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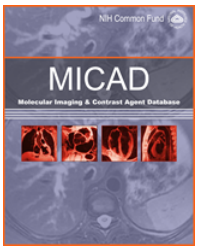

4-[18F]Fluorobenzenecarbohydrazide-methotrexate

[18F]-Folate-MTX-8

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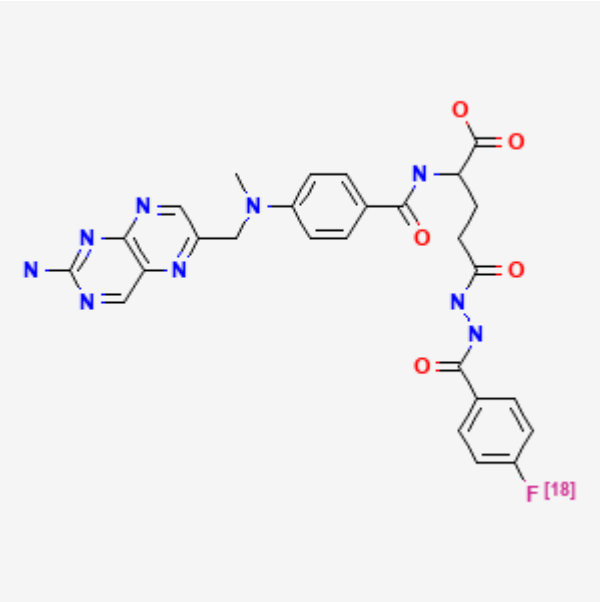
Chemical name:	4-[18F]Fluorobenzenecarbohydrazide-methotrexate
Abbreviated name:	[18F]-Folate-MTX-8
Synonym:	[18F]-**8**
Agent Category:	Compound
Target:	Folate receptor
Target Category:	Receptor
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	18F
Activation:	No
Studies:	• *In vitro* • Rodents



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Background

[PubMed]

Folic acid (FA; also known as folate or vitamin B_9) is a water-soluble vitamin that is required for the synthesis and repair of cellular DNA. FA also acts as a cofactor for many biological reactions and has an important role in cell maintenance and proliferation. Although the folate receptor (FR) is expressed at low levels in normal cells, this receptor is known to be upregulated in cancers such as those of the ovary, lung, breast, brain, colon, and the hematopoietic lineage cells (1). Therefore, the FR system has been targeted with radiolabeled folate and its derivatives, such as ^{67}Ga-deferoxamine (DF)-folate, ^{111}In-diethylenetriamine pentaacetic acid-folate, ^{99m}Tc-mercaptoacetyldiglycine-folate-methotrexate, etc., for the noninvasive detection of malignancies with single-photon emission computed tomography (2). Mathias et al. showed that $^{66/67}$Ga-DF-folate can be used with positron emission tomography (PET) for the imaging of FR-positive cancerous tumors in mice (3). However, although these tracers could detect the FR-rich tumors, they were deemed unsuitable for imaging lesions in the

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abdominal regions because high levels of radioactivity were observed to accumulate in the liver and intestines of the animals (4). In addition, $^{66/67}$Ga-labeled tracers are known to produce low-resolution images because they generate high positron energy (4.15 MeV and 1.89 MeV for ^{66}Ga and ^{67}Ga, respectively. ^{67}Ga-labeled agents are used for single photon emission computed tomography imaging) compared to ^{18}F (0.64 MeV), which generates superior images and is often used to radiolabel PET imaging agents in the clinic (4). In another study, it was shown that ^{18}F-fluorobenzylamine derivatives of folate could detect tumors that had a high expression of FR, but the radioactivity from these labeled compounds accumulated mainly at the rim of the tumor, and large amounts of the label were retained in the liver and intestines of the animals (4, 5).

In an ongoing effort to generate radiolabeled agents for the imaging of tumors that express high levels of FRs that would be superior to those developed and evaluated earlier, a ^{18}F-fluorobenzene derivative of folic acid ([^{18}F]-folate-1), a pyridinecarbohydrazide-folate derivative of folic acid ([^{18}F]-folate-2), a ^{18}F-fluorobenzene/methotrexate (MTX) conjugate of folic acid ([^{18}F]-folate-MTX-8), and a ^{18}F-pyridinecarbohydrazide-folate/methotrexate conjugate of folic acid ([^{18}F]-folate-MTX-9) have been synthesized (2). The biodistribution of these tracers was investigated in healthy mice. On the basis of results obtained from these studies, [^{18}F]-folate-2 was evaluated for the PET imaging of human KB cell line xenograft tumors (have a high expression of FR) in mice. This chapter describes the results obtained with [^{18}F]-folate-MTX-8. Separate chapters in MICAD (www.micad.nih.gov) discuss the studies performed with [^{18}F]-folate-1 (6), [^{18}F]-folate-2 (7), and [^{18}F]-folate-MTX-9 (8).

