Evaluation of 3′-Deoxy-3′-^{18}F-Fluorothymidine for Monitoring Tumor Response to Radiotherapy and Photodynamic Therapy in Mice

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3′-Deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT) has been suggested as a new PET tracer for imaging tumor proliferation. We investigated the use of ^{18}F-FLT to monitor the response of tumors to radiotherapy and photodynamic therapy (PDT) in mice. **Methods:** C_3H/He mice bearing an SCCVII tumor were treated with single-dose x-ray irradiation of 20 Gy. Tumor uptake was examined for ^{18}F-FLT, ^{3}H-thymidine (^{3}H-Thd), ^{18}F-FDG, and ^{14}C-deoxyglucose (^{14}C-DG) at 6 h, 12 h, 24 h, 3 d, and 7 d after radiotherapy. BALB/c *nu/nu* mice bearing a HeLa tumor were treated with PDT. Tumor uptake was examined for the 4 tracers at 24 h after PDT. Expression of proliferating cell nuclear antigen (PCNA) was determined in untreated and treated tumors. **Results:** In the biodistribution study, considerable uptake of ^{18}F-FLT was observed in both tumor types. Tumor volumes decreased to 39.3% ± 22.4% at 7 d after radiotherapy. The PCNA labeling index was reduced in x-ray–irradiated tumors (control, 53.2% ± 8.7%; 6 h, 38.5% ± 5.3%; 24 h after radiotherapy, 36.8% ± 5.3%). ^{18}F-FLT uptake in tumor expressed as the percentage of the injected dose per gram of tumor (%ID/g) decreased significantly at 6 h and remained low until 3 d after radiotherapy (control, 9.7 ± 1.2 %ID/g; 6 h, 5.9 ± 0.4 %ID/g; 24 h, 6.1 ± 1.3 %ID/g; 3 d after radiotherapy, 6.4 ± 1.1 %ID/g). ^{18}F-FDG uptake tended to gradually decrease but a significant decrease was found only at 3 d (control, 12.1 ± 2.7 %ID/g; 6 h, 13.3 ± 2.3 %ID/g; 24 h, 8.6 ± 1.8 %ID/g; 3 d after radiotherapy, 6.9 ± 1.2 %ID/g). PDT resulted in a reduction of the PCNA labeling index (control, 82.0% ± 8.6%; 24 h after PDT, 13.5% ± 12.7%). Tumor uptake of ^{18}F-FLT decreased (control, 11.1 ± 1.3 %ID/g; 24 h after PDT, 4.0 ± 2.2 %ID/g), whereas ^{18}F-FDG uptake did not decrease significantly after PDT (control, 3.5 ± 0.6 %ID/g; 24 h after PDT, 2.3 ± 1.1 %ID/g). Changes in the uptake of ^{18}F-FLT and ^{18}F-FDG were similar to those of ^{3}H-Thd and ^{14}C-DG, respectively. **Conclusion:** In our model system, changes in ^{18}F-FLT uptake after radiotherapy and PDT were correlated with those of ^{3}H-Thd and the PCNA labeling index. The decrease in ^{18}F-FLT uptake after treatments was more rapid or pronounced than that of ^{18}F-FDG. Therefore, ^{18}F-FLT may be a feasible PET tracer for monitoring response to therapy in oncology.

Key Words: 3′-deoxy-3′-^{18}F-fluorothymidine; PET; radiotherapy; photodynamic therapy

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Deregulated proliferation is one of the key features of malignant tumors and imaging of tumor proliferation is expected to improve the management of patients with cancer. Morphologic imaging techniques, which have been the standard method to identify treatment efficacy, depict tumor response as changes in tumor size and composition. Changes in size, however, are often delayed and it is difficult to evaluate early response to treatment by morphologic imaging techniques.

