[^{18}F]-Fluoro-2-deoxy-D-glucose-folate

[^{18}F]-5

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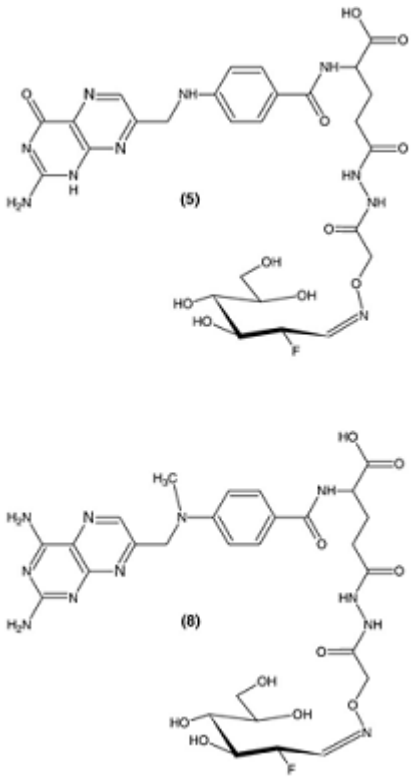
Chemical name:	[^{18}F]-Fluoro-2-deoxy-D-glucose-folate
Abbreviated name:	[^{18}F]-5
Synonym:	
Agent Category:	Compounds
Target:	Folate receptors
Target Category:	Receptors
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	^{18}F
Activation:	No
Studies:	• *In vitro* • Rodents



Structures of [^{18}F]-5 and [^{18}F]-8 (1).

Background

[PubMed]

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[^{18}F]-Fluoro-2-deoxy-D-glucose ([^{18}F]FDG)-folate, abbreviated as [^{18}F]-5, is a conjugate of [^{18}F]-fluorodeoxyglucose and folate, which was synthesized by al Jammaz et al. for use with positron emission tomography (PET) of tumors by targeting glucose metabolism and folate receptor (1).

2-Deoxy-D-glucose (2DG) was originally synthesized to inhibit glucose utilization by cancer cells and was later labeled with ^{18}F for imaging purposes (2, 3). [^{18}F]FDG, like glucose, is transported into cells by glucose transporters and converted to [^{18}F]FDG-6-phosphate ([^{18}F]FDG-6-P) within cells. Because [^{18}F]FDG-6-P cannot be further metabolized, it is then trapped within cells. Tumor cells are highly proliferative and have a high rate of glucose metabolism compared to normal tissue. Consequently, tumor cells accumulate much more [^{18}F]FDG-6-P than normal cells.

PET imaging with [^{18}F]FDG ([^{18}F]FDG-PET) has been widely accepted as a standard modality in the detection, staging, and therapy response monitoring of various malignant tumors. However, there are several limitations for the clinical use of [^{18}F]FDG-PET (4, 5). First, [^{18}F]FDG can accumulate in several important organs, including the brain and heart, which not only limits its use in these organs but also causes toxicity to them. Second, [^{18}F]FDG-PET is not suitable for imaging well-differentiated, low-malignant, and slow-growing tumors because these tumors often have low glucose utilization. Third, activated inflammatory cells have been found to take up considerable [^{18}F]FDG; thus, false-positivity may become an issue when inflammatory cell infiltrate is prominent in tumors or other diseases.

In an effort to develop new [^{18}F]FDG-based imaging probes, al Jammaz et al. synthesized two conjugates, [^{18}F]-5 and [^{18}F]-8, by conjugating [^{18}F]FDG with folate and methotrexate (MTX), respectively, using the readily available oxime-reactive [^{18}F]FDG building block (1). Folate is vital for the rapid proliferation of tumor cells, and folate receptor is highly affinitive and overexpressed in various types of human tumors (6, 7). In contrast, healthy cells are severely restricted in possessing folate receptors. In addition, folate conjugates have well been characterized as drugs, drug carriers, and imaging agents (8, 9). MTX is a folic acid analog but possesses a relatively low affinity for folate receptor. Studies by al Jammaz et al. showed that [^{18}F]-5 was more suitable for tumor imaging than [^{18}F]-8 (1). This chapter summarizes the data obtained with [^{18}F]-5 and [^{18}F]-8.

Related Resource Links:

- Folate-based chapters in MICAD
- Folate receptor gene information in NCBI
- Folate receptor-related articles in Online Mendelian Inheritance in Man (OMIM)
- Folate receptor-associated clinical trials

Synthesis

[PubMed]

The amide-linked reference compounds **5** (2-fluoro-2-deoxy-D-glucose-folate) and **8** (2-fluoro-2-deoxy-D-glucose-methotrexate) were synthesized by conjugating FDG with the γ-isomers of folate carbohydrazide oxime and methotrexate carbohydrazide oxime, respectively (1). The overall chemical yields for compounds **5** and **8** were 62% and 38%, respectively, which appeared to decompose beyond 185°C. The calculated molecular mass for compounds **5** and **8** were 692.61 and 705.65, respectively. The base peak m/z = 325 corresponds to the γ-moiety-(CH_2-CH_2-CO-NH-NH-oxime-FDG) of the conjugated folic acid and MTX. Chemical purities for both **5** and **8** were found to be >97%.

