Positron-Labeled Angiotensin-Converting Enzyme (ACE) Inhibitor: Fluorine-18-Fluorocaptopril. Probing the ACE Activity In Vivo by Positron Emission Tomography

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To evaluate the feasibility of probing the distribution of angiotensin-converting enzyme (ACE) in vivo using positron emission tomography (PET), 4-cis-[^{18}F]fluorocaptopril (^{18}FCAP) was prepared by the reaction of the triflate 2 with K^{18}F/Kryptofix 222 in MeCN followed by hydrolysis (2 N NaOH). The synthesis time was 1 hr with an average radiochemical yield (EOS) of 12% and a specific activity of >300 Ci/mmol. In vivo biodistribution in rats at 30 min after administration showed high uptakes into organs known to have high ACE concentration (lung, kidney and aorta) and faster clearance of ^{18}FCAP for lung and kidney, compared to the clearance from the aorta. When different amounts of unlabeled 4-cis-fluorocaptopril (SQ 25750) were coinjected in rats, a dose of > 5 μg/kg decreased the lung uptake by one-half while only 1 μg/kg decreased the kidney uptake by one-half. In general, the binding in the four tissues studied was saturable with the expected capacity. ^{18}FCAP was administered to a human and displaceable uptake observed in the lung and kidney. The results demonstrate the feasibility of probing ACE in vivo using PET.

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Renin-angiotensin system is one of the complicated mechanisms involved in blood pressure regulation (*1–4*) (Scheme 1). Renin, an enzyme produced in kidney, acts on the circulating protein angiotensinogen, produced in liver, to give a decapeptide, angiotensin I. Angiotensin I has no biologic activity and is the target of the angiotensin-converting enzyme (ACE), which is present in large quantities in lung.

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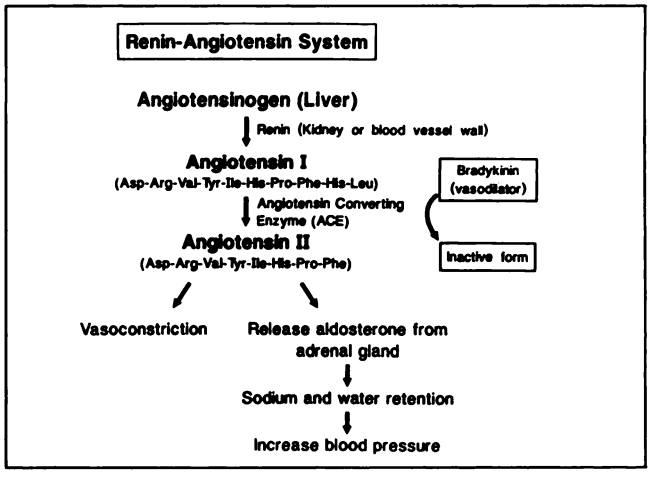
ACE is a zinc ion mediated peptidyldipeptide hydrolase (EC 3.4.15.1). It catalyzes conversion of the inactive decapeptide angiotensin I to the potent vasoconstrictor octapeptide angiotensin II, which also stimulates the release of aldosterone from the adrenal glands and leads to the sodium and water retention. ACE also catalyzes the inactivation of the naturally occurring vasodepressor bradykinin. The importance of these reactions in hypertensive disease has been clarified by the development of potent and specific inhibitors of ACE, e.g., the first orally active ACE inhibitor captopril (SQ14225) (*3,4*).

The design and development of captopril (2-D-methyl-3-mercaptopropanoyl-L-proline, K_i = 1.7 n*M*)[3] led to an exciting new treatment for hypertension. At present, ACE inhibitors are an important class of therapeutic drugs in treating hypertension.

The distribution, biotransformation, and excretion of captopril in animals has been studied with captopril labeled with ^{35}S, ^{2}H, ^{3}H, and ^{14}C (*5–9*). From these studies,



SCHEME 1. Role of ACE in the resin-angiotensin system.

it was concluded that the high concentration of radioactivity in kidney and liver were due to the excretory role of these organs, and the high concentration of radioactivity in lung is due to the high affinity of captopril for ACE, present in large quantity in lung (*8*). Using ^{3}H-captopril in tissue binding study, Strittmatter et al. found high ACE concentration in the homogenized tissues of choroid plexus, lung, kidney, and pituitary. For example, the ACE concentrations in the tissues of lung and choroid plexus were 2.7 and 0.88 nmol/mg of protein, respectively (*10*). In these tissues, the binding of ^{3}H-captopril to ACE closely paralleled the relative levels of ACE activity. Wilson et al. also demonstrated that ACE levels vary in accordance with levels of plasma-renin activity in the lungs, aorta, arteries, and adrenal medulla, but inversely with levels in pituitary, kidney, testis, and choroid plexus (*11*).

To investigate the feasibility of probing and mapping the ACE activity in vivo using positron emission tomography (PET), we chose to label FCAP with ^{18}F ($t_{1/2}$ = 110 min). In vitro inhibition studies using ACE obtained either from rat or rabbit lung has demonstrated that FCAP is a potent ACE inhibitor with an in vitro ID_{50} value of 0.30 μg/kg compared to 0.16 μg/kg for captopril (*12*). Therefore the use of fluorinated captopril to probe various ACE inhibitors in vivo is well justified. This paper reports the preparation and the biodistribution of [^{18}F]fluorocaptopril (^{18}FCAP).

