Cancer Chemother Pharmacol (2002) 49: 419–424
DOI 10.1007/s00280-002-0433-7

ORIGINAL ARTICLE

**Hui Wang · Patsy Oliver · Li Nan · Shuyi Wang
Zhi Wang · Julie K. Rhie · Ruiwen Zhang
Donald L. Hill**

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

Received: 10 September 2001 / Accepted: 4 January 2002 / Published online: 8 March 2002


**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-$^{14}$C]FAU, [2-$^{14}$C]FMAU, and [2-$^{14}$C]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 [$^{14}$C]thymidine. At 4 h after dosing, the concentrations of radioactivity from [$^{14}$C]thymidine and [$^{14}$C]FMAU were relatively high in spleen and tumor tissue, and that from [$^{14}$C]FAU was highest in tumor tissue. The tumor/skeletal muscle concentration ratios were $2.25 \pm 0.69$ and $3.07 \pm 0.42$ for [$^{14}$C]FAU and [$^{14}$C]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 [$^{14}$C]FMAU were two- to threefold greater than those from [$^{14}$C]thymidine or [$^{14}$C]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 [$^{14}$C]FMAU in tumor DNA was 45% greater than that from [$^{14}$C]thymidine and about threefold greater than that from [$^{14}$C]FAU. *Conclusions:* The extent of accumulation of [$^{14}$C]FMAU in tumor tissue and incorporation into tumor DNA indicate that [$^{18}$F]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. [$^{3}$H]Thymidine has been used as an imaging agent in experimental animals [4, 15, 18]. Further, [2-$^{11}$C]thymidine and [*methyl*-$^{11}$C]thymidine have been similarly evaluated in clinical studies [4, 6, 19, 29], but for this

This work was presented in part at the 92nd Annual Meeting of the American Association for Cancer Research, New Orleans, 24–28 March 2001.

This work was supported by NIH, NCI contract N01-CM-07111.

H. Wang · P. Oliver · L. Nan · S. Wang · Z. Wang
R. Zhang · D.L. Hill (✉)
Department of Pharmacology and Toxicology,
University of Alabama, Birmingham, AL 35294, USA
E-mail: Don.Hill@ccc.uab.edu
Tel.: +1-205-9347479
Fax: +1-205-9755082

J.K. Rhie
National Cancer Institute, 6130 Executive Blvd.,
Room 8042, Rockville, MD 20852, USA

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′-fluorodeoxyuracil-$\beta$-D-arabinofuranoside (FAU) and 2′-fluoro-5-methyldeoxyuracil-$\beta$-D-arabinofuranoside (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 specifically toxic 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 ($\beta$-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.). [2-$^{14}$C]Thymidine (53 mCi/mmol), [2-$^{14}$C]FAU (54 mCi/mmol), and [2-$^{14}$C]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 $C_{18}$ reversed-phase Hypersil ODS 5-μm column (250×5 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 m*M* $NH_4H_2PO_4$ + 4 m*M* $(NH_4)_2HPO_4$ (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 $2\times10^7$ 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 [$^{14}$C]FMAU, and 1.56 mg/kg (0.34 μCi/g) for [$^{14}$C]thymidine. 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 $H_2O_2$) 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

proteinase K, extraction of DNA with phenol/chloroform/isoamyl alcohol (25:24:1 v/v/v), and precipitation of DNA with 7.5 *M* ammonium acetate and 100% ethanol. The purity of the isolated DNA was calculated from the ratio $OD_{260}/OD_{280}$. The concentration of DNA was determined from the absorption at $OD_{260}$.