[^{18}F]-5 and [^{18}F]-8 were prepared by reacting the building block of [^{18}F]FDG with folate and methotrexate carbohydrazide oximes in acetate buffer (pH ~4.5) for 10–15 min at 60°C (1). The overall radiochemical yields for [^{18}F]-5 and [^{18}F]-8 were >80% (based on the starting [^{18}F]-FDG), with a total synthesis time of ~20 min. The radiochemical purities of [^{18}F]-5 and [^{18}F]-8 were >98%. The calculated partition coefficients for [^{18}F]-5 and [^{18}F]-8 were –1.54 ± 0.03 and –1.59 ± 0.02, respectively, representing a low lipophilic characteristic for both conjugates. The amount of cold compounds in the final products was below the detection limit of ultraviolet-visible spectroscopy (<1 μg). The specific activities for [^{18}F]-5 and [^{18}F]-8 were >9.25 GBq/μmol (250 mCi/μmol). The calculated charged density for [^{18}F]-5 was found to be around –1.67, which is 4 and 12 times higher, respectively, than that of the corresponding fluoropyridine and benzene carbohydrazide-folate conjugates (8).

Proteolytic degradation analysis showed that both [^{18}F]-5 and [^{18}F]-8 remained sufficiently stable (>99%) in human plasma during incubation for at least 4 h at 37°C, demonstrating a high *in vitro* stability of the two radioconjugates (1).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The binding affinities (K_d) of [^{18}F]-5 and [^{18}F]-8 were evaluated with saturation assays using folate receptor-positive human oral carcinoma KB cell line (1). The results demonstrated that [^{18}F]-5 had a higher binding affinity to KB cells than [^{18}F]-8 (1.83 ± 0.15 *versus* 4.74 ± 0.71 nM), comparable to the native folic acid.

Cellular internalization studies were performed with acidic buffer to determine the extent of internalization of [^{18}F]-5 and [^{18}F]-8 by KB cancer cells (1). A rapid and significant internalization was observed for both [^{18}F]-5 and [^{18}F]-8, with 25.92 ± 0.59% and 21.50 ± 0.10%, respectively, of the cell surface-bound conjugates internalized after incubation in acidic buffer for 20 min at 37°C, indicating that both conjugates maintained their integrity and displayed high affinity toward folate receptor.

Animal Studies

Rodents

[PubMed]

Biodistribution of [^{18}F]-5 and [^{18}F]-8 was investigated in normal Balb/c mice at 10, 60, and 120 min after tail vein injection (20 μCi, 740 kBq) (n = 4 mice/time points per agent) (1). The results for [^{18}F]-5 generally demonstrated fast and efficient clearance from blood and most organs (Table 1). [^{18}F]-5 displayed elevated radioactivity uptake and retention in the kidneys and lungs even at 120 min after injection, suggesting that the major route of [^{18}F]-5 elimination was the urinary system, which might be attributed to the presence of the hydrophilic [^{18}F]-FDG prosthetic group. [^{18}F]-8 exhibited almost equal radioactivity accumulation in most organs, and clearance from blood and nearly all organs was fast except for the intestines, where significant radioactivity accumulation was observed. Low bone radioactivity was observed for both [^{18}F]-5 and [^{18}F]-8. Thus, the high *in vivo* stability was probably due to the presence of a strong carbon-fluorine bond (1).

Table 1. Biodistribution of [^{18}F]-5 and [^{18}F]-8 in normal Balb/c mice.

Organs	[^{18}F]-5 (% ID/g)			[^{18}F]-8 (% ID/g)		
	10 min	60 min	120 min	10 min	60 min	120 min
Blood	3.26 ± 0.75	0.40 ± 0.17	0.22 ± 0.04	3.06 ± 0.57	1.30 ± 0.25	0.82 ± 0.21
Liver	2.85 ± 0.34	0.54 ± 0.07	0.47 ± 0.14	3.58 ± 0.39	1.45 ± 0.27	0.69 ± 0.16
Lung	2.24 ± 0.69	2.08 ± 0.16	1.20 ± 0.13	4.02 ± 0.71	1.29 ± 0.26	0.59 ± 0.14
Kidney	5.80 ± 1.38	1.83 ± 0.16	1.03 ± 0.32	3.23 ± 0.48	1.83 ± 0.55	0.78 ± 0.29
Intestine	2.35 ± 0.69	0.78 ± 0.25	0.72 ± 0.05	2.91 ± 0.59	2.56 ± 0.53	1.52 ± 0.05
Heart	3.26 ± 0.90	0.69 ± 0.26	0.45 ± 0.09	5.09 ± 0.92	0.91 ± 0.22	0.71 ± 0.16
Muscle	0.76 ± 0.03	0.35 ± 0.04	0.37 ± 0.06	4.71 ± 0.38	0.98 ± 0.23	0.55 ± 0.20
Bone	0.83 ± 0.40	0.50 ± 0.07	0.44 ± 0.18	0.93 ± 0.36	0.62 ± 0.19	0.59 ± 0.18
Spleen	1.42 ± 0.18	0.73 ± 0.25	0.83 ± 0.11	2.42 ± 0.36	1.19 ± 0.33	0.81 ± 0.33