MATERIALS AND METHODS

Fluorine-18-fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction on enriched ^{18}O-water using the Washington University CS-15 cyclotron (*13*). All chemical reagents used in this work were obtained from Aldrich Chemical Co., Milwaukee, WI. HPLC solvents were obtained from Fisher Scientific. The HPLC system consists of a Spectra Physics SP8700 pump, a Rheodyne injector (5-ml sample loop), a HPLC column, a Waters UV detector (Lambda-Max Model 480), a well-type NaI(Tl) scintillation detector and associated electronics, and a fraction collector. The HPLC conditions used in this work were the following: (1) for the purification of radiolabeled product, a Whatman Partisil semipreparative column (M9, 10/50, ODS-3, 10 × 500 mm; column A) eluted with solvent A, a mixture of 10% EtOH and 90% water (with 0.3% glacial acetic acid), at a flow rate of 3 ml/min; and (2) for the determination of specific activity of the radiolabeled product, a reversed-phase analytical column (YMC A-302, S-5, 120A column, 4.6 × 150 mm; column B) eluted with solvent B, a mixture of 18% MeOH and 82% water (with 0.5% phosphoric acid), at a flow rate of 1 ml/min.

Preparation of the Alcohol 1 (SQ 32980)

4-trans-Hydroxy-L-proline methyl ester hydrochloride (6.3 g, 34.7 mmol) was suspended in 45 ml of methylene chloride (distilled from phosphorus pentoxide). Diisopropylethyl amine (12.1 ml, 69.4 mmol) was added and the mixture cooled to 0°C. (R)-3-(Acetylthio)-2-methylpropanoyl chloride (5.18 ml, 34.7 mmol) was added, and the reaction was allowed to warm to room temperature and stir for 16 hr. The reaction was then quenched by addition of 10 ml of saturated sodium bicarbonate and the organic phase was washed with saturated sodium bicarbonate, 1 *M* hydrochloric acid (HCl) and saturated sodium chloride solutions. The organic layer was dried over magnesium sulfate, filtered, and the solvent removed to provide 6.8 g of the crude product as a yellow oil. Recrystallization from diethyl ether yielded 4.03 g (40%) of the desired product as a white solid. mp 76°–76.5°C; TLC (80% ethyl acetate, 20% hexane) R_f = 0.20; $[\alpha]_D$ (c = 1.51, MeOH) −177°. Analysis calculated for $C_{12}H_{19}NO_5S$: C, 49.81; H, 6.62; N, 4.84; S, 11.08. Found: C, 50.08; H, 6.50; N, 4.79; S, 10.86.

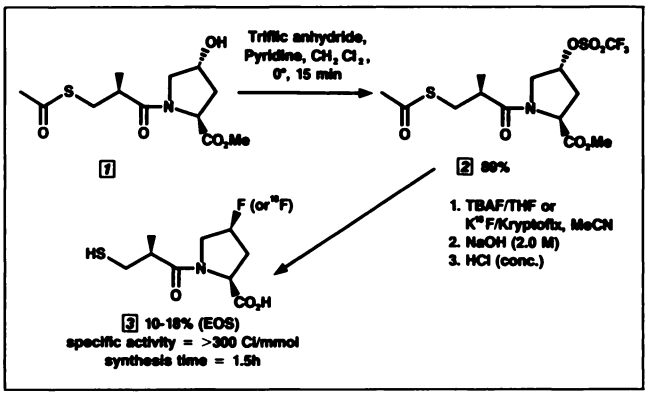
Preparation of the Triflate of SQ32980 (2) and cis-fluorocaptopril, FCAP

Alcohol 1 (SQ 32980, 5 mg, 17.3 nmol) was placed in a flask under nitrogen and dissolved in 0.5 ml of methylene chloride (Scheme II). The solution was cooled at 0°C, and to it was added pyridine (6 μl, 74 nmol, freshly distilled from calcium hydride) and triflic anhydride (6 μl, 36 nmol). After stirring for 15 min at 0°C, the clear yellow reaction mixture was transferred to a silica gel column and purified by flash chromatography (1.2 g silica gel; EtOAc/hexane = 4/6) to provide 5 mg (68%) of the pure triflate as a clear oil.

A solution of the triflate *2* (292 mg, 0.69 mmol) and tetra-n-butylammonium fluoride (0.35 ml of 1 M solution in THF, 0.35 mmol) in 5 ml THF was stirred for 50 min. The solvent was removed by a stream of argon and the residue suspended in aqueous sodium hydroxide (3.0 *M*, 1 ml) and stirred for 45 min. Another 1 ml of NaOH was added and the mixture stirred for 1 hr, then acidified to pH 1 with concentrated HCl. The solution was extracted with ethyl acetate, the extracts dried over magnesium sulfate, and the solvent removed to give 269 mg of a pale yellow oil. Preparative HPLC (YMC S345 ODS column, 20% MeOH/water/0.1% trifluoroacetic acid, flow rate 18.6 ml/min, UV detection at 220 nm) yielded 27.4 mg (33%) FCAP as a white solid, mp 130–132°C. TLC (80% methylene chloride/10% MeOH/10% acetic acid) R_f = 0.60. Analysis calculated for $C_9H_{14}FNO_3S$ 0.65 H_2O 0.1 CF_3CO_2H: C, 42.77; H, 6.01; N, 5.42; F, 9.56; S, 12.41. Found: C, 42.30; H, 5.57; N, 5.30; S, 12.41.

