18F-Fluorodeoxythymidine PET for Evaluating the Response to Hyperthermic Isolated Limb Perfusion for Locally Advanced Soft-Tissue Sarcomas

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Locally advanced soft-tissue sarcomas of an extremity can be treated either by amputation of the limb or by hyperthermic isolated limb perfusion (HILP) followed by resection of the tumor. In this study, the response to HILP was measured by PET with 18F-fluorodeoxythymidine (18F-FLT). Methods: Ten patients with primary nonresectable soft-tissue sarcomas of an extremity underwent HILP with tumor necrosis factor-α and melphalan. Before and after HILP, all patients underwent PET with 18F-FLT for response evaluation. Results: Before HILP, all tumors were clearly visible on 18F-FLT PET; for the maximum standardized uptake value (SUVmax), the mean was 3.5 (range, 1.0–6.7), and for the mean standardized uptake value (SUVmean), the mean was 1.9 (range, 0.7–2.7). After HILP, all but 1 tumor showed necrosis ranging from 10% to 95%. 18F-FLT PET after HILP revealed significantly decreased uptake of the tracer. The mean SUVmax decreased to 1.7 (P = 0.008), and the mean SUVmean decreased to 0.8 (P = 0.002). One small axillary lymph node metastasis was not visible on 18F-FLT PET. Conclusion: 18F-FLT PET revealed high uptake in soft-tissue sarcomas. 18F-FLT uptake was correlated with the mitotic index of the tumors (r = 0.82 and P = 0.004 for SUVmax; r = 0.87 and P = 0.001 for SUVmean). After HILP, the uptake of 18F-FLT decreased significantly (P = 0.008 and P = 0.002 for SUVmax and SUVmean, respectively). Tumors with initially high 18F-FLT uptake showed a better response to HILP (r = 0.64, P < 0.05). Software fusion of PET images with images from conventional imaging modalities revealed the heterogeneity of the tumors before and after HILP. Such data can help a surgeon in planning the resection of a tumor.

Key Words: 18F-FLT PET; soft-tissue sarcomas; hyperthermic isolated limb perfusion


Soft-tissue sarcomas are relatively rare, accounting for fewer than 1% of all cancers in adults, but they are responsible for approximately 2% of all cancer-related deaths. The numbers of patients presenting with soft-tissue sarcomas each year are approximately 8,300 in the United States and about 500–600 in The Netherlands (1). The majority of soft-tissue sarcomas occur in the upper or lower extremities. The usual treatment protocols include limbsaving surgery, often followed by adjuvant radiotherapy (2). Locally advanced soft-tissue sarcomas of the extremities may require ablative surgical procedures (amputation of the limb). Hyperthermic isolated limb perfusion (HILP) with cytostatic agents has the potential to render the majority of these tumors resectable, thereby preventing the need for amputation. With HILP, chemotherapeutic concentrations in tissues up to 20 times higher than those achieved with systemic chemotherapy can be achieved (3). HILP with tumor necrosis factor-α (TNF-α) and melphalan has resulted in overall response rates of 63%–91% and limb salvage rates of 73%–86% (4–8). However, HILP with TNF-α and melphalan is an expensive and intensive treatment with possible serious side effects (9).

PET is an imaging modality that offers the possibility of visualizing and quantifying metabolic pathways in a noninvasive way. 18F-FDG is the most widely used PET tracer in oncology. 18F-FDG uses the increased glycolytic activity of cancer cells for PET visualization. 18F-FDG also shows increased uptake in inflammatory cells, a property that limits the specificity of this tracer in monitoring cancer therapy.

In 1998, a new PET tracer, 18F-fluorodeoxythymidine (18F-FLT), was developed (10,11). 18F-FLT is a pyrimidine analog that uses the salvage pathway of DNA synthesis for PET visualization. 18F-FLT is taken up through facilitated transport and diffusion and is phosphorylated by thymidine kinase 1 (TK1) into 18F-FLT-monophosphate. TK1 is a cell cycle-regulated enzyme, and its activity is high in the stationary phase of normal cells. TK1 activity is higher in
malignant cells than in normal cells and is present throughout the cell cycle, leading to increased phosphorylation of \( ^{18}F\)-FLT in malignant tissues. After phosphorylation, \( ^{18}F\)-FLT is trapped intracellularly; therefore, the uptake of \( ^{18}F\)-FLT is a reflection of the proliferation of tissue cells. In a previous study, we showed that \( ^{18}F\)-FLT PET clearly visualizes soft-tissue sarcomas and has the potential to differentiate between low-grade and high-grade soft-tissue sarcomas (12).

Recent research data on the use of \( ^{18}F\)-FLT PET for the visualization of different tumor types indicated that the sensitivity of \( ^{18}F\)-FLT PET for most tumor types is lower than that of \( ^{18}F\)-FDG PET. However, we showed that the specificity of \( ^{18}F\)-FLT in a tumor/inflammation animal model is higher than that of \( ^{18}F\)-FDG (13). To date, conventional imaging techniques (CT and MRI) have been used to monitor the response to HILP in patients with locally advanced soft-tissue sarcomas. With these imaging modalities, excellent anatomic information can be obtained, and the growth or shrinkage of soft-tissue sarcomas can be monitored. However, images from CT and MRI provide only scant information about tumor aggressiveness and biologic response to therapy.

