Evaluation of 4′-[Methyl-^{14}C]Thiothymidine for In Vivo DNA Synthesis Imaging

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We evaluated 4′-[methyl-^{11}C]thiothymidine ([methyl-^{11}C]S-dThd) to obtain a thymidine analog that might prove simpler to use for imaging DNA synthesis and that might follow the same biochemistry as its surrogate. **Methods:** [methyl-^{14}C]S-dThd was synthesized by rapid methylation of 5-trimethylstannyl-4′-thio-2′-deoxyuridine via a palladium-mediated Stille coupling reaction with ^{14}C-methyl iodide. Degradation of [methyl-^{14}C]S-dThd, when incubated in human blood, was analyzed by high-performance liquid chromatography (HPLC). The in vivo potential of [methyl-^{14}C]S-dThd was evaluated by studying its distribution in EMT-6 mammary carcinoma–bearing mice. 2-Fluoro-2′-deoxycytidine, a potent inhibitor of DNA synthesis, was used to modulate cell proliferation. Tissue extraction was also performed to investigate the incorporation of [methyl-^{14}C]S-dThd into DNA. **Results:** [methyl-^{14}C]S-dThd was obtained in a 31%–41% radiochemical yield (calculated from ^{14}C-methyl iodide) at 130°C, 5-min reaction in *N,N*-dimethylformamide followed by semipreparative HPLC purification. The radiochemical purity of [methyl-^{14}C]S-dThd was >99% and the specific activity was 2.04 GBq/mmol (according to the specific activity of ^{14}C-methyl iodide). [2-^{14}C]Thymidine, when incubated with human blood, demonstrated rapid degradation. In contrast, [methyl-^{14}C]S-dThd was stable with <3% degradation at 60 min. An in vivo distribution study showed the accumulation of radioactivity in proliferating tissues (spleen, thymus, duodenum, and tumor). On the other hand, the radioactivity of nonproliferating tissues (lung, liver, kidney, and muscle) was rapidly cleared in parallel with the clearance of blood radioactivity. The tumor uptake of [methyl-^{14}C]S-dThd was high (8.8 percentage injected dose per gram at 60 min) and selective (tumor-to-blood ratio, 12.2 at 60 min). 2-Fluoro-2′-deoxycytidine pretreatment significantly reduced the tumor uptake of [methyl-^{14}C]S-dThd. Relative blood flow as measured by the uptake of 4-[*N*-methyl-^{14}C]iodoantipyrine was similar between the treated and untreated groups. Tissue extraction studies showed that most of the total tissue radioactivity of rapidly proliferating tissues was recovered in the DNA fraction at 60 min after [methyl-^{14}C]S-dThd injection. **Conclusion:** The labeling procedure is rapid and suitable for ^{11}C labeling. Positron-labeled 4′-thiothymidine should be useful for imaging DNA synthesis by PET.

Key Words: DNA synthesis; 4′-thiothymidine; proliferation; tumor; PET

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During the past 4 decades, deoxynucleoside analogs have grown increasingly important in the drug treatment of cancers and viral diseases. These nucleoside analogs are a type of prodrug and they are transformed in cells by 5′-phosphorylation into active nucleotides that can act as an inhibitor of viral and cellular replication. Therefore, when labeled with a suitable radionuclide, some of these nucleosides may have potential use in tumor imaging based on cell proliferation. To be effective in noninvasive imaging, the derivatives should be stable in the blood and be rapidly transported across cell membranes; they should be selectively phosphorylated by thymidine kinase 1, thereby accumulating in tumor cells; and they should be labeled with a positron-emitting or a single-photon-emitting radionuclide. Today, several nucleosides have been developed to visualize tumor cell proliferation by using the 2′-arabino-F (*1–4*) or the 3′-F strategy (*5–7*). These tracers have strengths and limitations for PET proliferation probes because of their differences in metabolism and biochemistry. Therefore, a thymidine (dThd) analog that might prove simpler to use for imaging DNA synthesis and that might follow the same biochemistry as dThd is still needed.

