2′-Fluoro-5-([^{11}C]-methyl)-1-β-D-arabinofuranosyluracil

[^{11}C]FMAU

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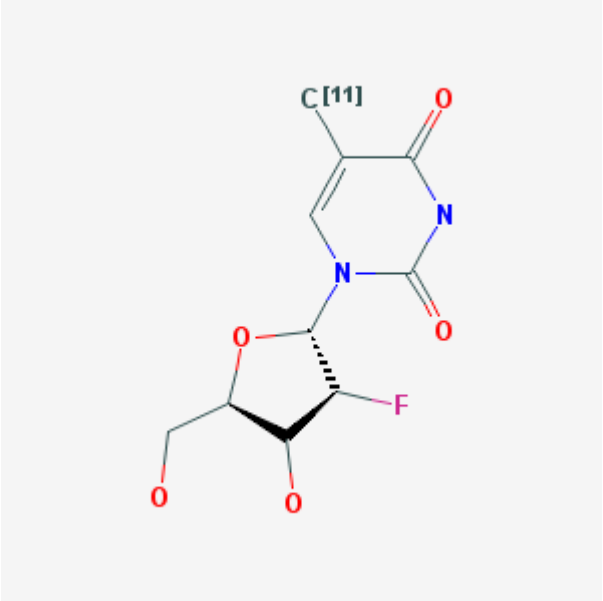
Chemical name:	2'-Fluoro-5-([^{11}C]-methyl)-1-β-D-arabinofuranosyluracil
Abbreviated name:	[^{11}C]FMAU
Synonym:	
Agent Category:	Compound
Target:	DNA
Target Category:	Incorporation into DNA
Method of detection:	Positron emission tomography (PET)
Source of signal:	^{11}C
Activation:	No
Studies:	• *In vitro* • Rodents • Non-primate non-rodent mammals



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Background

[PubMed]

Thymidine radiolabeled with radioactive C-14 or H-3 (TdR) is routinely used *in vitro* to measure cell proliferation and cell growth in various biological systems because it is a necessary and exclusive precursor for the synthesis of DNA (1). However, TdR can be easily catabolized by enzymes after *in vivo* administration and has resulted in the development of TdR derivatives that are not degraded and can be used to follow DNA synthesis to measure cell proliferation and growth (2, 3). Initially, a C- or H-labeled TdR was produced for imaging studies with positron emission tomography (PET); however, because this compound was catabolized and produced recirculating labeled products that reduced the tumor/normal tissue contrast, it was difficult to interpret results after its incorporation into DNA (4).

As a consequence, to circumvent the catabolite issue observed with TdR, some investigators developed TdR analogs that could be used for tumor imaging but were not catabolized (3, 5). Among the various TdR derivatives, those labeled with radioactive fluorine (^{18}F) or ^{11}C were developed to image and monitor tumors because, compared to normal tissue, rapid cell proliferation is a characteristic feature of these cancer lesions (6). Although [^{18}F]-3'-deoxy-3'-fluorothymidine ([^{18}F]FLT) has been used in the clinic to study different cancers, it has a limitation in that, within the cell, it is only converted into a triphosphate nucleoside, is not incorporated into the growing strand of DNA, and leads to DNA chain termination (7). The phosphorylation of FLT is taken to be an indicator of cytosolic thymidine kinase (TK_1) activity rather than DNA synthesis, and the use of TK_1 activity as a measure of DNA synthesis is not well established (8). Also, [^{18}F]FLT is not a suitable agent to detect malignancies in the liver and bone marrow because it produces a high background in these tissue (9). In addition, because [^{18}F]FLT is excreted through the urinary system, pelvic lesions may not be detected with this radiochemical (1).

Another analog of TdR, 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)thymidine (FMAU) has been labeled with either ^{18}F or ^{11}C and used to study cell proliferation or image tumors *in vivo* (2, 10, 11). The only difference between FMAU and TdR is the substitution of a hydrogen atom with fluorine at the 2'-position of the deoxyarabinose sugar moiety in the molecule (2). FMAU was shown to possess the same biological properties as TdR, including transport across the cell membrane, enzymatic phosphorylation, and incorporation into cellular DNA (12). Compared to TdR, clearance of ^{11}C-labeled FMAU ([^{11}C]FMAU) from the blood was observed to be slower and showed a higher incorporation in tumors (12).