In this study, the potential of \( ^{18}F\)-FLT PET to measure the metabolic response to treatment in patients with soft-tissue sarcomas was investigated.

**MATERIALS AND METHODS**

**Patients**

In this prospective study, 10 consecutive patients with locally advanced soft-tissue sarcomas of an extremity were included from May 2002 through November 2004. All tumors were considered nonresectable because of size, multicentricity, or localization near bone or neurovascular structures on MRI. To render these tumors resectable, patients were treated by use of HILP with TNF-\( \alpha \) and melphalan followed by delayed resection. All patients were treated at the University Medical Center Groningen and gave written informed consent. For patients to be included in the study, hematologic parameters and liver and kidney function tests had to be within normal limits. Pregnant patients and patients with psychiatric disorders were excluded from the study. The study protocol was approved by the Medical Ethics Committee of the University Medical Center Groningen.

**HILP**

The perfusion technique used at the University Medical Center Groningen is based on the technique developed by Creech et al. (14). The major artery and vein of the limb are clamped, and cannulas are inserted and connected to an extracorporeal circuit. A tourniquet is applied to minimize leakage into the systemic circulation. Leakage is measured continuously during perfusion with \( ^{131}I \)-albumin and a precordial scintillation detector as described by Daryanani et al. (15). The limb is wrapped in a thermal blanket to reduce heat loss and to achieve mild hyperthermia (39\(^\circ\)C–40\(^\circ\)C). Perfusion is performed with a roller pump, a DIDEOCO 902 (DIDEOCO) membrane oxygenator, and a heat exchanger. The perfusate consists of 250 mL of dextran 40 in 0.9% saline (NPBI International BV), 250 mL of white cell–reduced (filtered) packed red cells, 30 mL of 8.4% NaHCO\(_3\), and 0.5 mL of heparin (5,000 IU/mL). Subsequently, TNF-\( \alpha \) (Boehringer-Ingelheim GmbH) at 3 mg (upper limb) or 4 mg (lower limb) and melphalan (Glaxo-SmithKline) at 10 mg/L (leg volume) or 13 mg/L (arm volume) are administered intraarterially. After 60–90 min of perfusion, the limb is extensively flushed with 4–6 L of saline and then filled with 250 mL of white cell–reduced packed red cells. After another 60 min, the limb is flushed with 3,000–6,000 mL of dextran 40 in 5% glucose and 500 mL of blood (250 mL of red cells and 250 mL of plasma) (16). After removal of the cannulas, the procedure is concluded with a fasciotomy to prevent compartmental syndrome. On day 1 after surgery, the patient is observed closely in an intensive care unit, because serious complications can arise, especially when leakage into the systemic circulation has occurred (9,17).

**Histopathologic Examinations**

The histopathologic diagnosis was established after examination of either incision biopsy or true-cut biopsy specimens. Tumors were graded according to the French grading system as described by Coindre et al. (18). With this system, the differentiation grade of tumors, the number of mitotic figures per 2 mm\(^2\), and the amount of necrosis are scored. All tumors were divided into grade 1, grade 2, and grade 3 according to the French grading system. Grading of tumors was done both before and after HILP. The largest diameter before and after HILP was measured by MRI. The largest diameter of tumors before HILP was measured by MRI. Tumors were classified as low grade (grade 1), medium grade (grade 2), or high grade (grade 3).

### TABLE 1

**Patient and Tumor Characteristics**

<table>
<thead>
<tr>
<th>Patient (sex/age [y])</th>
<th>Histologic findings</th>
<th>Tumor grade</th>
<th>Localization</th>
<th>Largest diameter before HILP (cm)*</th>
<th>Mitotic index (mitotic figures/2 mm(^2))</th>
<th>Largest diameter after HILP (cm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (F/47) Pleomorphic leiomyosarcoma</td>
<td>3 Upper arm</td>
<td>2.8</td>
<td>43</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (F/56) Pleomorphic sarcoma</td>
<td>3 Upper arm</td>
<td>6.0</td>
<td>22</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (F/58) Pleomorphic sarcoma</td>
<td>3 Lower leg</td>
<td>6.0</td>
<td>36</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (M/63) Synovial sarcoma</td>
<td>3 Upper leg</td>
<td>6.0</td>
<td>30</td>
<td>5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (M/53) Myxofibrosarcoma</td>
<td>3 Upper leg</td>
<td>12.0</td>
<td>18</td>
<td>12.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (M/28) Synovial sarcoma</td>
<td>2 Popliteal fossa</td>
<td>3.0</td>
<td>6</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (M/70) Pleomorphic sarcoma</td>
<td>2 Lower leg</td>
<td>10.0</td>
<td>18</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (M/27) Epithelioid sarcoma</td>
<td>2 Lower arm</td>
<td>3.5</td>
<td>16</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 (F/37) Myxoid liposarcoma</td>
<td>1 Upper leg</td>
<td>12.2</td>
<td>2</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 (M/71) Myxofibrosarcoma</td>
<td>1 Lower arm</td>
<td>2.0</td>
<td>9</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Established by MRI.
grade 2, or grade 3 tumors. The mitotic index (number of mitotic figures per 2 mm²) was determined on hematoxylin- and eosin-stained sections of the tumors; the areas with the highest rates of mitosis were selected.