We have been attracted by the chemical structure of 4′-thiothymidine (S-dThd) because of its close similarity to dThd. Structural studies have shown that the conformation of S-dThd closely resembles that of dThd (*8*). In addition, an in vitro study demonstrated that the enzymes involved in the phosphorylation of dThd and its incorporation into DNA readily accept 4′-[methyl-^{3}H]thiothymidine and its metabolites as substrates (*9*). We also reasoned that 4′-[methyl-^{11}C]thiothymidine could be obtained by a 1-pot rapid methylation of 5-trimethylstannyl-4′-thio-2′-deoxyuridine via a palladium-mediated Stille coupling reaction with ^{11}C-methyl iodide. Therefore, we evaluated 4′-[methyl-^{14}C]thiothymidine ([methyl-^{14}C]S-dThd) as a model compound of a potential ^{11}C-labeled proliferation marker. In this study, we demonstrated preliminary evidence of S-dThd as a potential DNA synthesis imaging

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agent for PET through radiosynthesis and the biologic evaluation in vitro and in vivo using tumor-bearing mice.

MATERIALS AND METHODS

Materials

All chemical reagents were of the highest grade and were purchased from Sigma-Aldrich Japan K.K., Wako Pure Chemical Industries, Ltd., and Tokyo Kasei Kogyo Co., Ltd. ^{14}C-Methyl iodide (specific activity, 2.04 GBq/mmol) and [2-^{14}C]dThd (specific activity, 2.13 GBq/mmol) were purchased from Amersham Biosciences. 4-[*N*-methyl-^{14}C]Iodoantipyrine (4-[*N*-methyl-^{14}C]IAP; specific activity, 2.08 GBq/mmol) was purchased from PerkinElmer Life Sciences, Inc. ^{1}H-NMR spectra were recorded either on a JNM-GX-270 spectrometer (JEOL) or on a Varian Unity Inova 599.9 MHz (Varian Inc.) with tetramethylsilane as an internal standard. All chemical shifts (δ) were represented in parts per million (ppm) downfield from the standard. Nuclear Overhauser effect spectroscopy (NOESY) spectra were recorded on a Unity Inova 599.9 MHz (Varian Inc.). Fast-atom-bombardment mass spectra (FAB-MS) were obtained on a JEOL NMS-SX102 spectrometer. Column chromatography was performed using Wakogel C-200 (75–150 μm; Wako Pure Chemical Industries, Ltd.). For analytic thin-layer chromatography (TLC), silica gel 60 F_{254} plates (Merck Ltd.) were used. High-performance liquid chromatography (HPLC) was performed using a JASCO HPLC system for nonradioactive runs or a Hitachi HPLC system (Hitachi High-Technologies Co.) for radioactive runs. The radioactivity was measured by a Tri-Carb 1900TR β-spectrometer (PerkinElmer Japan K.K.). The animal experiments were performed according to the recommendations of the Committee for the Care and Use of Laboratory Animals, National Institute of Radiological Sciences.

Chemistry

All isolated materials were characterized by ^{1}H nuclear magnetic resonance spectroscopy and mass spectrometry.

4′-Thiothymidine ***5***. 4′-Thiothymidine **5** (S-dThd) was synthesized by adapting a procedure described previously (*10,11*). Figure 1 shows a schematic diagram for the synthesis of **5**. Benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-erythro-pentofuranoside **3** was prepared in a 7-step synthesis starting from 2-deoxy-D-erythropentose by the procedure of Dyson et al. (*12*). To a stirred suspension of thymine **1** (570 mg, 4.52 mmol) in dry acetonitrile (25 mL) was added bis(trimethylsilyl)acetamide (1.84 g, 2.23 mL, 9.04 mmol). The mixture was stirred at room temperature for 2 h and was then placed in a nitrogen atmosphere followed by the addition of crushed 4-Å molecular sieves (~0.3 g). The mixture was stirred for 5 min before the addition of benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-erythro-pentofuranoside **3** (1.49 g, 4.52 mmol) in dry acetonitrile (10 mL). Finally, a solution of *N*-iodosuccinimide (NIS) (1.02 g, 4.52 mmol) in dry acetonitrile (13 mL) was added. After 20 h, the reaction was quenched by the addition of saturated aqueous sodium thiosulfate (30 mL) followed by filtration, and the residue was washed with dichloromethane. The organic layer was separated, washed with saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$, and then filtered. The solvent was removed in vacuo and the crude product was purified by column chromatography (silica, ethyl acetate-hexane, 1:1), yielding a syrup of an α-/β-anomeric mixture of 3′,5′-di-*O*-benzyl-4′-thiothymidine (1.3 g, 87%). Separation by preparative liquid chromatography (ethyl acetate-hexane, 4:6) with a prepacked silica gel column [Ultra Pack; 300 × 50 (inner diameter [ID]) mm; Yamazen Co.] provided the pure β-anomer **4** (1.1 g, 57%).

