4-[^{18}F]Fluorobenzoyl-endothelin-1

[^{18}F]ET-1

The MICAD Research Team

Created: March 12, 2007; Updated: April 12, 2007.

Chemical name:	4-[^{18}F]Fluorobenzoyl-endothelin-1	
Abbreviated name:	[^{18}F]ET-1	
Synonym:	[^{18}F]Endothelin-1	
Agent Category:	Polypeptide	
Target:	ET receptor	
Target Category:	Receptor binding	
Method of detection:	PET	
Source of signal:	^{18}F	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on the above structure for additional information in PubChem.

Background

[PubMed]

Endothelin-1 (ET-1) is a 21 amino acid polypeptide that contains two disulfide bonds located closer to the N-terminus. It is believed to have an important role in a variety of physiological processes and contributes to the development of diseases such as atherosclerosis, hypertension, chronic heart failure, pulmonary hypertension, acute and chronic renal failure, etc (1-3). All these effects are mediated through a receptor-ligand mechanism. Two endothelin receptors, ET_A and ET_B, have been identified in mammals (4, 5). Each receptor type is expressed in a variety of tissues, with some tissues expressing both types (6). Various cytokines are known to regulate ET-I expression under physiological conditions (1).

In humans, stimulation of the ET_A receptors by ET-1 on underlying smooth muscles of the endothelium causes vasoconstriction that leads to an elevation of blood pressure and

NLM Citation: The MICAD Research Team. 4-[^{18}F]Fluorobenzoyl-endothelin-1. 2007 Mar 12 [Updated 2007 Apr 12]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

the development of hypertension (7, 8). Stimulation of the ET_B receptors on the endothelium results in the release of nitric oxide and prostacyclins, which culminates in vasodilation (9). As a result of the involvement of ET-1 in a variety of physiological processes in both normal and diseased states, it is necessary to elucidate the exact role of the ET receptor system *in vivo*.

Positron emission tomography (PET) is a very sensitive imaging technique, and recent improvements in equipment design have enabled researchers to use it for investigation of the ET receptor system in a small animal model (9-12). ^{18}F-Labeled ET-1 ([^{18}F]ET-1) was among the first ligands developed for PET study of these receptors *in vivo* (10, 13).

Synthesis

[PubMed]

Labeling of ET-1 with ^{18}F was performed using *N*-succinimidyl[4-^{18}F]fluorobenzoate ([^{18}F]SFB) as described by Johnstrom et al. (11). The synthesis of [^{18}F]SFB was performed as before (13-15), purified by reverse-phase high-performance liquid chromatography (HPLC) and concentrated in diethyl ether. The ether solution was dehydrated over a bed of magnesium sulfate and evaporated to dryness, and the [^{18}F]SFB was dissolved in acetonitrile. ET-1 dissolved in sodium bicarbonate was added to the solution, and the mixture was left at room temperature for 30 min. Radioactive ET was isolated by reverse-phase HPLC and reformulated by the addition of phosphate buffer. The resulting solution was loaded on a C18 SepPak Plus cartridge and the retained labeled ET-1 was eluted in ethanol. The ethanol was evaporated, and [^{18}F]ET-1 was dissolved in saline for use in the various studies.

The entire procedure was performed in 207 ± 3 min (n = 20) with a yield of 5.9 ± 0.7%. The final product had a specific activity of 220–370 GBq/μmol (5.94–10 Ci/ μmol) at the end of synthesis and was >95% pure. [^{18}F]SFB was shown to label peptides, proteins, and antibodies with ^{18}F in the N-terminus or the $\mathcal{E}$-amino group of the lysine residue (11). Depending on the pH, a mixture of two radiolabeled products may be obtained, with the label either on the N-terminus or the lysine residue at position nine from the N-terminus. With ET-I, performing the reaction under basic conditions (pH 8.6) yielded a single product labeled only at the lysine residue (11).

