2'-Fluoro-2'-deoxy-5'-[123/124/125/131I]iodo-1β-D-arabinofuranosyluracil

[123/124/125/131I]FIAU

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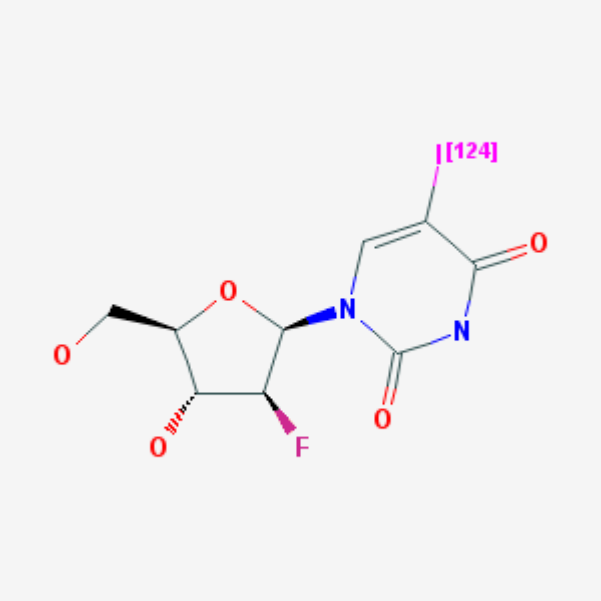
Chemical name:	2'-Fluoro-2'-deoxy-5'-[123/124/125/131I]iodo-1β-D-arabinofuranosyluracil
Abbreviated name:	[123/124/125/131I]FIAU
Synonym:	
Agent category:	Compound
Target:	Herpes simplex virus thymidine kinase (HSV-TK)
Target category:	Phosphorylation
Method of detection:	Positron emission tomography (PET)/ Single-photon emission computed tomography (SPECT)
Source of signal:	123/124/125/131I
Activation:	No
Studies:	• *In vitro* • Rodents • Non-primate non-rodent mammals • Humans



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Background

[PubMed]

A potential approach to the treatment of diseases and genetic disorders in humans is gene therapy [PubMed]. This is a technique whereby the absent or malfunctioned gene is replaced by a working gene to produce an enzyme or protein to correct the progression of the disease. Noninvasive molecular imaging technologies play an important role in the fields of gene therapy by monitoring gene expression continuously in living animals (1). Magnetic resonance imaging (MRI), optical imaging, ultrasound imaging, and radionuclide imaging (positron emission tomography (PET)/single photon emission computed tomography (SPECT)) modalities have been applied for gene therapy of cancer as well as cardiovascular, neurological, musculoskeletal, hepatic, immunological, diabetic, and inherited diseases in small animals (2-10). These noninvasive imaging technologies allow quantitative assessments of the magnitude, location, and duration of the transgene expression. Gene therapy can be achieved either *ex vivo* or *in vivo*. The gene can be delivered using a viral or nonviral vector, such as liposome and naked DNA. Retrovirus, adenovirus, adeno-associated virus, and herpes simplex virus have been used as gene transfer vectors. Imaging transgene expression can help to optimize gene therapy. There are two imaging strategies, direct and indirect. Direct imaging uses a target-specific probe directly to the target gene, such as receptor, enzyme, and antigen. For example, herpes simplex virus type 1 thymidine kinase (HSV1-tk) vector is transduced in cells first. Labeled FIAU is introduced later to monitor its expression. Indirect imaging involves coupling the target gene to a reporter gene, the expression of which can be tracked by a specific reporter gene probe. For example, the VEGF gene is linked with HSV1-tk in a vector, which is transduced in cells. Labeled FIAU is then introduced to monitor the expression of VEGF via the expression of the reporter (HSV1-tk).

Currently, there are two approaches to image transgene expression by using either a reporter enzyme using a substrate probe or a reporter receptor using a receptor ligand probe. The enzymes include cytosine deaminase, β-galactosidase, tyrosinase, and HSV1-tk. The Na^+/I^- symporter (NIS) gene has been studied as a potential reporter gene as well as a therapeutic gene (11). The most extensively studied reporter enzyme gene is HSV1-tk (8, 12). The reporter receptors are dopamine D_2 receptor (D_2R) (13) and somatostatin receptor subtype-2 (hSSTR2) (14).