After conversion to a triphosphate, FMAU was shown to have an inhibitory effect on the hepatitis B virus polymerase and is approved by the United States Food and Drug Administration for evaluation in clinical trials to treat this infection (13).

This chapter details the preclinical studies performed with [^{11}C]FMAU. Studies performed with [^{18}F]FMAU are presented in a separate chapter in MICAD (www.micad.nih.gov).

Synthesis

[PubMed]

The synthesis of [^{11}C]FMAU was described by Conti et al. (14). Initially, FMAU was prepared from 3'-5'-O-bis-(tetrahydropyranyl)-2'-fluoro-5-iodo-1-β-D-arabinofuranosyluracil (FMAU precursor). To obtain the FMAU precursor, 2'-fluoro-5-iodo-1-β-D-arabinofuranosyluracil (FIAU) was dissolved in dry tetrahydrofuran. *p*-Toluenesulfonic acid was added to this solution as a catalyst, followed by the addition of 2,3-dihydropyran. The mixture was stirred at room temperature for 2 h, and complete formation of the product was confirmed by thin-layer chromatography. The reaction was stopped with triethylamine. The solvent was subsequently evaporated, and the crude product was purified by preparative liquid chromatography on a silica gel column with acetone in hexane as an eluent. The solvent was evaporated to obtain the FMAU precursor as a white solid with a reaction yield of 93%.

For the synthesis of [^{11}C]FMAU, the FMAU precursor was dissolved in dry tetrahydrofuranand cooled to -78°C, and *n*-butyllithium was added to it (14). Subsequently ^{11}C-labeled methyl iodide was bubbled through the cold solution for 2 min, and then the reaction mixture was warmed to room temperature. After the addition of 2 M hydrochloric acid, the mixture was refluxed for 3 min in a heating block at 110°C. The residual solvent was then evaporated with argon, and the reaction was neutralized with 2 M sodium hydroxide after cooling. The crude product was separated by semipreparative high-performance liquid chromatography (HPLC), and purity of the product was checked by HPLC. On the basis of ^{11}C-labeled methyl iodide, the product yield was 53% in 30–35 min from the end of bombardment. Radiochemical purity of [^{11}C]FMAU was reported to be routinely >99% with a specific activity up to 100 Ci/mmol (3,700 Bq/mmol) (14). Stability of the product was not reported by the investigators (14).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

[^{11}C]FMAU and [^{18}F]-9-[(3-fluoro-1-hydroxy-2-propoxy)methyl]guanine ([^{18}F]FHPG) incorporation into cellular DNA were evaluated to measure herpes simplex virus thymidine kinase (HSV-tk) enzyme activity after gene transfer into cells for the detection of human cytomegalovirus (HCMV) infections (15). Significantly higher amounts of both [^{11}C]FMAU and [^{18}F]FHPG were observed to accumulate in cells that express HSV-tk compared to the control cells. However, [^{18}F]FHPG uptake in the cells that express HSV-tk was cell line–dependent, probably because the uptake depended either on the cell membrane transport characteristics or the cell enzyme substrate specificity. Both radiochemicals were reported to have a higher accumulation in HCMV-infected human

umbilical vein endothelial cells compared to the uninfected control cells. A higher uptake of [^{11}C]FMAU, compared to [^{18}F]FHPG, was evident in the control cells and was postulated to be the result of host kinase phosphorylation activity (the subcellular identification of FMAU phosphates was not reported by the investigators). With results from this study, the investigators concluded that [^{18}F]FHPG appeared to be more suitable than [^{11}C]FMAU for prediction of HSV-tk gene therapy outcome and detection of HCMV infections with PET (15).

