Prodrugs to Improve the Oral Bioavailability of a Diacidic Nonpeptide Angiotensin II Antagonist

Bruce J. Aungst,[1,2] Judy A. Blake,[1] Nancy J. Rogers,[1] Hiroshi Saitoh,[1] Munir A. Hussain,[1] Carol L. Ensinger,[1] and James R. Pruitt[1]

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DMP 811 is a diacidic angiotensin II antagonist. It has relatively low oral bioavailability in rats. A prodrug approach to improving oral bioavailability was tested. Five esters were synthesized and their stability in rat plasma in vitro was determined. The hydrolysis rates of these five esters ranged from almost immediate to negligible. A simple n-propyl ester was hydrolyzed very slowly (<10% in 24 hr) in rat plasma in vitro, and after oral dosing in rats plasma prodrug concentrations were much greater than DMP 811 concentrations. A pivaloyloxymethyl ester (**1**) was hydrolyzed relatively rapidly in rat plasma in vitro. Prodrug **1** was rapidly hydrolyzed by the intestine in vitro, and the intestinal permeation of DMP 811 was increased. DMP 811 oral bioavailability was 47% in rats dosed with 10 mg/kg **1**, compared to 11% for rats dosed with 10 mg/kg DMP 811. However, DMP 811 bioavailability was only 27% after a 2 mg/kg dose of **1**. In vitro plasma hydrolysis of **1** was highly species-dependent, with a half-life of 13 hr in human plasma but only 1 min in rat plasma. The prodrug approach has potential for improving the oral bioavailability of DMP 811, but selection of the optimal prodrug must be done in humans or in a species, such as dogs, with hydrolysis characteristics closer to humans.

KEY WORDS: angiotensin II antagonist; prodrug; oral bioavailability; intestinal permeation; DMP 811.

INTRODUCTION

Angiotensin II (Ang II) antagonists block the interaction of Ang II with its receptors and inhibit the actions of the renin-angiotensin system. Nonpeptide Ang II receptor antagonists have recently been discovered (1, 2), and the prototype, losartan, is in advanced clinical trials. Results of those trials suggest that losartan will be effective in treating hypertension and congestive heart failure (3). The therapeutic benefits of interrupting the renin-angiotensin system with angiotensin converting enzyme inhibitors are well established. Ang II antagonists should provide similar benefits with some possible advantages, and represent a new class of therapeutic agents.

Losartan and many of the newer Ang II antagonists have as a part of their structure a biphenyl tetrazole (4). DMP 811 and DuP 532[3] are two second generation compounds, and in preclinical studies these afforded the advantages of greater affinity for the Ang II receptor and increased antihypertensive potency, compared to losartan (5, 6). A structural feature of DMP 811 and DuP 532 that contributes to their increased receptor affinity is a carboxylic acid group, which with the acidic tetrazole, makes these compounds diacidic.

Both DMP 811 and DuP 532 have limited oral absorption, as indicated by their i.v./oral ED_{50} ratios in animals. The diacidic structure is clearly related to reduced oral absorption, since changing the carboxylic acid to carboxaldehyde results in complete oral absorption, although the diacids have greater intrinsic potency (5). Other studies have shown that the diacids, DMP 811 and DuP 532, have low lipophilicity and poor intestinal permeation in vitro and in situ, relative to other related biphenyl tetrazoles (Aungst et al., unpublished). This seems to be responsible for their poor oral bioavailability. A logical approach to increase the lipophilicity and intestinal permeation of these diacids is using the prodrug approach. Prodrugs have been successfully employed to increase the lipophilicity and oral bioavailability of various diacidic angiotensin converting enzyme inhibitors (7, 8). Similarly, a classic use of prodrugs to improve lipophilicity and oral bioavailability is with β-lactam antibiotics (9, 10).

We have tested the possibility of using prodrugs to improve the oral bioavailability of DMP 811. Intestinal permeation was examined in vitro, and bioavailability was evaluated in rats, measuring both prodrug and DMP 811 plasma concentrations. Since the performance of any prodrug will be dependent on its rate of conversion to the active drug, various prodrugs having a range of hydrolysis rates were prepared and evaluated. Hydrolysis rates were measured using plasma from rats, dogs, and humans, since prodrug hydrolysis rates can be highly species-dependent (11).

