The Potential of 2-Deoxy-2[^{18}F]Fluoro-D-Glucose (FDG) for the Detection of Tumor Involvement in Lymph Nodes

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To assess the potential of FDG for PET imaging of nodal tumor metastases, we evaluated its uptake into normal lymph nodes, tumor-involved lymph nodes, and subcutaneous tumor xenografts in rodents. Normal lymph nodes in mice and rats accumulate FDG moderately, developing node/blood ratios of 1.3–11.9/1 at 2 hr following i.v. injection. By contrast, FDG given subcutaneously to healthy Sprague Dawley rats developed very high normal draining lymph node/blood ratios (272/1) versus 7.7/1 by i.v. injection. In nude mice, subcutaneous human ovarian cancer xenografts had 1.27-fold more uptake relative to blood than did normal popliteal lymph nodes. Subcutaneous tumor xenografts of rat breast cancer developed tumor/normal node uptake ratios of 4.91 ± 0.43/1 and tumor/blood ratios of 6.6 ± 0.9 at 2 hr postinjection. Mouse nodes involved with 38C13 murine B-cell lymphoma had mean node/blood ratios of 42.9 ± 6.7/1 and tumored node/normal lymph node uptake of 6.3/1. Thus, FDG given intravenously but not subcutaneously (due to high normal nodal uptake) has potential as an agent for the detection of metastatic tumors in regional lymph nodes using PET scanning.

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FDG (2-deoxy-2[^{18}F]fluoro-D-glucose) has been shown to accumulate vigorously into normal brain, normal and some abnormal myocardium, as well as to brain tumors. FDG has been extensively utilized in these organs as a research tool and more recently as a clinical diagnostic tool (*1–4*). It has been recognized for many years that tumors commonly metabolize glucose excessively, apparently due to enhanced anaerobic and aerobic glycolysis (*5*). The increased utilization of glucose is paralleled by increased accretion of 2-deoxyglucose, an agent structurally similar to glucose, but which is largely trapped intracellularly following phosphorylation so it cannot be further metabolized, save for limited dephosphorylation (*6*). 2-deoxyglucose labeled with carbon-14 has been shown to accrete vigorously into animal tumors and when labeled with fluorine-18 (^{18}F) has been shown to accumulate into some human tumors including colorectal carcinoma to an extent sufficient to allow for tumor imaging in patients (*7–10*). We have recently reported our preliminary experience indicating that a broad spectrum of human tumor xenografts accumulate FDG (*11*). With the increasing availability of high resolution PET scanning devices and simpler medical cyclotrons, it is highly likely that FDG will be more widely used as a tumor scanning agent.

Since many human tumors metastasize first to regional lymph nodes prior to systemic spread, it is essential to know what the normal degree of uptake of FDG is into normal lymph nodes, since successful imaging of nodal metastases is dependent upon tumor uptake being greater than that in normal lymph nodes. Thus, we studied FDG uptake to the normal lymph nodes of laboratory rodents following intravenous or subcutaneous injection of FDG. Further, to gain a better understanding of the meaning of the values for normal nodal uptake of FDG, we evaluated FDG uptake following i.v. injection into two groups of mice and another group of rats with neoplasms (*12–14*). These animal studies support the feasibility of detecting lymph node metastases of tumors using FDG.

METHODS

FDG was synthesized using a nucleophilic exchange fluorination method on a quaternary 4-amino-pyridium resin to produce FDG with a very high specific activity (>3,000 Ci/mmol) (*15*). Fifty microcuries of FDG was injected via the femoral vein or subcutaneously (s.c.) into the left hind foot pad in healthy female Sprague Dawley rats or by tail vein in the mice. In one normal rat study, animals were injected with 15 μCi FDG i.v. or s.c. and killed at 20 min, 1 or 2 hr postinjection. Multiple normal tissues, including the popliteal lymph nodes, were removed, weighed, and counted in a gamma counter at the 511 keV window. All other animals

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were killed at 2 hr post-i.v. injection. Corrections for physical decay allowed for the determination of percent kilogram of injected dose per gram (% Kg ID/g) of ^{18}FDG reaching the tissues (*16*).