Preparation of ^{18}FCAP

An aliquot of the [^{18}F]fluoride solution in ^{18}O-water was added to a Reacti-vial containing K_2CO_3 (1 mg) and Kryptofix 222 (6 mg) (*14,15*). Water was azeotropically removed with MeCN under a stream of nitrogen. Potassium [^{18}F]fluoride was resolubilized into 0.3 ml of anhydrous MeCN containing 3 mg of the



SCHEME II. Synthetic scheme for unlabeled and ^{18}FCAP.

triflate *1*. The solution was stirred at ambient temperature for 5 min. To remove unreacted [^{18}F]fluoride, the solution was passed through a short silica gel column (40 mg), which was eluted with another 0.2 ml of MeCN. To the combined MeCN solution was added 0.5 ml of 2N NaOH solution (degassed with helium). The mixture was stirred under a stream of nitrogen at room temperature. After 30 min, the reaction mixture was acidified with 0.2 ml of concentrated HCl, diluted to a total volume of 3 ml with HPLC solvent A, and purified by HPLC (condition 1). The retention time of ^{18}FCAP is 21 min. After HPLC purification, the ^{18}FCAP solution was adjusted to pH 6 (30 μl of 2N NaOH per 3 ml of HPLC fraction), filtered through a 0.22-micron filter, and used for animal studies. For the human study, the chromatographic column was conditioned with ethanol followed by water for injection and finally the column was equilibrated with 10% ethanol in injectable water. The final preparation has been tested for sterility and pyrogens and representative preparations have been shown to be sterile and pyrogen-free. Sterility and pyrogen testing were performed according to the USP XXII with pyrogen testing being carried out using the Whittaker Bioproducts (Walkersville, MD) Limulus Amebocyte Lysate test kit.

The effective specific activity of the product was determined by HPLC to be >300 Ci/mmol using standards of authentic FCAP. 4-cis-Hydroxy-captopril (formed from the hydrolysis of the triflate of SQ32980) eluted at 18.5 min as a broad peak. A small fraction of the hydroxy captopril is eluted with the first fraction of ^{18}FCAP.

In Vivo Studies

General. Female Sprague-Dawley rats were used in all the biodistribution studies. Each rat was anesthetized with ether in an open system and injected with ^{18}FCAP in a surgically exposed femoral vein, the wound clipped to close, and allowed to recover. The animals were allowed free access to water and food at all times while awake. At the specified times after the administration of ^{18}FCAP, the animals were reanesthetized with ether and killed by decapitation.

Biodistribution Studies. Twenty rats (average weight = 173 ± 7 g) were divided into four groups and the animals were killed at 0.5, 1, 2, and 4 hr after the administration of ^{18}FCAP. The following were removed, weighed, and counted: blood, lungs, liver, spleen, kidney, heart, brain, bone, adrenal, aorta, and abdominal muscle.

Titration Studies. Twenty-eight rats (average weight = 150 ± 5 g) were divided into seven groups. The animals were co-injected with ^{18}FCAP and different amounts of unlabeled FCAP, none, 0.001, 0.005, 0.025, 0.125, 0.625, and 3.25 mg/kg respectively. The animals were killed at 2 hr postinjection. The following were removed, weighed, and counted: blood, lungs, spleen, kidney, heart, aorta.

Pretreatment Studies. Twenty rats (average weight = 169 ± 9 g) were divided into four groups, and the animals were pretreated with captopril (3.25 mg/kg) at 48, 24, 12, and 3 hr, respectively, prior to the administration of ^{18}FCAP. The animals were killed at 2 hr postinjection. The following were removed, weighed, and counted: blood, lungs, kidney, heart, aorta.

Displacement Studies. Twenty rats (average weight 183 ± 7 g) were divided into four groups. The animals were first injected with ^{18}FCAP and 30 min later a dose of FCAP (3.25 mg/kg) was administered to all three groups of animals except the control group. The animals in the control group were killed at 30 min postinjection of ^{18}FCAP. The animals in other three groups were killed at specified times, 1, 2 and 4 hr after the administration of ^{18}FCAP. The following were removed, weighed, and counted: blood, lungs, kidney, heart, aorta.

Human Study. A healthy male was injected with 7 mCi of ^{18}FCAP and imaged with Super PET II B, a time-of-flight machine with a resolution of 5 mm inplane and 7 mm in thickness (*16*). Following administration of ^{18}FCAP, images were taken at the level of the subject's lungs and kidneys. After 90 min, 25 mg of captopril was administered p.o. and after allowing 66 min for absorption two more images were taken at each level. The animal and human studies were carried out with the approval of the appropriate committees at Washington University.

RESULTS

The unlabeled standard FCAP was prepared by the reaction of triflate *2* and tetrabutylammonium fluoride followed by base hydrolysis (Scheme II). ^{18}FCAP was similarly prepared from *2* and K^{18}F/Kryptofix 222 in a synthesis time of 1 hr with an averaged radiochemical yield of 12% (n = 16) at EOS and a specific activity of > 300 Ci/mmol.

