Discovery of a Well-Absorbed, Efficacious Renin Inhibitor, A-74273


The development of orally active renin inhibitors has been plagued by limited bioavailability in animals and humans. A-74273 is a novel, potent nonpeptide inhibitor of human renin (IC\textsubscript{50}=3.1 nM). This compound was absorbed into the portal and systemic circulations of anesthetized rats, ferrets, monkeys, and dogs after intraduodenal dosing. This favorable pattern also was observed after oral dosing in conscious animals, except in monkeys. Hepatic extraction of A-74273 was more efficient in rats and monkeys than in dogs or ferrets. A-74273 modestly inhibits dog renin, and when given orally as the base (0, 0.3, 1, 3, 10, and 30 mg/kg; n=8 per dose) to conscious, salt-depleted dogs it induced dose-related reductions in mean arterial pressure and plasma renin activity. Peak falls in mean arterial pressure from normotensive baselines were —14±1, —26±3, and —44±3 mm Hg for the 3, 10, and 30 mg/kg groups, respectively (p<0.05). Baseline plasma renin activity values (10.9±1.1-12.7±1.1 ng angiotensin I/ml/hr) were maximally inhibited, ranging from 43±8% at 0.3 mg/kg to 98±1% at 30 mg/kg. Bioavailability in this model was estimated to be 54±13% when plasma drug levels were determined by a renin inhibitory activity assay, but bioavailability was lower when compared with high-performance liquid chromatographic analysis of A-74273. This discrepancy was accounted for by the identification of structurally similar metabolites that are as active as the parent drug against human renin but much less potent against dog renin. Despite overall lower in vitro activity in dog plasma than in human plasma, A-74273 is an active prodrug that is orally efficacious in dogs. (Hypertension 1992;20:768–775)

KEY WORDS • blood pressure • renin • antihypertensive agents • dog studies

The renin-angiotensin system (RAS) is recognized as a pivotal hormonal axis in the regulation of blood pressure and fluid volume\(^1\) and may play a key role in cellular proliferation.\(^2\) The effector hormone angiotensin II (Ang II) is primarily formed as a result of two sequential reactions catalyzed by renin and angiotensin converting enzyme (ACE), in that order. The inhibition of ACE is of notable therapeutic value in the treatment of hypertension and congestive heart failure.\(^3,4\) More than likely, the hemodynamic activity of ACE inhibitors is mediated through inhibition of the RAS,\(^5,6\) but the observed side effects associated with this line of therapy (e.g., cough and angioedema) may be attributed to the promiscuous nature of ACE in recognizing a host of functionally active peptide substrates such as bradykinin, neuropeptide Y, and substance P.\(^7\)

The inhibition of renin as the site of RAS blockade has several virtues. Renin inhibition interrupts the synthesis of Ang II at the first and rate-limiting step of the biochemical cascade, precluding the accumulation of bioactive products. Further, the high degree of specificity of renin to catalyze the hydrolysis of only one naturally occurring substrate, angiotensigen, theoretically suggests the potential for an improved side effect profile over currently available antihypertensive therapies. In addition, like ACE inhibitors, plasma and perhaps tissue Ang II levels are reduced during renin inhibition, obliterating or minimizing the interaction of Ang II with various receptor subtypes.\(^8,9\) Finally, studies in humans support the role of renin inhibitors as therapeutic agents. Clinical experience with, for example, the intravenously active renin inhibitor enalkiren (A-64662) proved that renin inhibition is a safe and effective means of lowering blood pressure in persons with essential hypertension\(^10\) and of improving the hemodynamic status of patients with congestive heart failure.\(^11\)

The search for an orally active renin inhibitor has been a formidable challenge. The development of renin inhibitors as orally administered therapeutic agents has shared the difficulties of other peptide-based molecules with respect to poor oral bioavailability.\(^12\) The present study illustrates progress in examining this issue. A-74273 is one representative of a new class of nonpeptide renin inhibitors that is capable of crossing the intestinal mucosa, surviving its journey through the liver, and making its way into the general circulation for a significant period of time. The result, as illustrated in the dog, is predictable dose-related pharmacological activity.
Methods