Studies in animal tumors included several tumor types. HTB77 human ovarian carcinomas (ATCC) were grown subcutaneously following the injection of 10 million tumor cells in the shoulder region of athymic Swiss nude mice. The 38C13 murine B-cell lymphomas were grown following the subcutaneous injection of a single cell suspension of 10^5 tumor cells into ~12-wk-old C3H/HeN mice in the left flank (*13*). Several weeks following injection for the ovarian tumor, and one to two weeks postinjection for the lymphomas, subcutaneous tumors developed and were used for experiments. Nodal metastases were palpable in the axillae in the 38C13 system before FDG injection. Rat mammary tumors were established in Lewis rats by subcutaneous injections of disaggregated rodent mammary tumors into the breast fat pad as previously described (*14*). These tumors were then studied when they reached 1–2 cm in diameter. Injections of FDG and sacrifice were performed as discussed above. Tumor/normal tissue ratios and the mean and variability were determined.

RESULTS

The time course of FDG activity in normal Sprague-Dawley rat lymph nodes and other normal tissues following i.v. or s.c. administration is shown (Fig. 1A–D). Following i.v. injection, the uptake of FDG in normal nodes is substantial, reaching 3.29 ± 0.85 and 7.75 ± 1.24 times blood levels, at one and two hours, respectively (Fig. 1D). These levels are greater than those in the liver and are similar to those in the kidney and lung. Of interest is that FDG uptake in the diaphragm is substantial, approaching that seen in the brain in this study (Fig. 1A–C).

When FDG was given s.c., draining lymph nodes at 1 and 2 hr postinjection had far greater activity/g than any lymph node or normal tissue in the above i.v. experiment. Indeed this normal nodal uptake was greater than that in any normal tissue/g in the s.c. experiment, including the heart and brain (Fig. 1A–C). After 1 and 2 hr, the first draining lymph node proximal to the s.c. injection site had 126 ± 41 and 272 ± 92 fold greater FDG activity than the blood in this study (Fig. 1D).

The uptake of FDG by tumors was studied in three systems. In nude mice, FDG accumulated into human HTB 77 ovarian cancer xenografts with a tumor/blood uptake ratio of 6.45 ± 0.18 at 2 hr postinjection. Tumors were ~1.5 cm in diameter, a size which has been demonstrated to have little necrosis (unpublished observation). In comparable nude mice without tumors, normal lymph nodes had a node/blood uptake ratio of 5.08 ± 1.36 for a tumor/normal node uptake (normalized to blood levels) ratio of 1.27/1 (Table 1). In normal BALB/c mice, normal nodal uptake was somewhat greater than in the nude mice, with node/blood uptake ratios of 11.94 ± 2.95/1 (Table 1).

To compare tumor uptake with normal nodal uptake in a syngeneic situation within individual animals, rat mammary carcinomas were established in Lewis rats (*14*). These subcutaneous syngeneic tumors developed tumor/normal popliteal lymph node uptake ratios of 4.91 ± 0.54/1. In this system, the normal popliteal lymph nodes, far removed from the primary upper thoracic tumor, had considerably lower uptake of FDG relative to blood (node/blood 1.33 ± 0.17) and liver than in the mice or Sprague Dawley rats (Table 1).

To determine whether lymph nodes involved with metastatic syngeneic tumor accreted FDG to a greater extent than normal lymph nodes, FDG uptake was assessed in the 38C13 murine lymphoma system. In this system, a primary tumor is established subcutaneously which then metastasizes to regional lymph nodes systemically and tumor-involved nodes accumulate FDG more than in any normal or tumor-involved tissue except for the heart (Fig. 2). Tumor involved/normal lymph node (from a normal control group) uptake ratios were ~6.3/1 with tumor involved node/blood uptake ratios of >42.9 ± 6.7/1 (Fig. 2). By contrast, normal node/blood uptake was 5.58 ± 1.26/1.