PET can aid in this task, because metabolic and physiologic changes precede size change. The fluorine-labeled glucose analog ^{18}F-FDG has been the most widely used agent in PET tumor imaging. However, increased glycolysis is not an essential property of proliferating cells and ^{18}F-FDG is taken up by inflammatory cells such as macrophages (*1*). Therefore, considerable efforts have been invested in seeking more suitable PET tracers for imaging tumor proliferation (*2*). Recently, a fluorine-labeled thymidine analog, 3′-deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT), has been developed as a candidate for imaging cell proliferation. ^{18}F-FLT is phosphorylated by thymidine kinase 1, the key enzyme of the pyrimidine salvage pathway of DNA synthesis, and metabolically trapped as a phosphorylated form (*3*).

^{18}F-FLT was firstly applied for PET in 1998 (*4*) and, since then, ^{18}F-FLT has been shown to accumulate in a variety of tumors and its uptake could reflect the tumor proliferation (*4–9*). However, there have been few reports on the use of ^{18}F-FLT for monitoring tumor response to therapy.

The aim of this study was to investigate the use of ^{18}F-FLT for monitoring response to anticancer treatments in mouse models. We clarified that the change of ^{18}F-FLT

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uptake after radiotherapy or photodynamic therapy (PDT) correlated well with the proliferative activity of transplanted tumors.

MATERIALS AND METHODS

Radiopharmaceuticals

^{18}F-FLT and ^{18}F-FDG were produced by the Central Research Laboratory, Hamamatsu Photonics K.K. ^{18}F-FLT was synthesized by nucleophilic substitution of nosylate precursor (precursor FLT [1-(2-deoxy-3-*o*-(4-nitrobenzenesulfonyl)-5-*o*-(4,4′-dimethoxytrityl)-D-threopentofuranosyl)-3-(2,4-dimethoxybenzyl)thymine]) according to the method developed by Grierson and Shields with minor modification (*10*). ^{18}F-Fluoride was produced with the cyclotron, using the ^{18}O (p, n)^{18}F nuclear reaction by irradiation of a water target containing ^{18}O-enriched water. ^{18}F-FDG was synthesized with an automated FDG synthesis module. Methyl-^{3}H-thymidine ([^{3}H-Thd]; specific activity, 2.22–3.2 TBq/mmol) and 2-deoxy-D-1-^{14}C-glucose ([^{14}C-DG]; specific activity, 1.85–2.29 GBq/mmol) were purchased from Amersham Biosciences Corp.

Animals and Tumor Models

Five- to 7-wk-old female C_3H/He mice (Japan SLC, Inc.) and BALB/c *nu/nu* mice (CREA Japan, Inc.) were used. Subcutaneous tumors were established in the thigh of C_3H/He mice with SCCVII, a murine squamous cell carcinoma cell line, and in the thigh of BALB/c *nu/nu* mice with HeLa, a human uterine cervical adenocarcinoma cell line. When the size of tumor reached 6–10 mm in diameter at 10–14 d after the injection of 5×10^6 cells, the mice were used for experiments. In all animal experiments, the mice were not anesthetized after tracer injection. The experimental protocol was fully accredited by the laboratory Animal Care Committee of the Hamamatsu University School of Medicine.

^{18}F-FLT Uptake in Untreated Tumor and Normal Tissue

The biodistribution of ^{18}F-FLT was assessed in untreated tumor-bearing mice. Twelve C_3H/He mice transplanted with SCCVII and 4 BALB/c *nu/nu* mice transplanted with HeLa were injected intravenously with 1.8–2.5 MBq ^{18}F-FLT via lateral tail vein. SCCVII-bearing mice were sacrificed at 0.5, 1, and 2 h after radiotracer injection. HeLa-bearing mice were sacrificed at 1 h. Blood, normal tissue (heart, lung, kidney, spleen, muscle, femur, small intestine), and tumor samples were rapidly excised. All samples were weighed, and the radioactivity was measured in an auto-well γ-counter (Aloka ARC-2000), applying a decay correction. Accumulation of tracers in tumor or normal tissues was expressed as the percentage of the injected dose per gram of tumor per 20 g of mouse weight (%ID/g).

