Quantitative [F-18]Fluorodeoxyglucose Positron Emission Tomography in Pretreatment and Grading of Sarcoma

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ABSTRACT
The purpose of this study was to determine the relationship between sarcoma tumor grade and the quantitative tumor metabolism value for [F-18]fluorodeoxyglucose (FDG) determined by positron emission tomography (PET) imaging. Seventy patients with bone or soft-tissue sarcomas underwent PET scanning with quantitative determination of tumor FDG metabolic rate (MRFDG) before treatment. MRFDG (µmol/g/min) for each tumor was compared with National Cancer Institute tumor grade, S-phase percentage, and percentage of aneuploidy of the tumor population. The pretreatment quantitative determination of tumor MRFDG by PET correlates strongly with tumor grade but not with the other selected histopathological tumor correlates. In addition, overlap of MRFDG PET values with tumor grade suggests that PET, an objective tumor measurement, may provide an alternative means of assessing tumor biological potential or may have the potential to overcome some of the limitations of traditional pathological evaluation. FDG PET can uniquely provide a metabolic profile of a diverse group of sarcomas noninvasively and provide clinically relevant tumor biological information.

INTRODUCTION
Sarcomas comprise ~1% of malignant tumors in adults but represent a significant diagnostic and therapeutic challenge. Diagnosis and treatment planning depend significantly on histological grade because it is expected that this descriptor, along with tumor size, will predict the biological aggressiveness of the tumor. However, the accuracy of tumor diagnosis and histological grading may suffer from sampling error, particularly in large, heterogeneous tumors. Incisional biopsy or multiple core-needle biopsies, the location of which has been determined by anatomical and surgical principles, may miss the most biologically significant regions of the tumor, thus compromising assessment of tumor grade by standard histology, as well as ancillary studies such as flow cytometry and cell cycle analysis.

In this group of tumors, there is a need for techniques that provide better assessment of tumor heterogeneity, and which provide pretreatment information on the biological aggressiveness of tumor beyond that provided by traditional means. We have obtained pretreatment quantitative data on tumor metabolism with FDG PET and have investigated whether these data correlate with histologically determined tumor grade.

We proposed that PET imaging with FDG of sarcoma before treatment could contribute important treatment planning information and hypothesized that FDG PET assessment of the tumor metabolism would correlate with its biological aggressiveness or grade. Previous experience with FDG tumor imaging is now substantial for many different tumors. FDG imaging has proven to be valuable for determining the presence or absence of malignancy and, in some cases, the histological grade of tumors. Adler et al. (3) and Griffeth et al. (4) showed that the semiquantitative DUR (5) obtained from static FDG images could distinguish between high- and low-grade soft-tissue sarcomas and between benign and malignant tumors. The method was less accurate in distinguishing intermediate- from low-grade tumors. Kern et al. (6) and Nieweg et al. (7) reported quantitative imaging results in small groups of sarcoma patients and demonstrated that the glucose metabolic rate could distinguish between sarcomas of low, intermediate, and high grades as defined by the NCI grading system (8, 9).

We hypothesized that a quantitative metabolic rate determination could more accurately assess tumor “biological aggressiveness” in a large series comprised of patients with bone and soft-tissue sarcomas (n = 70). FDG PET values should correlate with NCI histological grade and when different, might be a clue to inaccurate tumor grading because of sampling bias in the pretreatment biopsy. Potentially, the determination of tumor FDG uptake rate in a large, diverse sarcoma patient population could set a higher level of sensitivity and specificity for imaging. The method could delineate differences in behavior among tumors of different histological grades and types and establish FDG PET imaging as a clinical practice in evaluation of this patient group.

PATIENTS AND METHODS

Patients. Patients with soft-tissue or bone sarcomas presented to the University of Washington Multi-Disciplinary...
They underwent quantitative FDG PET imaging as a part of the routine work-up of the biopsies or resection specimen. A decision was made regarding preoperative chemotherapy. FDG PET scanning, other imaging (MRI, CT, and bone scan), and biopsy, patients were officially staged, and a tumor had not been treated for at least 6 months. After informed consent was obtained, an i.v. line was placed in each arm of the patient, one for FDG injection and the other for blood sampling. A blood sample was taken for glucose determination. Patients were then positioned in the tomograph. A 20–30 min attenuation scan was acquired over the tumor site. FDG (3–10 mCi) was infused i.v. over 2 min with a syringe pump, followed by a 60-min emission scan of the tumor. During the emission scan, serial venous blood samples were obtained every minute for up to 7 min after injection and then less frequently. Venous blood sampling was used as a means for determining MRFDG. Blood samples were centrifuged, and plasma was counted in a well counter (Packard Instruments Company, Meriden, CT). Blood glucose level was determined from at least three samples taken at intervals over the 60-min imaging time with a calibrated glucose analyzer (Beckman Instrument, Inc., Wakefield, MA). Images were reconstructed with the Hanning filter after scatter correction, resulting in a reconstructed resolution of ~10 mm (13). Two 500-ml reference vials containing 100 and 400 μCi F-18 were imaged in the tomograph on the same day as each study for cross-calibration of image data with blood sample counts. Aliquots of the reference vial contents were counted in triplicate in the well counter.