Related Resource Links

FR-related chapters in MICAD

Adult human FR protein and mRNA sequences

Human FR gene (Gene ID: 2348)

FR clinical trials

FR in Online Mendelian Inheritance in Man (OMIM) database

FR pathway in Kyoto Encyclopedia of Genes and Genomes (KEGG)

Folic acid information on Dailymed site

Synthesis

[PubMed]

Folate-MTX-8 was obtained by the conjugation of the γ-isomer of *N*-succinimidyl-methotrexate carboxylate with 4-fluorobenzenecarbohydrazide in presence of triethylamine and labeled the resulting product with ^{18}F as described by al Jammaz et al. (2). The synthesis and labeling of [^{18}F]-folate-2 and [^{18}F]-folate-1 have been detailed elsewhere (9). The total time of synthesis for each of the tracers was ~45 min, and the radiochemical yield and radiochemical purity of the final labeled products were >80% (based on the initial ^{18}F concentration) and >97% (without high-performance liquid chromatographic purification), respectively. The specific activity of the different radiolabeled compounds was reported to be >11.11 MBq/μmol (300 mCi/μmol) (2).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Using an *n*-octanol-water mixture (pH 7.3), the partition coefficient of [^{18}F]-folate-MTX-8 was determined to be 0.43. The partition coefficients of [^{18}F]-folate-1 and [^{18}F]-folate-2 were reported to be 0.38 ± 0.02 and 0.14 ±

0.01, respectively, indicating that these labeled compounds had a low lipophilicity and that, among the three tracers, [^{18}F]-folate-2 was least lipophilic (9).

[^{18}F]-Folate-MTX-8, [^{18}F]-folate-1, and [^{18}F]-folate-2 were reported to be stable in human plasma for at least 4 h at 37°C (data not presented) (2).

From a saturation assay with KB cell membranes, the FR binding affinities of [^{18}F]-folate-MTX-8, [^{18}F]-folate-1, and [^{18}F]-folate-2 were determined to be 22.52 ± 1.79 nM, 13.08 ± 0.83 nM, and 15.51 ± 1.80 nM, respectively (9). A cell internalization assay of [^{18}F]-folate-MTX-8, [^{18}F]-folate-1, and [^{18}F]-folate-2 with KB cells (using an acidic buffer, pH not mentioned) showed that 23.42 ± 1.53%, 25.85 ± 0.95%, and 11.86 ± 0.43% of the tracers were respectively internalized by the cells within 20 min at 37°C.

Animal Studies

Rodents

[PubMed]

The biodistribution of [^{18}F]-folate-MTX-8, [^{18}F]-folate-1, and [^{18}F]-folate-2 was studied in normal Balb/c mice as described by Jammaz et al. (2). The animals (n = 4 mice/time point for each tracer) were injected with 749 kBq (20 μCi) of the tracer through the tail vein and euthanized at 10, 60, and 120 min postinjection (p.i.) to determine the amount of radioactivity accumulated in the various organs. All data were presented as percent of injected dose per gram tissue (% ID/g). The label from [^{18}F]-folate-MTX-8 was cleared rapidly from the blood (5.64 ± 0.95% ID/g at 10 min p.i., 1.90 ± 0.28% ID/g at 60 min p.i., and 1.08 ± 0.25% ID/g at 120 min p.i.). The kidneys accumulated high levels of radioactivity (8.93 ± 1.49% ID/g at 10 min p.i. and 6.40 ± 1.35% ID/g at 120 min p.i.), and a similar trend was noted with the intestine (6.33 ± 0.19% ID/g at 10 min p.i. and 7.17 ± 1.56% ID/g at 120 min p.i.). This indicated that the tracer was excreted through the urinary and the hepatobiliary routes in the animals. With [^{18}F]-folate-1, rapid clearance of radioactivity from the blood was observed (4.41 ± 0.60% ID/g at 10 min p.i., 2.55 ± 1.50% ID/g at 60 min p.i., and 1.13 ± 0.51% ID/g at 120 min p.i.). High levels of label from [^{18}F]-folate-1 were observed in the kidneys (22.50 ± 4.42% ID/g at 10 min p.i. and 17.88% ID/g ± 0.10 at 120 min p.i.) and the intestine (5.95 ± 2.11% ID/g at 10 min p.i. and 5.86 ± 0.26% ID/g at 120 min p.i.) of these animals, indicating that this tracer also was excreted through the urinary and the hepatobiliary route. Radioactivity from [^{18}F]-folate-2 was rapidly cleared from circulation (1.22 ± 0.61% ID/g at 10 min p.i., 0.10 ± 0.05% ID/g at 60 min p.i., and 0.04 ± 0.01% ID/g at 120 min p.i.). A high accumulation of label from [^{18}F]-folate-2 was observed in the kidneys (13.8 ± 5.9% ID/g at 10 min p.i. and 2.92 ± 0.51% ID/g at 120 min p.i.), but the intestines accumulated a low level of the tracer (0.76 ± 0.20% ID/g at 10 min p.i. and 0.03 ± 0.01% ID/g at 120 min p.i.). This indicated that radioactivity from [^{18}F]-folate-2 was excreted primarily through the urinary route. The accumulation of low levels of radioactivity with [^{18}F]-folate-2 in the various organs suggested that this tracer had a superior pharmacokinetic profile compared to [^{18}F]-folate-MTX-8 and [^{18}F]-folate-1. No blocking studies with [^{18}F]-folate-MTX-8 were reported.