During the course of this work, other groups interested in developing nonpeptide Ang II antagonists have also explored prodrugs as a means of improving the bioavailability of their diacidic lead compounds. Results of these efforts, some of which have recently been published (12-14), are consistent with ours in suggesting that the use of prodrugs to increase oral bioavailability of nonpeptide Ang II antagonists is feasible.

MATERIALS AND METHODS

Preparation of Prodrugs

DMP 811 (4-ethyl-2-propyl-1-[[2′-(tetrazol-5-yl)biphenyl-4yl]methyl]imidazole-5-carboxylate) was supplied by The DuPont Merck Pharmaceutical Company. The prodrugs whose structures are shown in Figure 1 were prepared from DMP 811.

For prodrug **1**, 0.2490 g potassium iodide in one portion was added to a mixture of 0.6588 g DMP 811, 0.2049 g chloromethylpivalate, and 0.1382 g potassium carbonate in 3 ml dimethylformamide. The mixture was stirred overnight at room temperature under argon. The reaction was then partitioned between 8 ml water and 40 ml ethyl acetate. The organic layer was washed once with ice cold 0.1 N sodium thiosulfate, once with water, once with brine, and dried with

[1] DuPont Merck Research Laboratories, P.O. Box 80400, Wilmington, Delaware 19880-0400.

[2] To whom correspondence should be addressed.

[3] DuP 532 is 2-propyl-4-pentafluoroethyl-1-[[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]imidazole-5-carboxylate.

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anhydrous magnesium sulfate. After suction filtration and evaporation of the filtrate, flash chromatography with 25% ethyl acetate/hexanes provided 0.46 g of the desired product. ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.18 (s, 9H), 1.22 (t, 3H), 1.65 (m, 2H), 2.5 (m, 2H), 2.9 (q, 2H), 5.41 (s, 2H), 5.81 (s, 2H), 6.75 (d, 2H), 7.04 (d, 2H), 7.2-7.35 (m, 2H), 7.44 (m, 1 H), 7.9 (m, 1 H). Anal: calculated for $C_{29}H_{34}N_6O_7$: C, 65.64%; H, 6.46%; N, 15.84%. Found: C, 65.04%; H, 6.42%; N, 15.50%.

Prodrug **2** was prepared as follows. A mixture of 7.5 g of DMP 811, 5.17 g trityl chloride, and 1.92 g triethylamine was stirred in 50 ml methylene chloride overnight. After water addition and acidification with 1 N HCl to pH 3, the mixture was separated and the organic phase was extracted with methylene chloride/ethyl acetate (1:1). The organic extract was dried with anhydrous sodium sulfate, suction filtered, and the solvent was evaporated. After flash chromatography with ethyl acetate, 6.46 g of 4-ethyl-2-propyl-1-[[2′-(N-triphenylmethyl(tetrazole-5-yl))biphenyl-4-yl]methyl]-imidazole-5-carboxylate was obtained. 1.31 g of this product, 0.39 ml iodopropane, and 0.55 g potassium carbonate were added to 6 ml dimethylformamide. The reaction was allowed to stir for 24 hr, and then was diluted with 12 ml water and 90 ml ethyl acetate. The organic layer was separated and washed five times with water, once with brine, and dried with anhydrous magnesium sulfate. Chromatography with a gradient of 5 to 50% ethyl acetate in hexane gave 0.96 g of propyl 4-ethyl-2-propyl-1-[[2′-(N-triphenylmethyl(tetrazol-5-yl))biphenyl-4-yl]methyl]imidazole-5-carboxylate. This was dissolved in 22 ml of methanol and then 4.8 g silica gel and 6 drops of 6 N HCl were added. The gel was filtered away after 3 days, and the resulting silica was washed with methylene chloride and then ethyl acetate. The combined organic solutions were evaporated and the residue was chromatographed with a gradient of 0 to 5% methanol in chloroform to provide 0.25 g of prodrug **2**. ^{1}H-NMR ($CDCl_3$) δ 0.8-0.95 (m, 9H), 1.45-1.65 (m, 4H), 2.15 (t, 2H), 2.48 (t, 2H), 4.07 (t, 2H), 5.4 (s, 2H), 6.74 (m, 2H), 7.05 (m, 2H), 7.27 (m, 1 H), 7.48 (m, 2H), 7.77 (m, 1 H). Anal.: Calculated for $C_{26}H_{30}N_6O_2$; C, 68.10%; H, 6.59%; N, 18.33%. Found: C, 68.19%; H, 6.45%; N, 17.69%.