To a solution of 3′,5′-di-*O*-benzyl-4′-thiothymidine **4** (986 mg, 2.25 mmol) in dried toluene (8 mL) was added $TiCl_4$ (0.72 mL, 6.77 mmol) in dry toluene (3 mL). After 90 min, the mixture was cooled in an ice bath, and then methyl ethyl ketone (MEK) (8 mL) and citric acid (1.42 g) in water (10 mL) were added. The organic and aqueous phases were separated and the toluene layer was extracted with water (3 × 10 mL). The combined aqueous extracts were cooled in an ice bath and neutralized with aqueous ammonia, after which the aqueous phase was extracted with MEK (10 × 25 mL). The combined MEK extracts were dried ($MgSO_4$), filtered, and taken to dryness in vacuo. The white solid residue was purified by HPLC (acetonitrile/water/trifluoroacetic acid, 7.5:92.5:0.1, v/v/v) with a semipreparative ODS column (Mightysil RP-18 GP Aqua; 250 × 10 [ID] mm, Kanto Chemical, Co. Inc.), yielding a white solid **5** (65.2 mg, 11.2%). The β-anomer assignment of the compounds was based on its NOESY spectrum. The NOE relationships between H-6 (pyrimidine proton) and H-2′, H-3′, and H-5′ protons were observed, whereas the H′-1 and H-4′ protons, not being in the same plane, were not observed. Further, the NOE relationships between H′-1, H-2′, and H-4′ protons were observed. The NOESY results indicated a β-configuration of the *N*-glycosidic linkage at C-1′ of the thiosugar component. The purity of **5** was assessed by analytic HPLC (retention time, 9.2 min; purity, >99%). HPLC was performed with a C18 (5 μm) analytic column (150 × 4.6 [ID] mm, Mightysil RP-18 GP Aqua; Kanto Chemical, Co., Inc.). Elution was conducted by acetonitrile/water/trifluoroacetic acid (8:92:0.1, v/v/v) at a flow rate of 1 mL/min and monitored at 260 nm.

^{1}H NMR (600 MHz, CD_3OD), δ 7.99 (s, 1H, H-6), 6.41 (t, 1H, H-1′, J = 7.2 Hz), 4.48 (dd, 1H, H-3′, J = 4.0, 7.3 Hz), 3.73–3.81 (m, 2H, H-5′), 3.42 (dd, 1H, H-4′, J = 2.0, 5.3 Hz), 2.32–2.36 (m, 1H, H-2′), 2.23–2.28 (m, 1H, H-2′), 1.90 (s, 3H, CH_3); MS (FAB) m/z [M+H] calcd for $C_{10}H_{15}N_2O_4S$ 259, found 259.

4′-[methyl-^{14}C]Thiothymidine ***7***. 5-Trimethylstannyl-4′-thio-2′-deoxyuridine **6** was synthesized as reported previously (*13*). The rapid methylation of **6** via a palladium-mediated Stille coupling reaction with ^{14}C-methyl iodide was performed by adapting a previously described procedure (Fig. 2) (*14*). A solution of tris(dibenzylideneacetone)dipalladium(0) (0.9 mg, 1 μmol), tri(*o*-tolyl)phosphine (1.2 mg, 4 μmol), and **6** (0.8 mg, 2 μmol) in *N*,*N*-dimethylformamide (DMF) (0.3 mL) in a 5.0-mL oven-dried septum-equipped vial was prepared at room temperature. The

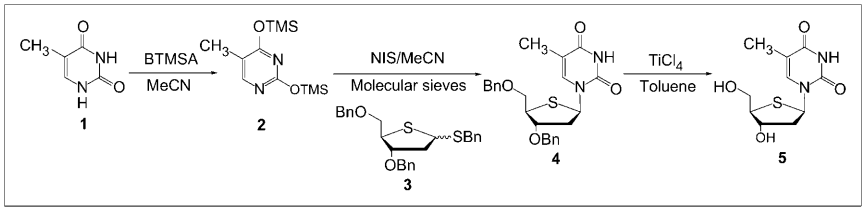

FIGURE 1. Scheme for preparation of 4′-thiothymidine (S-dThd). BTMSA = bis(trimethylsilyl)acetamide; OTMS = trimethylsilyloxy; NIS = *N*-iodosuccinimide.

