2′-[^{18}F]Fluoroflumazenil

[^{18}F]FFMZ

The MICAD Research Team

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Chemical name:	[^{18}F]fluoroethyl 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo-[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate
Abbreviated name:	[^{18}F]FFMZ
Synonym:	2'-[^{18}F]fluoroflumazenil; 3-(2'-[^{18}F]fluoro-FMZ)
Agent Category:	Compound
Target:	Benzodiazepine receptor
Target Category:	Ligand binding
Method of detection:	PET
Source of signal:	^{18}F
Activation:	No
Studies:	• *In vitro* • Rodents • Humans

Background

[PubMed]

Benzodiazepines are used for their sedative, anxiolytic, and muscle-relaxant properties. Their mechanism of action involves the binding of a ligand to a specific benzodiazepine receptor. Alteration of the central benzodiazepine receptor (CBR) has been reported in various diseases and pathologic conditions such as Alzheimer's disease, epilepsy, or cerebral ischemia (1-4).

CBRs have been studied *in vivo* by positron emission tomography (PET) and single photon emission computed tomography, and the existence of a specific benzodiazepine receptor linked to the γ-aminobutyric acid (GABA) receptor/chloride ionophore has been shown by use of diazepam (4).

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[^{11}C]Flumazenil, a highly selective benzodiazepine antagonist, has been the most widely used agent for PET imaging of CBRs, but the short half-life of ^{11}C (20 min) limits its use. To overcome this great disadvantage, the flumazenil analog [^{18}F]fluoroethyl 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo-[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate ([^{18}F]fluoroflumazenil; [^{18}F]FFMZ) has been developed and is currently under study. It can be conveniently synthesized and labeled, and preliminary research shows some favorable specific binding properties for CBRs.

Synthesis

[PubMed]

A one-pot, one-step synthesis method for [^{18}F]FFMZ was developed by Yoon et al. (5). In that procedure, the two precursors 2′-tosyloxyflumazenil (TFMZ) and 2′-mesyloxyflumazenil (MFMZ) were synthesized in three steps, labeled with no-carrier-added [^{18}F]fluoride, and then activated by repeated azeotropic distillation with Kryptofix 2.2.2./potassium carbonate in acetonitrile (MeCN).

An automated process for the labeling and purification of [^{18}F]FFMZ was also developed by Yoon et al. (5). This process involved adding 10 mg of the precursor TFMZ or MFMZ (in 2.5 ml of MeCN) to dry [^{18}F]fluoride (produced with ^{18}O-enriched (>95%) water)), and heating the mixture at 85°C for 12 min while purging with He. Purification was obtained by successive filtrations through polyvinylidene difluoride filters and high-performance liquid chromatography (HPLC), with gradient elution of [^{18}F]FFMZ taking 24.4 min (flow rate 5 ml/min). The entire automated procedure (after production of [^{18}F]fluoride) took 97 min.

The highest labeling efficiency was obtained with 4 mg of TMZ at 110°C for 10 min; the obtained specific activity of [^{18}F]FFMZ was 5.92×10^9 MBq/mol (1.6×10^5 Ci/mol). The reported labeling efficiency and radiochemical purity of [^{18}F]FFMZ after synthesis by the automated system were 68% and 98%, respectively. Stability tests performed by Yoon et al. (5) showed that 98% of the produced [^{18}F]FFMZ was intact at 37°C in human serum after 1 h.

Chang et al. (6) synthesized [^{18}F]FFMZ, using a procedure similar to the one described previously by Yoon et al. (5). The radiochemical purity and specific activity (determined by analytical HPLC) reported by Chang et al. (6) were >98% and approximately 4.5×10^9 MBq/mol (1.2×10^8 mCi/mol), respectively.

