Synthesis and Cellular Uptake of Radioiodine labeled 2'-deoxyuridine derivatives with HSV1-*TK*.

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1. Introduction

Several different radiolabeled probes have been developed to image Herpes Simplex Virus Type-1 thyrimidine kinase gene (HSV1-*TK*) expression. The nucleoside prodrugs under investigation for HSV1-*TK* imaging generally fall into two main chemical and radioisotope categories: the pyrimidine nucleosides, primarily radioiodinated, and the purin nucleosides, primarily radiofluorinated, and their respective analogues. In non-invasive imaging of the HSV1-*TK* system, many nucleoside derivatives have been recommended as HSV1-*TK* substrates. Most of these nucleoside derivatives have been developed as prodrugs for tumor proliferation imaging or as anti-viral drugs. For example, 5-fluorouracil (5-FU) and IUdR have been used as tumor agents and acyclovir (ACV), ganciclovir (GCV) and (E)-5-(2-bromovinyl)-2'- deoxyuridine (BVDU) as an anti-viral agents for virus infection several 5-substituted uracil nucleoside derivatives have been identified to have high sensitivity and selective accumulation in HSV1-*TK* expressing cells Of those, IVDU was shown to be rapidly accumulated in HSV1-*TK* expressing cells in vitro. Imaging of the HSV1-*TK* reporter gene along with various reporter probes is of current interest. In contrast to the mammalian kinase, which phosphorylates thymidine preferentially, HSV1-tk is less discriminative and phosphorylates a wide range of nucleoside analogues such as acycloguanosines and 2'-fluoro-2'-deoxyuridine derivatives that are not phosphorylated efficiently by the native enzyme. More specifically, 5-substituted 2'-fluoro-2'-deoxy-arabinofuranosyluracil nucleosides are efficiently phosphorylated by HSV- *TK*. This property, together with the presence of fluorine in the 2'-arabino-position, endows the 2'-fluoro-2'-deoxyuridines with antiviral activity against HSV.

2. Methods and Results

2.1. Synthesis and Radioiodination

5-Iodo-2'-deoxyuridine was purchased from the Aldrich Chemical Co. 5-Iodo-2'-fluoro-2'-deoxyuridine were prepared using literature procedures [2]. (E)-2-(Tributylstannyl)-1-(trimethylsilyl)ethene was prepared as previously described [5].

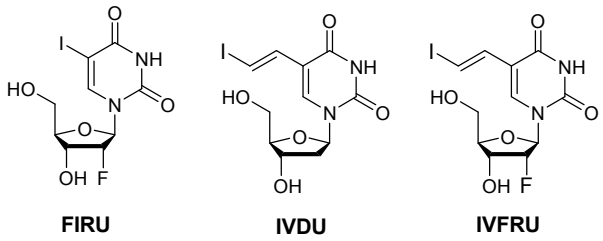

Figure 1. Structures of 5-iodo- and 5-(2-iodovinyl)-2'-deoxy-pyrimidine nucleosides.

Radioiodinated FIRU, IVDU and IVFRU were synthesized by the method of Morin et al. (1997), with minor modifications. Structures of intermediate compounds in each synthetic step were confirmed by 300 MHz ^{1}H-NMR proton nuclear magnetic resonance. Briefly, Na^{125}I (370 MBq; 30 μl) was placed in a reaction vial and ICl (0.154 mmol; 20 μl in 20% acetic acid) in acetonitrile were added to the above as carrier. Carrier-free radioiodinated IVDU, FIRU and IVFRU were also prepared by iododestannylation using Na^{125}I and H_2O_2 oxidizing agent. Subsequently, 5-(2-trimethylsilylvinyl)-2'-deoxyuridine ($SiMe_3$-VDU) or 5-Tributyl-stannyl-2'-fluoro-2-deoxyuridine ($SnBu_3$-FDU) or (Z)-5-(2'-trimethyl-silylvinyl)-2'-fluoro deoxyuridine ($SiMe_3$-VFRU) was added. The reaction was allowed to proceed for 15 min at 25 ℃.