In vivo biodistribution studies in rats at time from 30

TABLE 1
Biodistribution of ^{18}FCAP in Sprague-Dawley Rats* (Data Given as mean ± s.d., n = 5)

	%ID/g			
	30 min	1 hr	2 hr	4 hr
Blood	0.236 ± 0.095	0.129 ± 0.034	0.096 ± 0.014	0.066 ± 0.016
Lung	33.947 ± 6.232	32.713 ± 5.089	26.813 ± 3.094	19.785 ± 3.030
Liver	0.199 ± 0.047	0.131 ± 0.014	0.116 ± 0.018	0.113 ± 0.031
Spleen	0.837 ± 0.244	1.007 ± 0.105	0.838 ± 0.087	0.633 ± 0.114
Kidney	6.399 ± 1.779	4.073 ± 0.999	3.366 ± 0.867	2.323 ± 0.670
Heart	0.389 ± 0.068	0.432 ± 0.040	0.287 ± 0.036	0.208 ± 0.047
Brain	0.069 ± 0.015	0.076 ± 0.009	0.0635 ± 0.005	0.055 ± 0.008
Bone	0.125 ± 0.021	0.131 ± 0.018	0.117 ± 0.019	0.100 ± 0.023
Adrenal	0.289 ± 0.081	0.286 ± 0.077	0.211 ± 0.059	0.175 ± 0.043
Aorta	1.269 ± 0.232	1.211 ± 0.069	1.217 ± 0.151	1.145 ± 0.194

* Average weight = 173 ± 7 g.

min to 4 hr after administration of no-carrier-added ^{18}FCAP indicated high uptakes into organs known to have high ACE concentration (lung, kidney and aorta) (Table 1). Among these organs, the net efflux of ^{18}FCAP was faster for kidney and lung compared to the clearance from aorta. The washout from organs known to have high ACE levels is slow (Fig. 1). Low brain and heart uptakes of ^{18}FCAP were also observed. More importantly, the low bone uptake of radioactivity indicated there was no in vivo defluorination of ^{18}FCAP.

In titration studies where ^{18}FCAP and different amounts of unlabeled FCAP were coinjected, a dose of > 5 μg/kg decreased the lung and spleen uptake by one-half while only 1 μg/kg decreased the kidney uptake by one-half (Table 2). On the other hand, a dose of > 25 μg/kg was needed to decrease the aorta uptake by one-half. These studies clearly demonstrated that uptake of ^{18}FCAP is saturable.

In displacement studies, the uptakes of ^{18}FCAP into target organs were chased with an intravenous dose of cold FCAP (Table 3). After the chasing dose, the net efflux of tracer from all target organs was much faster than the corresponding no-carrier-added case (Table 1). The result shows that the binding of ^{18}FCAP to ACE is displaceable and hence FCAP is a reversible ACE inhibitor.

In pretreatment studies, the animals were blocked with CAP prior to the tracer administration. As expected the uptake of ^{18}FCAP into target organs was decreased by captopril pretreatment, and the shorter the pretreatment time, the higher the ACE-captopril complex concentration, and hence the lower the ^{18}F organ uptake (Table 4). This pretreatment study demonstrated the long lasting effect of captopril; for example, a dose of captopril at 12 hr prior to the administration of tracer decreased the uptake of tracer into organs such as lung, heart, and aorta by 61%, 62%, and 52%, respectively, and the kidney uptake by 86% compared to those of pretreated at 48 hr.

Human Study

Images of the lung and kidney at various times, postadministration of ^{18}F-CAP are shown in Figure 2. Quantitative time activity curves for the two organs are shown in Figure 3.

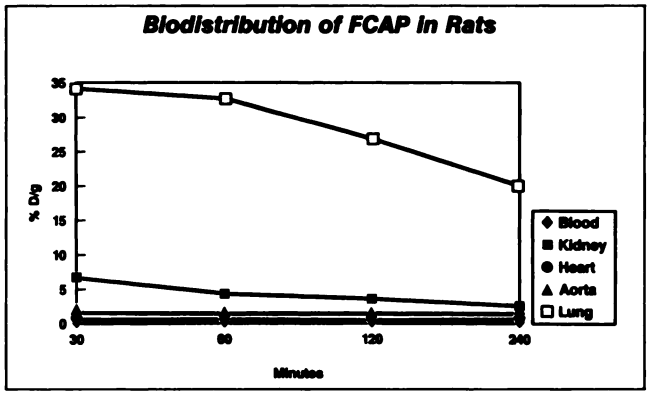


FIGURE 1. Clearance of ^{18}FCAP from blood, kidney, heart, aorta and lung over a 4-hr time period.

DISCUSSION

Chemistry

The thermal instability of the starting triflate *2* requires the triflate to be prepared from the alcohol *1* (SQ 32980) right before the labeling reaction with a synthesis time of 40 min. Once *2* is available, the labeling procedure was straightforward.

It is known that captopril undergoes oxidative decomposition to give the disulfide SQ 14551[6] (because of the free thio group) and forms an adduct with extraction solvents like methylene chloride. Therefore extra caution must be used to avoid the oxidative decomposition of ^{18}FCAP and the adduct formation between ^{18}FCAP and organic solvents.

