4-[^{18}F]Fluoro-2-D-methyl-3-mercaptopropanoyl-L-proline

[^{18}F]FCAP

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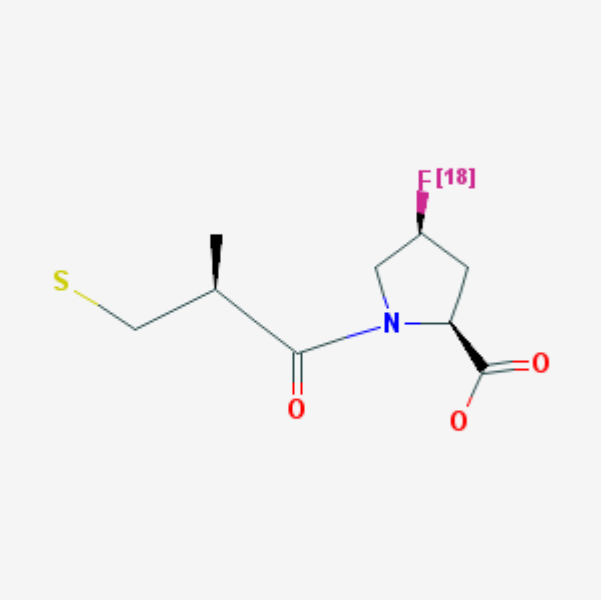
Chemical name:	4-[^{18}F]Fluoro-2-D-methyl-3-mercaptopropanoyl-L-proline
Abbreviated name:	[^{18}F]FCAP
Synonym:	4-cis-[^{18}F]-Fluorocaptopril
Agent Category:	Compound
Target:	Angiotensin-converting enzyme (ACE)
Target Category:	Inhibition of ACE
Method of detection:	PET
Source of signal:	^{18}F
Activation:	No
Studies:	• *In vitro* • Rodents • Humans



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Background

[PubMed]

The renin–angiotensin system (RAS) plays a very important role in blood pressure regulation (1-4) in humans. Renin is an enzyme produced in the kidneys and it cleaves circulating angiotensinogen (a protein produced in the liver) to yield angiotensin I, an inactive decapeptide. The angiotensin-converting enzyme (ACE), found primarily in the lung, converts angiotensin I to angiotensin II, an active vasoconstrictor octapeptide. Angiotensin II also stimulates the production of aldosterone from the adrenal glands that promotes sodium and water retention. ACE is also responsible for inactivating bradykinin, a vasodilator (5). RAS and angiotensin II also play a role in interstitial fibrosis, cardiac remodeling and fibrosis, and heart failure (6, 7). It has also been shown that RAS operates in the heart, and the upregulation of this system is related to heart failure (8-10). Inhibition of ACE in patients with heart failure has often resulted in a favorable outcome for the patient (11). In this regard, captopril (2-D-methyl-3-mercaptopropanoyl-L-proline) was developed as an orally active ACE inhibitor and is considered an important drug for the treatment of hypertension. Subsequently, [^{18}F]fluorocaptopril ([^{18}F]FCAP) was developed for use with PET (12) to understand the *in vivo* dynamics of ACE activity.

Synthesis

[PubMed]

[^{18}F]FCAP was synthesized by Hwang et al. (12) as described here briefly. Diisopropylethyl amine was added to 4-trans-hydroxy-L-proline methyl ester hydrochloride suspended in methylene chloride, and the mixture was allowed to cool to 0^{o}C. Then (R)-3-(acetylthio)-2-methylpropanoyl chloride was added to the cold mixture, and the reaction was allowed to warm to room temperature and stirred for 16 h. The reaction was quenched with sodium bicarbonate and the organic phase was dried over magnesium sulfate after washing with saturated sodium bicarbonate, 1 M hydrochloric acid, and saturated sodium chloride. The organic solvent was removed to obtain the crude product, which was then recrystallized from diethyl ether as a white solid. This solid (5 mg) was then dissolved in methylene chloride, cooled to 0^{o}C, and combined with freshly distilled pyridine with triflic anhydride. The mixture was stirred for 15 min at 0^{o}C, and the resulting clear, yellow, reaction mixture was transferred to a silica gel column for purification of the triflate analog by flash chromatography. A clear, oily, pure triflate (5 mg) was obtained.

The reference compound, nonradioactive *cis*-fluorocaptopril (FCAP), was prepared by Hwang et al. (12) by mixing the triflate analog of captopril with tetra-*n*-butylammonium fluoride followed by preparative high-performance liquid chromatography (HPLC) to obtain the pure standard material.To prepare [^{18}F]FCAP, the triflate was solubilized in anhydrous acetonitrile (MeCN) and potassium ^{18}F-labeled fluoride was added to it. The

mixture was stirred for 5 min at room temperature. A short silica gel column was used to remove excess ^{18}F-labeled fluoride and the reaction product was eluted with MeCN. Then 2 N NaOH (degassed with helium) was added to it. The mixture was acidified with concentrated HCl and diluted for purification by HPLC. After purification, the [^{18}F]FCAP solution was adjusted to pH 6 with NaOH, filtered through a 0.22-micron filter, and used for animal studies. For human studies, the column was conditioned with ethanol followed by water for injection, and finally equilibrated with 10% ethanol (prepared in water for injection). The final purified preparation was tested for sterility and pyrogenicity.