DISCUSSION

The data from the nude mouse ovarian xenograft, the rat breast cancer, and the murine lymphoma models all indicate that tumor uptake of FDG/g is greater than normal nodal uptake/g in the same species. These observations together with the knowledge that nodal metastatic tumors commonly enlarge lymph nodes, and the excellent contrast resolution of PET, suggest that PET imaging should be able to detect nodal foci of metastatic cancer in man over normal lymph node background activity levels (*6*).

While tumor FDG uptake is greater than that in normal lymph nodes following i.v. injection, s.c. injection results in intense accumulation in normal draining nodes, which accumulate the most FDG of any tissue in the body. The internal uptake in the draining nodes is almost certainly due to direct lymphatic delivery of FDG to the draining nodes. While the mechanism of this uptake in nodes is unknown, it is probably related to FDG accumulation in lymphocytes in the nodes. It seems clear that this high background activity in normal nodes after s.c. delivery would almost certainly preclude tumor imaging. In fact, this phenomenon should be noted in planning the injection site for clinical FDG tumor imaging studies to avoid inadvertent s.c. injection and resulting false-positive nodal visualization. Subcutaneous FDG delivery may be considered if PET mapping of regional normal nodal drainage is planned. However, in this study, only the nodes closest to the injection site developed very high FDG activity levels.