**Pathological Evaluation.** All sarcomas were graded as part of the routine work-up of the biopsies or resection specimens, and the pathologists were unaware of the PET findings. The soft-tissue tumors were graded according to the NCI grading system (8, 9). In brief, under this system, well-differentiated sclerosing and lipocytic liposarcoma, and myxoid liposarcoma without a “round-cell” component, are regarded as grade I tumors. Rare smooth muscle tumors and myxoid fibrosarcomas (myxofibrosarcoma or myxoid malignant fibrous histiocytoma) with only minimal atypism may also be regarded as grade I under this system (8, 9). Almost all other soft-tissue sarcomas are regarded as being either grade II (<15% necrotic) or grade III (15% or more necrotic). The NCI system does not specifically address Ewing’s sarcoma and primitive neuroectodermal tumor; at this institution, these are regarded as grade III sarcomas. Sarcoma subtypes (cell-specific diagnoses) were similar to those of other reported sarcoma series (10). Osteo- and chondrosarcomas were graded in accordance with the grading system of Unni (11), with grades III and IV combined into a single grade III.

For sarcoma patients who received neoadjuvant chemotherapy before definitive resection, grading was based on the initial needle-core biopsy. All other tumors were graded after evaluation of multiple sections from various regions of the resected specimen, and a single grade was given, reflecting the highest grade areas. In difficult cases, intradepartmental consultation was used to arrive at a final grade. After surgical resection and pathological dissection, samples of fresh tumor were submitted for flow cytometric analysis of percentage of cells in S-phase (actively synthesizing DNA) and tumor ploidy. Forty-eight of the tumors were located in the lower extremities, 8 in an upper extremity, 6 in the abdomen, 3 in the pelvis, and 4 in other sites. Tumor sizes ranged from 4–22 cm in greatest diameter.

**PET Imaging.** All scans were performed on an Advance Tomograph (GE Medical Systems, Waukesha, WI) operating in two-dimensional, high sensitivity mode with 35 imaging planes covering a 15-cm axial field of view. Patients prepared for the scan with an overnight fast. After informed consent was obtained, an i.v. line was placed in each arm of the patient, one for FDG injection and the other for blood sampling. A blood sample was taken for glucose determination. Patients were then positioned in the tomograph. A 20–30 min attenuation scan was acquired over the tumor site. FDG (3–10 mCi) was infused i.v. over 2 min with a syringe pump, followed by a 60-min emission scan of the tumor. During the emission scan, serial venous blood samples were obtained every minute for up to 7 min after injection and then less frequently. Venous blood sampling was used as a means for determining MRFDG. Blood samples were centrifuged, and plasma was counted in a well counter (Packard Instruments Company, Meriden, CT). Blood glucose level was determined from at least three samples taken at intervals over the 60-min imaging time with a calibrated glucose analyzer (Beckman Instrument, Inc., Wakefield, MA). Images were reconstructed with the Hanning filter after scatter correction, resulting in a reconstructed resolution of ~10 mm (13). Two 500-ml reference vials containing 100 and 400 μCi F-18 were imaged in the tomograph on the same day as each study for cross-calibration of image data with blood sample counts. Aliquots of the reference vial contents were counted in triplicate in the well counter.

**Image Analysis.** The 30–60 min summed images were used for ROI generation and subsequent image analysis. ROIs were intended to be representative of the most metabolically active portion of the tumor in these often large heterogeneous masses. ROIs were therefore drawn around the section of the tumor with maximal counts on summed images. They were placed over the tumor in three adjacent 4.25 mm image planes. The average counts within the regions were used in subsequent calculations. ROIs included the area on slices with maximum counts on the summed image and ranged in size from 2 to 16 cm². Time-activity curves were generated from application of the ROIs to the dynamic image sets and were corrected for physical decay of F-18.

