at least 50% of patients over 24 hours. Insufficient blood pressure–lowering effects were observed in healthy subjects and hypertensive patients due to inadequate ACE and NEP inhibition with once-daily oral doses of up to 50 mg of M100240. Divided doses might provide target ACE inhibition in more patients.

Keywords: Vasopeptidase inhibitor; NEP inhibition; ACE inhibition; angiotensin; hypertension; NONMEM; modeling; simulation

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From Drug Innovation and Approval, Aventis Pharmaceuticals, Bridgewater, New Jersey (Dr. Pfister, Dr. Haskell); University of New Jersey Medical and Dental School, New Brunswick (Dr. Martin); and the Children’s Hospital of Philadelphia, Pennsylvania (Dr. Barrett). Submitted for publication November 8, 2003; revised version accepted March 15, 2004. Address for reprints: Marc Pfister, MD, FCP, Aventis Pharmaceuticals, 1041 Route 202-206, Bridgewater, NJ 08807. DOI: 10.1177/0091270004265365

Angiotensin-converting enzyme (ACE) inhibition is a well-established treatment in hypertension. Recently, inhibition of neutral endopeptidase 24.11 (NEP) in addition to inhibition of ACE has gained increased interest in the treatment of hypertension, and renoprotection. NEP or blood pressure responses were evident. Given a target 90% ACE inhibition, simulation reveals that (1) 50 mg M100240 once daily produces adequate ACE inhibition 24 hours postdose in only 20% of subjects, and (2) higher and/or more frequent doses on the order of 25 mg three times daily or 50 mg twice daily are required to achieve the target ACE inhibition in at least 50% of patients over 24 hours. Insufficient blood pressure–lowering effects were observed in healthy subjects and hypertensive patients due to inadequate ACE and NEP inhibition with once-daily oral doses of up to 50 mg of M100240. Divided doses might provide target ACE inhibition in more patients.

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Angiotensin-converting enzyme (ACE) inhibition is a well-established treatment in hypertension. Recently, inhibition of neutral endopeptidase 24.11 (NEP) in addition to inhibition of ACE has gained increased interest in the treatment of hypertension, and renoprotection. NEP catalyzes the degradation of a number of endogenous vasodilator peptides, including α-atrial natriuretic peptides (ANP), brain natriuretic peptide, C-type natriuretic peptide, adrenomedullin, substance P, angiotensin-(1-7), and bradykinin. NEP is also involved in the catabolism of vasoconstrictor peptides, including endothelin-1 and angiotensin II. NEP inhibitors, in contrast to ACE inhibitors, are effective in animal models of low renin activity and have been found to produce diuresis and natriuresis in animals and humans. Dual ACE and NEP inhibitors, a new class of agents termed vasopeptidase inhibitors, may show superior efficacy in the treatment of high blood pressure, especially in low-renin, salt-sensitive hypertensive individuals in whom ACE inhibitors are less effective. Besides the antihypertensive effect of dual ACE/NEP inhibition, vasopeptidase inhibitors might be superior to ACE inhibitors in improving endothelial function in cardiovascular diseases.

M100240 is the thioester of the vasopeptidase inhibitor MDL100,173. In vivo, M100240 is rapidly converted to MDL100,173. MDL100,173 was previously...
reported to be a potent and balanced inhibitor of both ACE and NEP as the \( K_i \) appeared to be in the subnanomolar range and similar for ACE and NEP (0.08 and 0.11 nmol/L, respectively).\(^{20,21}\) Results of preclinical studies have shown M100240 to be effective at lowering blood pressure and protecting the heart from secondary changes in hypertensive, transgenic (\( mRen-2 \)) rats.\(^ {22,23}\) The terminal half-life and duration of effect observed in these preclinical studies suggested that once-daily administration of M100240 in humans was possible based on animal extrapolations.