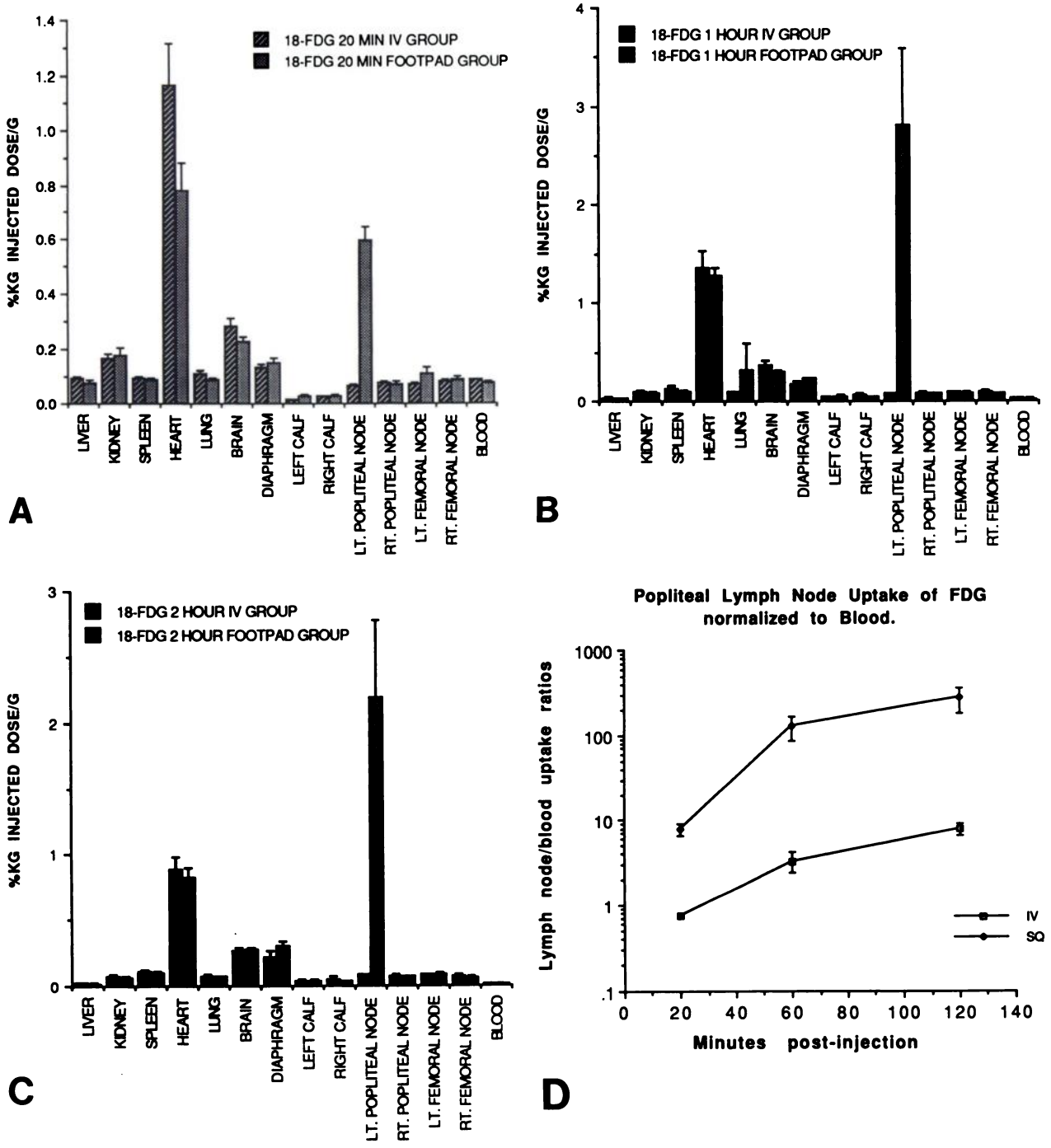

FIGURE 1
(A-C) FDG uptake in normal rat lymph nodes at 20 min, 1 hr, or 2 hr following i.v. or s.c. administration. Note that after i.v. delivery, FDG uptake is similar to other tissues, but after s.c. delivery to rat footpad, draining popliteal nodal uptake is dramatic (n = 4 animals/time point/group). (D) Normal lymph node/blood FDG uptake ratios following i.v. or s.c. delivery to rat footpad. Note intense FDG accumulation in normal lymph nodes following s.c. administration (n = 4 animals/time point/group).

In summary, while FDG does accumulate in normal lymph nodes to a moderate extent, tumor accumulation is greater per gram following i.v. injection in the three model systems studied, particularly in the syngeneic and metastatic syngeneic tumors. These data indicate that the detection of tumor metastases of sufficient size in regional lymph nodes should be possible using FDG PET scanning. Additional studies in patients with cancer and inflammatory adenopathy will be of interest to further evaluate the feasibility of this approach.

TABLE 1
FDG Uptake at Two Hours Post-Intravenous Injection (%kg injected dose/g)

	HTB77 OV CA n = 4	NI NUDE MICE n = 5	NL BALB/C MICE n = 5	LEWIS RATS/ (RMT TUMORS) n = 6
Tumor (s.q.)	0.0364 ± 0.0052	n/a	n/a	0.351 ± 0.090
NI popliteal lymph nodes	n/d	0.032 ± 0.007	0.082 ± 0.024	0.088 ± 0.025
Blood	0.0056 ± 0.0001	0.008 ± 0.002	0.005 ± 0.001	0.065 ± 0.018
Liver	0.0112 ± 0.0002	0.016 ± 0.001	0.012 ± 0.001	0.082 ± 0.014
Spleen	0.0285 ± 0.0086	0.005 ± 0.006	0.043 ± 0.006	0.136 ± 0.031
Heart	0.4653 ± 0.0599	0.352 ± 0.065	0.942 ± 0.251	0.200 ± 0.085
Lung	0.0382 ± 0.0042	0.083 ± 0.011	0.068 ± 0.025	0.113 ± 0.027
Muscle	0.0366 ± 0.0153	0.042 ± 0.008	0.058 ± 0.013	0.029 ± 0.011
Small bowel	0.0160 ± 0.0007	0.040 ± 0.006	0.049 ± 0.007	0.218 ± 0.046

n/a = not applicable.
n/d = not determined.