Confirmation of the purified [^{18}F]ET-1 product was performed using mass spectroscopy.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

In vitro binding studies were performed by Johnstrom et al. in human heart and kidney tissues (10) as described by Davenport and Kuc (16). The left ventricle tissue was incubated with 1 nM-labeled ET-1 for an increasing time, 0–120 min, to determine the association rate constant (K_{obs}). In the saturation study, the tissue was incubated with

increasing concentrations of [^{18}F]ET-1 (5 pM–2.5 nM) for 90 min. From these studies it was determined that [^{18}F]ET-1 had K_{obs} of 0.045 ± 0.004/min and a half time for association of 17 min. The dissociation constant (K_D), maximum density of receptors (B_{max}), and the Hills coefficient (n_H) for [^{18}F]ET-1 were determined to be 0.43 ± 0.05 nM, 27.8 ± 2.1 fmol/g protein, and 0.95 ± 0.04, respectively.

Kidney sections were used for the competition study (10). The sections were exposed to a fixed concentration of [^{18}F]ET-1 in the presence of either FR139317, a selective ET_A antagonist, or BQ3020, a selective ET_B antagonist. The two antagonists reduced [^{18}F]ET-1 binding to the kidney by 33.7 ± 13.3% and 73.3 ± 2.5%, respectively (P<0.05). This indicated that the ET_B receptor was the predominant receptor type in the kidney.

Animal Studies

Rodents

[PubMed]

Johnstrom et al. (11) used microPET to study the *in vivo* distribution of [^{18}F]ET-1 in the rat. For a typical imaging study, the animals were injected with a 0.2-ml bolus of 3.3 MBq-labeled ET-1. The specific activity at the time of each study was usually ~200 GBq/μmol (5.4 Ci/μmol). The lungs, heart, liver, and kidney of the animals were monitored for [^{18}F]ET-1 uptake. Only the lungs and kidneys showed a high uptake of the label. In the liver, only a moderate uptake was observed. Results for the heart were not presented (11). These observations indicated that organs showing an accumulation of the tracer have ET receptors, and, among these, the lungs and kidneys were possibly responsible for clearing ET-1 from circulation (11). A similar suggestion was made earlier by Fukuroda et al (17), who used ^{125}I-labeled ET-1 in competition studies with selective ET_A and ET_B receptor antagonists. Statements about specific binding to ER receptors can only be made if the authors did blocking studies with cold ET.

In another *in vivo* study in rats from the same laboratory (10), which was followed by *ex vivo* analysis, an accumulation of radioactivity was observed mainly in the lung, liver, kidney, and bladder. The thyroid, pituitary, and salivary glands showed low uptake. No uptake was observed in the brain or bone. In this study, [^{125}I]ET-1 was used for comparison. Observations from the *in vivo* study correlated with the *in vitro* detection of receptors in the lung, liver, and kidney as observed with [^{125}I]ET-1. The binding of [^{18}F]ET-1 in the lung could not be displaced by BQ788, a selective ET_B receptor antagonist. However, infusion of BQ788 prior to treatment with [^{18}F]ET-1 significantly reduced the uptake of radioactivity in the lung (85% reduction) and kidney (55% reduction). Under these conditions some uptake was observed in the heart. Only in the heart was the binding lower than expected, as observed with [^{125}I]ET-1 during *in vitro* studies (18). Evidently, [^{18}F]ET-1 had a rapid clearance from circulation with a half-life of 0.43 min, and a simultaneous increase in radioactivity was observed in the liver and lungs. These organs showed high levels of radioactivity up to 2 hr after administration. In the

kidney the uptake was initially rapid and after 20 min it increased slowly. The investigators suggest this could be caused by the accumulation of [^{18}F]ET-1 metabolites in the organ.

Other Non-Primate Mammals

[PubMed]

No publications are currently available.

Non-Human Primates

[PubMed]

No publications are currently available.

Human Studies

[PubMed]

No publications are currently available.

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