In healthy subjects, M100240 exerted a dose-dependent ACE-blocking activity, expressed by the inhibition of the pressor responses to exogenous angiotensin I challenges.\(^ {24}\) ACE inhibition,\(^ {25}\) together with increased urinary volume, atrial natriuretic peptide (ANP), and cyclic guanosine 3′5′-monophosphate (cGMP) excretion after intravenous administration in healthy volunteers, suggests that M100240 has dual ACE/NEP inhibition.\(^ {26}\)

Dose-biomarker response analyses can be useful in the establishment of the appropriate regimen and efficacy of most drugs.\(^ {27}\) Within the context of ACE inhibitors, it was noted that while differences in structural factors, pharmacokinetic properties, potencies, and putative differences in tissue penetration have been observed, few of these have demonstrated to have major clinical relevance.\(^ {28}\) Despite such statements, it has also been contended that both pharmacokinetic and pharmacodynamic factors play a role in determining the optimal dosage regimen of an antihypertensive agent for the individual patient.\(^ {29}\) The incorporation of modeling and simulation techniques to this problem offers the promise of establishing linkages between the fundamental assumptions regarding the assignment of clinical benefit to pharmacokinetic and pharmacodynamic (e.g., biomarker activity, blood pressure) outcomes. The extension of such relationships to both candidate selection and the design of more informative clinical trials is the topic of much research across therapeutic areas. The successful deployment of such an approach in diabetes\(^ {30}\) and oncology\(^ {31}\) both validates the approach and offers promise for cardiovascular disease targets.\(^ {32}\)

With respect to ACE inhibition alone, there is reasonable precedence for the pharmacokinetic/pharmacodynamic (PK/PD) properties, which constitute a potentially effective agent. Generally, a trough to peak blood pressure ratio of greater than 50% is required to support once-daily dosing (ICH-E1 guidelines). Likewise, the studies required to support a claim in hypertension would have to encompass 300 to 600 patient exposures for 6 months and at least 100 patients for 1 year, for a total dossier of >1500 patients evaluated. These targets are less certain for an agent exhibiting both ACE and NEP inhibition.

In an effort to assess the magnitude and duration of renin-angiotensin-aldosterone modulation as a function of various doses of M100240 administered in healthy volunteers, we recently conducted a multiple-dose, parallel-group study with M100240. Dose selection for the clinical program was based on the following three assumptions: (1) bioavailability (half-life time) of the oral administration of M100240 in healthy volunteers is 85% (7.5 h),\(^ {25}\) (2) approximately 60% ACE inhibition is observed at the trough (i.e., 24 h postdose) at oral doses of 10 and 20 mg once daily,\(^ {25}\) and (3) M100240 is a balanced inhibitor of both ACE and NEP with similar \( K_i \) for ACE and NEP.\(^ {20,21}\) The results of this study were pooled with other recent studies in healthy volunteers and hypertensive patients to enrich our data set. The main objectives of this model-based analysis were threefold: first, to characterize the relationship between individual drug exposure and neurohormonal response (i.e., ACE and NEP inhibition) across a wide dose range; second, to test the aforementioned PK/PD assumptions; and, third, to apply simulation to evaluate dose scenarios for potential clinical studies in hypertensive patients.\(^ {33,34}\)

**MATERIALS AND METHODS**

**Study Designs and Populations**

The designs of the four relevant M100240 phase I/II studies with available PK data are summarized in Table I. Biomarkers (i.e., quantifiable surrogate markers measuring in vivo drug effect) for ACE and NEP activity were measured in study A only. In this study, subjects were on a fixed-sodium diet (7 g of NaCl per day). The diet began 3 days before drug administration and was maintained during the next 12 days. Subjects received multiple doses of once-daily placebo or 2.5 mg, 10 mg, 25 mg, or 50 mg M100240 during that period. M100240 was administered orally with approximately 240 mL of water after an overnight fast at the same time every morning. A light breakfast was provided 30 to 60 minutes after drug intake, and lunch was provided 4 hours after drug intake. At baseline and on days 1 and 12, serial blood and urine samples were collected for PK and biomarker profiles.