X-Ray Irradiation

C_3H/He mice transplanted with SCCVII were anesthetized with 1 mg sodium pentobarbital intraperitoneally and then fixed with adhesive tape to place the tumor-bearing thigh in the field of irradiation. The other parts of the body were left outside of the radiation field. Tumors were exposed to a single dose of 20 Gy at a dose rate of 2.25 Gy/min.

Changes in Tumor Volume After Radiotherapy

Eight C_3H/He mice transplanted with SCCVII were randomly assigned to 2 groups: 1 treatment group and 1 control group. Each group consisted of 4 mice. From the day of the irradiation, tumor size was determined with a caliper measuring the largest diameter (a) and the perpendicular diameter (b). Tumor volume was calculated according to the formula $0.5 \times a \times b^2$, assuming an elliptic geometry. Growth curves were generated as a change of relative tumor volume based on the volume on the day of irradiation.

PDT

BALB/c *nu/nu* mice transplanted with HeLa were injected intravenously with 6 mg/kg of ATX-S10(Na) (13,17-bis[1-carboxypropionyl]carbamoylethyl-8-ethenyl-2-hydroxy-3-hydroxyiminoethylidene-2,7,12,18-tetramethylporphyrin sodium salt) via tail vein. ATX-S10(Na) is a second-generation photosensitizer, which was developed to reduce the hyperphotosensitivity of porfimer sodium (*11*). Three hours later, the mice were restrained for light exposure. A 10- to 14-mm-diameter area encompassing the tumor was irradiated with a semiconductor diode laser of 670-nm wavelength (LD670C; Hamamatsu Photonics K.K.) at a fluence of 100 J/cm^2. Tumors were inspected until 7 d after PDT in 5 mice.

Expression of Proliferating Cell Nuclear Antigen (PCNA)

Untreated SCCVII and HeLa tumors, SCCVII tumors at 6 and 24 h after radiotherapy, and HeLa tumors at 24 h after PDT were fixed in formalin, embedded in paraffin, and cut into 5.0-μm sections. Tumors were obtained from 4 mice in each group. One section per tumor was examined. Sections were incubated with biotinylated PCNA monoclonal antibody (Zymed Laboratory Inc.); streptoavidin peroxidase was used as a signal generator and diaminobenzidine tetrahydrochloride was used as a choromogen to stain PCNA-containing nuclei a dark brown. All sections were counterstained with hematoxylin for counting the total cell number. In each run, sections of small intestine were stained as positive controls and sections of brain were used as negative controls. First, the PCNA-stained section was scanned at low power to select areas exhibiting a high concentration of PCNA-positive cells and high cellular density. Then, the numbers of PCNA-positive and hematoxylin-positive cells were counted in 5 randomly selected fields of view per section using a BH2 microscope (Olympus Optical) at ×400 magnification. At least 500 cells were counted in each field. The PCNA labeling index was established as the percentage of PCNA-positive cells.

Tumor Uptake of Radiopharmaceuticals After Radiotherapy

In SCCVII-bearing mice, tumor uptake of ^{18}F-FLT or ^{18}F-FDG was assessed at 6 h, 12 h, 24 h, 3 d, and 7 d after radiotherapy. Untreated mice served as controls. Twenty-seven mice were used for ^{18}F-FLT (untreated control, $n = 4$; 6 h, $n = 5$; 12 h, $n = 5$; 24 h, $n = 4$; day 3, $n = 5$; day 7, $n = 4$). Twenty-nine mice were used for ^{18}F-FDG (untreated control, $n = 5$; 6 h, $n = 5$; 12 h, $n = 5$; 24 h, $n = 5$; day 3, $n = 5$; day 7, $n = 4$). The mice received 1.8–2.5 MBq ^{18}F-FLT or 1.8–2.5 MBq ^{18}F-FDG.