## Results

### Tissue distribution

The radioactivity in tissues (blood, liver, kidney, heart, lung, spleen, brain, skeletal muscle, and tumor) was measured and expressed as a percentage of the injected dose per gram of tissue (%ID/g). At 1 h after dosing, spleen and tumor contained the highest concentrations of radioactivity derived from [$^{14}$C]thymidine (Table 1). The content in spleen was about four times greater than that in tumor. The tumor/skeletal muscle ratio was 9.46 ± 8.18. In the remaining tissues sampled, except for blood, the concentrations of [$^{14}$C]FMAU-derived radioactivity were greater than those derived from either [$^{14}$C]thymidine or [$^{14}$C]FAU. The blood concentrations of [$^{14}$C]FAU and [$^{14}$C]FMAU were calculated to be 1.5 and 3.0 μ*M*, respectively. An average uptake of 7.22 ± 0.66%ID/g in tumor was found for [$^{14}$C]FMAU versus 3.38 ± 0.38 and 2.06 ± 1.22%ID/g for [$^{14}$C]FAU and [$^{14}$C]thymidine, respectively. Overall, at 1 h after dosing, both [$^{14}$C]FAU and [$^{14}$C]FMAU demonstrated substantial uptake into various tissues, although in a generally nonspecific manner.

At 4 h after dosing, [$^{14}$C]FAU and [$^{14}$C]FMAU presented a considerably different tissue distribution profile than at 1 h (Table 2). The concentrations of radioactivity from [$^{14}$C]FAU were highest in the tumor tissue and greatest from [$^{14}$C]FMAU in tumor and spleen. The ratio of the concentration in tumor tissue to that in smooth muscle for [$^{14}$C]FMAU was 3.07 ± 0.42. The greatest accumulation of radioactivity in tumor was observed with [$^{14}$C]FMAU and was 2.5-fold greater than that associated with [$^{14}$C]thymidine or [$^{14}$C]FAU. The distribution of [$^{14}$C]thymidine remained consistent with the earlier profile.

At 24 h after dosing (Table 3), substantial amounts of radioactivity were present only in spleen and tumor for all the three $^{14}$C-labeled compounds. In blood, the concentrations of [$^{14}$C]FAU and [$^{14}$C]FMAU were only 0.02 and 0.03%ID/g (25 and 30 n*M*), respectively. Between 1 and 24 h, the radioactivity concentrations in tumor from [$^{14}$C]FAU decreased by 48%, whereas the levels from [$^{14}$C]FMAU declined by less than 20%, and those from [$^{14}$C]thymidine did not change substantially. The tumor/skeletal muscle ratio increased with time for all the compounds, but most dramatically for [$^{14}$C]FMAU. The ratios for [$^{14}$C]thymidine were 9.46 ± 8.18 at 1 h and 54.5 ± 10.4 at 24 h after dosing. The ratios for [$^{14}$C]FAU and [$^{14}$C]FMAU increased from 1.25 ± 0.21 and 1.25 ± 0.30 at 1 h to 24.0 ± 14.1 and 41.6 ± 9.5, respectively, at 24 h, indicating accumulation of these compounds in tumor tissue.

**Table 1.** Tissue distribution of radioactivity in SCID mice at 1 h after dosing with [$^{14}$C]thymidine, [$^{14}$C]FMAU, or [$^{14}$C]FAU. Values are means ± SD and (except for the ratios) are percent of injected dose per gram of tissue

| Tissue | [$^{14}$C]thymidine | [$^{14}$C]FMAU | [$^{14}$C]FAU |
|---|---|---|---|
| Blood | 0.17 ± 0.05 | 1.95 ± 0.19 | 1.79 ± 0.27 |
| Liver | 0.58 ± 0.06 | 6.35 ± 0.38 | 2.72 ± 0.54 |
| Kidney | 0.48 ± 0.09 | 8.22 ± 0.46 | 3.47 ± 0.91 |
| Heart | 0.44 ± 0.06 | 5.34 ± 1.21 | 2.69 ± 0.59 |
| Lung | 0.44 ± 0.10 | 5.18 ± 0.74 | 1.79 ± 0.88 |
| Spleen | 8.10 ± 0.15 | 6.14 ± 1.34 | 3.26 ± 0.84 |
| Brain | 0.32 ± 0.03 | 4.39 ± 0.73 | 2.16 ± 0.46 |
| Tumor | 2.06 ± 1.22 | 7.22 ± 0.66 | 3.38 ± 0.38 |
| Skeletal muscle | 0.26 ± 0.10 | 6.08 ± 1.91 | 2.75 ± 0.49 |
| Tumor/muscle ratio[a] | 9.46 ± 8.18 | 1.25 ± 0.30 | 1.25 ± 0.21 |