**Pharmacokinetic data and methods.** To enhance the accuracy of PK parameter estimates (e.g., clearance, volume of distribution, etc.), all available MDL100,173 PK data were pooled and used for the PK analysis. PK
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Dose(s)</th>
<th>PK Sample Times (h)</th>
<th>Number of PK Samples</th>
<th>ACE/NEP Biomarkers</th>
<th>Blood Pressure</th>
<th>Study Population</th>
<th>Number of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Parallel</td>
<td>2.5, 10, 25, and 50 mg</td>
<td>0, 1, 2, 4, 8, 12, 24, 48, and 72</td>
<td>468</td>
<td>Yes</td>
<td>Cuff</td>
<td>Healthy volunteers</td>
<td>32</td>
</tr>
<tr>
<td>B</td>
<td>Crossover</td>
<td>50 mg</td>
<td>0, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 18, 24, 30, 36, and 48</td>
<td>865</td>
<td>No</td>
<td>Cuff</td>
<td>Healthy volunteers</td>
<td>30</td>
</tr>
<tr>
<td>C</td>
<td>Parallel</td>
<td>2.5, 5, 10, and 25 mg</td>
<td>0, 0.5, 1, 2, 3, 4, 6, and 8</td>
<td>3373</td>
<td>No</td>
<td>Cuff</td>
<td>Hypertensive patients</td>
<td>156</td>
</tr>
<tr>
<td>D</td>
<td>Sequential</td>
<td>10, 25, and 50 mg</td>
<td>Trough</td>
<td>129</td>
<td>No</td>
<td>Cuff ABPM</td>
<td>Hypertensive patients</td>
<td>33</td>
</tr>
</tbody>
</table>

PK, pharmacokinetic; ACE, angiotensin-converting enzyme; NEP, neutral endopeptidase 24.11; ABPM, ambulatory blood pressure monitoring.
data from four M100240 studies were included in the analysis: 1333 concentrations from 62 healthy subjects and 3502 concentrations from 189 hypertensive patients.

In all four studies, MDL100,173 plasma concentrations were quantified using a validated liquid chromatography tandem mass spectrometry method (LC/MS/MS).35

**Biomarker data and methods.** Biomarker data from study A subjects (n = 32) included plasma ACE activity, plasma renin activity (PRA), angiotensin II/angiotensin I ratio, bradykinin, urine ANP, and cGMP. The ACE inhibition profile was assessed in parallel with the PK profile (i.e., same sampling times). The NEP inhibition profile was assessed from urinary ANP and cGMP excretion (daily and fractionated amounts) at baseline and day 12. Fractional urine was collected over the following time intervals: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours. Plasma ACE activity was measured with a validated ACE-trap method.36 PRA, plasma angiotensin I and II, urinary ANP, and cGMP were measured by radioimmunoassay as described previously.37-39

**Blood pressure data.** Three supine blood pressure measurements were taken at 2-minute intervals after a rest period of at least 10 minutes. The arithmetic mean of the three measurements was considered the blood pressure value at a given time point. A standard mercury sphygmomanometer was used for all blood pressure measurements unless specified otherwise.

In study A, blood pressure was measured before drug administration, at 2-hour intervals up to 8 hours, and at 12 hours after drug intake. In study B, serial blood pressures were collected at 4-hour intervals. In study C, serial cuff blood pressure assessments were obtained in parallel with the PK samples. In study D, 24-hour ambulatory blood pressure monitoring (ABPM) and cuff blood pressure assessments at baseline and at the end of the dosing interval were used. These readings were assessed, both pooled and independently of the blood pressure measurement technique.

**Data Analysis Methods**

A two-step strategy was used for the PK-biomarker response analysis: a PK model was first fit to the MDL100,173 plasma concentration data. PK parameter estimates for each individual were derived from this model (and individuals’ data) and then used in a second step as fixed covariates in a subsequent PK-biomarker response model.

**Pharmacokinetic models.** Both one- and two-compartment PK models with first-order absorption were contrasted. A group effect (healthy subjects vs. hypertensive patients) on clearance (CL), volume of distribution (V), and relative bioavailability (F) was also tested.

The group effect (GE) on PK parameters was modeled as follows:

\[ P_i = (1 + \text{GE}) \exp(\eta) \]

where \( P \) is a vector of PK parameters such as \( V, CL, F \), and the rate constant of absorption (\( K_a \)). \( P_i \) is a parameter estimate for the \( i \)th individual, and \( \eta \) is the interindividual variance of the PK parameters. The indicator \( \text{GE} \) is coded as 1 (healthy volunteers) or 0 (hypertensive patients).