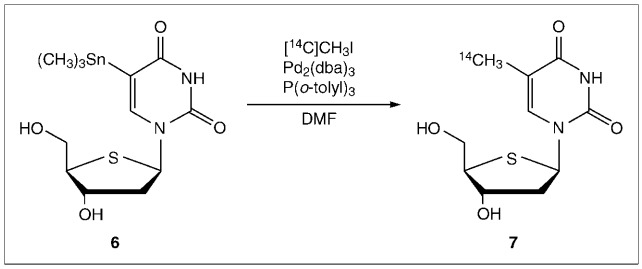

FIGURE 2. Scheme for preparation of [methyl-^{14}C]S-dThd. $Pd_2(dba)_3$ = tris(dibenzylideneacetone)dipalladium(0); P(*o*-tolyl)$_3$ = tri(*o*-tolyl)phosphine.

solution was purged with nitrogen gas for 10 min. Then, a solution of 4.06 MBq (2 μmol) of ^{14}C-methyl iodide in 0.24 mL of DMF was added, and the sealed vial was heated at 130°C in an oil bath. After 5 min, the mixture was cooled in an ice bath, after which water (3.5 mL) was added. The mixture was filtered by prefilter Millex-AP (Millipore Co.) and injected into HPLC with a semi-preparative column (Mightysil RP-18 GP Aqua, 250 × 10 [ID] mm; Kanto Chemical Co. Inc). The elution was started with a mobile phase of acetonitrile/water/trifluoroacetic acid (7.5:82.5:0.1, v/v/v) at a flow of 4.7 mL/min. After 15 min, the mobile phase composition was changed to 80:20:0.1 and kept there for 40 min (to elute lipophilic by-products). The radioactive fraction, eluted with a retention time corresponding to nonradioactive **5** (~9.0 min) was collected. After HPLC purification, the solvents were evaporated at 60°C and dissolved in physiologic saline solution. The radiochemical purity was analyzed by HPLC with a Mightysil RP-18 GP Aqua column (250 × 4.6 [ID] mm; Kanto Chemical Co. Inc.). The column was eluted at 1 mL/min with acetonitrile/water/trifluoroacetic acid (8:92:0.1, v/v/v).

Column-eluted fractions were collected and quantitated by scintillation spectroscopy to measure the amount in the [methyl-^{14}C]S-dThd (~9.0 min) and the amounts of impurities. The radiochemical purity of ([methyl-^{14}C]S-dThd **7** was >99% and the specific activity was 2.04 GBq/mmol (according to the specific activity of ^{14}C-methyl iodide).

Blood Degradation Study

To analyze the degradation of [methyl-^{14}C]S-dThd in blood, we performed a blood degradation study by adapting a previously described procedure (*15*). [methyl-^{14}C]S-dThd (0.18 nmol) or [2-^{14}C]dThd (0.17 nmol), each 0.37 kBq, was added into 1 mL of fresh, heparinized, complete human blood. Samples were incubated at 37°C, and 0.4-mL aliquots were removed at 20, 40, and 60 min and at 5 and 18 h. The samples were mixed with 0.8 mL of 0.5N perchloric acid, vortexed, centrifuged, and filtered. Aliquots were analyzed by HPLC with a Mightysil RP-18 GP Aqua column (250 × 4.6 [ID] mm). The column was eluted at 1 mL/min with acetonitrile/water/trifluoroacetic acid (5:95:0.1, v/v/v) for [2-^{14}C]dThd and acetonitrile/water/trifluoroacetic acid (8:92:0.1, v/v/v) for [methyl-^{14}C]S-dThd. Column-eluted fractions were collected and quantitated by scintillation spectroscopy to measure the amount in the parent compound and the amounts of degradation products.

