Radiolabeled 2′-fluorodeoxyuracil-β-D-arabinofuranoside (FAU) and 2′-fluoro-5-methyldeoxyuracil-β-D-arabinofuranoside (FMAU) as tumor-imaging agents in mice

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Abstract Purpose: The purpose of the present study was to evaluate, in conjunction with the National Cancer Institute, the feasibility of using two thymidine analogs, 2′-fluorodeoxyuracil-β-D-arabinofuranoside (FAU, NSC-678515) and 2′-fluoro-5-methyldeoxyuracil-β-D-arabinofuranoside (FMAU, NSC-678516), as 18-fluorine-labeled positron emission tomography (PET) imaging agents. Methods: The in vivo distribution and DNA incorporation of [2-14C]FAU, [2-14C]FMAU, and [2-14C]thymidine (as a control) were studied in SCID mice bearing human xenografts of T-cell leukemia CCRF-CEM. Levels of drug-associated radioactivity in blood, tumor and normal tissues including liver, kidneys, heart, lungs, spleen, brain, and skeletal muscle were determined. Results: At 1 h after dosing, radioactivity from all three compounds was distributed in a generally nonspecific manner, except that spleen and tumor tissue had relatively high concentrations of radioactivity from [14C]thymidine. At 4 h after dosing, the concentrations of radioactivity from [14C]thymidine and [14C]FMAU were relatively high in spleen and tumor tissue, and that from [14C]FAU was highest in tumor tissue. The tumor/skeletal muscle concentration ratios were 2.25±0.69 and 3.07±0.42 for [14C]FAU and [14C]FMAU, respectively. At 24 h after dosing, only spleen and tumor tissues contained appreciable amounts of radioactivity from either compound. In tumor tissue, the levels of radioactivity from [18F]FMAU were two- to threefold greater than those from [14C]thymidine or [14C]FAU. Examination of purified genomic DNA from tumor, liver, kidneys, brain, and skeletal muscle showed that, at 24 h after dosing, only DNA from tumor tissue contained appreciable concentrations of radioactivity. Radioactivity from [18F]FMAU in tumor DNA was 45% greater than that from [14C]thymidine and about threefold greater than that from [13C]FAU. Conclusions: The extent of accumulation of [18F]FMAU in tumor tissue and incorporation into tumor DNA indicate that [18F]FMAU could be useful as a functional PET tumor-imaging agent.

Keywords FAU · FMAU · Positron emission tomography · Functional imaging agent · Human tumor xenograft

Introduction There is considerable interest in the development of imaging agents for the direct measurement of tumor proliferation. A positron emission tomography (PET) radiotracer capable of assessing the functional status of a tumor would be useful in the evaluation of tumor progression, and the effectiveness of chemotherapeutic agents, and other therapeutic approaches including both cytotoxic and cytostatic agents. At present, however, there is a lack of a suitable clinical probe to assess tumor-specific antiproliferative effects. The model compound for several suggested agents has been thymidine since it is readily phosphorylated and incorporated into DNA, and, in its radiolabeled form, can provide information on the proliferative status of tumor cells. [3H]Thymidine has been used as an imaging agent in experimental animals [4, 15, 18]. Further, [2-11C]thymidine and [methyl-11C]thymidine have been similarly evaluated in clinical studies [4, 6, 19, 29], but for this...
purpose the utility of thymidine is limited due to its rapid catabolism and the 20-min half-life of the radionuclide [4]. Efforts are now underway to develop, for use as PET radiotracers, radiolabeled analogs of thymidine that have longer radionuclide half-lives, are resistant to enzymatic degradation, and are incorporated into DNA with higher specificity and affinity. Two such analogs are $^{18}$F-labeled 2'-deoxyuridine (FAU) and 2'-fluoro-5-methyluracil (FMAU)

Both FAU and FMAU labeled with $^{18}$F (which has a radionuclide half-life of 110 min) appear to have many of the desirable physical characteristics of a functional probe for tumor proliferation, and their use could eventually eliminate the need for an on-site cyclotron facility. A radiosynthesis method is available for $[^{18}F]$FAU and is readily adaptable to $[^{18}F]$FMAU [28]. Although these thymidine analogs are similar in structure, differences in their biochemical pathways might be utilized to effect differentially cells that express thymidylate synthase (TS). Colorectal or breast cancer, tumors with high levels of TS, are generally difficult to treat, resulting in a poor prognosis for these patients [13, 21]. TS levels correlate with the conversion rates of uridine analogs to methylated nucleotides [3]. FAU strongly inhibits the growth of tumor cells with high TS activity [3]. This compound may be toxic specifically to such cells, since its nucleotide derivative, FAUMP, can be readily methylated to FMAUMP [14]. After further phosphorylation, the FMAU moiety is incorporated into DNA, and cell replication is affected. In contrast, incorporation of administered FMAU, which contains the appropriate methyl group added by TS, is not related to the levels of TS. Such incorporation, nevertheless, reflects cellular proliferation.

