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# Effects of structural differences between radioiodine-labeled 1-(2′-fluoro-2′-deoxy-D-arabinofuranosyl)-5-iodouracil (FIAU) and 1-(2′-fluoro-2′-deoxy-D-ribofuranosyl)-5-iodouracil (FIRU) on HSV1-TK reporter gene imaging

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ABSTRACT

The goal of this investigation was to evaluate the effects of structural differences between FIAU and FIRU on their ability to serve as a potential tracer for reporter gene imaging. To examine the characteristics of different configurations of FIAU and FIRU, a series of evaluations were done on HSV1-TK gene-expressing cells and on mice with HSV1-TK gene-expressing tumor. The results showed that, as an imaging agent for HSV1-TK-expressing cells, radiolabeled FIAU was more efficient for in vivo imaging than FIRU.



## 1. Introduction

Herpes simplex virus type 1 thymidine kinase (HSV1-TK) is a multifunctional protein that can phosphorylate pyrimidine and purine nucleoside analogs (Ahn et al., 2007b; Choi et al., 2005; Hsieh et al., 2006). In contrast, human cellular thymidine kinase, because of restricted changes in the sugar moiety (Choi et al., 2005; Hsieh et al., 2006), has a higher specificity, phosphorylating only pyrimidine substrate analogs. Generally, HSV1-TK exhibits higher activity with unnatural nucleoside analogs at the first two steps of phosphorylation than the cellular kinase, which is activated at the final step (Marquez et al., 2004, 2005; Tae et al., 2001). Recently, the reporter gene concept for molecular imaging has become a standard in various molecular biology protocols using the HSV1-TK gene (Blasberg and Tjuvajev, 1999; Gambhir et al., 1999; Haberkorn and Altmann, 2001; Serganova et al., 2007). HSV1-TK is the most widely used "reporter gene" for radiotracer-based molecular imaging using micro-positron emission tomography (microPET), single photon emission computed tomography (SPECT), and gamma camera. It has been also utilized as a suicide gene for gene therapy for cancer (He et al., 2008; Iyer et al., 2005; Serganova et al., 2007; Urbain, 2001; Wang et al., 2006). Small animal imaging of in vivo expression of HSV1-TK using the corresponding reporter probes provides valuable information for monitoring gene therapy of cancers (Iyer et al., 2005; Urbain, 2001; Wang et al., 2006). Several nucleosides, especially those with a 3′- or 2′-nucleoside substitution having a radiolabeled pyrimidine or purine, have been reported to be potent imaging agents for detecting the expression of the HSV1-TK gene (Ahn et al., 2007b; Alauddin et al., 2007; Miyagawa et al., 2008; Nanda et al., 2002; Tjuvajev et al., 2002). It is well known that the substitution of the sugar moiety at the 3′ or 2′ position with an electronegative fluorine will stabilize the C–N glycosidic bond of nucleosides (Toyohara et al., 2002).

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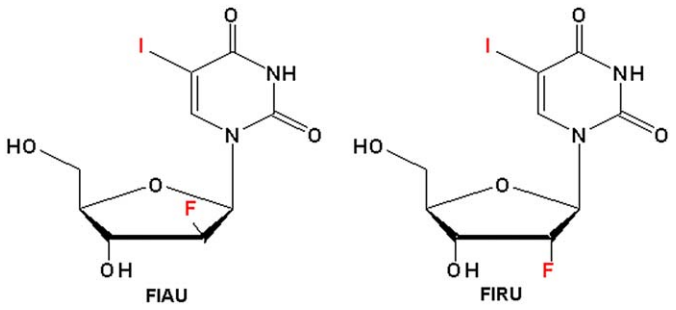