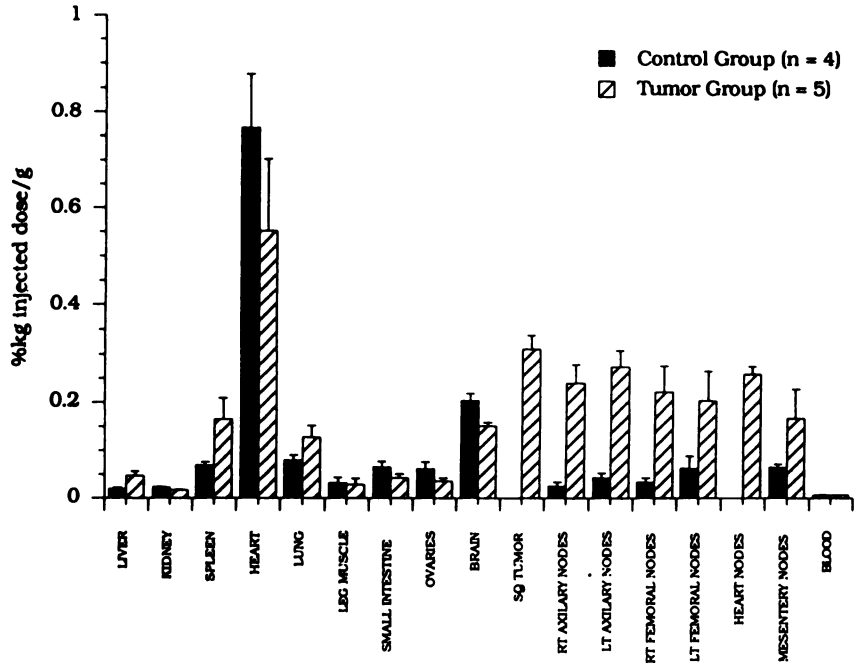

FIGURE 2
FDG uptake 2 hr post-i.v. injection in normal C3H/HeN mice and those with s.c. or primary and metastatic 38C13 lymphoma. Note intense uptake in tumor-involved lymph nodes versus normal nodes and other normal tissues.

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Editorial

Axillary Lymphoscintigraphy for Breast Cancer: Should We Do It? Can We Do It?

When cancer is diagnosed, identification of micrometastases is important in patients for whom effective adjuvant systemic therapy is available, since patients with micrometastases are more likely to relapse distantly in spite of local therapy. Although several malignancies may be successfully treated in the adjuvant setting, breast cancer has become the paradigm candidate disease for this approach. Evaluation for micrometastases to the axillary lymph nodes has been performed in patients with breast cancer since the advent of surgical approaches for local treatment (*1*). For decades, full axillary lymph node dissections were performed in an effort to surgically "sterilize" what was felt to be the primary regional drainage area. The objective of this aggressive surgical approach was to prevent the secondary escape of malignant cells to distant organs. Subsequent randomized trials comparing more to less aggressive local therapy have demonstrated that breast cancer is in most cases a systemic disease in which distant metastases occur simultaneously with those to local/regional lymph nodes (*2–5*). These studies led to the adoption of less aggressive surgery and to the investigation of adjuvant systemic therapies. Nonetheless, sampling of the axillary lymph nodes to determine whether metastases were present remains a very sensitive indicator of the metastatic potential of each patient's cancer, and axillary lymph node status has been recognized as one of the most important prognostic factors in patients with newly diagnosed primary breast carcinoma (*6*). Retrospective studies have suggested that patients with pathologically negative axillary lymph nodes have a recurrence rate of only 20%–40% over a 10-yr period (*6–10*), while those with one to three involved axillary lymph nodes have a recurrence rate of almost twice that (*6*). Moreover, of patients with 10 or more positive nodes, less than 20%–30% remain relapse-free after only five years of follow-up (*6*).

Most early trials of adjuvant systemic therapy focused on newly diagnosed breast cancer in patients with relatively poor prognoses, particularly those with positive axillary nodes. The successes of these early trials have now been well documented (*11*), although not all patients with positive lymph nodes benefit from adjuvant systemic therapy. In this regard, combination chemotherapy is indicated for premenopausal node-positive women, while antiestrogen therapy (tamoxifen) is appropriate adjuvant therapy for postmenopausal node-positive women with estrogen receptor positive tumors (*11*). The benefit of adjuvant systemic therapy in node-negative patients remains controversial (*12–14*). Four recently published studies in which node-negative patients were randomly assigned to some form of chemo- or hormonal therapy or to observation only have demonstrated two rather surprising findings (*15–18*):

1. Node-negative patients in these trials (treated or not) had a worse prognosis than expected from historical reviews.

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