Distribution Study

Five-week-old BALB/c mice were obtained from Japan SLC Inc. and held for 1 wk before the study. EMT-6 murine mammary carcinoma cell lines were obtained from the American Type Culture Collection and were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Tumor-bearing mice were established by subcutaneous injection of 2×10^5 cells to the right hind leg in 6-wk-old BALB/c mice. The experiments on the tumor-bearing mice were performed at least 10 d after inoculation, by which time the tumors had grown to about 5–10 mm in diameter. A saline solution of 0.1 mL containing 37 kBq of [methyl-^{14}C]S-dThd (0.18 μmol) was administered as a bolus through the tail vein. The mice were sacrificed by cervical dislocation at predetermined time intervals of 5, 15, 30, and 60 min. Four animals were used for each time interval. The tissues were removed afterward and weighed together with the blood samples. Tissues removed included the thymus, blood, lungs, heart, spleen, duodenum, right kidney, left liver lobe, quadriceps muscle, and tumor. The tissues and the blood samples were solubilized with Soluene 350 (PerkinElmer Japan K.K.) at 50–60°C for 4–5 h, and then ^{14}C activity was measured by a Tri-Carb 1900TR β-spectrometer (PerkinElmer Japan K.K.). All scintillation counts were corrected for quenching by the external standard method, and the results were expressed as a percentage of injected dose per gram tissue (%ID/g) without body weight normalization.

Tissue extraction was also performed as described previously (*16*). For extracted tissues, the activity levels present in the acid-soluble fraction, RNA, DNA, and protein were quantitated. The protein fraction, which also contained lipid, was obtained by solubilization of the residual pellet after removal of all fractions.

Effect of DNA Synthesis Inhibitor

2-Fluoro-2′-deoxycytidine (dFdC) (Gemcitabine; Eli Lilly), a potent inhibitor of DNA synthesis, was used to modulate cell proliferation as described previously (*17*). The dFdC was injected intraperitoneally at a dose of 5.0 or 40 mg/kg to inhibit DNA synthesis. The animals were divided into 3 groups. The first group, considered as a control, received no dFdC. The second group was treated with 5.0 mg/kg dFdC for 30 min before [methyl-^{14}C]S-dThd injection, and the third group received 40 mg/kg dFdC for 3 h before [methyl-^{14}C]S-dThd injection. In all 3 groups, the animals were sacrificed at 60 min after [methyl-^{14}C]S-dThd injection. The spleen, blood, and tumor were removed and processed to determine radioactivity as described. To determine whether the dFdC treatment altered blood flow in the spleen and tumor, a separate group of tumor-bearing animals was used to assess tissue blood flow by the uptake of 4-[*N*-methyl-^{14}C]IAP by adapting a previously described procedure (*18*). The animals were sacrificed 40 s after injection of 4-[*N*-methyl-^{14}C]IAP. The groups were compared statistically using the ANOVA program in Statcel for Windows (OMS Publishing Inc.). The Fisher protected least significance test was used to check for significance at the 5% level.

RESULTS

Chemistry

2,4-Bis(trimethylsilyloxy)thymine **2** was prepared by silylating thymine **1** with bis(trimethylsilyl)acetamide (BTMSA), which was then reacted in situ with benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-erythro-pentofuranoside **3** using NIS to activate the thiobenzyl glycoside. An α-/β-anomeric mixture of 3′,5′-di-*O*-benzyl-4′-thiothymidine **4** was obtained in good yield (87%). Separation by preparative liquid chromatography with a prepacked silica gel column

provided the pure β-anomer (57%). Debenzylation of the β-anomer was successfully deprotected in 33% yield with $TiCl_4$ in toluene, to give an overall yield of 4′-thiothymidine **5** (S-dThd) of 5.6%.

The rapid methylation of 5-trimethylstannyl-4′-thio-2′-deoxyuridine **6** via a palladium-mediated Stille coupling reaction with ^{14}C-methyl iodide was performed by adapting a previously described procedure (Fig. 2) (*14*). The 4′-[methyl-^{14}C]thiothymidine **7** ([methyl-^{14}C]S-dThd) was obtained in a 31%–41% radiochemical yield (calculated from ^{14}C-methyl iodide) at 130°C, 5-min reaction in DMF. The purification of [methyl-^{14}C]S-dThd **7** was performed by HPLC within 10 min.

Blood Degradation Studies

As expected from previous reports (*13,19*), [methyl-^{14}C]S-dThd was rather stable and more resistant to catabolism than [2-^{14}C]dThd. Incubation with human blood demonstrated rapid degradation of [2-^{14}C]dThd, which was cleaved to thymine (Fig. 3). In contrast, [methyl-^{14}C]S-dThd was stable, with <3% degradation in 60 min.