Mitterhauser et al. (7) synthesized [^{18}F]FFMZ by reaction of 3-desethylflumazenil with 2-bromo-1-[^{18}F]lfuoroethane. They reported a radiopurity of [^{18}F]FFMZ >98%. When starting with 52 ± 8 GBq of [^{18}F]fluoride, they obtained 52.3 GBq for [^{18}F]FFMZ. Specific radioactivity exceeded 89.4 GBq/μmol (2418.9 Ci/mmol).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

An assessment of the *in vitro* stability of [^{18}F]FFMZ in rat and human sera was made by Chang et al. (6). The experimental procedure involved incubating 25 µl of rat serum or 325 µl of human serum for 1 h in CO_2 at 37°C. After addition of absolute ethanol (775 µl) to precipitate serum proteins, the mixture was centrifuged for 5 min at 3000 rpm, and the supernatant was analyzed by HPLC. Results showed that, in rat serum, [^{18}F]FFMZ was hydrolyzed to FFMZ acid and [^{18}F]fluoroethanol, with 39% of [^{18}F]FFMZ degraded to [^{18}F]fluoroethanol at 10 min and only 8% of [^{18}F]FFMZ intact at 60 min. [^{18}F]FFMZ appeared to be stable at least for 1 h in the case of human serum.

Animal Studies

Rodents

[PubMed]

Mitterhauser et al. (7) evaluated the biological activity of [^{18}F]FFMZ with respect to the GABA(A) receptor, using male Sprague-Dawley rats/Him:OFA. Biodistribution studies were performed by injecting 1.58-2.72 MBq ($4.3\text{-}7.3 \times 10^{-2}$ mCi) of [^{18}F]FFMZ (in 180-225 µl of physiologic phosphate buffer) into the tail vein. After sacrifice at 5, 15, 30, and 60 min post injection, organs were weighed and counted.

The organ showing the highest uptake of [^{18}F]FFMZ was the pituitary gland (0.95 ± 0.35% injected dose (ID)/g at 15 min), followed by the cortex (0.73 ± 0.07% ID/g at 5 min) and the liver (0.70 ± 0.11% ID/g at 60 min). The lowest uptake was observed in the femur (0.35 ± 0.02% ID/g at 5 min and 0.36 ± 0.05% ID/g at 60 min), reflecting low levels of fluoride accumulation and showing that [^{18}F]FFMZ is metabolically stable. The tissue/blood ratio for the whole brain was 1.46. In comparison, the ratio for [^{18}F]fluoroethylflumazenil ([^{18}F]FEFMZ) in previous studies was 0.73 (8). Peak uptake was obtained at 5 min post injection for all sampled brain regions. Those results showed a kinetic process comparable to the one observed for [^{18}F]FEFMZ, but much faster than for [^{11}C]FFMZ (9).

Ex vivo studies using a similar experimental protocol were performed by Yoon et al. (5). [^{18}F]FFMZ (518 MBq (14 mCi); 0.35 ml) was injected intravenously into Sprague-Dawley rats. Receptor-blocking studies were performed with cold flumazenil (0.33 µmol; 0.3 ml). Phosphoimaging of the rat brain showed high uptake in the cortex, thalamus, and hippocampus, but those uptakes were blocked by pre-injection of cold flumazenil.

Biodistribution studies using male ICR mice have also been reported in the literature (6). The animals were sacrificed at 10, 30, and 60 min after an intravenous injection of 0.37 MBq (0.01 mCi) of [^{18}F]FFMZ. Results showed a relatively uniform distribution among organs (between 2 and 3% ID/g), including the heart, lung, spleen, and muscle. Maximum

uptakes for the intestine (5.7% ID/g) and bone (3.6% ID/g) were reported at 60 min post injection.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

Human Studies

[PubMed]

The only human experiment reported to date was performed by Chang et al. (6), using a healthy human volunteer (age 47 years; no medication at the time of the study). After intravenous injection of 370 MBq (10 mCi) of [^{18}F]FFMZ, static PET image acquisition was performed for a post-injection time period of 20 to 60 min. High uptakes in the frontal cortex, temporal cortex, occipital cortex, cerebellum, parietal cortex, and thalamus were observed (decreasing order). On the other hand, the brain stem showed low uptake of the tracer. Those results appeared to match known relative benzodiazepine receptor densities for humans.

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