[a]Calculated by dividing, for each animal, the tumor concentration by the muscle concentration and then determining the mean and standard deviation

**Table 2.** Tissue distribution of radioactivity in SCID mice at 4 h after dosing with [$^{14}$C]thymidine, [$^{14}$C]FMAU, or [$^{14}$C]FAU. Values are means ± SD and (except for the ratios) are percent of injected dose per gram of tissue

| Tissue | [$^{14}$C]thymidine | [$^{14}$C]FMAU | [$^{14}$C]FAU |
|---|---|---|---|
| Blood | 0.05 ± 0.01 | 0.54 ± 0.15 | 0.68 ± 0.46 |
| Liver | 0.37 ± 0.05 | 2.24 ± 0.27 | 1.12 ± 0.75 |
| Kidney | 0.18 ± 0.02 | 2.62 ± 0.42 | 1.68 ± 1.12 |
| Heart | 0.12 ± 0.01 | 1.85 ± 0.20 | 1.31 ± 0.91 |
| Lung | 0.28 ± 0.08 | 1.57 ± 0.62 | 1.08 ± 0.60 |
| Spleen | 12.6 ± 3.26 | 7.18 ± 1.38 | 1.19 ± 0.66 |
| Brain | 0.09 ± 0.00 | 1.25 ± 0.43 | 0.87 ± 0.50 |
| Tumor | 2.46 ± 0.60 | 6.12 ± 0.93 | 2.46 ± 0.85 |
| Skeletal muscle | 0.22 ± 0.10 | 2.02 ± 0.63 | 1.21 ± 0.71 |
| Tumor/muscle ratio[a] | 13.2 ± 7.4 | 3.07 ± 0.42 | 2.25 ± 0.69 |

[a]Calculated by dividing, for each animal, the tumor concentration by the muscle concentration and then determining the mean and standard deviation

### Radioactivity in genomic DNA

Of the five tissues from which DNA was extracted, only DNA from tumor tissue contained appreciable amounts of radioactivity derived from [$^{14}$C]FAU, [$^{14}$C]FMAU, or [$^{14}$C]thymidine at 1, 4, and 24 h after dosing (Table 4). Calculated as percentage of the injected dose per mg of DNA (%ID/mg DNA), the amounts of radioactivity in liver, kidneys, brain, and skeletal muscle were relatively small, although detectable. At 1 h after dosing, the radioactivity concentrations from both [$^{14}$C]FAU and [$^{14}$C]FMAU in tumor DNA was about one-fourth that from [$^{14}$C]thymidine. At 4 h after dosing, the radioactivity concentration in DNA from [$^{14}$C]FMAU was equivalent to that from [$^{14}$C]thymidine and about threefold greater than that from [$^{14}$C]FAU. At 24 h after dosing, the radioactivity concentration in tumor DNA from [$^{14}$C]FMAU was about 45% greater than that

**Table 3.** Tissue distribution of radioactivity in SCID mice at 24 h after dosing with [$^{14}$C]thymidine, [$^{14}$C]FMAU, or [$^{14}$C]FAU. Values are means ± SD and (except for the ratios) are percent of injected dose per gram of tissue

| Tissue | [$^{14}$C]thymidine | [$^{14}$C]FMAU | [$^{14}$C]FAU |
|---|---|---|---|
| Blood | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.00 |
| Liver | 0.21 ± 0.02 | 0.13 ± 0.03 | 0.06 ± 0.01 |
| Kidney | 0.12 ± 0.01 | 0.18 ± 0.02 | 0.07 ± 0.01 |
| Heart | 0.07 ± 0.01 | 0.10 ± 0.03 | 0.14 ± 0.10 |
| Lung | 0.32 ± 0.04 | 0.09 ± 0.02 | 0.10 ± 0.01 |
| Spleen | 6.86 ± 1.01 | 2.67 ± 0.98 | 0.32 ± 0.06 |
| Brain | 0.03 ± 0.00 | 0.08 ± 0.02 | 0.05 ± 0.01 |
| Tumor | 2.36 ± 0.50 | 5.82 ± 1.48 | 1.77 ± 0.37 |
| Skeletal muscle | 0.05 ± 0.01 | 0.14 ± 0.04 | 0.28 ± 0.38 |
| Tumor/muscle ratio[a] | 54.5 ± 10.4 | 41.6 ± 9.5 | 24.0 ± 14.1 |