Distribution Study

As shown in Figure 4, the in vivo distribution study showed the accumulation of radioactivity in proliferating tissues (spleen, thymus, duodenum, and tumor). On the other hand, the radioactivity of nonproliferating tissues (lung, liver, kidney, and muscle) was cleared rapidly in parallel with the clearance of blood radioactivity. The tumor uptake of radioactivity was high (8.8 ± 1.2 %ID/g at 60 min) and selective (tumor-to-blood ratio, 12.2 ± 5.9 at 60 min). The tissue extraction studies showed that most of the radioactivity was present in the DNA fractions of the rapidly proliferating tissues, such as the thymus, spleen, and duodenum (Fig. 5).

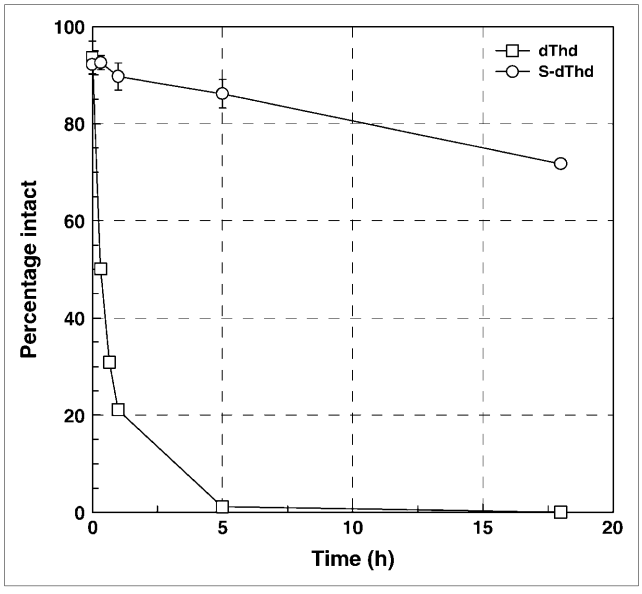

FIGURE 3. Degradation of [2-^{14}C]dThd and [methyl-^{14}C]S-dThd in human blood.

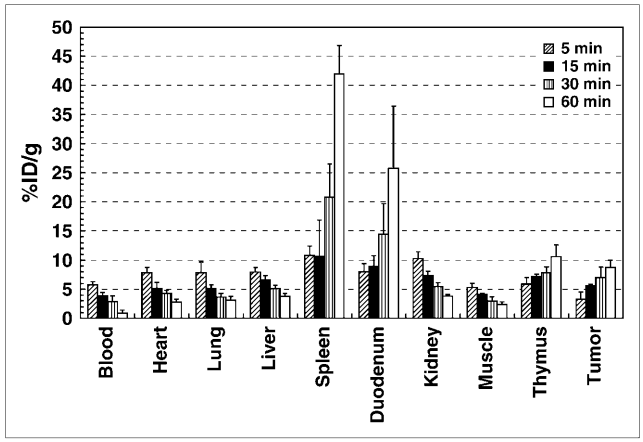

FIGURE 4. Distribution of [methyl-^{14}C]S-dThd in tumor-bearing mice. Results (%ID/g) are expressed as mean ± SD (n = 4).

On the other hand, in the tissues with low rates of cell turnover, such as liver, most of the radioactivity was present in the acid-soluble fraction. In the tumor tissues, much of activity was present in the DNA and acid-soluble fractions.

Effect of DNA Synthesis Inhibitor

As shown in Figure 6A, dFdC pretreatment significantly reduced the spleen and tumor uptake of [methyl-^{14}C]S-dThd. At 5.0 mg/kg treatment, the spleen and tumor uptake of [methyl-^{14}C]S-dThd showed 4.1-fold and 1.6-fold decrease, respectively. Moreover, at 40 mg/kg treatment, the spleen and tumor uptake of [methyl-^{14}C]S-dThd showed 2.5-fold and 3.1-fold decrease, respectively. The uptake of 4-[*N*-methyl-^{14}C]IAP did not differ between the treated and control groups (Fig. 6B).