In another *in vitro* study, Davoodpour et al. determined that the incorporation of [^{11}C]FMAU in prostate cancer cell aggregates was not a suitable marker to investigate the cytotoxicity of 2-methoxyestradiol, a metabolite of 17β-estradiol (16).

Animal Studies

Rodents

[PubMed]

[^{11}C]FMAU, [^{18}F]FLT, and 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-[^{76}Br]bromouracil ([^{76}Br]BFU) were evaluated to determine tissue proliferation using DNA incorporation and excretion modulation as a readout in a rat model (3). Three groups (n = 4–7 animals per treatment group) of Sprague-Dawley rats were treated with the PET agents, and a subgroup of each treatment group was also administered cimetidine (a drug that was previously shown to increase the cellular DNA incorporation of [^{76}Br]-BFU) to investigate whether it would increase the cellular incorporation of [^{11}C]FMAU and [^{18}F]FLT (17). The investigators observed that, among all the organs examined, organs with a high rate of DNA synthesis, i.e., the spleen and the intestines, had a maximum incorporation of the radiolabels (3). Although a gradual increase in [^{76}Br]BFU in DNA was observed in the cimetidine-treated group, this drug did not affect the uptake of either [^{11}C]FMAU or [^{18}F]FLT. From these results the investigators concluded that, because [^{76}Br]BFU was incorporated predominantly into the DNA, it could potentially be used as a PET agent for the measurement of *in vivo* cell proliferation. Similarly, [^{11}C]FMAU could be used to determine cell proliferation, but, because its label had a short half-life, this radiochemical could not be used for an extended period of observation. They investigators also concluded that [^{18}F]FLT was not suitable to measure cell proliferation because it was not incorporated into cellular DNA (3).

Bading et al. performed pharmacokinetic measurements with [^{14}C]FMAU and PET studies with [^{11}C]FMAU in rats bearing different types of syngeneic tumors (Dunning R3327-AT-2.1 and R3327-H prostate adenocarcinomas, and rat colorectal carcinomas) (12). With [^{14}C]FMAU, the relative cell proliferation rates could be determined in normal tissue. FMAU was incorporated into the small intestine and colon tumor DNA. Among tumors, the highest uptake was observed in the rapidly growing colon carcinoma, but uptake was low in both the rapid- and the slow-growing prostate tumors. The investigators concluded that, although tumor uptake was modest and did not always

correlate with the tumor growth rate, [^{11}C]FMAU may be useful to image DNA synthesis in tissue (12).

Other Non-Primate Mammals

[PubMed]

The uptake of [^{11}C]FMAU was measured with PET in a canine brain tumor model (18). Dynamic imaging with arterial sampling was performed in beagle dogs (n = 8) that were administered [^{11}C]FMAU after implantation of brain tumors. Six dogs were euthanized after a BUdR infusion and tumor time–activity curves (TACs) were obtained from computed tomography-defined regions of interest. The tumor volume occupied by viable cells was determined, and viable cells in the S-phase were identified by BUdR staining. Subsequently, a correlation between PET/[^{11}C]FMAU and BUdR was determined. The tumor standardized uptake values (SUVs) and tumor/contralateral brain uptake ratios were respectively determined to be 1.6 ± 0.4 and 5.5 ± 1.2 at 50 min. No ^{11}C-labeled metabolites of [^{11}C]FMAU were observed in the blood up to 60 min after administration. The clearance of [^{11}C]FMAU from plasma into the tumors correlated with the S-phase percent volume (P = 0.03), and the tumor SUV correlated significantly with both the S-phase and the cell percent volumes (P = 0.02 and 0.03, respectively). Tumor uptake and incorporation of [^{11}C]FMAU was observed to correlate with the volume density of dividing cells (P = 0.0003) and not the nondividing cells (P = 0.3). From these observations the investigators concluded that the incorporation of [^{11}C]FMAU correlated with tumor growth rate under the experimental conditions used for a canine brain tumor model, indicating that [^{11}C]FMAU could be used to image cell proliferation in cancers (18).

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

Supplemental Information

[Disclaimers]

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