The uptake of ^{3}H-Thd and ^{14}C-DG in x-ray–irradiated tumor was determined by the dual-tracer technique. A mixture of 0.0925 MBq ^{3}H-Thd and 0.037 MBq ^{14}C-DG was administered to untreated mice and to mice at 6 h, 12 h, 24 h, 3 d, and 7 d after radiotherapy. Thirty mice were used for this dual-tracer experiment (untreated control, $n = 6$; 6 h, $n = 4$; 12 h, $n = 5$; 24 h, $n = 5$; day 3, $n = 5$; day 7, $n = 5$).

The mice were killed at 1 h after radiotracer injection. Tumors were rapidly excised and weighed. Radioactivity was measured in an auto-well γ-counter for ^{18}F and in a liquid scintillation counter

using the double-window technique for ^{3}H and ^{14}C. Tumor uptake of radiotracers was calculated as in the biodistribution study.

Tumor Uptake of Radiopharmaceuticals After PDT

Tumor uptake of ^{18}F-FLT, ^{18}F-FDG, ^{3}H-Thd, and ^{14}C-DG was measured in HeLa-bearing BALB/c *nu/nu* mice at 24 h after PDT. Untreated mice served as controls. Eight mice were used for ^{18}F-FLT (control, $n = 4$; PDT, $n = 4$). Eight mice were used for ^{18}F-FDG (control, $n = 4$; PDT, $n = 4$). Twelve mice were used for the dual-tracer experiment of ^{3}H-Thd and ^{14}C-DG (control, $n = 6$; PDT, $n = 6$). The administered dose of radiopharmaceuticals and the methods for evaluating tumor uptake were the same as those in the experiments for radiotherapy.

Statistical Analysis

Statistical analysis was performed with SPSS for Windows software, version 11.0.1 (SPSS, Inc.). All data are expressed as mean ± SD. The differences between untreated controls and x-ray–irradiated groups with respect to radiotracer uptake and the PCNA labeling index were analyzed by the Kruscal–Wallis test with adjustment by the Bonferroni method for multiple comparisons. $P < 0.05$ was considered statistically significant.

Differences between untreated controls and PDT-treated groups with respect to radiotracer uptake and the PCNA labeling index were tested with the Mann–Whitney Wilcoxon test. $P < 0.05$ was considered statistically significant.

RESULTS

^{18}F-FLT Uptake in Untreated Tumor and Normal Tissue

Because tumor uptake of ^{18}F-FLT in SCCVII at 1 h after injection was not significantly different from that at 2 h (data not shown), we conducted the following experiments with ^{18}F-FLT at the 1-h time point. Figure 1 shows the tissue distribution of ^{18}F-FLT in SCCVII-bearing C_3H/He mice and in HeLa-bearing BALB/c *nu/nu* mice at 1 h after injection. High radioactivity was noted in the kidney and small intestine as well as the tumors. Splenic uptake was also high in C_3H/He mice.

Radiotherapy and Tumor Uptake of ^{18}F-FLT in SCCVII-Bearing Mice

The growth curve of SCCVII tumor in untreated and x-ray–irradiated mice is shown in Figure 2. Radiotherapy with 20 Gy resulted in tumor shrinkage and the relative tumor volume decreased to 39.3% ± 22.4% at 7 d after radiation. Tumors grew again after 14 d and the relative volume at day 21 was 98.6% ± 94.1%. There was a significant decrease in the PCNA labeling index in tumors obtained at 6 and 24 h after radiotherapy (control, 53.2% ± 8.7%; 6 h, 38.5% ± 5.3%; 24 h, 36.8% ± 5.3%, $P < 0.05$; Table 1).