Prodrug **3** was prepared by treating DMP 811 with Eschenmoser's salt in tetrahydrofuran at room temperature to give the N,N-dimethylamino ester. Anal.: Calculated for $C_{26}H_{31}N_7O_2$; C, 65.94%; H, 6.60%; N, 20.70%. Found: C, 68.30%; H, 7.30%; N, 20.75%.

To prepare prodrug **4**, 1.22 ml methanol was added slowly to a suspension of 1.29 chloromethyl chloroformate and 1.52 g potassium carbonate in 50 ml methylene chloride. Evaporation of the filtrate provided 1.12 g chloromethyl methyl carbonate as an oil which was used without further purification. After chromatography (ethyl acetate) from 0.66 g 4-ethyl-2-propyl-1-[[2′-(N-triphenylmethyl(tetrazol-5-yl))biphenyl-4-yl]methyl]imidazole-5-carboxylate, 0.19 g chloromethyl methyl carbonate, 0.14 g potassium carbonate, and 0.25 g potassium iodide in 3 ml dimethylformamide, 0.44 g of methoxycarbonyloxymethyl 4-ethyl-2-propyl-1-[[2′-(N-triphenylmethyl(tetrazol-5-yl))biphenyl-4-yl]methyl] imidazole-5-carboxylate was obtained. Prodrug **4**, the methoxycarbonyloxymethyl ester of DMP 811 (0.21 g), was obtained after chromatography (0% to 10% methanol/chloroform gradient) from 4-ethyl-2-propyl-1-[[2′-(N-triphenylmethyl(tetrazol-5-yl))biphenyl-4-yl]methyl]imidazole-5-carboxylate and 3 drops of 6 N HCl in 10 ml methanol. ^{1}H-NMR ($CDCl_3$) δ 0.83 (2t, 6H), 1.55 (m, 2H), 2.18 (t, 2H), 2.45 (q, 2H), 3.75 (s, 3H), 5.4 (s, 2H), 5.78 (s, 2H), 6.75 (m, 2H), 7.03 (m, 2H), 7.43-7.65 (m, 3H), 7.78 (m, 1H). Anal.: Calculated for $C_{26}H_{28}N_6O_5$; C, 61.89%; H, 5.34%; N, 15.90%. Found: C, 62.20%; H, 5.67%; N, 16.59%.

Prodrug **5** was prepared as follows. A mixture of 0.659 g DMP 811, 0.288 g 4-bromomethyl-1,3-dioxa-5-methyl-cyclopentene-2-one, and 0.138 g potassium carbonate was stirred in 3 ml dimethylformamide at room temperature under nitrogen for 4 hr. The reaction was partitioned between 8 ml water and 40 ml ethyl acetate. The organic extract was washed with water (6 × 10 ml) and brine, and dried with anhydrous magnesium sulfate. Filtration, evaporation, and flash chromatography of the residue with a 0% to 5% methanol/chloroform gradient provided 0.75 g of (1,3-dioxa-5-methyl-cyclopentene-2-one-4-yl)methyl 4-ethyl-2-propyl-1-[[2′-(N-triphenylmethyl(tetrazol-5-yl))biphenyl-4-yl]methyl]imidazole-5-carboxylate. To a solution of 0.75 g of the above product in 10.5 ml methanol in a nitrogen atmosphere was added 4 drops of 6 N HCl. The mixture was stirred at room temperature for 2 days. The volatiles were evaporated and the residue immediately chromatographed using a 0% to 6% methanol/chloroform gradient. Prodrug **5** (0.35 g) was obtained as a white foam. ^{1}H-NMR ($CDCl_3$) δ 0.80-0.93 (m, 6H), 1.50-1.62 (m, 2H), 2.05 (s, 3H), 2.18-2.20 (t, 2H), 2.40-2.55 (q, 2H), 4.83 (s, 2H), 5.40 (s, 2H), 6.65-6.75 (d, 2H), 7.00-7.11 (d, 2H), 7.43-7.50 (m, 1H), 7.50-7.65 (m, 2H), 7.80-7.83 (m, 1H). Anal.: Calculated for $C_{28}H_{28}N_6O_5$; C, 63.63%; H, 5.34%; N, 15.90%. Found: C, 63.16%; H, 5.41%; N, 15.60%.