In Vitro Pharmacology

Potency and specificity. Several in vitro techniques for the determination of potency and specificity based on inhibition of activity have been described previously: purified human renal renin, pH 6.0,22 human plasma renin, pH 7.4,14 bovine cathepsin D,13 porcine pepsin,13 human gastricsin,15 and human pepsin.15 The IC50 values for animal plasma renins determined at pH 7.4 were quantified by a modification of the human plasma assay in the presence of 3 mM ethylenediaminetetraacetic acid (EDTA), 1.4 mM phenylmethylsulfonyl fluoride (PMSF), and 1% dimethyl sulfoxide12; the respective incubation times at 37°C and the fractions of incubate used for the radioimmunoassay of angiotensin I (Ang I) were 1 hour and 100% for the monkey, 1 hour and 50% for the ferret, 1 hour and 40% for the dog, and 1 hour and 100% for the rat. To avoid possible overestimation of activity, 8-hydroxyquinoline was omitted from the assay.16 The IC50 measurements obtained in the dog were subsequent to furosemide treatment to raise the baseline plasma renin activity (PRA) values.

Inhibition studies of human cathepsin G derived from leukocytes (Sigma Chemical Co., St. Louis, Mo.) were performed with N-succinyl-Ala-Ala-Pro-Phe 4-nitroanilide as substrate.17 Human cathepsin D was isolated from spleen using hemoglobin affinity chromatography18 and assayed with hemoglobin substrate, similar to the assay for bovine cathepsin D.13

In Vivo Pharmacology Studies

All in vivo protocols in this article were reviewed and approved by the Institutional Animal Care and Use Committee of Abbott Laboratories.

Absorption and bioavailability. Plasma drug concentrations were determined after intravenous and intraduodenal dosing in fasted animals. The monkey served as the primate model, the rat and dog served as common species models, and the ferret was studied after pretreatment and were allowed free access to water. Each dog was instrumented previously with an abdominal aortic catheter advanced via the femoral artery under sterile conditions, using the tranquilizer acepromazine (10 mg/kg i.m.) (Aveco), atropine (0.05 mg/kg i.m.) (Phoenix Pharmaceuticals, St. Joseph, Mo.), and isoflurane anesthesia (Anaquest, Madison, Wis.). The recovery period before pretreatment spanned 1–2 weeks during which the dogs received daily antibiotic therapy with enrofloxacin (45 mg p.o.) (Mobay Corp., Shawnee, Kan.), which was discontinued 3 days before the experiment. Salt-depletion pretreatment was attained by administering furosemide (10 mg/kg i.m.) (Phoenix Pharmaceuticals) for 3 days before experimentation, in conjunction with feeding the animals a low (2–4 meq sodium per day) sodium diet (H/D, Hills Pet Products, Topeka, Kan.). To determine the pharmacodynamics and pharmacokinetics, on the day of the experiment (10–17 days after surgery) each dog was placed in a sling and the arterial catheter was connected to a sterile disposable transducer (Sorenson Transpac II, Abbott Laboratories) for the continuous, direct determination of mean arterial pressure (MAP) and heart rate. Data were collected at 3-minute intervals using the Modular Instruments computer-based acquisition system (Modular Instruments Inc., Southeastern, Pa.). After attaining steady baseline hemodynamic values for 90 minutes, each dog randomly received a single oral dose of vehicle or 0.3, 1, 3, 10, or 30 mg/kg A-74273 given as the unformulated dry base in a gelatin capsule. Hemodynamic responses were then followed up for 6 hours. Arterial blood was collected from the aortic catheter for the measurement of PRA at pH 6.0 (Instar Corp., Stillwater, Minn.) using EDTA and PMSF as angiotensinase inhibitors (without 8-hydroxyquinoline) and the determination of plasma drug levels (see above). Each dog received only one dose (n=8 per group).

