Intrapulmonary Delivery of Renin Inhibitory Peptides Results in Sustained Release Because of Saturable Transport

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INTRODUCTION

The clinical application of peptide drugs has been considered for the treatment of hypertension [renin inhibitors (1)], AIDS [HIV protease inhibitors (2)], and pain [enkaphalins and endorphins (3)]. A limitation of these compounds is their intrinsically poor absorption from the gut, even when protected from digestion (4). The intrapulmonary route of administration represents an alternative to oral delivery. Recent studies (5) have suggested that a series of model phenylalanine peptides is absorbed from the lungs more extensively than from the gut by a process that differs from gut absorption since it is independent of the hydrogen-bonding capacity of the peptide. To extend these observations to therapeutically useful peptides, renin inhibitory peptides (RIPs) were studied further in an intrapulmonary rat model.

MATERIALS AND METHODS

Peptides

Two RIPs, ditekiren and U-77436 (Fig. 1), were synthesized at the Upjohn Company as described previously (6,7). The pyridine and histidine of ditekiren had pK_a's of 4.0 and 6.3, respectively (Davio *et al.*, unpublished data).

Formulations

Intrapulmonary (IP). Peptides were dissolved in 0.1 *M* HCl. An equal volume of absolute ethanol was added, followed by dilution with 5% dextrose to give final concentrations of 10% ethanol with 8 m*M* HCl for Animal Procedure I and 10% ethanol with 10 m*M* HCl for Animal Procedure II. Increasing the acid concentration better accommodated the higher concentrations of peptides required for the second animal procedure. In some experiments, ^{51}Cr was added to the formulation so that the amount of peptide reaching the lungs could be monitored.

Intravenous (IV). Solutions of peptide were further diluted with 5% dextrose to lower the concentration for IV studies.

Dial-Urethane Anesthetic

The anesthetic was prepared by mixing 40 g urethane, 40 g ethylurea, and 10 g 5,5-diallyl barbituric acid. Next, a solution of 50 mg disodium calcium salt of EDTA dissolved in 10 mL sterile water was added, and the mixture was heated in water until completely dissolved. The solution was then cooled at room temperature and the volume brought up to 100 mL with sterile water.

Animals

Sprague Dawley male rats (400–550 g) from the Charles River Corporation were used. Animals were fasted overnight but allowed access to water.

Animal Procedure I. Bile and Urine Collection

Rats were anesthetized by intraperitoneal injection of 0.6 ml/kg of dial-urethane. Once unconscious, a longitudinal incision was made along the ventral side of the neck to expose the trachea and polyethylene tubing (I.D., 1.67 mm; O.D., 2.42 mm) was inserted through a transverse incision made between two cartilaginous rings. The cannula was then secured with a nylon ligature.

Next a midline incision was made into the abdomen to expose the bile duct, which was then cannulated with polyethylene tubing (I.D., 0.28 mm; O.D., 0.61 mm). The bladder was voided by gentle massage and a drop of cyanoacrylate adhesive was used to seal the urethral opening. After closing the abdominal incision with autoclips, rats were then placed on a padded benchtop and covered by towels to maintain core temperature throughout the study.

The intrapulmonary (IP) injection of the peptide was made by placing 25 μg of RIP in 50 μL of the formulation directly into the trachea cannula with a Hamilton syringe. The dose was then aerosolized and delivered to the lungs by a single actuation of a compressed air canister (32 psig; 50:50 mixture of P-11 and P-12). Intravenous injections of 75 μg/mL were given to each rat via the tail vein.

Bile was collected for 3–10 hr at half-hour or hourly increments. Urine was collected at sacrifice by aspiration of the bladder contents. Peptide in bile and urine were quantified by a previously reported activity assay (8) after diluting aliquots at least 10-fold to avoid interfering substances in these biological fluids.

