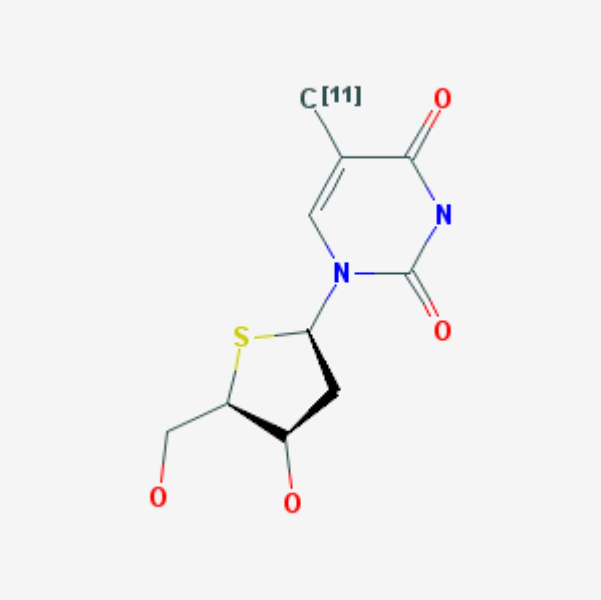
Methyl-[^{11}C]-4'-thiothymidine

[^{11}C]-4DST

Arvind Chopra, PhD[1]

Created: August 1, 2011; Updated: September 8, 2011.

Chemical name:	Methyl-[^{11}C]-4'-thiothymidine
Abbreviated name:	[^{11}C]-4DST
Synonym:	4'-[methyl-^{11}C]thiothymidine ([methyl-^{11}C]S-dThd)
Agent Category:	Compound
Target:	DNA
Target Category:	Nucleic acids
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	^{11}C
Activation:	No
Studies:	• *In vitro* • Rodents • Humans



Click on the above structure of [^{11}C]-DST for more information in PubChem.

Background

[PubMed]

Most anti-cancer drugs are aimed at curbing the processes of rapid cell proliferation and an increased rate of DNA synthesis, which are both necessary for the progression of all

[1] National Center for Biotechnology Information, NLM, Bethesda, MD 20894; Email: micad@ncbi.nlm.nih.gov.

NLM Citation: Chopra A. Methyl-[^{11}C]-4'-thiothymidine. 2011 Aug 1 [Updated 2011 Sep 8]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

cancers and the development of neoplastic tumors. Usually invasive procedures such as biopsies are used to confirm the presence of malignant tumors, but it can be difficult to take multiple biopsies from a patient if the cancer has metastasized to several different areas of the body (1). Therefore, non-invasive imaging methods that use radiolabeled thymidine analogs (a nucleoside present only in the DNA) in conjunction with positron emission tomography (PET) have been developed to detect tumors and to evaluate the efficacy of anti-cancer treatments in animals and humans (1). Initially, ^{11}C-labeled thymidine was developed to visualize cancerous tumors, but this radiolabeled compound is quickly metabolized in the cell and has been determined to have limited application in the clinic (2). Subsequently, ^{18}F-labeled PET agents with a thymidine base, such as 3'-deoxy-3'-[^{18}F]fluorothymidine ([^{18}F]-FLT) and 1-(2'-deoxy-2'-[^{18}F]fluoro-β-D-arabinofuranosyl) thymine ([^{18}F]-FMAU), were generated and evaluated for the visualization of neoplastic tumors. Among these, FLT is phosphorylated by thymidine kinase 1 (a cytosolic isozyme of thymidine kinase), but the phosphorylated molecule is not incorporated into the cellular DNA and is trapped within the cell (2). Hence, [^{18}F]-FLT is considered to be a surrogate marker for the visualization of cell proliferation under *in vivo* conditions. In addition, this cell proliferation tracer has several limitations that have been discussed elsewhere (3). The second tracer, [^{18}F]-FMAU, has to be phosphorylated by thymidine kinase 2 (TK2), a mitochondrial enzyme, before it is incorporated into the DNA (2). However, [^{18}F]-FMAU is considered suitable only for the detection of neoplasia in the pelvic region or for visualization of tumors that have metastasized to the bone, because with this radiolabeled compound there is a high retention of label in the liver, kidneys, and the myocardium (2). It has also been reported that there is no correlation between the uptake of a TK2-selective arabinothymidine substrate and the proliferation of cells in a malignant tumor (3).