Statistics

Within each treatment group one-sample t tests were conducted at each posttreatment time compared with

baseline. Between-group differences were analyzed using one-way analysis of variance and Tukey's multiple comparison procedure. Statistically significant differences were accepted at \( p<0.05 \). Results are given as mean±SEM.

### Results

#### Structure and In Vitro Activity

The structure of the parent drug, A-74273, \( N-(3-(4-

morpholino)propyl-5(S)-(2(S)-(4-methoxymethoxy)\\
Piperidin-1-yl)carboxy1-2-phenyl)ethoxyhexanamido-6-
cyclohexyl-4(S)-hydroxy-2(S)-isopropylhexanamid, \) is shown in Figure 1. By conventional definition this molecule does not contain an amide bond between two \( \alpha \)-amino acids and can be classified as a nonpeptide with a molecular weight of 787.21. The proteolytic inhibition profile of A-74273 is shown in Table 1. A-74273 is a potent inhibitor of human renin in the purified assay or in plasma at physiological pH. Although renin inhibitors are generally primate-selective in their activity, A-74273 as the parent drug is a modest inhibitor of dog renin so that under conditions of robust salt depletion the dog can serve as an efficacy model. Interestingly, when the dog assay was run in the presence of 8-hydroxyquinoline, the IQ\(_0\) dropped to 7.1 nM compared with 43 nM as reported in IQ\(_0\) (nM) (plasma renin, pH 7.4).

#### Absorption and Bioavailability Studies

The absorption potential and systemic availability of A-74273 were assessed after intraduodenal administration. A summary of the portal and systemic blood levels are shown in Table 2, and estimated bioavailabilities are contained in Table 3. Data for A-64662, a dipeptide with low oral bioavailability in humans,22 are included where appropriate for comparison.

Plasma drug levels were determined by a renin inhibitory activity assay measuring renin inhibitory activity since concentrations often were below 500 ng/ml and were difficult to quantify by routine HPLC methodology. The data in Table 2 indicate that A-74273 was absorbed well from the gastrointestinal tract of the rat, dog, and ferret as indicated by the detection of high levels in the portal circulation and was absorbed better than A-64662 in the monkey.

Hepatic extraction, reflected by the difference between the portal and arterial drug levels, was lower in the dog and ferret than in the rat and monkey (Table 2). Figure 2 illustrates the portal and arterial drug levels sampled at given intervals over the course of the experiment in the dog. Peak portal blood levels precede peak arterial levels, as expected. Peak arterial levels are fairly well maintained against a background of falling portal blood levels.

Estimated bioavailabilities of A-74273 listed in Table 3 are approximately 25-30% in the rat and ferret and slightly higher in the dog by both the oral and intraduodenal routes of administration. This compound was 16% bioavailable in the monkey after intraduodenal administration (compared with 1.7% bioavailable for A-64662), but only 2% bioavailable after oral administration. The superiority in oral bioavailability of A-74273 compared with A-64662 is demonstrated clearly in the dog, where multifold increases were observed.

#### Identification of Active Metabolites

Blood samples after 30 mg/kg p.o. doses in conscious, salt-depleted dogs were analyzed by HPLC for parent compound (A-74273) concentrations. This study was similar to but separate from the dose–response study and entailed intermittent measurements over 24 hours (n=6). A comparison of the results obtained from the renin inhibitory assay and HPLC analyses suggested the formation of active metabolites. The renin inhibitory assay consistently yielded higher values 1–4 hours after dosing. Metabolites were identified by treating the plasma with \( \text{Na}_2\text{CO}_3\), extracting with 70% ethyl acetate/hexane, reconstituting, and analyzing with HPLC–mass spectroscopy. Two metabolites were identified, the des-morpholino amine (A-78030) and the hydroxyethyl amine (A-78242), obviously derived from the C-terminal morpholino moiety (Figure 1). The results obtained for the blood levels of A-74273, A-78030, and A-78242 as measured by HPLC and the renin inhibitory assay values from conscious dogs dosed with 30 mg/kg p.o. are detailed as average values and variability (SEM) in Table 4. The lower limit of detection for the renin inhibitory activity assay was 11 ng/ml and that for HPLC analysis was 50 ng/ml. Blood levels were approximately at the lower level of detection at 30 minutes.