Analyses

Plasma samples from in vitro hydrolysis studies and from in vivo bioavailability studies, and tissue homogenate samples from in vitro hydrolysis studies were assayed for prodrug and DMP 811 concentrations. EXP3714 (2-n-butyl-4-chloro-1-[(2′(1 H-tetrazol-5-yl)biphenyl-4-yl)methyl]-imidazole-5-carboxylate) was used as an internal standard for each assay. The method for prodrugs **1**, **2**, **4**, and **5** and for simultaneous DMP 811 determination was as follows. The internal standard, 0.2 ml of 0.14 M phosphoric acid, and 8 ml ethyl acetate were added to 0.2 ml of sample or standard. After mixing and separating phases, the organic layer was collected and evaporated. The residue was redissolved in 0.2 ml mobile phase and assayed by HPLC. The chromatography utilized a 250 × 4 mm C8 LiChrospher 60 RP-select B column (EM Separations, Gibbstown, NJ). A two component gradient mobile phase was used, wherein the components were (A) 0.6% acetic acid in 0.01 M sodium acetate and (B) acetonitrile. For prodrug **1**, a gradient of 75% A: 25% B to 25% A: 75% B was run over 35 min, and the flow was 1.2 ml/min. For **2**, **4**, and **5**, the gradient was 75% A: 25% B to 35% A: 65% B over 25 min at 1.2 ml/min. Fluorescence detection with excitation at 235 nm and emission at 370 nm was used.

Some bioavailability studies in which only DMP 811 was measured used a modified version of this assay. Extraction

was into ether:isopropanol (95:5), and this was followed by back-extraction into 0.2 ml of 0.05 M NaOH, which was evaporated and reconstituted with mobile phase. A 150 × 4 mm C8 Nova-Pak column (Waters, Milford, MA) was used. The mobile phase components were as described previously, with a gradient of 81% A: 19% B to 55% A: 45% B run over 17 minutes. Detection was by uv absorbance at 246 nm.

In studies with prodrug 3, the assay was performed by adding 0.2 ml acetonitrile containing the internal standard, to 0.2 ml of sample, followed by vortexing, centrifugation, and HPLC on the supernatant. The column and detection were as for the other prodrugs. Isocratic conditions were used, with a mobile phase containing 77% A: 23% B.

In Vitro Hydrolysis Studies

Prodrug hydrolysis studies were performed using fresh rat, dog, and human plasma, and a rat intestinal homogenate containing 10% (w/v) tissue in pH 7.4 buffer. The medium was warmed to 37° and prodrug was added. At various times the reaction was quenched by adding the extraction solvent. Prodrug and DMP 811 concentrations were determined as described above.