[a]Calculated by dividing, for each animal, the tumor concentration by the muscle concentration and then determining the mean and standard deviation

from [$^{14}$C]thymidine and three-fold greater than that from [$^{14}$C]FAU. For [$^{14}$C]FAU, the radioactivity concentration in DNA rose 2.2-fold. The values for [$^{14}$C]thymidine remained stable over 24 h, whereas, in the same time frame, the levels of [$^{14}$C]FMAU increased 6.7-fold, indicating that the high tissue accumulation of FMAU-derived radioactivity was associated with its incorporation into DNA.

## Discussion

The rationale for studying the time course of tissue distribution and DNA incorporation of $^{14}$C-labeled thymidine analogs, FAU and FMAU, was to assess their potential as $^{18}$F-labeled PET imaging agents. The short half-life of the nuclide in [$^{11}$C]FMAU, which has been chemically prepared [5], greatly limits its usefulness. Testing as noninvasive probes for tumor proliferation was undertaken based on factors that included the favorable metabolic stability of these agents, a radiolabel half-life of 110 min, and biochemical similarities to thymidine. In the present study, the test compounds, [$^{14}$C]FAU and [$^{14}$C]FMAU, and the control compound, [$^{14}$C]thymidine, were administered to SCID mice bearing CCRF-CEM xenografts. Rodents have high circulating endogenous levels of thymidine (in the 1–2 $\mu M$ range) [20]. In contrast, the endogenous level of thymidine in human plasma is relatively low (about 40 n$M$) [20]. The mice were fed a diet deficient in thymidine to limit competition from this endogenous nucleoside. A limitation of the present study was that, with the dose of thymidine administered, the blood levels in mice immediately after dosing could have been as great as 60 $\mu M$, a concentration about 30 times the normal level and considerably greater than would be seen for a tracer with a high specific activity, such as $^{18}$F.

Some preclinical pharmacological studies of FAU and FMAU have been reported previously. Following cellular uptake, FMAU is phosphorylated by thymidine kinase to FMAUMP and is directly incorporated into DNA [1]. In studies involving rapid- and slow-growing rat prostate tumor cells in vitro, cellular kinetics for [$^{14}$C]FMAU have been shown to be comparable to those of thymidine in terms of uptake, cellular growth rate and saturability of cellular incorporation [1]. In dogs dosed with FAU, most of the dose is excreted unchanged in the urine within 24 h [16]. In dogs, FAU infused intravenously at doses of 50–500 mg/kg per day is well tolerated; few toxic symptoms have been noted [2]. In mice, rats, and dogs given FMAU, most of the dose is excreted in the urine without change [22]. In mice, the concentration of FMAU in plasma decreases more rapidly in those dosed intravenously than in those dosed by oral gavage. In a phase I trial of FMAU as an anticancer agent, doses of $\leq 32$ mg/m$^3$ per day for

**Table 4.** DNA incorporation of radioactivity in selected tissues of SCID mice at 1, 4 and 24 h after dosing with [$^{14}$C]thymidine, [$^{14}$C]FAU, or [$^{14}$C]FMAU. Values are means ± SD and (except for the ratios) are percent of injected dose per gram of tissue