DISCUSSION

In this study we successfully synthesized and fundamentally evaluated [methyl-^{14}C]S-dThd. In particular, the higher stability and rapid DNA-incorporating nature of

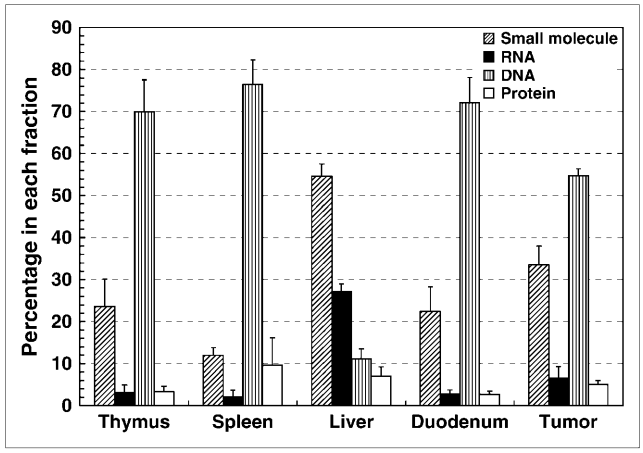

FIGURE 5. Extraction of ^{14}C activity in tissues of EMT-6 tumor–bearing mice at 60 min after injection of [methyl-^{14}C]S-dThd. Results (percentage of ^{14}C activity in each fraction) are expressed as mean ± SD (n = 5).

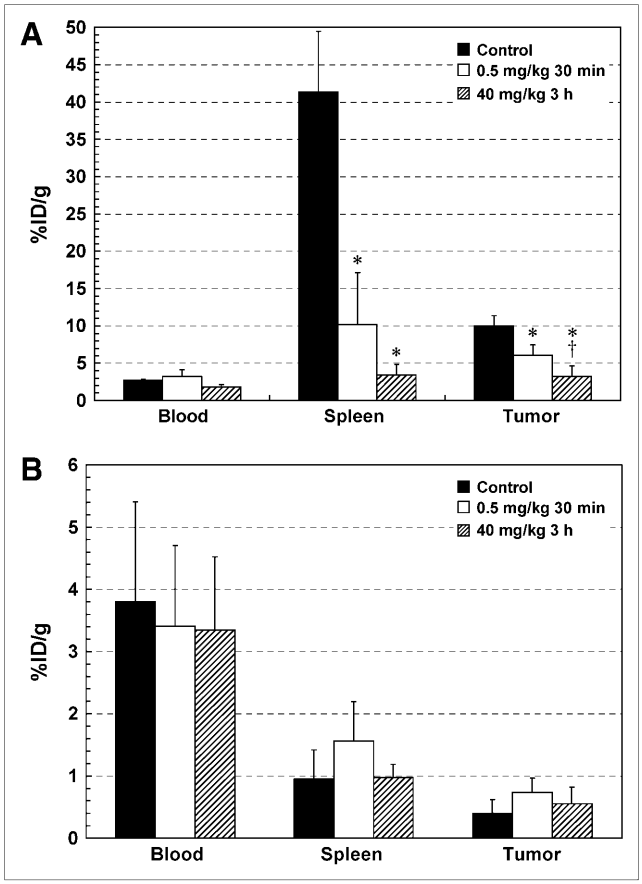

FIGURE 6. Effect of dFdC on [methyl-^{14}C]S-dThd (A) and 4-[*N*-methyl-^{14}C]IAP (B) uptake in spleen and tumor tissues (n = 5). Results (%ID/g) are expressed as mean ± SD (n = 4 or 5). *$P < 0.05$ vs. corresponding control. †$P < 0.05$ vs. corresponding 0.5 mg/kg treatment for 30 min.

[methyl-^{14}C]S-dThd caused a selective high uptake in the proliferating tissues.

We adopted a previously described palladium-mediated coupling reaction between trialkyltin compounds and methyl iodide for the radiosynthesis of [methyl-^{14}C]S-dThd. This reaction takes place under neutral conditions (*14*,*20*). Therefore, under these mild conditions, there is no need to protect the hydroxyl groups. In accordance with these previous descriptions, the radiosynthesis of [methyl-^{14}C]S-dThd was successfully and rapidly performed in a 1-pot reaction. HPLC purification was also successfully performed within 10 min. Therefore, we considered that the labeling and purification procedures were rapid and simple enough for ^{11}C-methyl iodide labeling and would be suitable for automation.

Distribution studies showed high levels of radioactivity accumulated in proliferating tissues, such as the thymus, spleen, duodenum, and tumor. The time-course studies demonstrated that [methyl-^{14}C]S-dThd worked differently between the proliferating and nonproliferating tissues. The proliferating tissues progressively accumulated radioactivity, which is consistent with a sustained input of [methyl-^{14}C]S-dThd. Blood degradation studies also support the in vivo stability of [methyl-^{14}C]S-dThd. Moreover, tissue extraction studies also showed that a dominant portion of tissue radioactivity was incorporated into DNA in the proliferating tissues. In contrast, the washout of [methyl-^{14}C]S-dThd by the nonproliferating tissues appears nearly exponential. In this situation, there is no incorporation of the radioactivity into DNA. A previous study reported that DNA-incorporated S-dThd was not removed from the DNA for up to 72 h (*9*). These data might indicate that the use of S-dThd may preclude the consideration of a loss of established activity in cells (reversible nucleotide metabolism within the salvage pathway) in the model. This situation may allow us to use a simple 3-compartment kinetic model to calculate a DNA synthesis rate constant.