With the information described above, in an effort to produce an alternative tracer that can be used to visualize tumor proliferation, Toyohara et al. synthesized 4'-thiothymidine (in this molecule, the 4'-oxo of thymidine is substituted with a 4'-sulfur) and showed that its methylated and ^{14}C-labeled derivative, 4'-methyl-[^{14}C]-thiothymidine ([^{14}C]-4DST), accumulated mainly in rapidly proliferating tissues in mice (spleen, duodenum, and thymus), including the xenograft tumors (4). It has also been shown that the label from [^{14}C]-4DST is present mainly in the DNA fraction of these tissues. On the basis of these observations, ^{11}C-labeled 4'-methyl-thiothymidine ([^{11}C]-4DST) was synthesized and evaluated for the visualization of cell proliferation with PET in mice bearing xenograft tumors generated from several different cell lines (3). The biodistribution and use of [^{11}C]-4DST for the imaging of brain tumors in humans with PET was investigated in a recent pilot study (5).

Other Sources of Information

Related chapters in MICAD

Clinical trials with [^{18}F]-FLT

Clinical trials with [^{18}F]-FMAU

Cell proliferation assay protocols

[3H]-Thymidine cell proliferation assay

Synthesis

[PubMed]

The synthesis of [11C]-4DST using two different precursors (a 5-trimethylstannyl-precursor (5-TMS) and a 5-tributylstannyl-precursor (5-TBS)) has been described by Toyohara et al. (3). The total time of synthesis with both precursors was 30 min from the end of bombardment, and the radiochemical yields (RCY) with 5-TMS and 5-TBS were reported to be 18.9 ± 4.1% (n = 10 synthetic reactions) and 14.5 ± 2.4% (n = 4 synthetic reactions), respectively. The radiochemical purity (RCP) of [11C]-4DST obtained with both precursors was >99% as determined with analytical high-performance liquid chromatography (HPLC). The specific activities of the final product synthesized with 5-TMS and 5-TBS were 47 ± 23 GBq/μmol (1.27 ± 0.62 Ci/μmol; n = 6 determinations) and 121 ± 28 GBq/μmol (3.27 ± 0.75 Ci/μmol; n = 4 determinations), respectively.

In another study, [11C]-4DST synthesized with the 5-TBS precursor had a RCP of >99% and a specific activity of >80 GBq/mmol (>2.16 Ci/mmol) (6).

When a palladium-mediated rapid C-[11C]methylation reaction was used to synthesized [11C]-4DST for animal and human use, the RCY, RCP, and specific activity of the labeled compound were reported to be 93%, ≥99.5%, and 89–200 GBq/μmol (2.4–5.4 Ci/μmol), respectively (7).

For use in humans a modified method was used for the synthesis of [11C]-4DST as described by Toyohara et al. (5). Briefly, a solution of tri(*o*-tolyl)phosphine in *N*,*N*-dimethylformamide (DMF) and the precursor in DMF was prepared and mixed with copper chloride, potassium carbonate, and Tris(dibenzylideneacetone)dipalladium. Freshly prepared [11C]-methyl iodide was then trapped in the reaction mixture of DMF and the reaction mixture was heated at 80°C for 5 min. [11C]-4DST was purified from the reaction mixture with high performance liquid chromatography and collected in a flask containing ascorbic acid, evaporated to dryness, dissolved in 0.9% sodium chloride and sterile filtered for use. The total time of synthesis was 25 min from the end of bombardment and the radiochemical yield and purity of the tracer was 73.4 ± 12.3% (n = 7 reactions) and always >99%, respectively. The specific activity of [11C]-4DST was reported to be 313 ± 200 TBq/mmol (8459 ± 5405 Ci/mmol) (n = 9 synthesis reactions)

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

No publication is currently available.

