SPECT Imaging of Herpes Simplex Virus Type1 Thymidine Kinase Gene Expression By [^{123}I]FIAU[1]

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Rationale and Objectives. Introduction of suicide genes, such as herpes simplex virus type1 thymidine kinase (*HSV1-tk*), in tumor cells has provided a useful method for tumor gene therapy. Several L-nucleosides, such as Lamivudine (3TC) and Clevudine (L-FMAU), have been successfully tested as high-potency antiviral agents. To investigate the potential differences between D- and L-isomers of nucleosides, [$^{125/123}$I]-2'-fluoro-2'-deoxy-1β-D/L-arabino-furanosy-5-iodo-uracil (D/L-FIAU) have been synthesized and evaluated as potential SPECT agents for imaging *HSV1-tk* gene expression.

Materials and Methods. [$^{125/123}$I]D- and L-FIAU were prepared by iododestannylation of the respective tin precursors with $^{125/123}$I-sodium iodide. In vitro cell uptake studies were performed by incubation of [^{125}I]D- and L-FIAU in RG2 cells expressing *HSV1-tk* (RG2TK+). In vivo studies including biodistribution and SPECT were performed in RG2TK+ and RG2TK− tumor-bearing nude mice using [^{123}I]D- and L-FIAU.

Results. Cell uptake and biodistribution studies indicated that [$^{125/123}$I]L-FIAU did not show any high accumulation (sensitivity) or uptake ratios (selectivity) in HSV1-TK–positive (RG2TK+) tumors as compared to control tumors. In contrast, [$^{125/123}$I]D-FIAU displayed both sensitivity and selectivity to RG2TK+ tumors. The selective in vivo accumulation of [^{123}I]D-FIAU increased with time and the tumor uptake ratios (RG2TK+/RG2TK−) for 2, 4, and 24 hours averaged 6.2, 22.7, and 58.8, respectively. High-resolution SPECT of four nude tumor-bearing mice demonstrated a very high uptake of [^{123}I]D-FIAU in the RG2TK+ tumor, while no significant tracer accumulation was observed in the RG2TK− tumor and other organs.

Conclusion. The data suggest that only the D-isomer of [^{123}I]FIAU is useful for imaging *HSV1-tk* gene expression in mice by high-resolution SPECT imaging.

Key Words. D-FIAU; L-FIAU; *HSV1-tk*; SPECT; molecular imaging.

Salvage pathways are important cellular routes for recycling nucleosides as starting material for the intracellular synthesis of DNA or RNA. It is particularly important for viruses that may not have a full spectrum of enzymes for the de novo synthesis of nucleosides. Thymidine kinase (TK) is a key enzyme in this de novo pathway (TK denotes the enzyme, while *tk* refers to the gene). This rate-limiting enzyme catalyzes the monophosphorylation of thymidine to dTMP. The monophosphate, dTMP, is subsequently converted to diphosphate, dTDP, and triphosphate, dTTP. Ultimately, the triphosphate, dTTP, is a substrate for DNA polymerases. The dTTP is in a unique position in this metabolic pathway, because it is a substrate useful only for DNA synthesis and not for RNA synthesis. Human TK enzyme is one of the key enzymes controlling the rate of DNA synthesis. Many viruses also use this salvage pathway to replicate themselves, and

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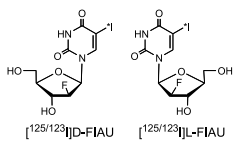
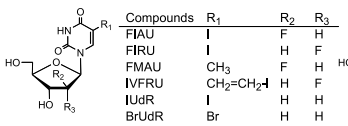
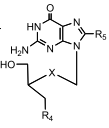
TABLE 1
Nucleosides Used to Study *HSV1-tk* Gene Expression





Compounds	R_1	R_2	R_3
FIAU	I	F	H
FIRU	I	H	F
FMAU	CH_3	F	H
IVFRU	CH_2=CH_2-I	H	F
IUdR	I	H	H
BrUdR	Br	H	H



Compounds	X	R_4	R_5
GCV	O	OH	H
PCV	CH_2	OH	H
FGCV	O	OH	F
FPCV	CH_2	OH	F
FHPG	O	F	H
FHBG	CH_2	F	H

more importantly, this pathway provides a rapid and direct link to DNA synthesis, which is critical for the survival of many viruses. Due to its pivotal role in virus survival, the viral TK enzymes are highly indiscriminating. Taking advantage of the differences in selectivity between human and viral TK enzymes, antiviral agents have been successfully developed. In this project, we propose to explore the differences in selectivity and develop labeled nucleosides for measuring HSV1-TK enzyme activity by in vivo imaging.