Animal Procedure II. Serum Collection

Rats were implanted with a superior vena cava cannula (9,10) and a trachea cannula as described above. Peptides were administered at doses and times specified in Figs. 3 and 4. At various times after dosing, 0.3 mL of blood was collected via the cannula. Sera was harvested and stored frozen

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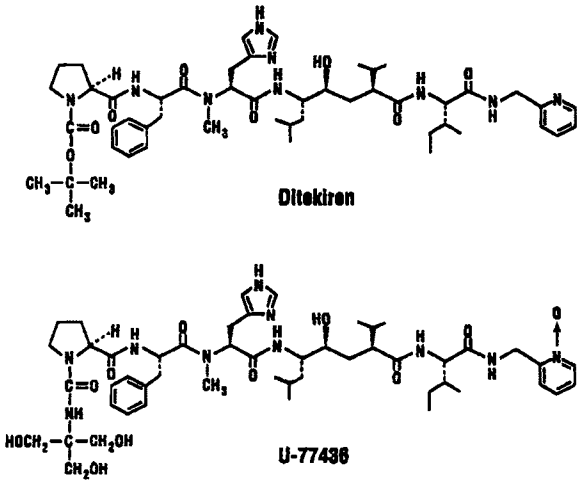

Fig. 1. Chemical structure of ditekiren and U-77436.

until the RIPs were quantified by a previously reported activity assay (8).

Organ Removal and Analysis

Once experimentation was concluded, animals were euthanized with an injection of 0.4 mL KCl directly into the jugular vein.

In studies where ^{51}Cr had been added to the intrapulmonary formulations, lungs, trachea, and cannula were excised and analyzed in a Minaxi Auto-Gamma 5000 series gamma counter. The percentage of the peptide dose reaching the lungs was then calculated.

Statistical Methods

Peptide absorption was calculated using the following formula:

$$\% \text{ Absorption} = \frac{\text{Total \% IP recovery}}{\text{Total \% IV recovery}} \times 100$$

The recovery after IV administration is reflective of 100% absorption; therefore, the recovery after IP administration is corrected to that of IV administration to find the overall percentage absorption.

The standard error of the mean (SE) was calculated for the percentage recoveries of each model peptide after both IV and IP administration for each study day using the following formula:

SE of absorption =

$$\sqrt{\left(\frac{\%\text{ IP Rec}}{\%\text{ IV Rec}}\right)^2 \left[\left(\frac{\text{SE of } \%\text{ IP Rec}}{\%\text{ IP Rec}}\right)^2 + \left(\frac{\text{SE of } \%\text{ IV Rec}}{\%\text{ IV Rec}}\right)^2\right]}$$

where Rec is recovery.

RESULTS AND DISCUSSION

Earlier HPLC studies with U-77436 had indicated that this peptide remains intact during its excretion into the bile and urine, such that the activity assay accurately quantifies this compound (Lakings *et al.*, unpublished results). Most of the U-77436 given intravenously was recovered in the bile during the first hour of collection (Fig. 2, IV), similar to previously reported results with ditekiren (11). The remainder was discovered in the urine, so that recovery was essentially complete (Table I).

After intrapulmonary administration, U-77436 was again excreted primarily in the bile, but in contrast to intravenously dosed peptide, recovery was incomplete 10 hr after dosing (Fig. 2, IP). This "sustained release" had not been observed with either ditekiren (11) or U-77436 (13) after oral administration.

Absorption from the lungs, as calculated from bile and urine recovery, was 52% (Table I), comparable to what had been found after intrapulmonary administration of methylated tripeptide analogues of phenylalanine (5). Thus, IP absorption was greater than the 6–16% observed after oral administration of U-77436 (12). In a separate study (n = 6), ^{51}Cr recovery in the lungs was 63 ± 11% (SE) of the administered dose. If this percentage of the U-77436 dose actually reached the lungs, approximately 81% (i.e., 52.1/63.2 × 100%) of it was absorbed. Had the experiment continued past 10 hr, complete absorption of the dose delivered to the lungs might have been expected. In summary, transport from