Pretreatment of animals with the DNA synthesis inhibitor dFdC reduced the uptake of proliferating tissues markedly to a level similar to that of nonproliferating tissues, such as heart, lung, liver, kidney, and muscle. Although 4-[*N*-methyl-^{14}C]IAP, like almost all blood-flow tracers, has its practical and theoretic limitations, we believe that it provides reasonable, qualitative data under conditions of use. The uptake of 4-[*N*-methyl-^{14}C]IAP did not differ between the treated and control groups; hence, there was no positive correlation between the change in [methyl-^{14}C]S-dThd uptake and the change in relative blood flow. Moreover, the blood radioactivity level of 4-[*N*-methyl-^{14}C]IAP did not differ between the treated and control groups; hence, there was no difference of the initial tumor delivery of the tracer. Therefore, we considered normal blood flow to have little influence on the ultimate distribution of [methyl-^{14}C]S-dThd.

Previous work from our group suggested that the dThd analog 5-^{125}I-iodo-4′-thio-2′-deoxyuridine (^{125}I-ITdU) could be used as a tracer for DNA synthesis (*13*,*19*). Although ^{125}I-ITdU exhibited a high resistance to the glycosidic bond cleavage reaction, the specific and significant uptake in proliferating tissues needs greater washout and clearance of the exchangeable fraction of background activity because of the small number of ^{125}I-iodide metabolites. On the other hand, the specific and significant uptake of [methyl-^{14}C]S-dThd was rapid (within 60 min). This might be attributed to the rather stable ^{14}C-C–C bonding of [methyl-^{14}C]S-dThd as compared with the ^{125}I-I–C bonding of ^{125}I-ITdU. In addition, the higher Michaelis–Menten constant/maximum velocity ratio (V_{max}/K_m ratio) of S-dThd to cytosolic thymidine kinase (13% that of dThd) also might cause early specific significant uptake (*9*).

The measurement of tumor growth and that of DNA synthesis are attractive imaging targets in oncology. Imaging with ^{11}C-dThd is of great interest, as it is the native pyrimidine base used in DNA synthesis. Although [2-^{11}C]dThd is useful in research and validation studies, the practical limitations of this tracer will preclude it from gaining wide acceptance, and it has been evaluated at only a few research centers. The arguments against the widespread

clinical use of [2-^{11}C]dThd PET include the combination of short physical half-life, the complexity of its synthetic route, the duration of synthesis, the number of different metabolites, and the complexity of data analysis. Consequently, recent research has focused on dThd analogs that are radiolabeled with the longer-lived ^{18}F, have a rapidly DNA-incorporating nature, and are resistant to degradation. However, we considered that, in spite of a short physical half-life, a dThd analog that might prove simpler to use for imaging DNA synthesis and that might follow the same biochemistry as dThd would be useful for specific clinical situations, such as discrimination of early clinical response in chemotherapy or radiotherapy. Our results suggest that [methyl-^{11}C]S-dThd closely resembles the biochemistry (though this resemblance might be merely anabolic) of dThd and might be used as an alternative to [2-^{11}C]dThd. In addition, [methyl-^{11}C]S-dThd synthesis would be simple and short enough for the short physical half-life of ^{11}C.

One limitation of the study of dThd analogs in mice is the major differences in metabolism and uptake seen in different species (*21,22*). Studies in mice are not necessarily very predictive of results in other mammals or humans. For that reason, ^{11}C labeling, pharmacokinetic, and dosimetric studies should be performed in animal models closer to human beings, such as dogs or monkeys. Further studies will be needed to evaluate the strengths and limitations of [methyl-^{11}C]S-dThd to determine the clinical situations in which it can be used.

CONCLUSION

The labeling procedure was rapid and was found to be suitable for ^{11}C labeling. Positron-labeled 4′-thiothymidine **5** (S-dThd) should be useful for imaging DNA synthesis by PET.

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