In nude mice bearing KB xenograft tumors (n = 4/group), [^{18}F]-5 displayed a rapid clearance from blood with 0.30 ± 0.23% injected dose per gram tissue (ID/g) remained at 60 min after injection (Table 2) (1). High tumor uptake was observed for [^{18}F]-5 (3.32 ± 0.32% ID/g) at 60 min after injection, and the tumor/blood and tumor/muscle ratios reached 11.07 and 9.22, respectively. Similar to the findings in normal mice, a moderate uptake of [^{18}F]-5 was also found in the kidneys (1.49 ± 0.05% ID/g), but this was much lower than the uptake of [^{18}F]-fluorobenzene and pyridine carbohydrazide-folate conjugates (19.7 ± 2.25 and 7.90 ± 1.25% ID/g, respectively) reported previously (8). The relatively low uptake in the kidneys may be attributed to the nature of [^{18}F]FDG as a

prosthetic group and the overall negative charge for [^{18}F]-5. [^{18}F]-5 also showed low accumulation in the liver and intestine (0.70 ± 0.61 and 0.75 ± 0.03% ID/g, respectively).

For blocking studies, each animal was intravenously injected with excess cold folic acid (~100 μg) 10 min before injection of the radiotracers (1). Animals were euthanized at 60 min after radiotracer injection (n = 4 mice/group). Cold folic acid markedly reduced the uptake of [^{18}F]-5 in the target organs and tumors. [^{18}F]-5 uptake was reduced in tumors by ~80% (3.32 ± 0.32 *versus* 0.72 ± 0.21% ID/g, $P < 0.05$), indicating receptor specificity of [^{18}F]-5. [^{18}F]-5 uptake by the kidneys was also considerably reduced by at least 50% (1.49 ± 0.05 *versus* 0.77 ± 0.05% ID/g, $P < 0.05$). The radioactivity in other organs either remained at the same degree or increased as observed for the liver and intestine.

Table 2. Biodistribution of [^{18}F]-5 and [^{18}F]-8 in mice bearing tumor xenografts.

Organs	[^{18}F]-5 (% ID/g)		[^{18}F]-8 (% ID/g)	
	60 min	60 min blocked[a]	60 min	60 min blocked[a]
Blood	0.30 ± 0.23	0.31 ± 0.29	0.37 ± 0.17	0.22 ± 0.03
Liver	0.70 ± 0.61	1.45 ± 0.30	0.61 ± 0.31	0.38 ± 0.07
Lung	1.30 ± 0.30	1.49 ± 0.19	1.16 ± 0.41	1.47 ± 0.21
Kidney	1.49 ± 0.05	0.77 ± 0.05	0.82 ± 0.05	0.78 ± 0.15
Intestine	0.75 ± 0.03	1.75 ± 0.38	0.90 ± 0.25	0.88 ± 0.16
Heart	0.20 ± 0.08	0.12 ± 0.04	0.21 ± 0.03	0.30 ± 0.15
Muscle	0.36 ± 0.10	0.38 ± 0.06	0.44 ± 0.05	0.52 ± 0.17
Bone	0.09 ± 0.05	0.15 ± 0.02	0.16 ± 0.06	0.17 ± 0.07
Spleen	1.30 ± 0.31	1.42 ± 0.35	1.37 ± 0.35	0.83 ± 0.28
Tumor	3.32 ± 0.32	0.72 ± 0.21	1.10 ± 0.21	0.37 ± 0.33

[a]Animals were injected with 100 μg folic acid 10 min before the administration of radiotracers.

[^{18}F]-8 also displayed fast clearance from blood and most of the other organs, but this clearance was slower than that of [^{18}F]-5 (Table 2) (1). In addition, [^{18}F]-8 exhibited two-fold lower kidney uptake (0.82 ± 0.05% ID/g) and almost four-fold lower tumor uptake (1.1 ± 0.21% ID/g) at 60 min after injection when compared with uptake of [^{18}F]-5. Cold folic acid also blocked the uptake of [^{18}F]-8 in tumors by ~60% (1.10 ± 0.21 *versus* 0.37 ± 0.33% ID/g; $P < 0.05$), suggesting receptor-mediated accumulation of this radioconjugate.

Analysis of the urine collected at 60 min after injection showed that a significant amount of radioactivity (>95%) was still associated with [^{18}F]-5 and [^{18}F]-8, suggesting that neither radiolabel is prone to rapid *in vivo* degradation (1).

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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