Tumor uptake of ^{18}F-FLT decreased significantly at 6 h after radiotherapy and remained low until 3 d ($P < 0.05$; Fig. 3). At 7 d after x-ray irradiation, there was a tendency for ^{18}F-FLT uptake to increase (control, 9.7 ± 1.2 %ID/g; 6 h, 5.9 ± 0.4 %ID/g; 12 h, 6.2 ± 0.6 %ID/g; 24 h, 6.1 ± 1.3 %ID/g; day 3, 6.4 ± 1.1 %ID/g; day 7, 9.3 ± 3.1 %ID/g). A decrease in ^{3}H-Thd uptake was observed at all time points (control, 9.0 ± 0.7 %ID/g; 6 h, 5.5 ± 0.4 %ID/g; 12 h, 5.9 ± 0.8 %ID/g; 24 h, 4.3 ± 0.3 %ID/g; day 3, 4.0 ± 0.3 %ID/g; day 7, 5.3 ± 0.6 %ID/g). The uptake of ^{18}F-FDG and ^{14}C-DG tended to gradually decrease. A statistically significant decrease in tumor uptake of ^{18}F-FDG was found only at 3 d (control, 12.1 ± 2.7 %ID/g; 6 h, 13.3 ± 2.3 %ID/g; 12 h, 9.8 ± 1.5 %ID/g; 24 h, 8.6 ± 1.8 %ID/g; day 3, 6.9 ± 1.2 %ID/g; day 7, 8.1 ± 2.1 %ID/g)

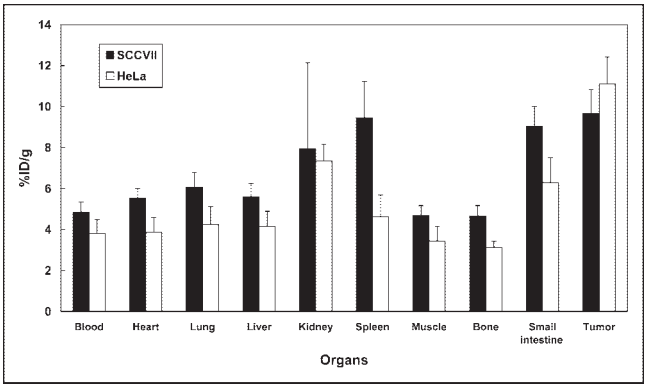

FIGURE 1. Biodistribution of ^{18}F-FLT in C_3H/He mice transplanted with SCCVII and in BALB/c *nu/nu* mice transplanted with HeLa at 1 h after injection. Data are expressed as mean ± SD.

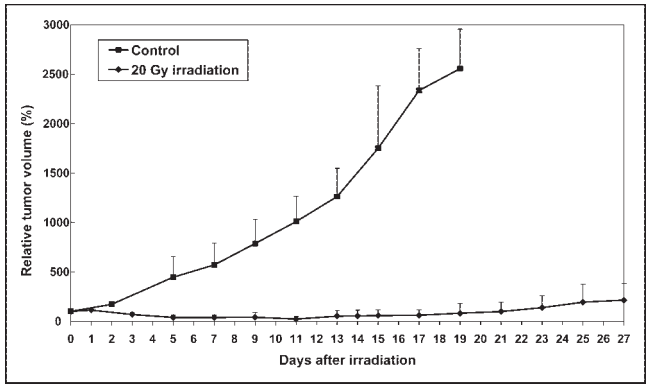

FIGURE 2. Changes in relative tumor volume in control mice and x-ray–irradiated mice. Mice bearing SCCVII received 20 Gy at day 0. Data are expressed as mean ± SD. Radiotherapy resulted in tumor shrinkage and no tumor regrowth was found until 14 d after radiation. Tumors grew again after 14 d.

TABLE 1
PCNA Labeling Index in Tumors After Radiotherapy and PDT

Group	SCCVII tumor treated with 20-Gy irradiation	HeLa tumor treated with PDT
Control	53.2 ± 8.7	82.0 ± 8.6
6 h	38.5 ± 5.3*	ND
24 h	36.8 ± 5.3*	13.5 ± 12.7*

*$P < 0.05$ vs. untreated control group.
ND = not done.
Data are expressed as mean ± SD.