![Figure 1. Schematic represents structure of parent drug, A-74273, and its two major metabolites, A-78242 and A-78030.](image)

### Table 1. Proteolytic Inhibition Profiles of A-74273 and A-64662

<table>
<thead>
<tr>
<th>Compound</th>
<th>( IC_{90} ) (nM)</th>
<th>( IC_{90} ) (plasma renin, pH 7.4)</th>
<th>% Inhibition (1.0x10(^{-5}) M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(human renin purified, pH 6.0)</td>
<td>Human</td>
<td>Monkey</td>
</tr>
<tr>
<td>A-74273</td>
<td>2.4</td>
<td>3.1</td>
<td>3.6</td>
</tr>
<tr>
<td>A-64662</td>
<td>0.75</td>
<td>14</td>
<td>5.1</td>
</tr>
</tbody>
</table>
after dosing and are reported as zero. Based on the time points sampled, peak blood levels of the parent drug and metabolites coincide at approximately 180 minutes. A-78242 is the predominant circulating metabolite. Renin inhibitory activity assay results suggest that active drug is circulating at low levels 24 hours after dosing. Figure 3 compares the area under the curve for blood levels using the two methods. The renin inhibitory assay results are in good agreement with the sum of the HPLC findings.

Table 5 summarizes the renin inhibitory activities of the parent compound and its metabolites. The activities of the derivatives compared with those of the parent compound in the purified human system at pH 6.0 (same as renin inhibitory assay conditions) reveal that the metabolites would be "counted as parent" in an amount equivalent to their actual concentrations. The IC₅₀ values of the derivatives in human plasma at pH 7.4 suggest that they would be markedly active as they are approximately half as potent as the parent compound. This is not the case for the dog since A-78030 and A-78242 are only approximately 1/7 and 1/6 as potent as the parent compound; thus it would be expected that the duration of the hypotensive effect in this species would be less than in humans, assuming similar biotransformation.

**Table 2.** Portal and Arterial Plasma Drug Levels of A-64662 and A-74273 Following Intraduodenal Administration of 10 mg/kg Dose to Various Species

<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>n</th>
<th>Portal Average</th>
<th>Portal Peak</th>
<th>Arterial Average</th>
<th>Arterial Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-74273</td>
<td>Monkey</td>
<td>6</td>
<td>1,700±400</td>
<td>2,900±600</td>
<td>200±40</td>
<td>400±90</td>
</tr>
<tr>
<td>A-64662</td>
<td>Monkey</td>
<td>7</td>
<td>120±90</td>
<td>570±380</td>
<td>35±11</td>
<td>220±90</td>
</tr>
<tr>
<td>A-74273</td>
<td>Dog</td>
<td>5</td>
<td>970±460</td>
<td>2,800±1,200</td>
<td>570±290</td>
<td>770±330</td>
</tr>
<tr>
<td>A-74273</td>
<td>Rat</td>
<td>5</td>
<td>1,300±200</td>
<td>2,200±300</td>
<td>170±60</td>
<td>330±170</td>
</tr>
<tr>
<td>A-74273</td>
<td>Ferret</td>
<td>6</td>
<td>3,900±500</td>
<td>5,800±500</td>
<td>2,600±600</td>
<td>4,200±900</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

