2′-Deoxy-2′-[^{18}F]fluoro-5-fluoro-1-β-D-arabinofuranosyluracil

[^{18}F]FFAU

The MICAD Research Team

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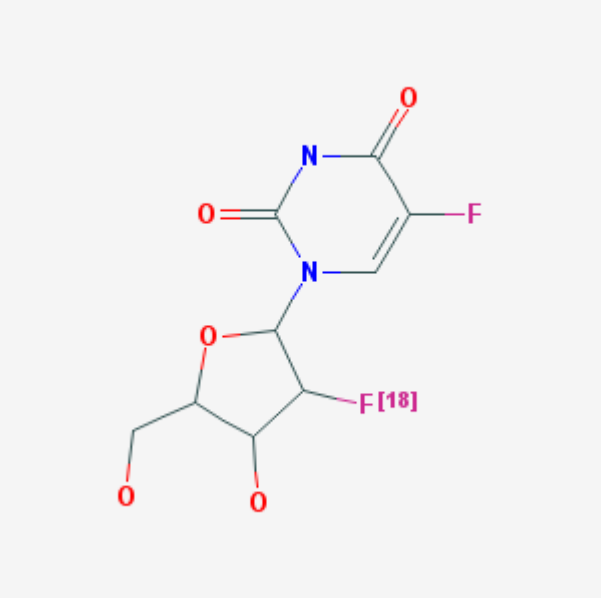
Chemical name:	2′-Deoxy-2′-[^{18}F]fluoro-5-fluoro-1-β-D-arabinofuranosyluracil
Abbreviated name:	[^{18}F]FFAU
Synonym:	
Agent Category:	Compound
Target:	Herpes simplex virus thymidine kinase (HSV-tk)
Target Category:	Phosphorylation
Method of detection:	Positron emission tomography (PET)
Source of signal:	^{18}F
Activation:	No
Studies:	• *In vitro* • Rodents



Background

[PubMed]

Herpes simplex virus type-1 thymidine kinase (HSV1-tk) can be used as a suicide gene for gene therapy. As of today, animal studies using HSV1-tk gene and ganciclovir (1) have brought some success in treating malignant tumors with suicide gene therapy; however,

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clinical results showed that such methods did not provide sufficient gene delivery to the tumor human cells for therapy (2).

HSV1-tk can phosphorylate a wide range of nucleoside analogs (e.g. acycloguanosines and 2'-deoxyfuranosyluracil nucleoside derivatives) that are not phosphorylated efficiently by the native enzyme (3). The presence of fluorine in the 2'-arabino position in a furanosyluracil nucleoside results in enhanced monophosphorylation by HSV-tk type 1 and type 2 compared with the host thymidine kinase.

In vivo imaging methods are essential tools for monitoring the gene expression as an indicator of gene delivery and for quantifying the level of HSV1-tk enzyme activity after gene transfer (4-6). Several probes for positron emission tomography (PET) of HSV1 -tk gene have been studied so far. Among them, [^{18}F]FMAU, [^{18}F]FHPG (7), [^{18}F]FHBG and [^{18}F]FIAU. [^{124}I]FIAU appears to be superior to the acycloguanosine derivatives FHPG and FHBG in some cell lines (8) with respect to total uptake and the uptake ratio (tk-positive to wild type), although it is susceptible to deiodination *in vivo*. 2'-deoxy-2'-fluoro-5-fluoro-1-α-D-arabinofuranosyluracil ([^{18}F]FFAU) may offer an advantage over [^{124}I]FIAU and other 5-substituted analogs for imaging gene expression, and is currently under investigation (9).

Synthesis

[PubMed]

Alauddin et al. (9) synthesized [^{18}F]FFAU by reaction of the respective triflate (2-deoxy-2-trifluoromethanesulfonyl-1,3,5-tri-O-benzoyl-α-D-ribofuranose with tetrabutylammonium [^{18}F]fluoride. This reaction produced 2-[^{18}F]-fluoro-1,3,5-tri-O-benzoyl-α-D-arabinofuranose, which was converted to its 1-bromo derivative by treatment with hydrogen bromide in acetic acid (HBr/AcOH). After coupling with protected 5-fluorouracil, the resulting product was hydrolyzed (in base) and purified by high-performance liquid chromatography (HPLC) to obtain the desired product [^{18}F]FFAU.