| Time after dosing (h) | Tissue | [$^{14}$C]thymidine | [$^{14}$C]FMAU | [$^{14}$C]FAU |
|---|---|---|---|---|
| 1 | Liver | 0.0085 ± 0.0040 | 0.0019 ± 0.0003 | 0.0003 ± 0.0002 |
| | Kidney | 0.0075 ± 0.0029 | 0.0004 ± 0.0000 | 0.0002 ± 0.0001 |
| | Brain | 0.0007 ± 0.0002 | 0.0000 ± 0.0000 | 0.0002 ± 0.0001 |
| | Tumor | 0.0987 ± 0.0317 | 0.0270 ± 0.0010 | 0.0238 ± 0.0169 |
| | Skeletal muscle | 0.0003 ± 0.0004 | 0.0006 ± 0.0004 | 0.0004 ± 0.0004 |
| | Tumor/muscle ratio[a] | 329 | 45 | 60 |
| 4 | Liver | 0.0081 ± 0.0058 | 0.0032 ± 0.0023 | 0.0006 ± 0.0003 |
| | Kidney | 0.0082 ± 0.0014 | 0.0019 ± 0.0017 | 0.0002 ± 0.0000 |
| | Brain | 0.0017 ± 0.0009 | 0.0010 ± 0.0013 | 0.0000 ± 0.0000 |
| | Tumor | 0.0826 ± 0.0176 | 0.1119 ± 0.0409 | 0.0262 ± 0.0084 |
| | Skeletal muscle | 0.0027 ± 0.0012 | 0.0020 ± 0.0023 | 0.0001 ± 0.0001 |
| | Tumor/muscle ratio[a] | 31 | 56 | 262 |
| 24 | Liver | 0.0082 ± 0.0009 | 0.0032 ± 0.0014 | 0.0009 ± 0.0005 |
| | Kidney | 0.0091 ± 0.0040 | 0.0011 ± 0.0002 | 0.0002 ± 0.0002 |
| | Brain | 0.0015 ± 0.0002 | 0.0004 ± 0.0002 | 0.0002 ± 0.0002 |
| | Tumor | 0.1070 ± 0.0152 | 0.1623 ± 0.0511 | 0.0691 ± 0.0202 |
| | Skeletal muscle | 0.0019 ± 0.0015 | 0.0004 ± 0.0002 | 0.0016 ± 0.0010 |
| | Tumor/muscle ratio[a] | 56 | 406 | 43 |

[a]Because the concentrations in skeletal muscle were low and variable, the tumor/muscle ratios were calculated by dividing the average concentration in tumor by the average concentration in muscle

5 days have been shown to cause little or no observable toxicity [8].

The data derived from tissues collected from mice at 1 h after dosing are consistent with observations of [*methyl*-$^{11}$C]thymidine as a PET tracer for proliferating cells in tumor-bearing patients [19]. Although [$^{11}$C]thymidine is incorporated readily into DNA of proliferating cells, its rapid catabolism and the background resulting from the formation of [$^{11}$C]$CO_2$ preclude its practical clinical utility [4]. The data relative to [$^{14}$C]thymidine distribution are useful to compare the time frame and extent of [$^{14}$C]FAU and [$^{14}$C]FMAU uptake and distribution. Since substantial uptake of radioactivity in all tissues was noted at 1 h following administration of [$^{14}$C]FAU and [$^{14}$C]FMAU, only thymidine would be adequate for tumor imaging at such early time points, due to the nonspecific nature of the tissue distribution of [$^{14}$C]FAU and [$^{14}$C]FMAU. The results with mice confirm previous observations with dogs dosed intravenously at 90 mg/kg [16], which demonstrated that FAU is rapidly distributed throughout the body.

At 4 h after dosing, appreciable selectivity was noted, with tumor:skeletal muscle ratios for [$^{14}$C]FAU and [$^{14}$C]FMAU of $2.25 \pm 0.69$ and $3.07 \pm 0.42$, respectively. Although, between 1 and 4 h, substantial washout of radioactivity occurred in other tissues, the radioactivity concentrations in tumor tissue remained relatively stable. At 4 h after dosing, the radioactivity concentration from [$^{14}$C]FMAU in tumor tissue was 2.5-fold greater than that from [$^{14}$C]thymidine and [$^{14}$C]FAU, which could be reflected as sensitivity during PET imaging. Since FAU and FMAU could be administered with a label of $^{18}$F, which has a half-life of 110 min, the values indicate that [$^{18}$F]FMAU in particular could serve as an effective probe for cell proliferation. Factors other than cell proliferation may be important in tissue accumulation of the fluorinated compounds, as suggested by the disparity in the extent of splenic uptake of [$^{14}$C]FAU and [$^{14}$C]FMAU. At 4 h after dosing, the spleen/tumor ratio for [$^{14}$C]FAU was 0.5, but the ratio was 1.2 for [$^{14}$C]FMAU.

