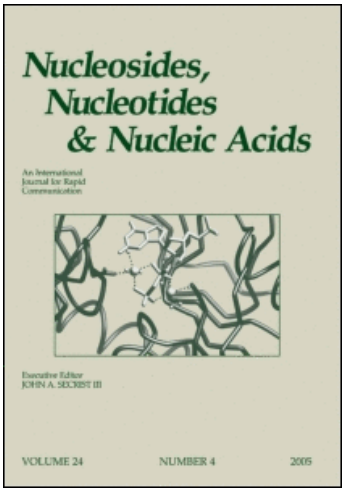

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Radiolabelled Pyrimidine Nucleosides to Monitor the Expression of HSV-1 Thymidine Kinase in Gene Therapy

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RADIOLABELLED PYRIMIDINE NUCLEOSIDES TO MONITOR THE EXPRESSION OF HSV-1 THYMIDINE KINASE IN GENE THERAPY

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ABSTRACT: Selective radiolabelling and imaging of transduced *HSV tk* expressing cells was studied using [^{123}I]IVFRU, [^{125}I]FIRU and [^{125}I]FIAU. Although all three radionucleosides accumulated in the KBALB-STK transduced murine tumour line *in vitro* and *in vivo*, [^{125}I]FIRU provided optimal performance in terms of selectivity for *HSV tk* expressing cells and % of injected dose accumulating in the tumor.

In vivo transfer of the herpes simplex virus type-1 thymidine kinase (*HSV tk*) gene and subsequent administration of antiviral drugs such as ganciclovir has emerged as a promising gene therapy protocol for proliferative disorders. The detection of *HSV tk* expression using non-invasive gamma scintigraphy is now reported.

In vitro uptake of radioiodinated (*E*)-5-(2-iodovinyl)-2'-fluoro-2'-deoxyuridine (IVFRU), 5-iodo-2'-fluoro-2'-deoxyuridine (FIRU) and 5-iodo-2'-fluoro-2'-arabinouridine (FIAU) was determined in the KBALB and KBALB-STK cells (transduced with a retroviral vector possessing the *HSV tk* gene [1]). [^{123}I]IVFRU, [^{125}I]FIRU or [^{125}I]FIAU (40 pmol; sp. Act. 63 GBq/mmol) was added, incubated at 37 °C, and the adherent cells were lysed (0.25 to 8 h) for radiometry (acid-insoluble and acid-soluble fractions).

KBALB-STK and KBALB cells were also grown as solid tumors in the flanks of male Balb/c mice. When the tumors reached approximately 700 mm^3, mice (n = 4) were dosed (i.v.) with [^{123}I]IVFRU, [^{125}I]FIRU or [^{125}I]FIAU (185 kBq, sp. act. 133 GBq/mmol). Mice were sacrificed after 8 h, and tissue radioactivity was determined upon necropsy. *In vivo* scintigraphic images ([^{131}I]FIRU; 3.7 MBq, sp. act. 118 GBq/mmol) were acquired [2].

Total cellular uptake of [^{123}I]IVFRU and [^{125}I]FIAU in *HSV tk*-expressing cells comprised both cytosolic and nucleic acid components (acid insoluble fraction). This study

provided evidence that IVFRU and FIAU are incorporated into the DNA of proliferating KBALB-STK cells, since more than 50% of the radioactivity was present in the acid-insoluble fraction of cell lysates. In contrast, FIRU was not incorporated into the acid-insoluble fraction, implying that incorporation into DNA was not the basis for its for metabolic entrapment. Uptake of IVFRU and FIRU in non-transduced KBALB cells was negligible *in vitro*, but uptake of FIAU was ten times higher (2 pmol/10^5 cells) than IVFRU or FIRU after incubation (40 pmol) for 8 h, indicating that FIAU is less selective than IVFRU or FIRU. Incorporation of FIAU in the acid-insoluble component of KBALB-STK lysates was also greater (20 pmol/10^5 cells) than for either IVFRU or FIRU.

Biodistribution studies in Balb/c mice bearing subcutaneous KBALB or KBALB-STK tumors demonstrated that the *HSV tk*-expressing tumors selectively accumulate radiolabelled IVFRU, FIRU and FIAU *in vivo*. Blood radioactivity was the highest in animals bearing KBALB tumors; this, together with low concentrations of radioactivity in the KBALB tumors and other organs/tissues, produced low tumor:blood and tissue:blood ratios. In contrast, the KBALB-STK tumors accumulated more radioactivity and had lower blood radioactivity, thereby providing increased tumor:blood ratios compared to animals bearing KBALB tumors. Low uptake of radioactivity by non-target tissues, and favorable tumor:blood ratios suggested that the KBALB-STK tumors could be scintigraphically imaged with these radiolabelled nucleosides, as shown in previous studies of *HSV tk* expression in transduced tumors in rats. [3,4] High uptake of [^{131}I]FIRU (14% of the injected dose/g of tumor) by KBALB-STK tumors *in vivo*, high uptake *in vitro* and high *in vitro* specificity for *HSV tk*-expressing cells make [^{131}I]FIRU the superior imaging *HSV tk* imaging agent of the three radionucleosides in this study.

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