Animal Studies

Rodents

[PubMed]

The biodistribution of [^{11}C]-4DST was investigated in groups of mice (n = 4–5 animals/group) bearing xenograft tumors derived from EMT-6 (a murine mammary carcinoma cell line), LL (a murine lung cancer cell line), Colon-26 (a murine rectum carcinoma cell line), S-180 (a murine sarcoma cell line), and C6 (rat glioma cell line) cells, respectively (3). The animals were administered a bolus of 37 MBq (1 mCi) [^{11}C]-4DST (~0.8 nmol) through the tail vein and euthanized 60 min later. All major organs, including the tumors, were then removed from the animals to determine the amount of radioactivity accumulated in the tissues, and the results were presented as standardized uptake values (SUV). Maximum uptake was observed in the spleen of animals of the various groups (SUVs between 2 and >10), followed by tumors derived from the different cell lines (SUVs between ~2 and ~7) and the thymus (SUVs between 1 and ~3). Non-proliferative tissues from organs such as liver, lung, kidneys, etc., had SUVs of <2. From this study, the investigators concluded that the uptake of [^{11}C]-4DST by the tumors was high and selective (3). No blocking studies were reported.

In the same study, the DNA-incorporated SUV (DNA_{SUV}) of [^{11}C]-4DST was compared to that of 2-[^{14}C]-4DST in five different tumor tissues (3). The mean DNA_{SUV} with [^{11}C]-4DST was reported to be the same as that of 2-[^{14}C]-4DST in the various tumor tissues (Pearson's correlation coefficient $r = 0.96$, $P < 0.01$), indicating that the two tracers had a similar behavior under *in vivo* conditions.

MicroPET imaging of mice between 60 and 70 min after an injection of [^{11}C]-4DST showed that there was high uptake of the tracer in the tumor, bone marrow, intestine, and the urinary bladder (3).

Tsuji et al. evaluated the use of [^{18}F]-fluorodeoxy glucose ([^{18}F]-FDG), [^{18}F]-FLT, and [^{11}C]-4DST for the imaging of H226 or 211H mesothelioma cell line tumors (the two cell lines are derived from epithelial mesothelioma and sarcomatoid mesothelioma, respectively) implanted subcutaneously and in the pleural cavity of nude mice (6). Histochemical staining confirmed that, compared to the 211H xenograft tumors, the H226 xenografts produced a very high level of mesothelin, a protein that is overexpressed in epithelial mesothelioma, ovarian cancer, and some squamous cell carcinomas. The animals (n = 3–6 mice/group) were injected with the respective tracers through the tail vein and euthanized 50 min later. The tumors and all major organs were removed to determine the amount of accumulated radioactivity. Compared to either [^{18}F]-FDG or [^{18}F]-FLT, a significantly higher uptake of [^{11}C]-4DST was observed in the H226 xenografts ($P < 0.05$). In contrast, the 211H tumors showed a significantly higher ($P < 0.01$) uptake of [^{18}F]-FDG compared to that of [^{11}C]-4DST. A similar trend was observed with the tumor/heart and tumor/lung ratios with both of these labeled compounds. The

differential uptake of the tracers in the two subtypes of mesothelioma was subsequently confirmed with PET imaging of mice bearing the H226 or 211H cell xenograft tumors.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

Toyohara et al. conducted a pilot study with PET to investigate the biodistribution and brain tumor imaging in humans with [^{11}C]-4DST (5). For the biodistribution study, three healthy volunteers were administered an intravenous bolus of 688 ± 47.9 MBq (2.2 ± 0.09 mmol) [^{11}C]-4DST, and the individuals were subject to whole-body dynamic PET scans for the next ~125 min as detailed elsewhere (5). From the images it was evident that maximum label was initially present (1–11 min of the scan) in the kidneys, followed by the liver; both organs showed a gradual decline in accumulated label during the subsequent scans (24–34 min and 78–98 min). The bone marrow showed a gradual increase in accumulated radioactivity during the entire period of the scan. Only a moderate amount of the tracer was detected in other proliferative tissues, such as the spleen and the intestines; very little uptake was evident in the non-proliferative tissues, such as the muscles and lungs. These observations indicated that the uptake of [^{11}C]-4DST was selective for tissue that had a high proliferation rate (5). The highest absorbed dose was estimated to be in the urinary bladder wall (17.6 ± 2.7 μGy/MBq), followed by the kidneys (17.6 ± 2.7 μGy/MBq), liver (12.4 ± 2.3 μGy/MBq), and spleen (6.9 ± 1.4 μGy/MBq); for all other organs, the dose ranged between 1.9 ± 0.1 μGy/MBq (skin) and 4.7 ± 0.2 μGy/MBq (osteogenic cells). The mean effective dose for the radiochemical was estimated to be 4.2 ± 0.3 μSv/MBq.