To avoid oxidative decomposition, all reagents and HPLC solvents were thoroughly degassed with helium, and the basic hydrolysis reaction, the second step of the labeling procedure, was also performed under an atmosphere of nitrogen. At the beginning of this work, a solvent mixture of 20% MeOH and 80% water (with 0.1% glacial acetic acid) was used for the purification of ^{18}FCAP by

TABLE 2
Biodistribution of ^{18}FCAP with Different Specific Activities* in Sprague-Dawley Rats 2 Hours Postinjection† (Data Shown as mean ± s.d., n = 4)

	%ID/g						
	3.25 mg/kg	0.65 mg/kg	125 μg/kg	25 μg/kg	5 μg/kg	1 μg/kg	NCA
Blood	0.041 ± 0.016	0.052 ± 0.018	0.055 ± 0.015	0.077 ± 0.017	0.094 ± 0.015	0.078 ± 0.015	0.073 ± 0.016
Lung	0.126 ± 0.029	0.320 ± 0.107	1.380 ± 0.417	5.39 ± 0.549	13.01 ± 2.03	16.25 ± 4.63	23.78 ± 2.72
Spleen	0.037 ± 0.009	0.043 ± 0.008	0.096 ± 0.012	0.292 ± 0.025	0.693 ± 0.107	0.933 ± 0.124	1.090 ± 0.060
Kidney	0.169 ± 0.042	0.254 ± 0.007	0.402 ± 0.033	0.730 ± 0.084	1.230 ± 0.282	1.70 ± 0.165	3.24 ± 0.806
Heart	0.031 ± 0.009	0.034 ± 0.007	0.050 ± 0.008	0.115 ± 0.013	0.256 ± 0.019	0.322 ± 0.036	0.369 ± 0.073
Aorta	0.135 ± 0.047	0.153 ± 0.025	0.412 ± 0.107	0.840 ± 0.071	1.28 ± 0.172	1.22 ± 0.075	1.35 ± 0.125

* 100 μCi of ^{18}FCAP was first mixed with the specified amount of FCAP and then administered to animals.
† Average weight = 150 ± 5 g.

TABLE 3
Displacement of ^{18}FCAP from Target Organs by ^{18}FCAP in Sprague-Dawley Rats*
(Data %ID/g Shown as mean ± s.d., n = 5)

	Control	Treated†		
	30 min	1 hr	2 hr	4 hr
Blood	0.156 ± 0.023	0.346 ± 0.051	0.139 ± 0.029	0.033 ± 0.001
Lung	32.042 ± 2.115	14.072 ± 2.278	4.659 ± 0.377	1.217 ± 0.070
Kidney	5.180 ± 0.571	3.292 ± 0.538	1.040 ± 0.159	0.235 ± 0.008
Heart	0.431 ± 0.056	0.261 ± 0.033	0.099 ± 0.014	0.034 ± 0.004
Aorta	1.777 ± 1.196	0.718 ± 0.180	0.315 ± 0.119	0.170 ± 0.076

* Average weight = 169 ± 9 g.
† Animals were injected intravenously with FCAP (3.25 mg/kg) at 30 min after the administration of ^{18}FCAP.

HPLC. After the purification, the solution of ^{18}FCAP was concentrated in vacuo and reconstituted into saline. During the concentration step, if a bath temperature exceeding 40°C was used, a decomposed compound, which is substantially more polar than ^{18}FCAP, was observed. To eliminate the concentration step a HPLC solvent mixture consisted of 10% EtOH and 90% water (with 0.3% glacial acetic acid) was used instead. After HPLC purification, the ^{18}FCAP solution was adjusted to pH 6 with 2 N NaOH, filtered and used for animal studies.

To prevent the adduct formation between ^{18}FCAP and organic solvents like methylene chloride and acetone, the reaction vessels were thoroughly cleaned and dried, the HPLC system was also thoroughly cleaned with MeOH followed by the desired HPLC solvents.

Although the decomposed radiolabeled by-product has not been fully characterized, with cautions like those mentioned above, the by-product formation could be effectively minimized to less than 1%. The specific activity of ^{18}FCAP was determined by HPLC to be >300 Ci/mmol (EOS). The variation of the specific activity of ^{18}FCAP was due to the presence of 4-cis-hydroxy-captopril which was formed by the base hydrolysis of the triflate of SQ32980.

The stability of ^{18}FCAP in HPLC solution at room temperature was carefully monitored by HPLC. At pH < 5 (product solution in HPLC solvents) and under inert atmosphere, the product solution was perfectly stable for more than 6 hr. However, at a higher pH (>7) a significant amount (25%) of tracer decomposition was observed in less than 30 min at room temperature, and the decomposition exceeded 50% after 2 hr at room temperature. Therefore, for all animal studies the pH of the product solution was carefully adjusted to pH 6 to avoid decomposition of the tracer. The radiochemical of the tracer was analyzed prior to the animal study and immediately after the last administration of the tracer. With the precaution mentioned above no decomposition of the tracer was observed in all cases.