The radioactivity concentration from [$^{14}$C]FMAU in tumor remained constant between 4 and 24 h, whereas considerable loss of radiolabel occurred in all other tissues evaluated. The tissue levels at 24 h likely reflect the incorporation of nucleotides (derived from administered nucleosides) into DNA. An $^{18}$F-labeled agent would not be practical for PET imaging at 24 h. Nevertheless, an analog of FMAU incorporating an isotope with a longer half-life, such as 5-bromo-FAU, even though its distribution might be different, could have potential. Although [$^{14}$C]FAU demonstrated a lower uptake in tumor, the specificity for tumor tissue over spleen and other normal tissue has implications for its use as an antitumor agent.

Over a 24-h period, radioactivity from the three compounds accumulated in DNA from tumor tissues. Even though the tumor cells contained high levels of TS activity, incorporation of radioactivity from [$^{14}$C]FAU, which proceeds through its methylated derivative, FMAU, occurred to a lesser extent than for [$^{14}$C]thymidine and [$^{14}$C]FMAU, which do not require methylation by TS. These findings are consistent with the observation that FMAU inhibits cell growth to a greater extent than FAU, an observation probably related to the fact that more FMAU is incorporated into cellular DNA [1, 3, 14]. Differences in tissue uptake, as observed in the spleen, may exist, and the additional methylation step accomplished by TS may limit the rate of FAU incorporation into DNA. The presence of more radioactivity from [$^{14}$C]FMAU and [$^{14}$C]thymidine in tumor DNA may be related to the fact that only the enzymatic steps of phosphorylation precede their incorporation. [$^{14}$C]FAU must be phosphorylated to the mononucleotide, methylated, and further phosphorylated to the di- and triphosphate derivatives prior to its incorporation into DNA. The activity of TS in CCRF-CEM xenografts may not be sufficient to make the tissue a target for the bioactivation of FAU.

In humans, lower levels of endogenous thymidine would reduce the competition for the uptake and DNA incorporation of thymidine analogs. For [$^{14}$C]FAU and [$^{14}$C]FMAU, the tumor/skeletal muscle ratios were greater for DNA-associated radioactivity relative to total $^{14}$C in tissue. The extraction procedure used did not allow complete recovery of tissue DNA; no such technique is available. Although calculated values of DNA-associated radioactivity would underestimate the total present, the recoveries should be similar for all three compounds. The fact that these radioactive species are incorporated into DNA is consistent with the concept of their specificity for rapidly proliferating tumor tissue and enhances the potential for development of a thymidine analog as a PET imaging agent.

The availability of a clinical noninvasive probe for tumor proliferation would be relevant in numerous applications including diagnosis and monitoring of antitumor agents during treatment. A PET probe for proliferation that is incorporated into DNA would differ from agents currently used or under clinical evaluation. $^{18}$F-Fluorodeoxyglucose is useful in tumors with high glucose metabolism, and $^{131}$I-iodine is useful in iodine-positive thyroid cancer [9, 12, 23, 26]. Two other compounds that have been evaluated are [$^{18}$F]3′-deoxy-3′-fluorothymidine, a chain terminator [25], and [$^{18}$F]fluoro-2′-deoxyuridine [24]. Their effects reflect primarily the activity of thymidine kinase rather than DNA synthesis.

The selective tissue uptake and DNA incorporation of [$^{14}$C]FMAU supports the feasibility of [$^{18}$F]FMAU as a functional PET imaging agent. The potential for [$^{18}$F]FAU is uncertain. From this study with xenografts, there remain several ambiguities, including the influence of circulating thymidine levels, dosimetry, optimal imaging time, and influence of tumor type. These issues can be resolved in a clinical setting, which is now prac-

ticable due to the availability of a method for synthesis of these compounds [28].