To investigate the use of [^{11}C]-4DST to visualize tumors in the brain with PET, the presence of tumor masses in six patients was initially confirmed with Gd-magnetic resonance imaging (Gd-MRI) or ^{11}C-methionine ([^{11}C]-Met) PET as described by Toyohara et al. (5). Each patient was then injected with 528–817 MBq/1.7–10.0 mmol (14.3–22.1 mCi/1.7–10.0 mmol) [^{11}C]-4DST, dynamic PET scans were acquired for the next 90 min, and the images from the scans were analyzed as detailed elsewhere (5). There was little uptake of [^{11}C]-4DST in the normal brain (the radiolabel is impermeable to the blood–brain barrier), but rapidly growing tumors (earlier visualized with Gd-MRI and [^{11}C]-Met PET) were clearly visible (because the blood–brain barrier is disrupted in these

patients). Although [^{11}C]-Met PET detected all the contrast-enhanced regions visualized with Gd-MRI, a clinically stable tumor visualized with Gd-MRI was not confirmed with [^{11}C]-4DST. In addition, the distribution pattern of [^{11}C]-Met in the tumor regions was not similar to that of [^{11}C]-4DST, even in those tumors that had a high accumulation of this label (5).

HPLC analysis of the plasma and the urine of the individuals revealed that several hydrophilic metabolites of [^{11}C]-4DST were produced in the patients, indicating that the radiolabeled compound was metabolized in the human system, although it was shown to be stable in mice (5).

From these studies, the investigators concluded that [^{11}C]-4DST was suitable for the imaging of brain tumors with PET; however, because it was evaluated in only a small group of humans, further studies are necessary to confirm the suitability of this labeled compound for application in the clinic (5).

Supplemental Information

[Disclaimers]

No information is currently available.

References

1. Bading J.R., Shields A.F. *Imaging of cell proliferation: status and prospects.* J Nucl Med. 2008;49 Suppl 2:64S–80S. PubMed PMID: 18523066.
2. Chen K., Chen X. *Positron emission tomography imaging of cancer biology: current status and future prospects.* Semin Oncol. 2011;38(1):70–86. PubMed PMID: 21362517.
3. Toyohara J., Okada M., Toramatsu C., Suzuki K., Irie T. *Feasibility studies of 4'-[methyl-(11)C]thiothymidine as a tumor proliferation imaging agent in mice.* Nucl Med Biol. 2008;35(1):67–74. PubMed PMID: 18158945.
4. Toyohara J., Kumata K., Fukushi K., Irie T., Suzuki K. *Evaluation of 4'-[methyl-14C]thiothymidine for in vivo DNA synthesis imaging.* J Nucl Med. 2006;47(10):1717–22. PubMed PMID: 17015909.
5. Toyohara J., Nariai T., Sakata M., Oda K., Ishii K., Kawabe T., Irie T., Saga T., Kubota K., Ishiwata K. *Whole-Body Distribution and Brain Tumor Imaging with 11C-4DST: A Pilot Study.* J Nucl Med. 2011;52(8):1322–1328. PubMed PMID: 21764794.
6. Tsuji A.B., Sogawa C., Sugyo A., Sudo H., Toyohara J., Koizumi M., Abe M., Hino O., Harada Y.N., Furukawa T., Suzuki K., Saga T. *Comparison of conventional and novel PET tracers for imaging mesothelioma in nude mice with subcutaneous and intrapleural xenografts.* Nucl Med Biol. 2009;36(4):379–88. PubMed PMID: 19423005.
7. Koyama H., Siqin Z. Zhang, K. Sumi, Y. Hatta, H. Nagata, H. Doi, and M. Suzuki, *Highly efficient syntheses of [methyl-11C]thymidine and its analogue 4'-[methyl-11C]thiothymidine as nucleoside PET probes for cancer cell proliferation by*

Pd(0)-mediated rapid C-[11C]methylation. Org Biomol Chem. 2011;9(11):4287–94. PubMed PMID: 21503302.