2.2 Analysis and purification

After the reaction was over, the final product was purified by reverse phase HPLC on mBondapak C18 column (3.9 × 300 mm, Waters, USA), using gradient elution with distilled water (D.W.) and acetonitrile. The concentration of acetonitrile was increased from 10% to 40% over a period of 25 min at a flow rate of 1.5 ml/min: 10% acetonitrile isocratic elution for 1-5 min, 40% acetonitrile gradient elution for 6-10 min, and 40% acetonitrile isocratic elution for 11-25 min. Retention time of radiolabeled compounds was determined from UV and radioactivity detector (Raytest, Germany) data.

2.3 Cellular uptake

The MCA cell line is a MCA RH7777 hepatoma cell line, and MCA-*TK* cells are a cell line derived from HSV1-*TK* expressind cells using aretroviral vector; both cell lines were gifts from Dr. Kwon of Molecular Oncology Laboratory.

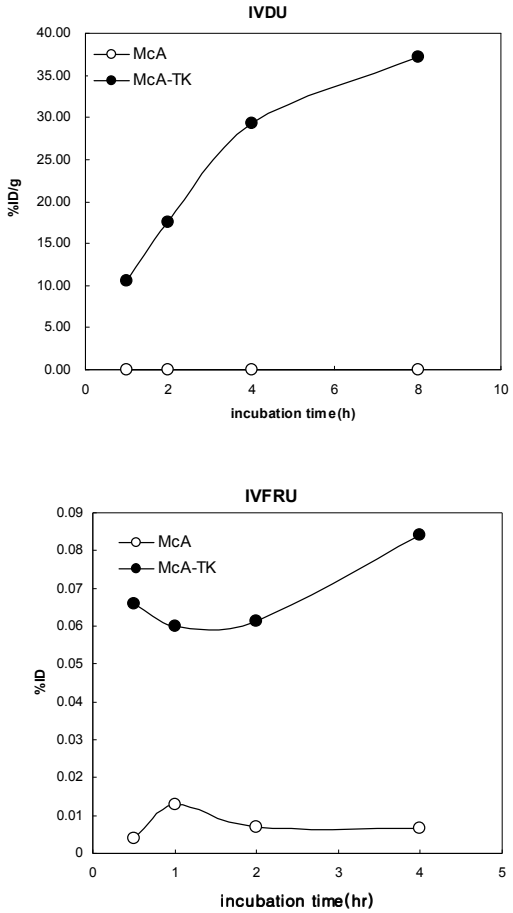

Figure 2. In vitro cellular uptake of no-carrier-added [^{125}I]IVDU and [^{125}I](Z)-IVFRU in MCA-TK and MCA cells.

Cells were grown to 2×10^5 cells/well in 6-well cultureplates and incubated 37 ℃ for 24 hr. [^{125}I]FIRU, [^{125}I]IVDU and [^{125}I]IVFRU were added to each well (1 μCi/2 ml). Thus the above mixture were incubated for 15, 30, 60, 120, 240, and 480 min at room temperature. The media were removed, the cells rinsed with PBS, and adherent cells were then lysed with 0.5 M perchloric acid (0.5 ml). The lysate was placed on ice for 30 min, vortex mixed and centifuged at 1500 rpm for 5 min in an Eppendorf microfuge. The supernatants were stored and the pellet washed with 0.5 M perchloric acid and again centrifuged 1500 rpm for 5 min. The radioactivity in the acid-insoluble fraction (nucleic acids and proteins) and acid-soluble fractions were determinded by gamma counting.

3. Conclusion

The objective of this study was to identify a suitable radiolabeled probe which could prove useful as a marker for cells transduced or transfected with the HSV1-*TK* gene during in vivo gene therapy. All of the compounds evaluated displayed very low uptake in KBALB cells that lack the HSV1-*TK* gene. In HSV1-*TK* gene-transduced tumor cells (KBALB-STK), the order of cellular uptake is [^{125}I]IVDU > [^{125}I]IVFRU > [^{125}I]FIRU.

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