**Models for humoral response.** Individual patient posterior Bayesian PK parameter estimates are provided by NONMEM for the second stage using the post hoc procedure.40,41 \( E_{max} \) and sigmoid-\( E_{max} \) models were contrasted to describe the relationship between measured MDL100,173 concentrations and measured biomarker response in plasma and urine.

Possible circadian variation of biomarker response was described by a cosine function as follows34:

\[ R_t = mR + A \cos \left( \frac{2\pi(t + S)}{tp} \right) \]

where \( R_t \) is the biomarker response at a given clock time \( t \) (i.e., where 24 h is midnight), \( tp \) is the time period of the cosine function (fixed to 6, 12, or 24 h), \( mR \) is the 24-hour mean value of the measured biomarker for humoral response, \( A \) is the amplitude of the circadian variation, and \( S \) is the phase shift of the variation.

**Residual variability for observations.** To model residual intraindividual variability for pharmacokinetic observations (e.g., measurement errors), both additive and proportional components of error were accounted for as follows:

\[ y = f + \epsilon_1 + \epsilon_2 \]

where \( y \) is the measured MDL100,173 concentration (or biomarkers in plasma), \( f \) is the model for its expectation, and the error \( \epsilon = (\epsilon_1, \epsilon_2) \) is distributed \( N(0, \Sigma) \), where \( \Sigma \) is diagonal.

**Model building.** The value of the objective function at convergence (approximately minus twice the maximized log-likelihood of the data)41 was used to evaluate model hierarchies—more complex (i.e., more paramet-
ters) over less complex submodels. This was accomplished by computing the log-likelihood ratio, the difference in objective function between the fits of the two models, and referencing it to a $\chi^2(df)$ distribution, where $df$ is the number of free parameters by which the larger model exceeds the smaller. The selection of an appropriate PK parameter model was based on (1) a significant reduction in the objective function value with the likelihood ratio test (for nested models, $p < 0.001$; i.e., increase in objective function value of 10.88 for 1 degree of freedom); (2) diagnostic plots, including observed versus predicted values and individual weighted residuals versus individual predictions; and/or (3) a decrease in standard error, interindividual variance of the PK parameters, and residual error.

Computations were performed with the software NONMEM (version V) for LINUX (NONMEM Project Group, University of California, San Francisco) and S-PLUS 6 for LINUX (Insightful Corporation, Seattle, WA). Parameter estimates are provided as the mean (interindividual variability, %).

**Simulation for dose-ACE inhibition relationship.** Model-based simulation was used to evaluate candidate dose regimens with regard to a desired biomarker response. Target biomarker response was defined as (1) 50%, 60%, 70%, 80%, or 90% ACE inhibition in at least 50% of patients at trough and (2) 90% ACE inhibition in at least 50% of patients over 24 hours. ACE activity profiles in plasma ($n = 500$) for various dose regimens (higher doses and more frequent administration schedules than those employed in the clinical program) were simulated with parameters drawn from estimated parameter distributions.

**RESULTS**

A two-compartment PK model fit of the MDL100,173 concentration data was superior to a one-compartment model, based on model selection criteria as previously described. Population estimates (percent standard errors) were 527 L/h (56%) for CL, 0.45 h$^{-1}$ (7.5%) for Ka, 636 L/h (15%) for intercompartmental clearance (Q), 200 L (51%) for central V, and 8020 L (9.8%) for peripheral V. Interindividual variability in peripheral V was set to zero, and interindividual variability in CL, Ka, and central V was estimated to be 42%, 71%, and 105%, respectively. Clearance was estimated to be lower in hypertensive patients than in healthy subjects (231 vs. 527 L/h). There was no group effect on V, the absorption rate constant (Ka), and bioavailability (F).