Evidence for ACE Binding of ^{18}FCAP

In 1982, Kuhar (*17*) discussed the criteria for receptor identification and the problems of extrapolating from in vitro to in vivo labeling. These criteria are equally applicable to binding of FCAP to ACE. Because the three-dimensional structure of ACE has not been detailed, just as it has not been for most receptor systems, an operational definition of specific binding must be employed. Kuhar suggested that the in vivo binding should follow the in vitro binding in terms of specificity, affinity, and correlation with known sites of action. In vivo biodistribution results show that ^{18}FCAP binds to the lung, kidney, aorta, heart and possibly other organs with a high affinity but low capacity. The saturability of ACE by ^{18}FCAP is clearly demonstrated by injection of decreasing specific activities

TABLE 4
Biodistribution (2 Hours Postadministration) of ^{18}FCAP in Sprague-Dawley Rats* Pretreated with Captopril† (Data %ID/g Shown as mean ± s.d., n = 5)

	Pretreated			
	48 hr	24 hr	12 hr	3 hr
Blood	0.094 ± 0.016	0.092 ± 0.013	0.104 ± 0.016	0.069 ± 0.006
Lung	18.362 ± 3.970	13.338 ± 5.283	11.255 ± 1.739	2.711 ± 0.322
Kidney	1.408 ± 0.298	1.331 ± 0.155	1.188 ± 0.182	0.601 ± 0.067
Heart	0.212 ± 0.033	0.183 ± 0.053	0.131 ± 0.015	0.060 ± 0.023
Aorta	0.987 ± 0.182	0.787 ± 0.214	0.521 ± 0.102	0.190 ± 0.024

* A dose of 3.25 mg/kg was used.
† Average weight = 183 ± 7 g.

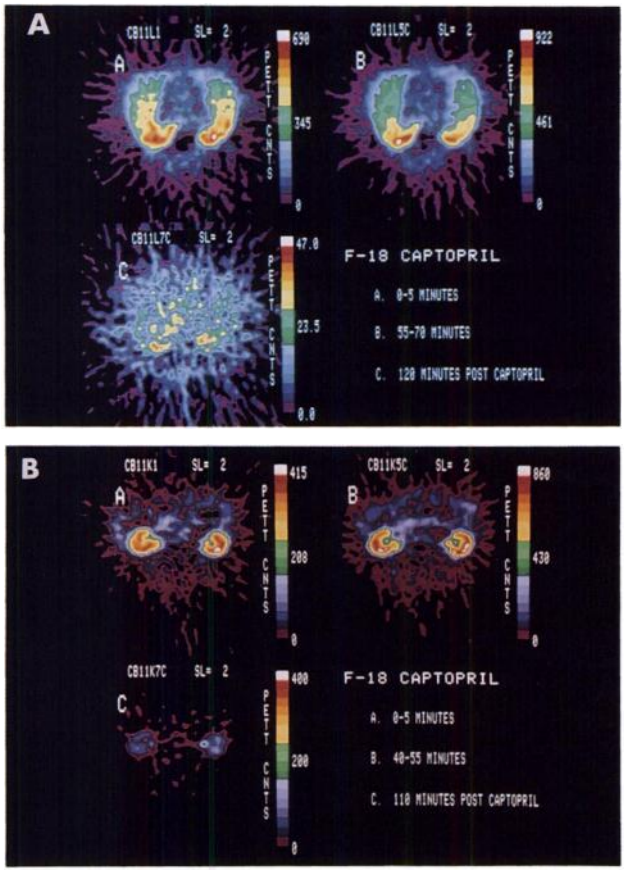


FIGURE 2. PET images at the level of the lung (A) and kidney (B) following administration of 6 mCi of ^{18}FCAP to a normal human subject. The images are taken at the times designated in the upper and lower right panels.

of ^{18}FCAP (Table 2). Further proof is given by the chase experiments where the bound ^{18}FCAP can be displaced by injecting non-radioactive FCAP intravenously at 30 min after the administration of ^{18}FCAP, which is distributed in the major organs in proportion to the known concentrations of ACE as determined by ex vivo experiments. This is further evidence of specific binding. Finally, the affinity constants can be shown to be the same order of magnitude as those observed in other ACE inhibitor binding. Assuming that the binding obeyed the equation governing ligand receptor binding in vitro, i.e., the system is at steady state, analysis of the Scatchard type was undertaken. The use of this equilibrium expression can only be justified if the system is at steady state, i.e., the rate of change of the concentration of ^{18}FCAP is zero. This is interpreted on a practical level in two ways. Patlak and Blasberg (*18*) state that when the change in the blood plasma concentration eventually becomes slow enough that its time constant is much longer than any of the time constants of the system, then the amount in the reversible compartment will be in effective steady state with the blood plasma. Gjedde et al. (*19*), on the other hand, state that if the plasma concentration continues to decline slowly but nonexponentially, then the tissue ratio never reaches its steady-state value, that is, the steady-state volume. We believe that ^{18}FCAP obeys the former definition at 2 hr after injection (Fig. 1).

The distribution at 2 hr after co-injection of various amounts of CAP and no-carrier-added ^{18}FCAP (Table 1) was analyzed using the LIGAND program. The specific activity of ^{18}FCAP was 2,200,000 dpm/pmol. The injected doses of CAP were 0,1,5,125,650, and 3250 μg CAP/kg. This amount was corrected for the weight of the rat (150 g) and used as the total dose in the LIGAND program (*20*). A reaction volume of 0.5 ml was used in the first data set, assuming that the reaction with ACE was a first-pass phenomenon and the intravenous bolus was approximately 0.5 ml. Based on the fact that CAP is reversibly protein bound, the data were recalculated using the rat plasma volume (5.4 ml in a 150-g rat) as the reaction volume. In all cases, the Scatchard plots were linear after correction for nonspecific background. The affinity constants and ACE concentration are listed in Table 5.

