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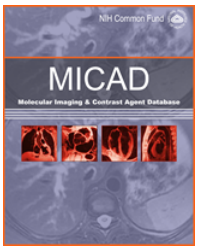

9-(4-[18F]Fluoro-3-hydroxymethylbutyl)guanine

[18F]FHBG

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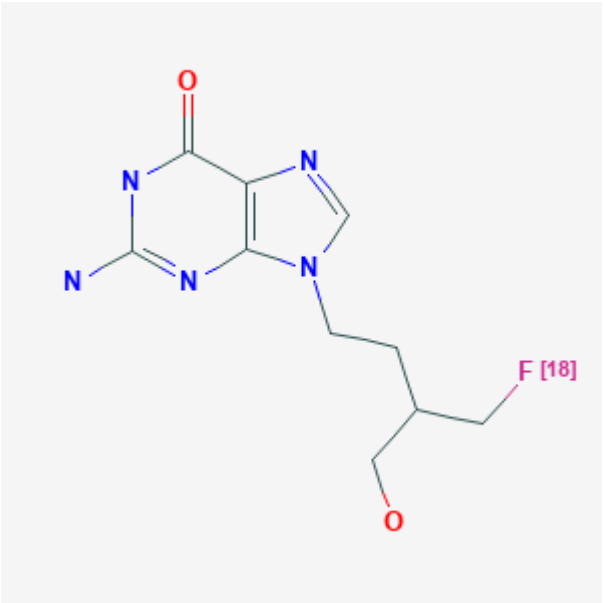
Chemical name:	9-(4-[18F]Fluoro-3-hydroxymethylbutyl)guanine
Abbreviated name:	[18F]FHBG
Synonym:	2-amino-9-[3-([18F]fluoromethyl)-4-hydroxy-butyl]-1,9-dihydropurin-6-one
Agent category:	Compound
Target:	Herpes simplex virus thymidine kinase (HSV-TK)
Target category:	Phosphorylation
Method of detection:	Positron emission tomography (PET)
Source of signal:	18F
Activation:	No
Studies:	• *In vitro* • Rodents • Non-primate non-rodent mammals • Non-human primates • Humans



Structure is currently not available in PubChem.

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 malfunctioning gene is replaced by a working gene to produce an enzyme or a protein to correct the progression of 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 (positon

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emission tomography (PET)/single photon emission computed tomography (SPECT)) modalities have been applied for gene therapy of cancer, 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 by *ex vivo* or *in vivo* methods. 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. Indirect imaging involves coupling the target gene to a reporter gene, the gene expression of which can be tracked by a specific reporter gene probe. Currently, there are two approaches to image transgene expression, either by reporter enzyme using substrate probe or reporter receptor using receptor ligand probe. The enzymes include cytosine deaminase, β-galactosidase, tyrosinase, and herpes simplex virus type 1 thymidine kinase (HSV1-tk) or its mutant, HSV1-sr36tk. The most extensively studied reporter enzyme gene is HSV1-tk (8, 11). The reporter receptors are dopamine D_2 receptor (D_2R) (12) and somatostatin receptor subtype 2 (hSSTR2) (13).

HSV1-tk is a commonly studied suicide gene for cancer therapy of glioma, prostate cancer, leukemia, and lymphoma (10, 14). Suicide gene therapy is based on the enzymatic conversion of a nontoxic prodrug into a lethal drug by a transgene. Anti-HSV nucleoside analogs, such as acyclovir, ganciclovir, and penciclovir, are converted to monophosphates by HSV thymidine kinase. The cellular enzymes converted 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 prodrug 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 (15).

[^{18}F]FHBG is a side-chain ^{18}F-labeled analog of penciclovir. [^{18}F]FHBG has been evaluated *in vitro* and in small animals and is a potential imaging agent for gene expression using PET. Noninvasive molecular imaging of therapeutic HSV1-sr36tk suicide gene therapy in mice was demonstrated with [^{18}F]FHBG and [^{18}F]fluorodeoxyglucose PET imaging (14). Coexpression of the reporter gene HSV1-tk and a therapeutic gene is opening up further opportunities for gene therapy (15).

Related Resource Links:

- Chapters in MICAD (HSV-TK)
- Gene information in NCBI (HSV-TK)

Synthesis

[PubMed]

9-(4-Hydroxy-3-hydroxymethylbutyl)-guanine (penciclovir) was converted to a methoxytrityl derivative followed by tosylation. The tosylate was reacted with either tetrabutylammonium [^{18}F]fluoride or [^{18}F]KF in the presence of kryptofix 2.2.2, followed by acidic hydrolysis to produce 9-(4-[^{18}F]fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]FHBG). [^{18}F]FHBG was isolated by high-performance liquid chromatography (HPLC) purification. The radiochemical yield was 8.0-22.3%, with an average of 12% (corrected for decay). Synthesis time was 90-100 min, including HPLC purification with radiochemical purity >99% and average specific activity of 11.8 GBq/mmol (320 mCi/mmol) (16). Similar syntheses were later reported by others (17, 18). One-pot and automated syntheses were achieved to provide 10-15% yield in about 60 min (17, 19).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

[^{18}F]FHBG was shown to accumulate in HSV1-tk expressing cells in cultures. HT-29 human colon cancer cells were infected with HSV1-tk (20). In vitro uptakes of [^{18}F]FHBG in the transduced cells were 31 and 71 times higher than the nontransduced cells at 1 and 5 h, respectively. HPLC analysis showed 10% intact [^{18}F]FHBG, 6% monophosphate, 54% diphosphate and 30% triphosphate in the transduced cells at 5 h.