HSV1-tk is a commonly studied suicide gene for cancer therapy of glioma, prostate cancer, leukemia, and lymphoma (10, 15). Suicide gene therapy is based on the enzymatic conversion of a nontoxic prodrug into a lethal drug by a transgene. Anti-HSV nucleoside

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analogs, such as acyclovir, ganciclovir, and penciclovir, are converted to monophosphates by HSV-TK. The cellular enzymes then convert the monophosphates to di- and triphosphates, which inhibit mammalian DNA polymerase. HSV1-tk has also been widely studied as a reporter gene. The HSV prodrugs have been explored for HSV1-tk reporter gene imaging. The selective phosphorylation of the produg probes by HSV1-TK leads to trapping within the transduced cells and not significantly in the other cells. Many radiolabeled acycloguanosine and thymidine derivatives have been studied for HSV1-tk reporter gene imaging *in vitro*, in small animals, and in humans (16). 2'-Fluoro-2'-deoxy-5'-iodo-1β-D-arabinofuranosyluracil (FIAU) has been tested as an anti-viral thymidine nucleoside analog against herpes and hepatitis viruses. Radiolabeled FIAU has been evaluated *in vitro* and in small animals to be a potential imaging agent for HSV1-tk gene expression using PET/SPECT imaging (17, 18).

Related Resource Links:

- Chapters in MICAD (HSV-TK)
- Gene information in NCBI (HSV-TK)
- Articles in Online Mendelian Inheritance in Man (OMIM) (MC1R)
- Clinical trials ([124I]FIAU)

Synthesis

[PubMed]

2'-Fluoro-2'-deoxy-1-beta-D-arabinofuranosyluracil (FAU) was generally labeled by direct iodination with [*I]NaI to form [*I]FIAU (19, 20). [*I] denotes [123I], [124I], [125I], or [131I]. [*I]FIAU was isolated by column chromatography or high-performance liquid chromatography (HPLC) purification. Radiochemical yield was 51-55% with radiochemical purity of >97%. In an alternative approach, 5-trimethylstannyl-1-(2'-fluoro-2'deoxy-1-beta-D-arabinofuranosyl)uracil (FTAU) was reacted with [*I]NaI to form [*I]FIAU (21, 22). The radiochemical yield was in the range of 85-95% with radiochemical purity of >98%.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

[131I]FIAU was stable in human serum and whole blood at 37°C over a period of 24 h. The uptake of [125I]FIAU in D-247 human glioma cells *in vitro* was 20-fold greater than that of [125I]IUdR. The uptake was blocked by an excess of the respective unlabeled compounds. More than 80% of the cellular radioactivity was incorporated into DNA (22).

FIAU was shown to be greatly accumulated in various HSV1-tk transduced cell lines over control cells (17, 18, 21, 23). In general, FIAU accumulation was better than FHBG and FHPG (17, 20). The transduced cells were more sensitive to ganciclovir inhibition as compared to the control cells *in vitro* (18, 23). Highly significant relationships were

observed between HSV1-tk expression and sensitivity to ganciclovir and FIAU accumulation in the transduced cell lines (8).

Animal Studies

Rodents

[PubMed]

Biodistribution of [^{131}I]FIAU was studied in normal mice. The initial tracer uptake was high at 0.25 h in liver, kidneys, muscle, blood, and urine. The uptakes in most organs decreased with time with the exception of the thyroid, which showed an increasing uptake with time (22). Biodistribution studies of FIAU in mice and rats bearing HSV1-tk-positive and -negative tumors showed excellent tracer uptakes in the positive tumors within 1 h, which persisted up to 24 h (18, 20). Clear PET and SPECT images of the HSV1-tk-expressing tumors were observed.