A sensitivity analysis was performed reestimating CL with modified healthy volunteer PK data sets (i.e., without MDL100,173 concentrations after 8, 12, or 24 h postdose), as the group effect on CL could be the result of differences between sampling schemes in healthy subjects and those in patients. MDL100,173 concentrations were measured up to 48 or 72 hours in healthy subjects, whereas MDL100,173 concentrations were measured up to 8 hours or at the dosing interval (i.e., 24 h postdose) in patients. However, the estimate for apparent clearance remained lower in healthy subjects versus patients with all three modified PK data sets, and lower apparent clearance could not be explained by removing PK samples after 8 hours postdose (reference data on file).

**ACE activity.** The direct inhibitory $E_{\text{max}}$ model adequately described the relationship between MDL100,173 concentration and measured ACE activity. There was no hysteresis in these PK-biomarker data, and adding an effect compartment did not improve the model (i.e., no delay of onset for the inhibitory effect on ACE activity). The calculated Hill response factor (sigmoid $E_{\text{max}}$ model) was close to 1 (i.e., 0.84); therefore, the simpler $E_{\text{max}}$ model was selected as the final ACE activity response model. A single cosine function with a fixed period ($t_p$) of 12 hours with an amplitude (A) of 5.8% of average maximum effect (i.e., 344 fmol/mL/min) described the circadian rhythm of

### Table II  Response Parameter Estimates for ACE Activity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measured ACE Activity</th>
<th>Angiotensin II/Angiotensin I Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{max}}$ response model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}$ (fmol/mL/min)</td>
<td>344 (8.8)</td>
<td>0.49 (0.4)</td>
</tr>
<tr>
<td>$E_{\text{max}}$ (ng/mL)</td>
<td>0.43 (34)</td>
<td>0.33 (9.8)</td>
</tr>
<tr>
<td>Cosine function (12-h time period)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude (fmol/mL/min)</td>
<td>20.1 (—)</td>
<td>0.031 (2.4)</td>
</tr>
<tr>
<td>Phase shift (h)</td>
<td>1.01 (—)</td>
<td>1.2 (—)</td>
</tr>
</tbody>
</table>

Data are given as mean (CV%, interindividual variability). $E_{\text{max}}$, maximum effect; $E_{\text{max}}$, MDL100,173 plasma concentration eliciting half-maximum effect; CV, coefficient of variation; ACE, angiotensin-converting enzyme.
ACE activity. Similar results were obtained with measured angiotensin II/angiotensin I ratios. Using angiotensin II/angiotensin I ratios, the amplitude of the single cosine function was 6.3% of the average maximum effect (i.e., 0.49). MDL100,173 plasma concentration eliciting the half-maximum effect (EC₅₀) was also comparable. Average EC₅₀ was estimated to be 0.43 ng/mL with the ACE activity response model versus an estimated average for the EC₅₀ of 0.33 ng/mL with the angiotensin II/angiotensin I response model. Response parameters and circadian rhythm parameters for ACE activity and angiotensin II/angiotensin I ratios are given in Table II. Figure 1 shows the goodness of fit of the final PK-response model to the ACE activity data.

Angiotensin I and II. The direct Eₘₐₓ model adequately described the relationship between MDL100,173 and angiotensin I concentrations as the Hill response factor (sigmoid Eₘₐₓ model) was estimated to be approximately 1. Maximum drug effect was estimated to be 300 fmol/mL, and EC₅₀ (i.e., MDL100,173 plasma concentration eliciting the half-maximum effect) was estimated to be 10.6 (33%) ng/mL.

The direct inhibitory sigmoid Eₘₐₓ model described the relationship between MDL100,173 and angiotensin II concentrations better than the direct inhibitory Eₘₐₓ model as the Hill response factor appeared to be greater than 1 (i.e., 2.9). The maximum drug effect was estimated to be 4.5 fmol/mL, and the EC₅₀ was estimated to be 2.8 (11%) ng/mL. Allowing a (MDL100,173 plasma concentration-independent) time effect on angiotensin II concentrations improved the model significantly. The concentration-independent increase in mean angiotensin II concentration was estimated to be 0.067 mol/mL per day (i.e., 1.5% of baseline angiotensin II concentration at day 1).