Tumor and normal tissue DUR were calculated as follows:

\[
DUR = \frac{\bar{A}}{ID/m} \times \frac{\text{Plasma glucose}}{100}
\]

where \(\bar{A}\) is mean tissue activity from 30 to 60 min (μCi/g), \(ID\) is injected dose (mCi), and \(m\) is patient weight (kg). Plasma glucose, the average of glucose samples obtained before imaging, is stated in mg/dl. Tumor and normal tissue MRFDG were
Table 2  PET imaging data on sarcoma grade

<table>
<thead>
<tr>
<th>Sarcoma grade</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range, MRFDG</td>
<td>15.0-0.4</td>
<td>35.3-2.0</td>
<td>38.7-3.1</td>
</tr>
<tr>
<td>Median</td>
<td>4.8</td>
<td>8.1</td>
<td>19.8</td>
</tr>
<tr>
<td>Range, DUR</td>
<td>1.7-1.0</td>
<td>11.0-1.3</td>
<td>12.4-2.1</td>
</tr>
<tr>
<td>Median</td>
<td>2.8</td>
<td>3.4</td>
<td>7.5</td>
</tr>
</tbody>
</table>

calculated according to the graphical analysis method of Patlak et al. (14) using the following equation:

\[
\frac{\bar{A}}{C_h} = K_i \frac{\int C_s \, dt}{C_h} + (V_0 + V_b)
\]  

(B)

MRFDG = Plasma glucose × K

(C)

where $\bar{A}$ is tissue concentration of the tracer (µCi/g), $C_h$ is blood concentration of the tracer (µCi/ml), $V_0$ and $V_b$ are tracer volume-of-distribution (ml/g) and blood partial volume (ml/g), respectively, and $K_i$ is tracer blood-tissue transfer constant (ml/min/g). $K_i$ is obtained from the slope of the fit of $\bar{A}/C_h$ versus $\int C_s \, dt$ from 20 to 60 min after injection. In the original description of the graphical approach of Patlak et al. (14), arterial blood served as the input function, $C_h$. To avoid the potential difficulties of arterial cannulation, we used venous blood samples, which results in an underestimation of blood tracer concentrations in the first 15-20 min after injection. We have shown that this results in a consistent 5-10% overestimation of the MRFDG (15). In this data analysis, an attempt was not made to account for the differences between tissue handling of FDG and glucose (14, 15). The results are, therefore, reported as the MRFDG (µmol/min/100 g).

Data Analysis. For each patient, tumor MRFDG and DUR were compared to NCI histological grade. Differences in the mean DUR and MRFDG between the high-, intermediate-, and low-grade tumor groups were tested for statistical significance using 2-tailed student’s $t$ test. DUR and MRFDG were also plotted against the percentage of S-phase cells. The percentage of cells with aneuploid DNA content was measured by flow cytometry. Correlation coefficients were generated for each of the data sets.

RESULTS

The MRFDG increases with higher grade of tumor, allowing FDG PET imaging to accurately distinguish among the three NCI tumor grades (Fig. 1A). Although there is overlap among the different groups, the means are significantly different. The $P$ for distinguishing high- from intermediate-grade tumors was $P < 0.001$, between high- and low-grade tumor was $P < 0.0001$, and between intermediate- and low-grade tumor was $P < 0.002$. A similar plot using DUR values shows that the test for difference between high- and intermediate-grade tumors was significant ($P < 0.003$), as well as between high- and low-grade tumors ($P < 0.0001$) and between intermediate- and low-grade tumors ($P < 0.002$; Fig. 1B). MRFDG values ranged from 0.79 to 38.7 µmol/min. For DUR values, the range was 0.8 to 12.4. The DUR range of values was more restricted than the range of MRFDG
values (Table 2). The plot of MRFDG versus percentage of tumor cells in S-phase shows no correlation ($r^2 = 0.221$); the MRFDG correlation with the percentage of aneuploid tumor cells had $r^2 = 0.007$ (Fig. 2).

There was also a suggestion of similarity of tumor metabolism values within particular histological subtypes within individual NCI tumor grades. For example, primitive neuroectodermal tumor and Ewing's sarcomas showed the lowest values of the high-grade tumor category (MRFDG, 4.7–15.1). The lowest FDG PET values were in the grade I liposarcomas (MRFDG, 0.7–4.5), and differentiation between these and the intermediate-grade liposarcomas was apparent (MRFDG, 2.3–7.3). Malignant fibrous histiocytoma, leiomyosarcoma, and osteosarcomas displayed the highest MRFDG values. Also as expected, intermediate-grade tumors were represented by several tumor types.

Visual interpretation of the FDG integrated images provided additional clinical information. Tumors often showed marked heterogeneity, with peripheral areas of higher metabolic activity and central areas of decreased or no activity, suggestive of tumor necrosis (Fig. 3). This was valuable clinically, because the presence of necrosis in tumors categorizes a tumor as high grade and a candidate for neoadjuvant chemotherapy. In making an observation of the whole tumor, these important biological characteristics were immediately apparent and available for clinical decision making.