The affinity constant and the ACE concentration vary depending on the reaction volume used because the same %ID/g would indicate a stronger affinity and a more dilute ACE in the larger volume. Because the two values covary,

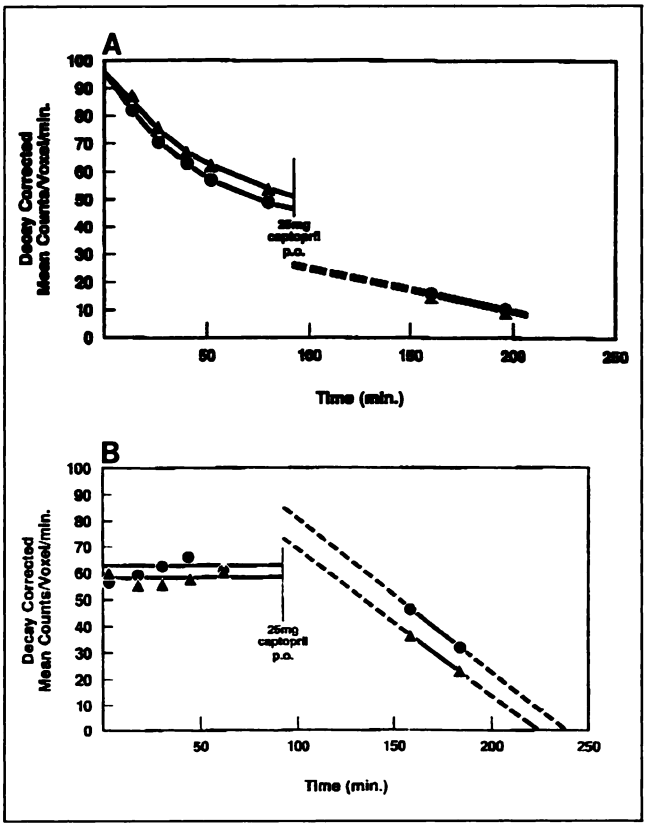


FIGURE 3. Decay-corrected time-activity curves generated from sequential PET images of the lung (A) and kidney (B) in a normal human subject following administration of ^{18}FCAP. ▲ designates the right organ and ● the left. Five images were taken prior to administration of unlabeled captopril and two were taken postadministration.

TABLE 5
The Affinity Constants (K_A) (M-1) and ACE Concentration (M) for Lung, Kidney, Heart, and Aorta Assuming a Reaction Volume of 0.5 ml or 5.4 ml Using Data of Table 2

	$K_A \times 10^7$	[ACE] $\times 10^{-12}$	K_A[ACE]*
Lung for 0.5 ml	7.68	2510	0.19
Lung for 5.4 ml	83.00	270	0.22
Kidney for 0.5 ml	64.2	53.5	0.034
Kidney for 5.4 ml	696	4.95	0.034
Heart for 0.5 ml	8.4	24.6	0.0021
Heart for 5.4 ml	91.1	2.27	0.0021
Aorta for 0.5 ml	7.9	93.3	0.0074
Aorta for 5.4 ml	85.6	8.6	0.0074

* Binding capacity.

the product of the two is a constant. This product is often called the "binding potential" under certain well defined conditions (*21*). The product of the affinity constant and the ACE concentration is highest for the lung, and lowest for the heart and the aorta, the likely site of reaction of ACE inhibitors. The 0.5-ml reaction volume is probably most appropriate for the lung because of the first-pass phenomenon, whereas the 5.4 ml reaction volume is probably most appropriate for the kidney, heart, and aorta.

Use of ^{18}FCAP as a Probe To Determine the Half-Lives of ACE Inhibitors

One of the most interesting uses of ^{18}FCAP is to determine the half-lives of other nonlabeled ACE inhibitors in man using PET. To better understand the behavior of FCAP in vivo, the kinetics of FCAP were compared under various conditions.

The distribution of no-carrier-added ^{18}FCAP at 30, 60, 120 and 240 min after intravenous injection was analyzed assuming the net efflux is a first order kinetic process. A "chase" experiment was also carried out wherein 3.25 mg/kg of unlabeled FCAP was injected 30 min after the injection of ^{18}FCAP. Finally, the net efflux of CAP was measured indirectly by preinjecting CAP and then measuring the concentration of ^{18}FCAP in the lung, kidney, heart, and aorta as a function of the time after injection of the CAP. The rats were killed 2 hr after the ^{18}FCAP injection. Therefore, the net efflux of CAP was measured indirectly by the increased uptake of ^{18}FCAP at the ACE. In the usual kinetic equation:

$$\mathrm{Ln}(CAP_t - CAP_\infty) = \mathrm{Ln}\,(CAP_o - CAP_\infty) - k_1 t$$

CAP is replaced by the indirect parameter %ID/g ^{18}FCAP. The CAP concentration at CAP_∞ becomes the ^{18}FCAP value in the absence of preinjected CAP. The net efflux for CAP is then obtained from the slope of the plot of %ID/g ($^{18}FCAP_{max}$) − (%ID/g $^{18}FCAP_t$) versus time. The net efflux for FCAP and CAP obtained from these three experiments are given in Table 6.