Intestinal Permeation

A segment of jejunum was excised from ether-anesthetized, fasted, male, Sprague-Dawley rats (Crl:CD (SD)BR, Charles River, Kingston, KY). The segment was rinsed and cut longitudinally to form a sheet. Segments of the sheet were mounted onto the pins of diffusion cells, and the other half-cells clamped into place. The surface area for diffusion was 1.78 cm^2. A prodrug or DMP 811 buffer solution (0.2 mM) was placed into the mucosal chamber, and drug-free buffer was placed into the serosal chamber. The mucosal and serosal solutions were bubbled with 95% O_2:5% CO_2, which also provided mixing, and they were maintained at 37°. Serosal samples (0.5 ml) were taken at various times and diluted with 0.5 ml of 0.1% trifluoroacetic acid. Prodrug and DMP 811 concentrations were determined by HPLC.

Bioavailability Studies

Fasted, male Sprague-Dawley rats were used in all studies. DMP 811 was administered intravenously and orally using aqueous dosing solutions at pH ≈ 8. Dosing volumes were 1 ml/kg, and the doses were 1 mg/kg i.v. and 10 mg/kg oral. Prodrugs 1 and 2 were dosed orally at 10 mg/kg and 2 mg/kg (for prodrug 1 only) using PEG 400 as the vehicle. Dosing volumes were 1 ml/kg. Blood samples were collected via an indwelling jugular vein cannula, which was surgically implanted on the day before the experiment. Heparin was used as an anticoagulant. The volume of blood samples was replaced with transfused blood from separate donor rats. Plasma was collected and frozen until analyzed using the methods described above. The assay was for both prodrug and DMP 811 when prodrugs were dosed. The area under individual plasma DMP 811 concentration vs. time curves (AUC) was calculated using the trapezoidal method, with extrapolation based on the slope of decay of the terminal linear portion of the semilog plot of plasma concentrations vs. time. DMP 811 bioavailability was calculated based on dose-corrected AUC values after oral and i.v. doses. Bioavailabilities of intact prodrugs were not calculated. There were 4-6 rats in each group. Results are presented as mean ± S.E.

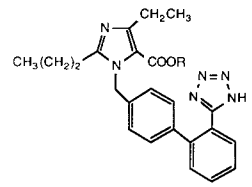
RESULTS

Hydrolysis in Rat Plasma

Five DMP 811 prodrugs with diverse ester substituents were prepared and evaluated. These were selected based on their expected broad range of susceptibilities to hydrolysis. Structures are shown in Figure 1. These prodrugs were initially tested for their stability in rat plasma in vitro. A comparison of the prodrug concentration vs. time profiles for the five prodrugs is given in Figure 2. The disappearance of each prodrug was associated with the appearance of an equimolar concentration of DMP 811 (which are not shown in Figure 2). First-order hydrolysis half-lives could be calculated for prodrugs 1 and 4, and these were 1.1 and 0.9 minutes (replicate determinations) for 1, and 0.8 min for 4. The simple esters 2 and 3 were very stable in rat plasma (<20% hydrolyzed in 24 hr), and prodrug 5 was hydrolyzed almost immediately. Based on its intermediate stability profile, prodrug 1 was the subject of further testing.

Intestinal Permeation in Vitro

The hypothesis proposed was that prodrugs could have greater lipophilicity and intestinal permeation than DMP 811, and that this could result in improved oral bioavailability. A comparison of intestinal permeation profiles of DMP 811 and prodrug 1 was made using excised rat intestine in diffusion cells. Prodrug 1 did not permeate through the rat intestine intact, and was apparently hydrolyzed by the brush border or during permeation through the membrane. A hydrolysis study using a homogenate of rat intestine (10% w/v) showed rapid hydrolysis of prodrug 1, with a half-life of 1.6



Compound	R
DMP 811	H
1	
2	
3	
4	
5	

Fig. 1. Structures of DMP 811 and the prodrugs prepared and evaluated.