**Table 3.** Estimated Bioavailabilities of A-64662 and A-74273 in Various Species

<table>
<thead>
<tr>
<th>Compound</th>
<th>Routes</th>
<th>Species</th>
<th>n</th>
<th>Bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-74273</td>
<td>i.d.</td>
<td>Monkey</td>
<td>6</td>
<td>16±4</td>
</tr>
<tr>
<td>A-64662</td>
<td>i.d.</td>
<td>Monkey</td>
<td>7</td>
<td>1.7±0.5*</td>
</tr>
<tr>
<td>A-74273</td>
<td>p.o.</td>
<td>Dog</td>
<td>4</td>
<td>1.9±1.5†</td>
</tr>
<tr>
<td>A-74273</td>
<td>i.d.</td>
<td>Dog</td>
<td>5</td>
<td>27±14</td>
</tr>
<tr>
<td>A-74273</td>
<td>p.o.</td>
<td>Dog</td>
<td>8</td>
<td>54±13‡</td>
</tr>
<tr>
<td>A-64662</td>
<td>p.o.</td>
<td>Dog</td>
<td>5</td>
<td>&lt;2.2</td>
</tr>
<tr>
<td>A-74273</td>
<td>i.d.§</td>
<td>Rat</td>
<td>5</td>
<td>24±10</td>
</tr>
<tr>
<td>A-74273</td>
<td>p.o.</td>
<td>Rat</td>
<td>6</td>
<td>26±15</td>
</tr>
<tr>
<td>A-74273</td>
<td>i.d.§</td>
<td>Ferret</td>
<td>6</td>
<td>28±6‡</td>
</tr>
<tr>
<td>A-74273</td>
<td>p.o.§</td>
<td>Ferret</td>
<td>5</td>
<td>31±15</td>
</tr>
</tbody>
</table>

Compound administered as 10 mg/kg dose given in solution; 5 (i.d.) or 6 (p.o.) hours experiment duration unless otherwise noted. Bioavailability compared with 1.0 mg/kg i.v. dose unless otherwise noted. i.d., Intraduodenal; p.o., oral.

*Bioavailability 1.5±0.5% compared with 0.3 mg/kg i.v. dose.
†Compared with 0.3 mg/kg i.v. dose.
‡Compared with 10 mg/kg i.v., oral dose given as dry base.
§2 hours duration.

**Oral Dose–Response Study in Conscious, Salt-Depleted Dogs**

Pharmacodynamics and endocrine profile. Baseline values of all parameters of interest were comparable among the groups of dogs studied. Vehicle control values for MAP and heart rate remained steady throughout the experiment. A-74273 induced statistically significant reductions in MAP and dose-related recoveries at the 3, 10, and 30 mg/kg p.o. doses (Figure 4, top panel). The respective peak changes in MAP were −14±1, −26±3, and −44±3 mm Hg from the normotensive baselines. At the highest dose, MAP remained suppressed by 22 mm Hg on average at 6 hours. Both the systolic and diastolic blood pressures were affected (data not shown). Modest decrements in MAP of 6 and 8 mm Hg were observed in the 0.3 and 1.0 mg/kg groups, respectively. The hypotensive responses did not induce reflex tachycardia at any dose (Figure 4, middle panel). Group baseline PRA values ranged between 10.9±1.1 and 12.7±1.1 ng Ang I/ml per hour, and maximal inhibition spanned between 43±8% at 0.3 mg/kg and 98±1% at 30 mg/kg despite a trend toward elevation with time noted in the vehicle-treated group (Figure 4, bottom panel). The recovery of PRA values was dose-related and qualitatively paralleled the decrements in MAP. However, PRA was relatively more sensitive to renin inhibition than was MAP.

The area under the curve for the blood pressure response to either 10 mg/kg i.v. or 10 mg/kg p.o. showed...
a close similarity of the overall response between the two routes of administration (Figure 5). The area under the curve following dosing was 87±11% mm Hg·hr⁻¹ for the intravenous group versus 61±13% mm Hg·hr⁻¹ for the oral group. As expected, the peak response after intravenous dosing exceeded that following oral dosing. The percent decreases from MAP baselines for intravenous versus oral dosing were 25±1% (peak change) versus 10±4% at 30 minutes after dosing, 23±2% versus 17±3% (peak change) at 45 minutes, and 13±3% for both routes by 180 minutes. The blood pressure responses were no longer significantly different from the control values at the end of the 6-hour observation period.