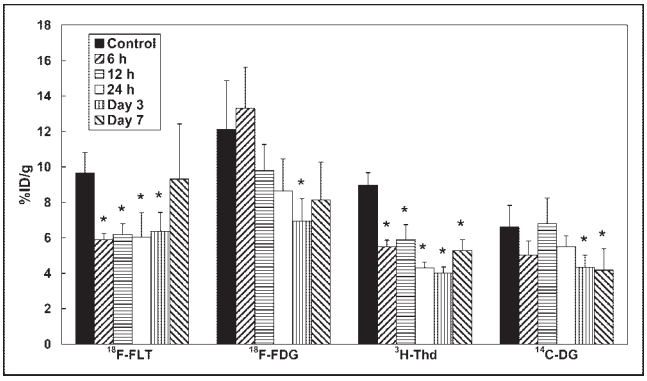

FIGURE 3. Tumor uptake of 4 tracers after radiotherapy. Data are expressed as mean ± SD. Asterisks indicate statistically significant differences compared with untreated controls ($P < 0.05$). Tumor uptake of ^{18}F-FLT decreased significantly at 6 h, 12 h, 24 h, and 3 d after radiotherapy compared with untreated controls. There was a significant decrease in ^{3}H-Thd uptake at 6 h, 12 h, 24 h, 3 d, and 7 d compared with untreated controls. Tumor uptake of ^{18}F-FDG and ^{14}C-DG did not show a statistically significant decrease at 6, 12, and 24 h after radiotherapy.

and that of ^{14}C-DG at 3 and 7 d after radiotherapy (control, 6.6 ± 1.3 %ID/g; 6 h, 5.0 ± 0.8 %ID/g; 12 h, 6.8 ± 1.4 %ID/g; 24 h, 5.5 ± 0.6 %ID/g; day 3, 4.3 ± 0.7 %ID/g; day 7, 4.2 ± 1.2 %ID/g).

PDT and Tumor Uptake of ^{18}F-FLT in HeLa-Bearing Mice

Massive edema was observed at 24 h after PDT. On the third day, tumors shrunk and their color turned blackish-brown, which represented necrosis. The PCNA labeling index decreased significantly at 24 h after PDT (control, 83.2% ± 8.6%; PDT, 13.5% ± 12.7%, $P < 0.05$; Table 1). A significant decrease in ^{18}F-FLT or ^{3}H-Thd uptake was observed in PDT-treated tumors (control, 11.1 ± 1.3 %ID/g; PDT, 4.0 ± 2.2 %ID/g for ^{18}F-FLT; control, 9.5 ± 1.0 %ID/g; PDT, 6.5 ± 0.9 %ID/g for ^{3}H-Thd, $P < 0.05$; Fig. 4). There was no significant difference between untreated controls and PDT-treated tumors in the uptake of ^{18}F-FDG and ^{14}C-DG (control, 3.5 ± 0.6 %ID/g; PDT, 2.3 ± 1.1 %ID/g for ^{18}F-FDG; control, 2.1 ± 0.6 %ID/g; PDT, 2.1 ± 0.5 %ID/g for ^{14}C-DG).

DISCUSSION

This study demonstrated that ^{18}F-FLT uptake by transplanted tumors showed a rapid response to radiotherapy and PDT preceding objective tumor shrinkage. Changes in ^{18}F-FLT uptake were similar to those of ^{3}H-Thd except at 7 d after radiotherapy. ^{3}H-Thd is rapidly incorporated into DNA and has been widely used as a marker of cell proliferation. Although ^{18}F-FLT is not incorporated into DNA, ^{18}F-FLT would be a reliable marker for cell proliferation as well as ^{3}H-Thd. There was a tendency for ^{18}F-FLT uptake to increase at 7 d after x-ray irradiation. Tumor size remained stable for a further 7 d and only a mild increase in tumor size was observed 21 d after radiotherapy. Further examination is needed to clarify the role of ^{18}F-FLT as an indicator of tumor regrowth.