Four stereoisomers, β-D, α-D, β-L and α-L, exist for nucleosides. Among them, β-D nucleosides are the natural building blocks for DNA and RNA synthesis. In the past 10 years, there has been significant progress in using L-nucleosides as anti-viral agents (1). Herpes simplex virus type 1 (HSV1) thymidine kinase (ATP, thymidine 5'-phosphotransferase; EC 2.7.1.21) is the first rate-limiting step in the salvage pathway for synthesis of triphosphate nucleoside precursors for viral DNA synthesis. HSV1-TK enzyme has been shown to phosphorylate β-L-thymidine as well as β-D-thymidine with equal efficiency. The K_i of β-L thymidine, 2 μM is almost as potent as the K_m of the natural substrate, β-D-thymidine (K_m = 2.8 μM). However, in vitro enzyme assays showed that β-L-thymidine is not recognized by human TK. Therefore, L-nucleosides appear to be more efficacious against HSV1 as compared to mammalian cells. Similarly, both β-D and β-L isoforms of carbocyclic analogs of 5-iodo- and 5-(2-bromovinyl)-2'-deoxyuridine showed potent inhibition of HSV replication and high binding affinity for HSV1-TK enzyme (2–4). L-FMAU (Table 1) is a thymidine analog that has demonstrated a high potency in antiviral activity against hepatitis B virus (HBV) and Epstein Barr virus (EBV). L-FMAU, like the corresponding D-nucleoside, is phosphorylated by viral TK to the monophosphate, L-FMAU-MP. Subsequently, it is phosphorylated to the diphosphate and triphosphate by nucleoside monophosphate kinase and nucleoside diphosphate kinase, respectively. The antiviral activity resides with L-FMAU-TP, which is a potent inhibitor of the DNA polymerase of HBV and EBV (5,6). The selectivity on viral DNA polymerase confers the high potency toward the viruses while sparing normal tissues and organs from toxic side effects. Upon entry into the cell, monophosphorylation of the nucleoside by HSV1-TK is the only enzymatic reaction responsible for its intracellular trapping. The selective trapping of the nucleoside by HSV1-TK could potentially be translated into an imaging signal that reflects the presence of HSV1-TK (Table 1).

The rationale for testing the L-isomer of FIAU as a potential imaging agent for *HSV1-tk* gene expression is based on the suitability of L-nucleosides for HSV1-TK due to the lack of enantioselectivity of the enzyme, in addition to allowing for the safer imaging of gene delivery (7). It is our objective to exploit the differences in membrane transportation, biochemical and metabolic processes between human and viral enzymes for the unnatural L-nucleosides. As such, it may be possible to develop labeled L-nucleosides that selectively accumulate in tumor cells expressing the *HSV1-tk* gene.

In the past 20 years, 1-(2'-deoxy-2'-flouro-1'-β-arabinofuranosyl)-5-iodouracil (FIAU) has been tested as an experimental antiviral agent (8,9). Unfortunately, it has shown a high toxicity in humans that limits its use as a therapeutic agent (10). More recently, radiolabeled D-FIAU (^{124}I or ^{131}I) has been used as an imaging agent for positron emission tomography (PET) or single photon emission computed tomography (SPECT) imaging of *HSV1-tk* gene expression. The imaging approach is very attractive in measuring gene expression in vivo and this topic has been extensively reviewed recently (11–18). The approach originated from cancer gene therapy based on *HSV1-tk* gene expression coupled with acyclovir (ACV) or ganciclovir (GCV) treatment. Several nucleoside-based–labeled pyrimidine and purine ribosyl or acyclic nucleosides, including FIAU, FHPG, FHBG, etc. (see Table 1), have been reported as potential imaging agents for detecting the expression of *HSV1-tk* gene. Recent reports have suggested that D-FIAU has several unique features making it an attractive tracer for imaging *HSV1-tk* gene expression in tumor tissues (19–21). Indeed, [^{124}I]D-FIAU shows excellent uptake in xenograft HSV1-TK+ tumor tissue, and the tracer accumulation is severalfold higher than that of FHPG. The signal to noise ratio for FIAU is also superior to that of FHPG (20). However, the major disadvantage of using [^{124}I]D-FIAU as a probe of gene expression is its radionuclide properties: I-124 has a