**Fig. 1.** The chemical structures of FIAU and FIRU, two substrates of the HSV1-TK enzyme.

2′-Fluoro-2′-deoxyuridine analogs were proved to be potent imaging agents (Choi et al., 2004; Miyagawa et al., 2008). Analogs of 2′-fluoro-2′-deoxyuridine are primarily phosphorylated by the HSV1-TK enzyme (Toyohara et al., 2002). Pyrimidine nucleoside analogs, such as radioiodine-labeled 1-(2′-fluoro-2′-deoxy-D-arabinofuranosyl)-5-iodouracil (FIAU), showed better uptake than purine analogs, such as 9-(4-[$^{18}$F]-fluoro-3-hydroxymethylbutyl)-guanine ([$^{18}$F]FHBG), for HSV1-TK gene expression imaging (Brust et al., 2001; Tjuvajev et al., 2002). In a number of studies, substitution of pyrimidine nucleoside analogs with fluorine at the 2′ and 3′ positions of deoxyribose has been studied as both therapeutic and imaging agents. Modifications within the sugar ring have been introduced to synthesize various nucleoside analogs such as FIAU and radioiodine-labeled 1-(2′-deoxy-2′-fluoro-D-ribofuranosyl)-5-iodouracil (FIRU) (Mercer et al., 1989; Wiebe et al., 1999). FIAU has a 2′-fluoro substituent in the arabino configuration while FIRU has a 2′-fluoro substituent in the ribo configuration (Fig. 1). According to previous studies, FIAU and FIRU are highly desirable substrates for the HSV1-TK enzymes (Nanda et al., 2002; Tjuvajev et al., 1998, 1999, 1996; Toyohara et al., 2002; Wiebe et al., 1999).

In this study, we evaluated the effectiveness of FIAU and FIRU as potential tracers for imaging HSV1-TK expression using microPET. Furthermore, the in vitro and in vivo biological characteristics of FIAU and FIRU, which have different configurations at the 2′-fluorine position, were compared.

## 2. Methods

1. *Preparation of radiolabeled nucleoside analogs*: The radioiodinated FIAU and FIRU were prepared using the iodination–destannylation method reported by Ahn et al. (2007a) with little modification. Carrier-free sodium [$^{124}$I]iodide was produced using the $^{125}$Te(p, 2n) $^{124}$I nuclear reactions on enriched $^{125}$TeO2 in MC-50 cyclotron at the Korea Institute of Radiological and Medical Sciences (KIRAMS, Seoul, Korea). [$^{131}$I]iodide was purchased from the Korea Atomic Energy Research Institute (KAERI, Daejeon, Korea). Na[$^{125}$I]I solution was obtained from PerkinElmer (Waltham, MA). The FIAU precursor was purchased from Future Chem (Seoul, Korea). [$^{124,125,131}$I]FIAU and [$^{124,125,131}$I]FIRU were prepared by electrophilic iodination reaction using their 5-tributylstannyl-precursors. Approximately 4.0 mg of the 5-tributylstannyl precursor was dissolved in 5.0 mL of MeOH. A 10 mCi (370 MBq) amount of the corresponding radiolabeled NaI in 0.01 N aqueous NaOH was added to the precursor solution (100 μL). The reaction mixture was acidified to pH 4–5 with 1.0 N aqueous HCl, and 30% $H_2O_2$ (50 μL) was added. After incubation for 30 min at ambient temperature, the reaction mixture was quenched with saturated $NaHSO_3$. The radioiodinated FIAU and FIRU were purified by high performance liquid chromatography (HPLC) separation using the following system; a Gilson 321 pump combined with Gastorr BG-14 in-line degasser, a Gilson ultraviolet/visible-151 (UV/VIS-151) detector (295 nm), and a Bioscan flow-count photomultiplier tube (PMT) radioactivity detector. The resulting products were loaded on a μ Bondapak$^{TM}$ C 18 column (3.9 mm × 300 mm) using Rhenodyne$^{TM}$ injector with 1.0 mL of sample loop and eluted at a flow rate of 1.0 mL/min. The initial mobile phase consisted of 10:90:0.1% acetonitrile/water/trifluoroacetic acid (TFA). This initial composition was kept for 15 min and the ratio of acetonitrile was increased up to 80% for next 5 min. The retention times of the radiolabeled compounds were determined using UV and radioactivity detectors (Raytest, Straubenhardt, Germany). The [$^{124,125,131}$I]FIAU and [$^{124,125,131}$I]FIRU fractions were collected and dried by nitrogen blowing on the heating block (40 °C). The radiochemical purity of each compound was determined by thin layer chromatography (solvent; dichloromethane–methanol 9:1 v/v). For cell uptake experiment, the product was reconstituted in 1 mL of Dulbecco's phosphate-buffered saline (DPBS), mixed with ascorbic acid (10 μL, 10 mg/mL) as a stabilizer against probable radiolysis, and filtered through a 0.22 μm syringe filter into a sterile glass vial.