The net efflux of the no-carrier-added ^{18}FCAP was slowest for the aorta, with a half-life of 27 hr (Table 6A). As would be expected, the relatively large chase dose of

TABLE 6
Net Efflux of ^{18}FCAP and CAP

	k_1 (min^{-1})	$t_{½}$ (min)	%D/g (t = 0)
(A) For nca ^{18}FCAP			
Lung	0.0026	266.5	37.3
	(0.002–0.003)*		(33.9–40.7)
Kidney	0.0043	161.2	6.2
	(0.00–0.014)		(1.9–11.5)
Heart	0.0034	203.8	0.47
	(0.00–0.008)		(0.25– 0.68)
Aorta	0.00042	1650	1.3
	(0.00–0.0009)		(1.18–1.36)
(B) For ^{18}FCAP (CHASE)			
Lung	0.015	46.2	39.6
	(0.012–0.038)		(34.2–99.9)
Kidney	0.015	46.2	7.6
	(0.012–0.021)		(6.8–10.4)
Heart	0.012	57.7	0.53
	(0.010–0.022)		(0.51–0.88)
Aorta	0.014	49.5	1.8
	(0.00–0.050)		(0–8.1)
(C) For CAP (pre)			
Lung	0.00087	800	18.4
	(0.0001–0.0002)		(8.8–34.3)
Kidney	0.0019	356	1.3
	(0.0017–0.0030)		(1.2–2.0)
Heart	0.0011	622	1.2
	(0.0008–0.0017)		(0.18–0.27)
Aorta	0.0015	452	1.3
	(0.0007–0.0015)		(0.9–1.4)
(D) For CAP (pre) taken from the data of Cushman et al. (*18*)			
Lung	—	—	—
Kidney	0.0010	693	112
Heart	0.0010	693	75
Aorta	0.0005	1386	81

* 95% confidence limits as determined using Systat Non Lin Program.

3.25 mg CAP/kg accelerates the net efflux from the four target tissues. All net efflux rates were similar (46–49.5 min.) (Table 6B). The net efflux of CAP as measured indirectly by the decrease of ^{18}FCAP %ID/g in the target tissue was much slower than for the two direct experiments except for the aorta which had a net efflux value of 1650 min for the no-carrier-added ^{18}FCAP and only 452 min for the preinjection of CAP with indirect measurement using ^{18}FCAP (Table 6C). The CAP was given at 3.25 mg/kg and as such saturated the system. In addition, no-carrier-added ^{18}FCAP is at a distinct disadvantage in the competition for ACE sites because of the relatively high concentration of CAP. Nevertheless, as a measure of free ACE, it reflects the rather long half-life of CAP when used as an antihypertensive agent. This latter method can be used to measure the half-lives of a series of ACE inhibitors without radiolabeling each pharmaceutical.

Recently Cushman et al. (*22*) measured the net efflux of captopril by using an ex vivo procedure to measure the

residual ACE activity. Briefly, tissue samples were taken at various times after oral administration and frozen for subsequent ACE activity assay. Before assay of ACE activity, the tissue was weighed and homogenized, then the uncentrifuged homogenate was diluted to the highest concentration that would permit accurate measurement of ACE activity by the fluorometric assay. The assay is based on the cleavage of the substrate Hip-His-Leu by ACE. Because of the dilution, the ex vivo ACE will always be less than the in vivo ACE. Cushman et al. suggest that a 20% inhibition of tissue ACE observed in diluted homogenates ex vivo usually represents nearly 100% inhibition in the intact tissue in vivo. The net efflux as measured by residual ACE is given in Table 6D in the same units used in Table 6C. The dose of captopril was 30 mg/kg po. The half-lives are in general agreement given the many assumptions made in both experiments.

Human Studies

One of the potential uses of ^{18}FCAP is to study the kinetics of CAP and other ACE inhibitors in vivo. The human studies show this to be possible. If one examines the PET numbers in Figure 3, the lung/kidney ratio decreases from ~1.6 to 0.8 over the 90 min time prior to administration of unlabeled captopril. In rats, the lung-to-kidney ratio increases from ~5.3 to 7.9 from 30 to 120 min postinjection. In the animal data, activity was expressed as % ID, whereas a volume element is used in the human study. The relative density of the lung to kidney can be determined from the transmission scan that had been carried out prior to the PET emission scan. The relative density was ~3.5; so in the human the absolute initial uptake on a weight-to-weight ratio in the lung was 4.8 times that in the kidney, a similar ratio to that obtained at the early time point in the rat. The clearance rate from the lung in the human is greater than that in rats while the opposite is true for the kidney. This observation points out the difficulty in extrapolating from animal to human data and the importance of being able to probe the effect of drug behavior at the site of action in humans. ^{18}FCAP appears to be a new radiopharmaceutical with properties that will allow the measurement of ACE levels under various therapeutic regimens in man and also will allow the determination of half-lives of various ACE pharmaceuticals without radiolabeling each day.

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