H9c2 rat embryonic cardiac cells were infected with Ad4tk, an adenovirus expression a mutant HSV1-sr39tk and vascular endothelial growth factor ($VEGF_{121}$) independently (21). Northern blot analysis showed increasing HSV1-sr39tk and VEGF RNA expression in the transduced cells. Accumulation of [^{18}F]FHBG and [^{14}C]FIAU increased with increasing HSV1-sr39tk and HSV1-tk expression, respectively. Secretion of VEGF also increased with the reporter gene expressions. There is also a significant correlation of reporter gene probe uptake with VEGF production *in vitro*. Therefore, it may be feasible in future for monitoring angiogenesis gene therapy *in vivo*.

Animal Studies

Rodents

[PubMed]

Tissue accumulation of [^{18}F]FHBG was studied in nude mice transplanted with HT-29 human colon cancer cells with or without HSV1-tk transduction at 2 and 5 h after injection of the tracer (20). Tumor uptake of [^{18}F]FHBG was 4-fold ($P = 0.05$) and 13-fold ($P = 0.001$) higher at 2 and 5 h, respectively, in tumors grown from transduced cells compared with control cells in individual animals. Bone was the only normal organ, with a high tracer uptake indicating defluorination. Transduced tumor/bone ratio of 2 stayed the same at 2 and 5 h. Transduced tumor/other normal tissue ratios were ranging from 5 to 25 at 2 h and 6 to 22 at 5 h. [^{18}F]FHBG was cleared rapidly from the blood with a biphasic decrease. About 0.04%ID/g was observed in the blood at 2 h.

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 (22). Dynamic PET was performed during the 2 h after injection of the tracer. A significant cardiac [^{124}I]FIAU accumulation of a 1.24-fold increase over the 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).

In another study, RG2 rat glioma tumor cells were transduced with HSV1-tk and transplanted subcutaneously in nude rats. 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 h and 24 h (23). Therefore, different cell types may handle reporter gene and reporter probe differently.

Wu et al. (24) observed that the expression of two genes (HSV1-sr39tk and $VEGF_{121}$) linked together had good correlations in cell assays *in vitro* and in rat infarcted myocardium imaging with [^{18}F]FHBG PET. The VEGF level in the myocardial tissue was confirmed by *ex vivo* measurements The induced VEGF expression did not improve myocardial contractility by echocardiography, perfusion by [^{13}N]ammonia imaging, and metabolism by [^{18}F]fluorodeoxyglucose imaging. However, new growth of capillaries and small blood vessels was observed

in the VEGF-expressing hearts. This demonstrated the feasibility of molecular imaging for monitoring angiogenic gene expression with PET reporter genes and probes.

Other Non-Primate Mammals

[PubMed]

Pigs were injected with similar titers of adenovirus expressing HSV1-sr39tk or control gene into the exposed myocardium, followed by [^{18}F]FHBG 2 days later. Dynamic PET was performed during the 2 h after injection of the tracer. There was a continuous increase of [^{18}F]FHBG uptake in areas infected with HSV1-sr39tk. At maximum, cardiac uptake was 2.64-fold greater than the control. About 19% of intact [^{18}F]FHBG remained in the plasma at 2 h, as measured by HPLC. Only one metabolite was detected and remained unknown (22).

Non-Human Primates

[PubMed]

Sequential, whole-body PET scans of two normal, cynomolgus monkeys were obtained over a period of 2 h after intravenous injection of 23-28 MBq/kg (0.62-0.76 mCi/kg) of [^{18}F]FHBG. Low uptake in bone was observed in the monkeys as compared with high bone uptake in the mice. The tracer was cleared rapidly in the monkeys. In the urine, 97% of recovered activity was intact [^{18}F]FHBG as compared to 69% in the plasma at 2 h after injection of the tracer (20).

Human Studies

[PubMed]

Human dosimetry was estimated in eight healthy volunteers by intravenous injection of 69-228 MBq (1.86-6.16 mCi) of [^{18}F]FHBG (25). The average effective dose is 0.0159 mSv/MBq (58.8 mrem/mCi). The organs receiving the highest absorbed doses were the urinary bladder (0.0942 mGy/MBq or 0.349 rad/mCi), kidneys (0.0511 mGy/MBq or 0.189 rad/mCi), gut (0.0457 mGy/MBq or 0.169 rad/mCi), and liver (0.0223 mGy/MBq or 0.0825 rad/mCi). In the urine, 83% of the total activity was intact [^{18}F]FHBG.

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