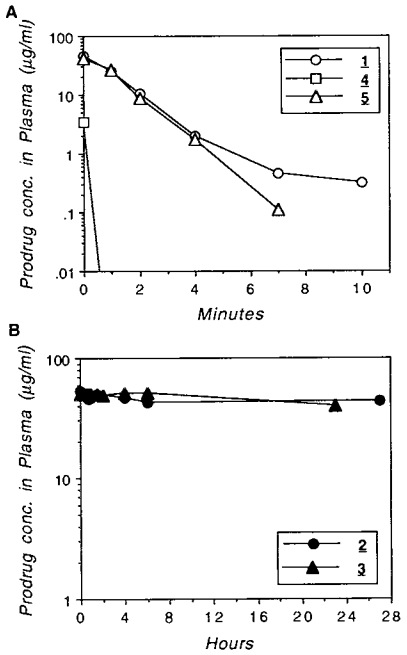


Fig. 2. Hydrolysis profiles of prodrugs 1-5 in rat plasma in vitro. Note that the time scale of A is minutes, and the time scale of B is hours.

min. DMP 811 appearance in the receiving chamber was greater when the donor solution contained prodrug 1 than when it contained DMP 811 (Figure 3). This confirmed the hypothesis that prodrugs have greater intestinal permeation than DMP 811 and can increase the absorption of DMP 811.

Bioavailability Studies

DMP 811 oral bioavailability was determined in rats, based on a comparison of AUC values after 1 mg/kg i.v. and 10 mg/kg oral doses. Bioavailability was 11.1% ± 1.2%. Prodrug 1 was administered to rats at a 10 mg/kg dose, which is equivalent to 7.8 mg/kg DMP 811. DMP 811 was soluble in an aqueous dosing vehicle at slightly alkaline pH, but prodrug 1 was administered in PEG 400. In a separate study there was no difference in DMP 811 bioavailability when it was administered in aqueous or PEG vehicles. Prodrug 1 was rapidly converted to DMP 811 in vivo, and no unchanged prodrug was detected in any plasma sample. Figure 4 shows the plasma DMP 811 concentration vs. time profiles after

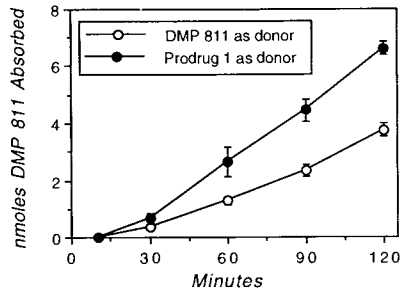


Fig. 3. Permeation of DMP 811 through rat intestine in vitro from donor solutions containing DMP 811 (○) or prodrug 1 (●). Each curve represents the mean ± S.E. of 3 experiments.

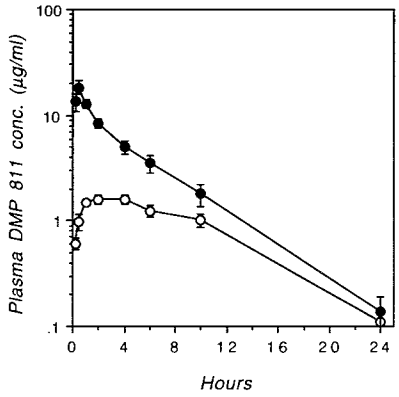


Fig. 4. Plasma DMP 811 concentrations in rats dosed orally with 10 mg/kg DMP 811 (○) or 10 mg/kg prodrug 1 (●), which is equivalent to 7.8 mg/kg DMP 811. These represent the mean ± S.E. of 4 and 6 rats.

prodrug 1 or DMP 811 dosing. Plasma DMP 811 concentrations were greatly increased using the prodrug, and the maximum concentration occurred much sooner after dosing. Bioavailability was increased 4-fold, to 46.7% ± 6.5%.

The bioavailability study was repeated using a lower dose of prodrug 1. Results of all studies are summarized in Table I. The increase in bioavailability was not as great with a 2 mg/kg prodrug dose as at the 10 mg/kg dose. Although the cause of this dose-dependence is not known, one possibility is concentration-dependent hydrolysis.

For comparison, the propyl ester, prodrug 2, which was very stable in rat plasma in vitro, was also dosed orally to rats. Figure 5 shows plasma concentrations of prodrug and DMP 811 after 2 dosing. In this case, plasma concentrations of the prodrug were much greater than DMP 811 concentrations formed by hydrolysis. Since this prodrug is less active than DMP 811 as an Ang II receptor antagonist, it is undesirable to have prodrug hydrolysis so slow that prodrug concentrations are greater than DMP 811 concentrations. From the rapid peak in DMP 811 concentrations, it appears that some hydrolysis occurred during absorption, most likely by the intestine, as in the in vitro intestinal permeation study.