Renin activity. A dose-dependent increase in plasma renin activity was only noted at the two highest doses (i.e., 25 and 50 mg once daily). However, a concentration-dependent increase or decrease in plasma renin activity could not be established.

NEP activity. A transient significant increase in ANP excretion was observed after the first dose of 25 and 50 mg of M100240. No appreciable increase in cGMP excretion was observed at 2.5-, 10-, and 25-mg doses. A transient significant increase was observed in subjects receiving 50 mg, however. The profiles of ANP and cGMP excretion after the 1st and the 12th doses were similar. There were no dose- or concentration-dependent effects on measured biomarkers for NEP activity.

Bradykinin. Measured bradykinin plasma concentrations after M100240 administration ranged from 2 to 166 fmol/mL. No clear dose-, concentration-, or time-dependent change in bradykinin plasma concentrations was found.

Blood pressure response. No appreciable blood pressure reductions were observed in healthy volunteers (studies A and B) following 2.5, 10, and 25 mg once-daily administration of M100240. In study C, only the 25-mg dose gave appreciable diastolic and systolic blood pressure–lowering effects at trough in hypertensive patients. In study D, there was a minimal stepwise effect on 24-hour mean blood pressure decreases from the 10- to the 25-mg dose. The 50-mg dose, however, did not add meaningfully to the response in 24-hour mean blood pressure compared to the 25-mg dose in hypertensive patients.

Dose–ACE inhibition relationship (based on simulation). Figure 2 shows that 50 mg M100240 administered once daily produces greater than 80% and greater than 90% ACE inhibition in 40% and 20% of subjects at trough (i.e., 24 h postdose) and indicates that divided doses (i.e., twice-daily dose regimens) provide > 90%
ACE inhibition in at least 5% more subjects than once-daily dose regimens with the same total daily dose (i.e., 49% vs. 44% of total population with 100 mg/day). To achieve a target > 90% ACE inhibition in more than 50% of subjects over a desired dose interval (i.e., 24 h), higher and/or more frequent doses on the order of 25 mg three times daily or 50 mg twice daily may be required (Figure 3).

DISCUSSION

This report presents the first model-based analysis of the relationship between individual drug exposure and biomarker response with the vasopeptidase inhibitor M100240. ACE activity and the angiotensin II/angiotensin I ratio measured at trough correlated well on day 1 ($R^2 = 0.95, p < 0.0001$; Figure 4), as previously reported.\textsuperscript{42} Mean daily increases in angiotensin II were estimated to be 1.5% of baseline (predose) values. Similar increases over time in angiotensin II concentrations have been observed with the ACE inhibitor fosinopril,\textsuperscript{43} whereas angiotensin II concentrations at trough after single and multiple administrations of lisinopril or the ACE/NEP inhibitor omapatrilat remained unchanged.\textsuperscript{13,43} A direct inhibitory $E_{\text{max}}$ model adequately described the relationship between MDL100,173 concentration and ACE activity or the angiotensin II/angiotensin I ratio. Of importance, there was no delay of the ACE inhibitory effect as the temporal rise and decline of MDL100,173 plasma concentrations mirror that of ACE inhibition. The circadian rhythm of both measured ACE activity and the calculated angiotensin II/angiotensin I ratio could be best described with a single cosine function with a fixed period of 12 hours and an amplitude of approximately 6% of the respective baseline values. This finding is in
accordance with previous studies reporting that not only blood pressure but also renin-angiotensin-aldosterone biomarkers follow a circadian rhythm.44-46 As expected, the dose-dependent decrease in angiotensin II was mirrored by a dose-dependent increase in plasma renin activity. Similar results have been reported for lisinopril13,47 and omapatrilat.13,43 Although modest changes in biomarkers for NEP inhibition were observed at the two highest doses, no concentration-dependent signals for NEP inhibition (i.e., increase in urinary excretion of ANP or cGMP) were evident across the dose range evaluated once daily. This is in contrast to previous studies with intravenous administration of M10024026 or oral administration of omapatrilat,9,43,48,49 in which significant increases in urinary volume, ANP, and cGMP excretion were observed. Despite the concentration-dependent changes in ACE inhibition and the nominal NEP inhibition, no overt dose- or concentration-dependent changes in measured bradykinin were found.