The biodistribution of [^{18}F]-folate-MTX-8, [^{18}F]-folate-1, and [^{18}F]-folate-2 was also investigated in nude mice (n = 4 animals/tracer) bearing KB cell tumors (2). The animals were injected with the radiotracers as described above and euthanized at 60 min p.i. With all the radiolabeled compounds, the uptake of radioactivity in the various organs of the mice at 60 min p.i. was similar to that observed earlier (see above). The tumor uptake of label with [^{18}F]-folate-MTX-8, [^{18}F]-folate-1, and [^{18}F]-folate-2 at 60 min p.i. was 0.81 ± 0.09% ID/g, 5.94 ± 1.16% ID/g, and 5.74 ± 0.16% ID/g, respectively, indicating that the tumor uptake of radioactivity with [^{18}F]-folate-MTX-8 was significantly lower ($P < 0.05$) than that observed with either [^{18}F]-folate-1 or [^{18}F]-folate-2. The tumor/blood and tumor/muscle ratios with [^{18}F]-folate-MTX-8, [^{18}F]-folate-1, and [^{18}F]-folate-2 at 60 min p.i. were 0.80 and 1.63, 14.70 and 28.70, and 6.39 and 25.83, respectively. For blocking studies, the mice were injected with 100 μg (0.22 μmol) FA 10 min before the administration of [^{18}F]-folate-MTX-8, [^{18}F]-folate-1, or

[^{18}F]-folate-2, and the rodents were subsequently treated as before (2). With [^{18}F]-folate-MTX-8, the tumors showed a significantly reduced ($P < 0.05$) uptake of radioactivity (0.19 ± 0.10% ID/g) at 60 min p.i., and a similar trend was evident in the kidneys, intestines, and liver (among these organs the kidney and the liver show a higher expression of FR compared with the other normal tissues). The tumors showed a significantly reduced accumulation of radioactivity (0.74 ± 0.17% ID/g, $P < 0.05$) with [^{18}F]-folate-1 at 60 min p.i., and the kidneys, liver, and the intestines of the animals also showed a reduced level of the label ($P < 0.05$). With [^{18}F]-folate-2, the uptake of radioactivity in the tumor at 60 min p.i. was reduced to 0.66 ± 0.10% ID/g ($P < 0.05$), and a similar trend was observed in the kidneys, liver, and intestines ($P < 0.05$). These studies indicated that only [^{18}F]-folate-1 and [^{18}F]-folate-2 had a high binding specificity for the FR in the tumors and the various organs of the animals.

Table: Tumor Uptake and Tumor/Blood and Tumor/Muscle Ratios of different ^{18} F-Labeled Folate and Folate-MXT Compounds

Tracer	Tumor Uptake (% ID/g)		Ratio (No Folate Pre-treatment)	
	No Folate Pre-treatment	With Folate (100 µg) Pre-treatment	Tumor/blood	Tumor/Muscle
[^{18} F]-Folate-1	5.94 ± 1.16	0.74 ± 0.17	6.39	25.83
[^{18} F]-Folate-2	5.74 ± 0.16	0.66 ± 0.10	14.72	28.70
[^{18} F]-Folate-MXT-8	0.18 ± 0.09	0.19 ± 0.10	0.90	1.80

From the studies and the results discussed above, the investigators concluded that [^{18}F]-folate-2 was probably superior to either [^{18}F]-folate-MTX-8 or [^{18}F]-folate-1 for the imaging of FR-expressing tumors in rodents (2).

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

Supplemental Information

[Disclaimers]

No information is currently available.

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