Species Dependence of Hydrolysis

The species-dependence of in vitro plasma hydrolysis of prodrug 1 was examined by comparing rat, dog, and human plasma. This prodrug was hydrolyzed much more slowly in dog and human plasma than in rat plasma. The apparent

Table I. DMP 811 Bioavailability (Mean ± S.E.) in Rats Dosed with DMP 811 or Prodrug 1

	10 mg/kg DMP 811 in pH 8 solution	10 mg/kg 1 (7.8 mg/kg DMP 811) in PEG 400	2 mg/kg 1 (1.6 mg/kg DMP 811) in PEG 400
N	4	6	8
C_{max} (μg/ml)	1.80 ± 0.09	19.10 ± 2.25	1.94 ± 0.32
t_{max} (hr)	2.8 ± 0.6	0.6 ± 0.1	0.9 ± 0.3
t½ (hr)	4.75 ± 0.44	3.76 ± 0.42	4.61 ± 0.72
F (% of Dose)	11.1 ± 1.2	46.7 ± 6.5	27.4 ± 2.4

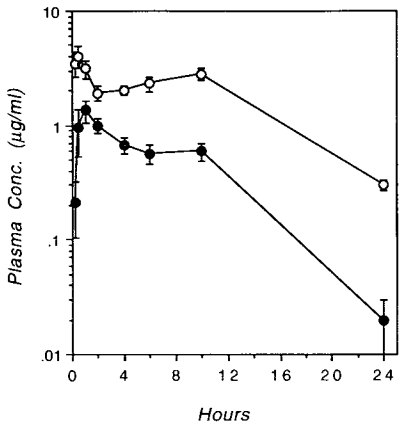


Fig. 5. Plasma concentrations of prodrug 2 (○) and DMP 811 (●) in a single group of 4 rats dosed with 10 mg/kg prodrug 2 orally. Mean ± S.E. are given.

half-life in human plasma was 13 hr, compared to the 1 min half-life in rat plasma. The time course of 1 hydrolysis in dog plasma was similar to that in human plasma, but since the dog plasma study only covered 3 hr, the half-life was not estimated.

DISCUSSION

This work demonstrates the feasibility of using prodrugs of DMP 811, a diacidic nonpeptide Ang II antagonist, to improve its oral absorption. Both the intestinal absorption rate of DMP 811 in vitro and oral bioavailability of DMP 811 in vivo were increased in rats with prodrug 1. This prodrug was relatively rapidly hydrolyzed in rat plasma or by rat intestine in vitro. The hydrolysis rates are critical determinants of the usefulness of any particular prodrug. Ideally, for this application the prodrug should be delivered to the intestinal membrane completely intact, it should have greater permeation than DMP 811, and it should then be rapidly hydrolyzed during absorption or in the plasma in vivo. Prodrug 1 seems to have these properties in rats. Prodrug 2, in contrast, is useful to illustrate the consequence of using a prodrug that is hydrolyzed too slowly. After oral dosing, this prodrug persisted in plasma and there was insufficient hydrolysis to DMP 811.

Prodrug hydrolysis rates are highly species-dependent. The rat may not be a good animal model for selecting the optimal prodrug within this series of compounds, because of rapid prodrug hydrolysis. But the rat is useful for feasibility studies such as these, and rat studies require the synthesis of relatively little compound for a prodrug to be evaluated. The results from rats suggest that a prodrug that is rapidly hydrolyzed in human plasma may be useful for improving bioavailability in humans. Further testing to identify the optimal prodrug should be done in a species, such as dogs, with hydrolysis characteristics closer to humans. From the rat plasma hydrolysis results, it is clear that simple esters like 2 and 3 will be much too stable. The double ester approach, as exemplified by prodrugs 1, 4, and 5, is more likely to be useful in humans.

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