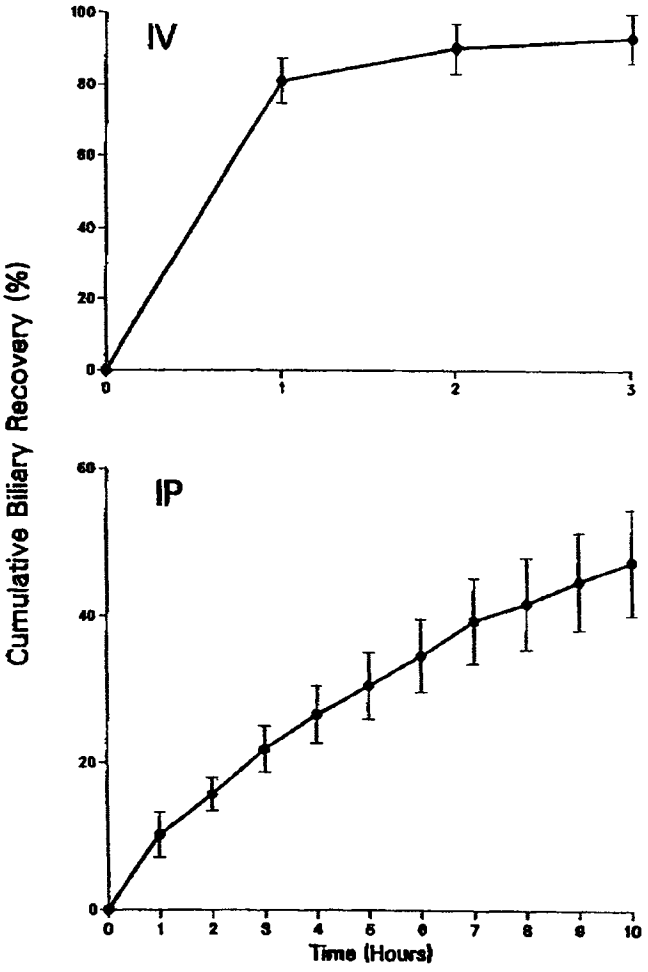

Fig. 2. Mean (±SE) cumulative biliary recovery of U-77436 after intravenous (IV) and intrapulmonary (IP) administration (n = 4).

Table I. Recovery of U-77436 (ng/mL ± SE) After Intravenous or Intrapulmonary Administration

Treatment	Percentage			
	Recovery in bile	Recovery in urine	Total recovery	Absorption
U77436 IV (75 μg/rat)	93.3 ± 6.9	11.6 ± 1.4	104.9 ± 6.3	—
U77436 IP (25 μg/rat)	48.0 ± 6.9	5.7 ± .9	53.7 ± 7.8	52.1 ± 8.0

the lungs appeared to be slower but more complete than absorption from the intestine.

One explanation for the slow absorption of U-77436 from the lungs might be saturable transport. The convergence of the serum profiles of U-77436 dose–response curves suggested this as well (Fig. 3B). Such convergence would not be expected if the slow absorption were due to binding to the lung or sequestration in macrophages. For example, if binding or sequestration acted as a sink, no absorption would occur except at high doses of the drug. Convergence would then be seen at low doses of peptide, not high ones, as currently observed. If a percentage of the delivered dose was bound or sequestered, higher doses would still give higher "free" drug levels and dose proportionality would be maintained. Instead, the serum profiles for the 250 and 500 μg/kg dose for both peptides were virtually identical (Fig. 3). Thus, sequestration and binding are unlikely explanations for the dose–response pattern observed, although these possibilities cannot be conclusively ruled out with the available data.