**DISCUSSION**

Confirming the findings by Nieweg et al. (7), determination of MRFDG was more sensitive than DUR for determining tumor grade. In our study, this relationship existed for both soft-tissue and bone sarcomas. Quantitative imaging protocols contribute clinically relevant information. Of particular interest, however, are these tumors in the intermediate- and low-grade categories (Fig. 1A), which showed high metabolic rate but only
low-intermediate NCI histological grade. This brings up the question of whether the tumors will behave clinically as more aggressive higher grade processes.

The overlap of MRFDG between categories contributes several other interesting findings. Within any one NCI grade, there is a wide range of variability in tumor metabolic rates. Although the NCI sarcoma grading system in most instances is useful in predicting outcome stratification for therapy, there still remains a subset of tumors in which the histological grade does not predict the biological behavior of tumors seen in clinical practice. Additionally, it may be difficult to apply the NCI system to tumors that are difficult to classify histologically and immunohistochemically. In these circumstances, flow cytometry and immunocytochemistry data do not contribute greatly to sorting out the dilemma. MRFDG PET measurements provide an objective measure of tumor metabolism that correlates with tumor grade. Clinical follow-up of these patients in terms of time to local recurrence and distant metastases will help to establish whether PET provides more accurate prognostic information than traditional grading. The data on clinical outcomes for this patient group are under analysis.

In our series, tumor MRFDG did not correlate with percentage of tumor cells in S-phase or with aneuploid DNA content. This lack of correlation suggests that these techniques measure different tumor parameters. One interpretation of our data may well be that the NCI grading classification of tumors may not be the "gold standard" in predicting tumor behavior. A correlation between percentage of tumor cells in S-phase or aneuploid DNA content and MRFDG might be expected. Previous experience with other PET imaging agents for tumor metabolism, such as [C-11]methionine in a breast cancer series, have shown associations with tumor S-phase fraction (16). Tumors with more active glucose metabolism might be expected to have a higher rate of proliferation as well. Determination of proliferation rate over ploidy is clinically relevant, because increased cell population aneuploidy is frequently associated with poor outcome, as is higher grade. This lack of correlation in our data suggests that FDG and a measure of tumor proliferation, such as [C-11]thymidine (17, 18), images may complement each other. Additionally, S-phase and aneuploidy measurements are made only on a small portion of the tumor, whereas FDG PET examines the whole tumor. Sampling differences or errors may also contribute to this lack of correlation, because the images and gross pathology of these tumors show a wide range of heterogeneity in metabolism and appearance.

The implications for routine clinical use of quantitative FDG imaging are several. To obtain a quantitative image, more time-consuming, dynamic imaging protocols are required than for FDG static imaging. The data analysis is also more complex. However, we have recently validated imaging procedures that reduce blood sampling and imaging time (15). Although modified quantitative FDG imaging procedures are still more difficult to perform than FDG static scans, the extra effort is justified because of the additional clinical information provided by quantitative analysis of MRFDG.

The goal of obtaining tumor metabolic information noninvasively for the whole tumor is an important one for the clinical use of quantitative PET imaging. Noninvasive assessment of tumor grade by metabolic imaging has a great deal of clinical potential. For the foreseeable future, neoadjuvant chemotherapy for sarcomas will continue to be offered to patients with large intermediate- and high-grade tumors per clinical practice based on standard clinical work-up. This is the uniform treatment approach for sarcoma patients at this institution and is the framework in which to continue to evaluate FDG PET imaging in this setting. Because it is believed that biological behavior of tumors is determined by the highest grade region of tumors, an accurate assessment of the entire tumor with identification of areas of highest biological activity is of value, both in determining a site for initial biopsy and identifying tumors where the
initial histological evaluation may not have provided an accurate reflection of the true biological potential.

The pretreatment quantitative determination of MRFDG PET correlates strongly with histological tumor grade in this preliminary study of a diverse group of sarcomas. FDG PET can uniquely define the metabolic profile of tumors, providing adjunctive data not obtainable by anatomical computed tomography or magnetic resonance imaging. Because the determination of tumor grade is a critical factor in individual patient treatment planning, quantitative PET imaging can play an important role in patient management. We continue to use FDG PET quantitative imaging for determination of tumor MRFDG in our evaluation of tumors on the University of Washington Sarcoma Clinical Service.

REFERENCES

Quantitative [F-18]fluorodeoxyglucose positron emission tomography in pretreatment and grading of sarcoma.