The dose range assessed in this study was selected based on the proposed balanced in vitro potency of M100240 for ACE inhibition and NEP inhibition.20 However, a recent in vitro study optimizing the stability of the analyte in the test system revealed that the IC50 value for NEP inhibition is significantly higher as compared to that for ACE inhibition (0.0015 vs. 0.000035 μM).50 This finding is consistent with other ACE/NEP inhibitors, such as gemopatrilat and CGS 30440, with different concentration-response curves for ACE and NEP.51,52 That is, at least 10-fold higher concentrations are required for NEP inhibition than for ACE inhibition. Furthermore, dose selection was based on an oral bioavailability of M100240 estimated to be not statistically different from 100%.25 Oral bioavailability of M100240 was, however, overestimated in this study as ACE inhibition was used as a surrogate for drug exposure by determining its profile after oral and intravenous administration. Indeed, when using a metric of drug exposure, the absolute oral bioavailability of M100240 was measured more accurately at approximately 49%.51 Therefore, we used plasma concentrations of the ACE/NEP inhibitor MDL100,17320 to assess individual drug exposure. Interestingly, area average drug exposure (i.e., area under the curve) appeared to be higher in healthy subjects than in hypertensive patients. This could be an artifact based on the measured sampling scheme as MDL100,173 concentrations were measured up to 48 or 72 hours in healthy subjects, whereas MDL100,173 concentrations were measured up to 8 hours or only at the dosing interval in patients. However, a sensitivity analysis showed that the estimate for apparent clearance remained lower in healthy subjects versus hypertensive patients with modified PK data sets (i.e., without MDL100,173 concentrations after 8, 12, or 24 h postdose). The apparent difference in drug exposure between healthy subjects and hypertensive patients is unlikely the result of a difference in drug adherence, as tested group effects on bioavailability were negligible.

It has been shown for ACE inhibitors that the relationship between blood pressure and ACE inhibition is flat up to 90% ACE inhibition, whereas between 90% and 100% ACE inhibition, the concentration-effect relationship correlation is steep.54 Most clinically available ACE inhibitors produce 80% to 90% ACE inhibition at trough (e.g., lisinopril,42 cilazapril54), and other ACE/NEP inhibitors such as omapatrilat and CGS 30440 produce 70% to 90% ACE inhibition at trough.48,49,52,55 However, it should be noted that different approaches used to assess ACE inhibition may result in slightly different values for ACE inhibition;56 moreover, in vitro and in vivo inhibition of the active sites of ACE may differ among ACE/NEP inhibitors.57 In addition, the long terminal phase observed for many ACE inhibitors typically reflects the dissociation of the
active moiety from plasma ACE, with the half-life of the free drug often much shorter. Hence, MDL100,173 is likely to exhibit a dissociation for ACE that does not favor once-daily administration.

NEP activity should be additive to the comparable ACE inhibition of available ACE inhibitors to provide better clinical outcomes in conditions in which dual ACE/NEP inhibition ultimately proves to be useful. Omapatrilat, a balanced ACE/NEP inhibitor with similar K_s values for ACE and NEP, produces a superior blood pressure–lowering effect than lisinopril at comparable ACE inhibition (i.e., 70%-90% ACE inhibition at the end of the dosing interval) due to the addition of NEP inhibition.

Simulation reveals that a dose of 50 mg M100240 once daily produces at least 90% ACE inhibition 24 hours postdose in only 20% of subjects. The fundamental assumptions that guided the initial dose selection for M100240 were not valid in the clinical setting. This most likely explains the suboptimal blood pressure–lowering effects in hypertensive patients with once-daily doses of up to 50 mg. To achieve a target 90% ACE inhibition at a dose interval in more than 50% of subjects, higher total daily doses on the order of 100 mg would be required. As the neurohormonal response (ACE activity) profile mirrors the drug exposure profile, divided doses (or an extended-release formulation) should provide better target ACE inhibition in more patients not only at trough but also over the entire desired dosing interval. These predictions would be useful to optimize dose and design selection for subsequent clinical trials in hypertension.

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