The present work, involving intravenous administration of $[^{14}$C]FAU, $[^{14}$C]FMAU, or $[^{14}$C]thymidine in tumor-bearing SCID mice, was intended to evaluate the potential of $^{18}$F-radiolabeled FAU and FMAU as clinical PET imaging agents. The CCRF-CEM cell line was chosen because of its relatively high expression of TS (β-tubulin ratio of 0.118) (Rustum Y, Roswell Park Cancer Institute, Buffalo, N.Y., and Alley M, NCI-FCRDC, Frederick, Md.; personal communication) and the capacity of these cells to form solid xenograft tumors in mice [11]. Based on differences in metabolism and circulating levels of pyrimidines in rodents and humans [20], a thymidine-restricted diet was selected. The emphasis on incorporating concepts of tumor specificity and developing functional imaging agents is relevant to the future diagnostic and prognostic evaluation of cancer patients.

Materials and methods

Cells, animals, and materials

CCRF-CEM cells were obtained from the Southern Research Institute (Birmingham, Ala.) and the SCID mice were supplied by the National Cancer Institute. The protocol for animal use and care was approved by the Institutional Animal Use and Care Committee of the University of Alabama at Birmingham and adhered to the “Principles of Laboratory Animal Care” (NIH publication 85-23, revised 1985). Matrigel was purchased from Becton Dickinson (Franklin Lakes, N.J.). $[^{14}$C]Thymidine (53 mCi/mmol), $[^{18}$F]FAU (54 mCi/mmol), and $[^{18}$F]FMAU (52 mCi/mmol) were purchased from Moravek Biochemicals (Brea, Calif.).

Determination of purities of radiolabeled FAU, FMAU and thymidine

For the determination of the purities, an HP 1050 computer-aided high-performance liquid chromatography (HPLC) system was used with a C18 reversed-phase Hypersil ODS 5-μm column (250x5 mm). For analysis of $[^{14}$C]thymidine, the mobile phase was water/acetonitrile (96:4 v/v), and for analysis of $[^{14}$C]FAU and $[^{14}$C]FMAU, the mobile phase was a mixture of 4 mM NH$_4$H$_2$PO$_4$ + 4 mM (NH$_4$)$_2$HPO$_4$ (solution A) and acetonitrile (solution B), B remaining at 0% for the first 5 min, increasing from 0 to 50% between 5 and 35 min, and remaining at 50% for 5 min. The flow rate was 1 ml/min. At 1-min intervals, chromatographic fractions were collected, and radioactivity was determined by liquid scintillation counting in a Beckman spectrometer equipped with an external standard. The radioactive purities of these compounds were 99.3 ± 0.3% for $[^{14}$C]thymidine, 99.6 ± 0.7% for $[^{14}$C]FAU, and 99.3 ± 0.7% for $[^{14}$C]FMAU.

Xenograft model

SCID mice were inoculated subcutaneously with 2×10$^7$ CCRF-CEM cells in a preparation of medium/Matrigel (2:1). The “take” rate 20 days later was about 70%, and mice with tumors were placed on a thymidine-deficient diet (Harlan, TD 94048). They were maintained on this diet for 1 week prior to dosing. The average tumor mass at the start of the study was 750 mg.

Dosing and sample collection

$[^{14}$C]FAU (in 25% ethanol), $[^{14}$C]FMAU (in 25% ethanol), and $[^{14}$C]thymidine (in sterile water) were administered to tumor-bearing SCID mice (23-29 g body weight) as an intravenous bolus injection via a tail vein. Doses (5 μl/g) were 0.74 mg/kg (0.16 μCi/g) for $[^{14}$C]FAU, 1.48 mg/kg (0.29 μCi/g) for $[^{18}$F]FAU, and 1.56 mg/kg (0.34 μCi/g) for $[^{18}$F]FMAU. At 1, 4, and 24 h after dosing, four mice in each group were killed for sample collection, except in the group dosed with $[^{14}$C]thymidine, in which only three mice were killed at the 24-h time-point.

Tissues collected at the specified time-points were blood, liver, kidneys, heart, lungs, spleen, brain, skeletal muscle, and tumor. Solid tissues were lightly blotted on filter paper. Tumors, livers, brain, skeletal muscle, and kidneys were divided into two approximately equal parts. The volume of blood was measured, and the samples of solid tissues were weighed. Tissue samples intended for radioactivity counting were homogenized in five volumes of 0.9% saline. The remaining samples were stored at −80°C for analysis of radioactivity incorporated into DNA.

Sample analysis

The radioactivity was determined in triplicate portions of blood (after clarification with H$_2$O$_2$ and tissue homogenates in a liquid scintillation spectrometer equipped with an appropriate set of external standards. The procedure was essentially the same as that previously reported [30]. The radioactivity in the DNA of the tissues (liver, kidneys, brain, skeletal muscle, and tumor) was also determined. The procedure, a modification of a published protocol [7, 10, 27], involved tissue digestion in a buffer containing...