## References


1. Bading JR, Shahinian AH, Bathija P, Conti PS (2000) Pharmacokinetics of the thymidine analog 2′-fluoro-5-[$^{14}$C]-methyl-1-$\beta$-D-arabinofuranosyluracil ([$^{14}$C]FMAU) in rat prostate tumor cells. Nucl Med Biol 27:361
2. Chang CJG, Smith AC, Fulton R, Farnell DR, Lin T-H, Emory MB, Page JG (2000) 120-Hour infusion dose range-finding study of 2′-fluoro-ara-deoxyuridine (FAU, NSC-678515) in beagle dogs. Proc Am Assoc Cancer Res 41:703
3. Collins JM, Klecker RW, Katki AG (1999) Suicide prodrugs activated by thymidylate synthase: rationale for treatment and noninvasive imaging of tumors with deoxyuridine analogues. Clin Cancer Res 5:1976
4. Conti PS, Hilton J, Magee CA, Anderson JH (1989) Tumor imaging with positron-emission tomography (PET) and [$^{11}$C]-thymidine: determination of radiolabeled thymidine metabolites by high-pressure liquid chromatography (HPLC) for kinetic data analysis. Radiology 173:P420
5. Conti PS, Alauddin MM, Fissekis JR, Schmall B, Watanabe KA (1995) Synthesis of 2′-fluoro-5-[$^{11}$C]-methyl-1-$\beta$-D-arabinofuranosyluracil ([$^{11}$C]-FMAU): a potential nucleoside analog for in vivo study of cellular proliferation with PET. Nucl Med Biol 22:783
6. Eary JF, Mankoff DA, Spence AM, Berger MS, Olshen A, Link JM, O'Sullivan F, Krohn KA (1999) 2-[C-11] Thymidine imaging of malignant brain tumors. Cancer Res 59:615
7. Enrietto PJ, Payne LN, Hayman MJ (1983) A recovered avian myelocytomatosis virus that induces lymphomas in chickens. Pathogenic properties and their molecular basis. Cell 35:369
8. Fanucchi MP, Leyland-Jones B, Young CW, Burchenal JH, Watanabe KA, Fox JJ (1985) Phase I trial of 1-(2′-deoxy-2′-fluoro-1-beta-D-arabinofuranosyl)-5-methyluracil (FMAU). Cancer Treat Rep 69:55
9. Goldberg MA, Lee MJ, Fischman AJ, Mueller PR, Alpert NM, Thrall JH (1993) Fluorodeoxyglucose PET of abdominal and pelvic neoplasms: potential role in oncologic imaging. Radiographics 13:1047
10. Gross-Bellard M, Oudet P, Chambon P (1973) Isolation of high-molecular-weight DNA from mammalian cells. Eur J Biochem 36:32
11. Houghton PJ, Mirro J Jr, Goorha RM, Raimondi SC, Fridland A, Houghton JA (1989) Growth and differentiation of a human T-cell leukemia cell line, CCRF-CEM, grafted in mice. Cancer Res 49:7124
12. Inokuma T, Tamaki N, Torizuka T, Magata Y, Fujii M, Yonekura Y, Kajiyama T, Ohshio G, Imamura M, Konishi J (1995) Evaluation of pancreatic tumors with positron emission tomography and F-18 fluorodeoxyglucose: comparison with CT and US. Radiology 195:345
13. Johnston PG, Fisher ER, Rockette HE, Fisher B, Wolmark N, Drake JC, Chabner BA, Allegra CJ (1994) The role of thymidylate synthase expression in prognosis and outcome of adjuvant chemotherapy in patients with rectal cancer. J Clin Oncol 12:2640
14. Klecker RW, Katki AG, Collins JM (1994) Toxicity, metabolism, DNA incorporation with lack of repair, and lactate production for 1-(2′-fluoro-2′-deoxy-$\beta$-D-arabinofuranosyl)-5-iodouracil in U-937 and MOLT-4 cells. Mol Pharmacol 46:1204
15. Larson SM, Weiden PL, Grunbaum Z, Rasey JS, Kaplan HG, Graham MM, Harp GD, Sale GE, Williams DL (1981) Positron imaging feasibility studies I: characteristics of [$^{3}$H] thymidine uptake in rodent and canine neoplasms: concise communication. J Nucl Med 22:869