Rats were percutaneously injected with similar titers of adenovirus-expressing HSV1-tk, HSV1-sr39tk, or control gene into the myocardium, followed by [^{124}I]FIAU (HSV1-tk rats and controls) or [^{18}F]FHBG (HSV1-sr39tk rats and controls) 2 days later (24). Dynamic PET was performed during the 2 h after injection of the tracer. A significant cardiac [^{124}I]FIAU accumulation of 1.24-fold increase over controls occurred in images obtained early (10–30 min) after the tracer injection. However, no difference between HSV1-tk-infected animals and controls was seen in the images obtained later because of tracer washout. For [^{18}F]FHBG, specific myocardial accumulation greater than background levels was detected in HSV1-sr39tk-infected animals at early imaging and increased over time until the latest imaging (105–120 min). At maximum, cardiac [^{18}F]FHBG uptake showed a 4.15-fold increase compared with controls (105-120 min). Co-expression of the reporter gene HSV1-tk and a therapeutic gene is opening up more opportunities for gene therapy of heart diseases (5).

In another study, RG2 rat glioma tumor cells were transduced with HSV1-tk and transplanted subcutaneously in nude rats (20). The *in vitro* and *in vivo* imaging results show that [^{124}I]FIAU is a better reporter probe than [^{18}F]FHBG for imaging HSV1-tk expression, with greater sensitivity and higher accumulation at 2 and 24 h. Therefore, different cell types may handle reporter gene and reporter probe differently.

Various preclinical studies showed that [^{124}I]FIAU can be used to monitor co-expression of HSV1-tk and a *cis*-linked transgene in mice and rats (25-28). This approach will facilitate further development of gene-therapy protocols in humans. [^{124}I]FIAU was reported to be a useful agent to image bacterial infections (29) and tumor-localized bacteria expressing HSV1-tk in small animals (30).

Other Non-Primate Mammals

[PubMed]

In cats, the kinetic analysis of [124I]FIAU radioactivity showed an early peak (1-2 min after injection) in heart and kidneys (0.20% injected dose (ID)/g; SUV, 4.0), followed by a second peak (10-20 min after injection) in liver and spleen (0.16% ID/g; SUV, 3.2) (19). Subsequent clearance from tissues and a late peak in the bladder were observed at 10-15 h after injection. In the unlesioned cat brain, no substantial [124I]FIAU uptake occurred throughout the PET measurements (0.02% ID/g; SUV, 0.4), indicating that FIAU cannot cross the intact blood-brain barrier (BBB).

Bengel et al. (31) reported that PET imaging of HSV1-tk expression with [124I]FIAU in pig hearts was limited to the first 30 min because of a rapid washout of [124I]FIAU from the hearts. However, Miyagawa et al. (24) showed that good images were obtained with [18F]FHBG in HSV1-sr39tk-expressing rat and pig hearts up to 120 min but not with [124I]FIAU in HSV1-tk-expressing hearts.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

In one recurrent glioblastoma patient, [124I]FIAU uptake in normal brain was very low (0.0002%ID/g; SUV, 0.16) after a bolus injection of 77.7 MBq or 2.1 mCi [124I]FIAU (19). In contrast, the recurrent glioblastoma showed relatively high uptake of radioactivity (5-10 min after injection; 0.001%ID/g; SUV, 0.8), which was cleared slowly over the 68-h imaging period. The presence of the tumor was confirmed by MRI, [11C]MET and [18F]FDG measurements. However, substantial uptake of [124I]FIAU may occur within areas of BBB disruption (e.g., glioblastoma), which is helpful for imaging clinically relevant levels of HSV1-tk gene expression in brain tumors after gene therapy.

HSV1-tk liposomal vector was infused intratumorally to five recurrent glioblastoma patients, followed by ganciclovir treatment for 14 days (32). Treatment responses were measured by MRI, [11C]MET PET and [18F]FDG PET as well as [124I]FIAU PET. One of five patients showed specific uptake of [124I]FIAU with signs of necrosis as observed by [11C]MET and [18F]FDG radioactivity within the volume of specific [124I]FIAU trapping after ganciclovir treatment. The other four patients with no specific [124I]FIAU uptake may be explained by their significantly lower number of proliferating tumor cells per voxel and therefore inefficient transfer of the HSV1-tk gene. [124I]FIAU PET imaging of HSV1-tk gene expression in patients is feasible and that the gene expression may predict the therapeutic effect.

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