^{18}F-FLT uptake in tumor was also validated by comparison with the PCNA labeling index. PCNA is a 36-kDa nuclear polypeptide that is related to the cell proliferation (*12*). A previous study indicated that PCNA, synthesized during the late G_1-to-S phase, is an auxiliary for DNA polymerase (*13*). Correlation between ^{18}F-FLT uptake and the PCNA labeling index after radiotherapy and PDT also suggests the usefulness of ^{18}F-FLT for monitoring cell proliferation.

PDT is a new treatment modality for solid tumors. The procedure consists of the administration of a photosensitizer that accumulates preferentially in the tumor, followed by local illumination of neoplastic tissues with red light (*14,15*). PDT has been shown to induce tumor necrosis through initial vascular damage and to direct tumor cell killing induced by singlet oxygen (*16*). Because ^{18}F-FLT is useful for monitoring the effect of PDT, which has a different antitumor mechanism from radiotherapy, ^{18}F-FLT could be applicable to other types of anticancer therapy.

In C_3H/He mice, high uptake was found in the spleen and small intestine, which are organs with a high proliferative activity in mice. High uptake in the kidneys suggests a renal excretion of ^{18}F-FLT. Uptake in the spleen was not high in BALB/c *nu/nu* mice in comparison with C_3H/He mice. Distribution of ^{18}F-FLT in normal tissues may be different among strains of mice.

A decrease in tumor uptake of ^{18}F-FDG was found only 3 d after radiotherapy, and ^{18}F-FDG uptake did not decrease significantly after PDT. ^{14}C-DG showed a change similar to that of ^{18}F-FDG. These results may be attributable to a minimal change in glycolysis in tumor cells or an increase in glucose uptake by inflammatory tissues early after radiotherapy or PDT. Work with animal tumors and cultured cells has suggested that radiolabeled

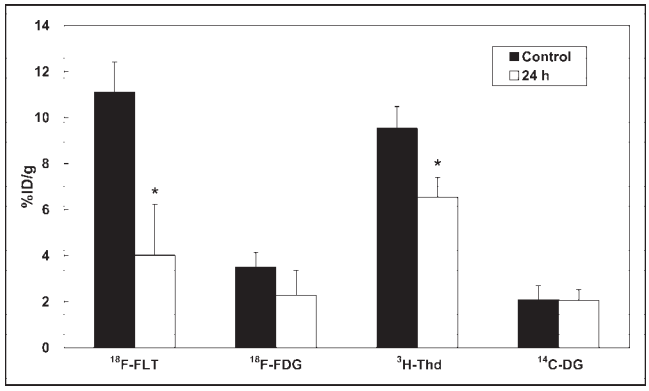

FIGURE 4. Tumor uptake of 4 tracers at 24 h after PDT. Data are expressed as mean ± SD. Asterisks indicate statistically significant differences compared with untreated controls ($P < 0.05$). A significant decrease of tumor uptake of ^{18}F-FLT and ^{3}H-Thd was observed in PDT-treated tumors. There was no significant difference between untreated controls and PDT-treated tumors in the uptake of ^{18}F-FDG and ^{14}C-DG.

thymidine is a better indicator of cell proliferation than ^{18}F-FDG (*17,18*). Furthermore, Barthel et al. showed an early decrease in ^{18}F-FLT uptake by RIF-1 tumors after 5-fluorouracil treatment, which was more pronounced than that of ^{18}F-FDG (*19*). Our results agree with the findings that the uptake of radiolabeled thymidine provided more accurate assessments of the early response to anticancer therapy than that of ^{18}F-FDG.

CONCLUSION

We have shown that the decrease in ^{18}F-FLT uptake after radiotherapy and PDT was more rapid or pronounced than that of ^{18}F-FDG. ^{18}F-FLT uptake correlated well with ^{3}H-Thd uptake and the PCNA labeling index. The change in ^{18}F-FLT uptake was supposed to reflect proliferative activity after anticancer treatment. Therefore, ^{18}F-FLT is expected to be a feasible PET tracer for monitoring the response to therapy in oncology.

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