Pharmacokinetics

Corresponding drug levels in the plasma were determined by renin inhibitory activity assay. The peak values were 146±72, 1,300±373, and 3,075±1,019 ng/ml for the 3, 10, and 30 mg/kg p.o. dose groups, respectively, between 60 and 180 minutes after dosing. At 6 hours 20±6, 248±48, and 1,411±588 ng/ml, respectively, were detected in the plasma samples from these three groups.

Discussion

Structurally diverse peptide-based renin inhibitors have been reported to demonstrate some degree of oral activity measured by various responses, such as lowered PRA or blood pressure in animal models.22-27 and humans.22,28 Because most current transition-state analogue inhibitors of renin are extremely potent, the elicitation of a response induced by low concentrations of a compound is not uncommon.22,27-31 However, renin inhibitors reported to date have displayed poor oral bioavailability.19,31 A clear demonstration of consistent, dose-related reductions in blood pressure of a sufficiently long duration, accompanied by a measurable and predictable pharmacokinetic profile in humans, will be necessary to establish a compound as an orally active drug. The first step in achieving this goal is to demonstrate these characteristics in animals.

A-74273 displays enhanced oral bioavailability in several species. Our experience with early compounds such as A-64662 revealed that poor absorption, marked by variable portal blood concentrations that peaked soon after drug administration (less than 30 minutes after dosing) in conjunction with rapid hepatic extraction, were the two causative factors of low and variable bioavailability.39 A-74273 represents a departure from that pattern. Following intraduodenal dosing all four species tested absorbed A-74273 into the portal circulation. However, the monkey and rat livers more efficiently extracted this compound than did those of the dog and ferret. The only model in which low and variable systemic drug blood levels were observed for A-74273 was the conscious monkey (oral dosing). These results were not due to formulation, technical problems, or the inability of the compound to be absorbed and to be efficacious in the monkey since intraduodenal administration was successful and significantly better than that for A-64662.21 This observation appears to be species-related since oral dosing results in the other three species paralleled the responses to intraduodenal administration. One explanation might lie in the nature of the monkey alimentary canal, which is lined with a sticky, mucus coating in which the renin inhibitor might embed.

Table 4. Blood Levels of A-74273 and Metabolites in Six Conscious Dogs After 30 mg/kg p.o. A-74273

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time after dose (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>A-74273</td>
<td>262±187</td>
</tr>
<tr>
<td>A-78030</td>
<td>960±105</td>
</tr>
<tr>
<td>A-78242</td>
<td>1,077±78</td>
</tr>
<tr>
<td>Total</td>
<td>383±306</td>
</tr>
</tbody>
</table>

Renin inhibitory activity assay

Total: 480±372, 4,747±700, 1,762±458, 953±279, 683±328, 217±75, 103±36, 80±30, 57±19

Values are mean±SEM ng/ml.

Table 5. Renin Inhibitory Activities of Metabolites of A-74273

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (nM) (purified renin, pH 6.0)</th>
<th>IC₅₀ (nM) (plasma renin, pH 7.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-74273</td>
<td>2.4</td>
<td>3.1</td>
</tr>
<tr>
<td>A-78030</td>
<td>3.2</td>
<td>5.1</td>
</tr>
<tr>
<td>A-78242</td>
<td>2.8</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Figure 3. Line graph compares plasma drug levels as analyzed by renin inhibitory activity bioassay (total active drug) and high-performance liquid chromatography (HPLC) (separation of parent drug and metabolites).
Which animal species best predicts human bioavailability of renin inhibitors? The answer to this question is unknown. Furthermore, the bioavailability values in our studies can be viewed as only estimates since our protocols were not designed as crossover studies and renin inhibitory activity assay methodology rather than HPLC analysis of parent drug only was employed to determine plasma drug levels. Although the HPLC value for the A-74273 concentration represents the true level of parental compound in the plasma, it is the renin inhibitory activity assay result that more accurately reflects the potential therapeutic efficacy.