2. *Cell lines and cultures*: The MCA-RH7777 (MCA, CRL1601) rat hepatoma cell line was purchased from the American Type Culture Collection (ATCC, Rockville, MD). Thymidine kinase (TK)-transduced MCA cells (MCA-TK) (Kwon et al., 2001) were kindly provided by Dr. Kwon, the Molecular Oncology Laboratory of KIRAMS. MCA and MCA-TK cells were grown in Dulbecco's modified eagle's medium (DMEM; Welgene, Seoul, Korea), supplemented with 20% horse serum (Gibco, Carlsbad, CA), 5% fetal bovine serum (FBS; JHR Biosciences, Lenexa, KS), and 1% penicillin–streptomycin (Gibco, Carlsbad, CA). The medium was changed twice or three times per week. The cells were cultured at 37 °C in a 5% $CO_2$ atmosphere. The MCA-TK cell line was selected in the presence of G418 (600 μg/mL; Gibco, Carlsbad, CA).

3. *Cytotoxicity and cell proliferation assays*: MCA and MCA-TK cells ($2 \times 10^3$ cells/50 μL) were seeded into 96-well plates. Ganciclovir (GCV, Sigma, St. Louis, MO), FIAU, or FIRU was added to each well (0–10 mM/0.1 mL). After incubation for 5 days at 37 °C in a 5% $CO_2$ atmosphere, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS, Promega, Madison, WI) was added to each well (20 μL/well). After an additional 2 h incubation, proliferation of the treated cells was quantified by measuring the absorbance of the culture media at 492 nm with a 96-well plate reader (GENios, TECAN Co., Boston, MA). The cytotoxicities of FIAU and FIRU were compared. GCV was used as a standard reference nucleoside analog. For studies of the viability of cells in GCV, FIAU, or FIRU containing solutions, the nucleoside analogs were solubilized in dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO) to a final concentration of 5%. The nucleoside analog solutions were diluted with cell culture media to reach a final DMSO concentration of 5%. The trypan blue dye (Sigma, St. Louis, MO) exclusion assay was used in conjunction with an examination of cellular density using a hemocytometer. All experiments were performed in triplicates.

4. *Cellular uptakes of radioactive iodine-labeled FIAU and FIRU*: MCA and MCA-TK cells were plated in 6-well plates at a density of $1 \times 10^6$ cells/well and incubated at 37 °C for 24 h. When 80–100% confluence was reached, each well was incubated with [$^{125}$I]FIAU and [$^{125}$I]FIRU (1 μCi/2 mL) for 0.5, 1, 2, 4 h at 37 °C in 5% $CO_2$ atmosphere. The cells were rinsed with DPBS without $Mg^{2+}$ and $Ca^{2+}$ cations (Welgene, Seoul, Korea), and lysed with ice-cooled 0.5 M perchloric acid (Sigma, St. Louis, MO). The lysate was incubated on ice for 30 min, vortexed and centrifuged at 15,000 rpm for 5 min at room temperature. The pellet was washed twice with ice-cooled 0.5 M perchloric acid, centrifuged at 15,000 rpm for 5 min. Subsequently, the radioactivity was counted