The synthesis took 1 h and the purified product had a specific activity of >300 Ci/mmol with an average yield of 12% at the end of synthesis.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

[^{3}H]Captopril ([^{3}H]CAP) binding has been studied *in vitro* in membranes of various rat tissues (13). The binding to these membranes was saturable and reversible with a K_D of 2.4 nM. In this study, Strittmatter et al. observed that [^{3}H]CAP and ACE were distributed in parallel in the various tissues and the regions of the brain. The highest binding was observed in the coratoid plexus, lung, and corpus striatum, which strongly indicates that captopril selectively bound to ACE.

Other investigators have also studied the distribution of ACE in various animal tissues using radioactively labeled captopril, and the radiation was primarily detected in the kidney, lungs, and pituitary (14, 15). Radiation levels were shown to vary in different tissues, including the arteries, aorta, and adrenal medulla, depending on renin activity (16).

Animal Studies

Rodents

[PubMed]

[^{18}F]FCAP biodistribution studies were performed in female Sprague-Dawley rats (12). The animals were killed 0.5, 1, 2, and 4 h after administration of [^{18}F]FCAP. Blood, lungs, liver, spleen, kidney, heart, brain, bone, adrenal gland, aorta, and abdominal muscles were removed weighed, and incorporated radioactivity was measured. Maximum uptake was noted in the lungs, kidneys, and aorta. These organs are also known to have high concentrations of ACE.

In a titration study by the same investigators (12), the animals were co-injected with [^{18}F]FCAP and differing amounts of unlabeled FCAP. The animals were killed 2 h postinjection. Blood, lungs, spleen, kidneys, heart, and aorta were removed form the animals and weighed, and incorporated radioactivity was measured. Compared to

controls, low concentrations of unlabeled FCAP reduced the radioactivity by one half in the kidney, lung, and spleen. However, a much higher concentration of unlabeled FCAP was necessary to reduce the uptake in the aorta. This suggested that the uptake of [^{18}F]FCAP binding with the ACE of this animal was saturable.

In a displacement study (12), the animals were first injected with [^{18}F]FCAP, and 30 min later unlabeled FCAP was administered. The control animals received only [^{18}F]FCAP. The animals were killed 1, 2, and 4 h after administration of [^{18}F]FCAP. The control animals were killed 30 min after [^{18}F]FCAP administration. The aorta, heart, lung, kidneys, and blood were removed from the animals and weighed, and incorporated radioactivity was measured. The displacement study demonstrated, in general, that tracer efflux from the various organs was very fast, which indicates that the binding of [^{18}F]FCAP to ACE was reversible.

Other Non-Primate Mammals: Dogs

[PubMed]

Markham et al. (17) developed a model in dogs to measure [^{18}F]FCAP kinetics with pulmonary ACE by positron emission tomography (PET). In a companion publication (18), it was shown that a reduction in pulmonary perfusion (due to regional alveolar hypoxia) reduced the amount of measurable ACE circulating in the lungs. From this data the authors concluded that the technique was suitable to investigate *in vivo* expression of ACE.

Non-Human Primates

[PubMed]

No publications are currently available.

Human Studies

[PubMed]

Hwang et al. (12) reported the use of [^{18}F]FCAP in a normal male volunteer. The individual was injected with 7 mCi of the tracer and imaged sequentially at the lung and kidney levels five times using PET. After 90 min, the subject was given cold captopril orally, and 66 min later two more sequential images were taken at both levels. The lung/kidney ratio decreased from ~1.6 to 0.8 over the 90-min period before unlabeled captopril was administered. In contrast, for rats this ratio increased from ~5.3 to 7.9 over a 90-min period post-injection. On a weight/weight ratio basis, the human and the animals showed a similar uptake. In the human, the lungs showed an uptake 4.8 times that of the kidneys. However, the clearance rate from the lung in the human was higher than in the rats, whereas the opposite was observed with the kidneys. From these observations the authors concluded that results obtained with animals may not necessarily reflect the exact effect of

a drug at the site of action in humans. According to these investigators, [^{18}F]FCAP would be an excellent tool to study ACE dynamics in humans.

It was reported that inhibition of ACE delayed the onset of pulmonary hypertension (PPH) in animals due to pulmonary vascular remodeling (19). To determine if this was also true for humans, a study on the binding and inhibition of pulmonary ACE in humans using [^{18}F]FCAP was initiated (20). For this study, the combined forward rate constant (CFRC) for pulmonary ACE in humans was determined. The CFRC is a product of the association constant (k_a) and concentration of the ACE binding sites (B_{max}); it is therefore proportional to the mass of ACE in the lungs. The CFRC decreased by 84% in five normal humans after they ingested 5 mg enalapril (an ACE inhibitor) daily for a week. In five patients with PPH who were given the same treatment, a decrease of 76% was observed in the CFRC. The investigators observed that individuals with PPH had a significantly reduced total mass of pulmonary ACE, and a low dose of an ACE inhibitor can delay vascular remodeling in such patients.

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