Studies are presently ongoing in our laboratories to better explain why compounds such as A-74273 demonstrate improved oral bioavailability compared with their predecessors. Which structural combination of features is required in a molecule of this general structural class to render it orally bioavailable? Although one desirable goal to be achieved in designing the next generation of renin inhibitors would be to reduce the molecular weight of the compounds, it may not be a necessary milestone. The absorption of A-74273, a compound with a molecular weight of 787, dispels the notion that renin inhibitors are too large to be absorbed. The combination of physical properties with an optimal balance between lipophilicity and water solubility coupled with a high dissolution rate may be key to designing an orally active compound. A-74273 is highly lipophilic, yet it is soluble in water (0.8 mg/ml, pH 7.7 and 0.21 mg/ml, pH 7.4 phosphate buffer), as are two of its metabolites, A-78030 (1.87 mg/ml, pH 7.4 phosphate buffer) and A-78242 (1.84 mg/ml, pH 7.4 phosphate buffer). A third metabolite has been detected recently in rat and dog bile. Clearly, one positive feature of this compound is that it could be administered as the unformulated dry base in a capsule, a rare characteristic among renin inhibitors. Although stabilizing the peptide bonds to enzymatic degradation in dipeptide renin inhibitors alone does not guarantee oral activity, it is certainly a minimal requirement for oral activity, and in the case of A-74273 the peptide bonds were eliminated completely. Based on the structure of the metabolites identified, A-74273 appears to be susceptible to metabolism only at the C-terminal morpholine moiety (Figure 1). The pattern of metabolism of A-74273 was studied in vitro (data not shown). Experiments in which carbon-14-labeled A-74273 was incubated with rat, dog, and human hepatic microsomes showed that the patterns of metabolism were parallel in these species (i.e., formation of the same metabolites), suggesting that the active metabolites identified in dog plasma may be formed and circulate in humans.

The pharmacodynamic and pharmacokinetic profile of A-74273 represents a new level of progress in achieving improved oral bioavailability that translates into predictable functional activities in animal models. Of note are the low variability and the dose-related consistency of the physiological responses, something traditionally not seen with compounds that have low (<5%) bioavailabilities. A-74273 is an efficacious, oral hypotensive agent in the conscious salt-depleted dog, even though the parent drug and its active metabolites are considerably less potent against dog renin than against human renin and the baseline blood pressures of the dogs in this study were normal and not elevated. For these reasons, high doses of A-74273 were used in the dog. The maximal hypotensive response to A-74273 occurred later during the present experiments and in experiments in monkeys than reported previously with other renin inhibitors, presumably reflecting the desirable pharmacokinetic activity of this compound. Note that using a pharmacological response to estimate oral bioavailability does not appear to be a viable
alternative to determining plasma drug concentrations. The results in Figure 5 suggest that the bioavailability of a renin inhibitor such as A-74273 could be greatly overestimated if the area under the curve of intravenous and oral hypotensive responses were compared as the basis for the calculation. As has been observed with other agents that interfere with the RAS,\(^\text{12}\) A-74273 had no significant effect on heart rate.

A dissociation between the blood pressure and PRA responses to renin inhibition has been described previously with peptide-based renin inhibitors.\(^\text{12,23}\) Although some degree of dissociation is evident in the present dose–response study with A-74273, the data suggest that the inhibition and recoveries of PRA values qualitatively paralleled the MAP response. Thus, the dissociation between the blood pressure response and the PRA inhibitory response observed with earlier reported compounds appears to be less of an issue with A-74273. This may be due to the nonpeptidic nature, physical properties, or both that allow this compound to attain a rapid distribution into tissues. The possibility that a tissue RAS\(^\text{6}\) coexists with the well-defined blood-borne system has been a recurrent explanation for the observed dissociation between the blood pressure and PRA responses to renin inhibition and still exists as a viable means to explain pharmacological durations of action that exceed the circulating half-life of renin inhibitors.

Based on the findings described in this article, the rational design of renin inhibitors with the potential for oral activity in humans may be feasible as the mystery of how to discover bioavailable molecules based on peptide structures slowly unfolds. However, we will not know whether we truly have made progress in addressing the bioavailability obstacle until the results demonstrated in preclinical studies are confirmed in human trials.

Acknowledgments

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