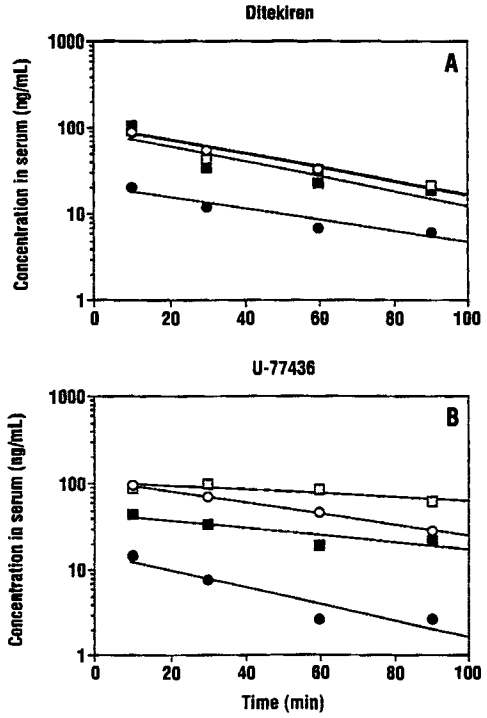

Fig. 3. Mean serum concentrations (n = 2–11) of U-77436 (A) and ditekiren (B) after intrapulmonary dosing with 62.5 (●), 125 (■), 250 (○), or 500 (□) μg/kg.

To investigate the possibility that the dose saturation observed was due to precipitation of U-77436 at high doses, ditekiren, a similar but less soluble RIP, was also studied. While U-77436 has a solubility of 20 mg/mL in water at 37°C, (13), ditekiren is over 100× less soluble [i.e., 0.165 mg/mL (14)]. Earlier HPLC studies had demonstrated that this peptide remains intact during excretion into the bile and urine and is reproducibly quantified by the activity assay utilized (11). Dose–response curves of ditekiren gave serum profiles similar to those observed with U-77436 (Fig. 3B).

If solubility were the factor responsible for saturable transport, the dose of ditekiren at which this phenomenon was first observed would be approximately 1/100th of the dose at which saturation occurred with U-77436. Instead, the dose–response profiles are quite similar. Therefore, precipitation of the peptides in lungs due to solubility considerations is an unlikely explanation for the observed saturation.

Multiple doses of ditekiren gave a profile different than the theoretical imposition of several single doses, providing further evidence for saturable transport. This is, in fact, what occurred (Fig. 4). Multiple doses of ditekiren allowed maintenance of steady-state levels similar to the maximum observed in the dose–response studies but lower than those which would have been predicted.

These studies suggest that RIPs are almost completely absorbed from the lungs over a much longer period than would be expected after oral administration. This "sustained release" appears to be due to saturable transport that is probably not related to solubility of the test compound. This finding differs from the dose proportionality observed in humans given intrapulmonary doses of leuprolide acetate (15), a peptide about the same size as the two RIPs reported herein. However, the human doses were less than one-fifth of the 125 μg/kg dose, which exhibited saturation in the rat studies reported herein.

In summary, intrapulmonary absorption of renin pep-

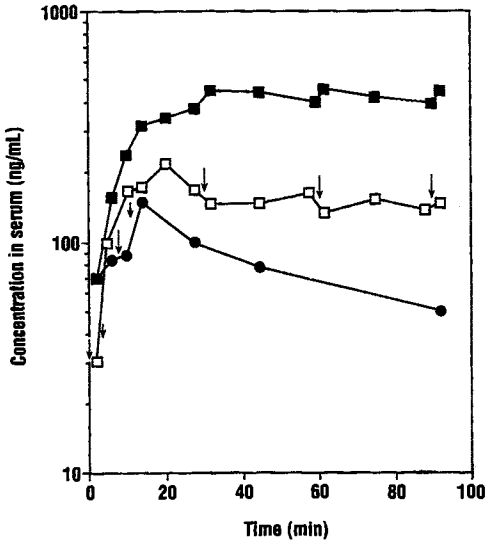

Fig. 4. Mean serum concentrations (n = 2) of intrapulmonary doses of ditekiren: single dose of 250 μg/kg (●); multiple doses at 0, 4, 8, 12, 30, 60, and 90 min as indicated by arrows (□); theoretical multiple dose prediction calculated by superimposing and summing the single dose curve using the multiple dosing time points (■).

tides is more complete and less rapid than oral dosing and independent of desolvation energy. Transport of these peptides from the lungs is saturable through a mechanism apparently independent of pulmonary precipitation or binding or sequestration into macrophages.

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