[^{18}F]FFAU was produced with a radiochemical yield of 20%-30% (decay-corrected with an average of 25% in 4 runs), and a radiochemical purity >99%. The average specific activity for [^{18}F]FFAU was 85 GBq/μmol (2,300 mCi/ μmol) at the end of the synthesis.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

In vitro studies performed by Alauddin et al. (9) showed that the total accumulation of [^{3}H]FFAU in HSV1-tk-expressing cells was significantly higher than that in wild-type control cells, and increased rapidly in the transduced cells, reaching a plateau by 60 min. Maximum incorporation of FFAU within a short time (30 min) suggested that the rate of monophosphorylation by the HSV1-tk was also very high. This was further supported by

dynamic PET scans, which showed that tumor uptake peaked within 10 min of injection and remained elevated throughout the time course studied (see Rodents section).

The *in vitro* uptake ratios obtained between transduced and wild-type control cells were 176, 126, and 81 at 30, 60, and 120 min respectively. Tumor of [^{3}H]FFAU in tk-positive cells was approximately 180-fold ($P < 0.001$) higher than those obtained in previous studies using 2'-[^{14}C]-deoxy-2'-fluoro-5-methyl-1-β-D-arabinofuranosyluracil ([^{14}C]FMAU) and 9-(4-[^{18}F]-fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]FHBG) in the same cell lines (7).

In their studies, Allaudin et al. (9) reported a gradual and slow accumulation of FFAU in wild-type cells likely representing minimal baseline monophosphorylation by host kinase and irreversible binding to thymidylate synthase (TS) (10) throughout the 2 h study period. However, no significant increase in uptake in transduced cells was observed after 60 min, and the ratio between tk-positive and wild-type cells decreased from 176 to 81 by 2 h.

Animal Studies

Rodents

[PubMed]

Allaudin et al. (9) performed *ex vivo* studies of [^{3}H]FFAU and *in vivo* PET studies of [^{18}F]FFAU on 6- week-old athymic tumor-bearing nude mice (Harlan). For the *ex vivo* studies, one group of mice (n = 6) was injected intravenously via a tail vein with [^{3}H]FFAU (37 kBq, 1 μCi, 200 μl), and blood samples were collected for 3 of them at 2, 5, 10, 20, 60, and 120 min post injection. The activity for each sample was calculated as a percentage of the injected dose per gram (% ID/g). At 2 h after injection, mice were sacrificed, and the activity was measured on excised tumors and organs using the external standard method of quench correction.

For *in vivo* PET studies of [^{18}F]FFAU, mice (n = 2) were injected with 7.4 MBq (0.2 mCi) of [^{18}F]FFAU, through the tail vein, and imaging was performed at 30 min, 1 h, and 2 h after injection. The radioactivity uptake in tumor (kBq/cm^{3}, μCi/cm^{3}) was converted to % ID/g and compared with the biodistribution data using [^{3}H]FFAU. The higher uptake obtained in transduced tumor suggested an accumulation of [^{18}F]FFAU, probably resulting from reversible binding to - or complex formation with- HSV-tk with net intracellular substrate trapping. Contrary to [^{18}F]FMAU, no significant uptake in wild-type tumors was observed, suggesting that [^{18}F]FFAU underwent only minimal phosphorylation by the host kinase during the 2 h time period.Results showed that tumor uptake in transduced cells was much higher than that in nontransduced cells. The values obtained for [^{3}H]FFAU in transduced and wild-type tumors were 30.75 ± 7.43%ID/g and 3.87 ± 1.80% ID/g, respectively. The uptake in other organs, including blood, was quite low, except kidney, which appeared slightly higher with renal clearance as the primary route of radiotracer excretion.

Comparative studies between FFAU, FMAU, and FHBG on the cell line HT-29 showed that the total uptake of [^{3}H]FFAU *in vivo* (% ID/g) was much higher compared with that of FMAU and FHBG, and that the uptake ratio between transduced and wild-type cells at 2 h for FFAU was also higher than that of FMAU and FHBG. On the other hand, the ratio was comparable between FHBG and FMAU. The high accumulation and the uptake ratio (specificity) of [^{18}F]FFAU in tk-positive cells - along with its *in vivo* stability and favorable biodistribution characteristics, suggested that this probe might be superior to other agents previously studied in the HT-29 cell line.

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.

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