16. Lin T-H, Chang CJG, Noker PE, Smith AC, Page JG (2000) Disposition of 2′-fluoro-ara-dexoyuridine (FAU, NSC-678515) in beagle dogs. Proc Am Assoc Cancer Res 41:703
17. Lu L, Bergstrom M, Fasth KJ, Langstrom B (2000) Synthesis of [$^{76}$Br] bromofluorodeoxyuridine and its validation with regard to uptake, DNA incorporation, and excretion modulation in rats. J Nucl Med 41:1746
18. Mankoff DA, Shields AF, Link JM, Graham MM, Muzi M, Peterson LM, Eary JF, Krohn KA (1999) Kinetic analysis of 2-[$^{11}$C]thymidine PET imaging studies: validation studies. J Nucl Med 40:614
19. Martiat P, Ferrant A, Labar D, Cogneau M, Bol A, Michel C, Michaux JL, Sokal G (1988) In vivo measurement of carbon-11 thymidine uptake in non-Hodgkin's lymphoma using positron emission tomography. J Nucl Med 29:1633
20. Nottebrock H, Then R (1977) Thymidine concentrations in serum and urine of different animal species and man. Biochem Pharmacol 26:2175
21. Pestalozzi BC, Peterson HF, Gelber RD, Goldhirsch A, Gusterson BA, Trihia H, Lindtner J, Cortes-Funes H, Simmoncini E, Byrne MJ, Golouh R, Rudenstam CM, Castiglione-Gertsch M, Allegra CJ, Johnston PG (1997) Prognostic importance of thymidylate synthase expression in early breast cancer. J Clin Oncol 15:1923
22. Philips FS, Feinberg A, Chou TC, Vidal PM, Su TL, Watanabe KA, Fox JJ (1983) Distribution, metabolism, and excretion of 1-(2-fluoro-2-deoxy-beta-D-arabinofuranosyl)thymine and 1-(2-fluoro-2-deoxy-$\beta$-D-arabinofuranosyl)-5-iodocytosine. Cancer Res 43:3619
23. Reske SN, Grillenberger KG, Glatting G, Port M, Hildebrandt M, Gansauge F, Berger HG (1997) Overexpression of glucose transporter 1 and increased FDG uptake in pancreatic carcinoma. J Nucl Med 38:1344
24. Seitz U, Wanger M, Vogg AT, Glatting G, Neumaier B, Greten FR, Schmid RM, Reske SN (2001) In vivo evaluation of 5-[$^{18}$F]fluoro-2′-dexoyuridine as tracer for positron emission tomography in a murine pancreatic cancer model. Cancer Res 61:3853
25. Shields AF, Grierson JR, Dohmen BM, Machulla HJ, Stayanoff JC, Lawhorn-Crews JM, Obradovich JE, Muzik O, Mangner TJ (1998) Imaging proliferation in vivo with [F-18] FLT and positron emission tomography. Nat Med 4:1334
26. Stokkel MP, de Klerk JH, Zelissen PM, Koppeschaar HP, van Rijk PP (1999) Fluorine-18 fluorodeoxyglucose dual-head positron emission tomography in the detection of recurrent differentiated thyroid cancer: preliminary results. Eur J Nucl Med 26:1606
27. Strauss WM (1998) Preparation of genomic DNA from mammalian tissue. In: FM Ausubel, et al (eds) Current protocols in molecular biology. Wiley, New York, pp 2.2.1–2.2.3
28. Sun H, Manger TJ, Muzik O, Collins JM, Shields AF (2001) Biodistribution and metabolism of F-18-FMAU: PET studies. J Nucl Med [Suppl] 42:308
29. Vander Borght T, Pauwels S, Lambotte L, Labar D, De Maeght S, Stroobandt G, Laterre C (1994) Brain tumor imaging with PET and 2-[carbon-11] thymidine. J Nucl Med 35:974
30. Wang H, Cai Q, Zeng X, Yu D, Agrawal S, Zhang R (1999) Antitumor activity and pharmacokinetics of a mixed-backbone antisense oligonucleotide targeted to the RI alpha subunit of protein kinase A after oral administration. Proc Natl Acad Sci USA 96:13989