At 6–8 wk after HILP, patients were scheduled for local excision of their tumors. The pathologist measured tumor size and determined the percentage of tumor necrosis in the specimens resected after HILP.

**PET Imaging**

18F-FLT was synthesized by the method of Machulla et al. (19). 18F-FLT was produced by fluorination with 18F-fluoride of the 4,4'-dimethoxytrityl-protected anhydrothymidine and then a deprotection step. After purification by reversed-phase high-performance liquid chromatography, the product was made isotonic and passed through a 0.22-μm filter. 18F-FLT was produced with a radiochemical purity of greater than 95% and a specific activity of greater than 10 TBq/mmol. The radiochemical yield was 8.8% ± 3.7% (decay corrected).

PET studies were scheduled shortly before and 39 d (range, 28–49 d) after perfusion, concurrently with MRI scans. A median dose of 399 MBq (range, 320–430 MBq) of 18F-FLT was injected intravenously before perfusion, and 363 MBq (range, 120–430 MBq) was injected after perfusion. At 60 min after injection, patients were placed in an ECAT EXACT HR+ PET scanner (Siemens/CTI Inc.) for imaging of the tumor in the emission–transmission–transmission–emission mode. Depending on the size of the tumor, 1–4 bed positions were used for 8 min per position (5 min for emission and 3 min for transmission). After imaging of the tumor, a whole-body scan was performed from the crown to half way through the femur for 5 min per bed position. Data from multiple bed positions were iteratively reconstructed (ordered-subset expectation maximization) (20).

**Data Analysis**

PET scans of the tumors were interpreted visually for regions of increased uptake. A 3-dimensional volume of interest was drawn around the tumor by use of the 70% isocontour of the maximum standardized uptake value (SUV) of the tumor. The maximum SUV (SUV_max) and the mean SUV (SUV_mean) within this volume of interest were determined automatically with a Leonardo workstation (Siemens/Leonardo; Siemens AG). For some patients, PET images were fused with MR images by use of fusion software on the Leonardo workstation.

Whole-body images were scored for the presence of absence of regions of increased 18F-FLT uptake, taking into account the pattern of physiologic uptake of 18F-FLT.

**Statistical Analysis**

The mean SUV_max and the mean SUV_mean before and after HILP were compared by use of the paired-sample t test. The SUV_max and the SUV_mean before HILP were correlated with the mitotic index by use of the Pearson correlation coefficient. P values of less than 0.05 were considered statistically significant.

**TABLE 2**

PET Results and Pathologic Response

<table>
<thead>
<tr>
<th>Patient</th>
<th>Before HILP</th>
<th>After HILP</th>
<th>Change in SUV_max (%)</th>
<th>Change in SUV_mean (%)</th>
<th>Necrosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SUV_max</td>
<td>SUV_mean</td>
<td>Interval (d)</td>
<td>SUV_max</td>
<td>SUV_mean</td>
</tr>
<tr>
<td>1</td>
<td>6.7</td>
<td>2.7</td>
<td>28</td>
<td>6.4</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>3.6</td>
<td>2.5</td>
<td>34</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
<td>2.7</td>
<td>30</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>3.6</td>
<td>2.3</td>
<td>43</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>4.3</td>
<td>2.1</td>
<td>49</td>
<td>2.4</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>1.0</td>
<td>0.7</td>
<td>34</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>7</td>
<td>6.0</td>
<td>2.5</td>
<td>41</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>8</td>
<td>1.8</td>
<td>1.2</td>
<td>45</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>9</td>
<td>1.3</td>
<td>0.9</td>
<td>47</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>1.3</td>
<td>0.8</td>
<td>43</td>
<td>0.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>
RESULTS

Ten patients (6 men and 4 women) with a mean age of 51 y (range, 27–71 y) were studied. Patient and tumor characteristics are shown in Table 1. For 9 patients, histologic diagnosis was made from an incision biopsy; for 1 patient (patient 9), multiple true-cut biopsies were performed. Patient 10 was diagnosed with a recurrent soft-tissue sarcoma.

Before perfusion, all tumors were clearly visible on 18F-FLT PET, with a mean SUVmax of 3.5 (range, 1.0–6.7) and a mean SUVmean of 1.9 (range, 0.7–2.7). Both SUVmax and SUVmean correlated with the mitotic index of the tumors ($r = 0.82$ and $P = 0.004$ for SUVmax; $r = 0.87$ and $P = 0.001$ for SUVmean) (Fig. 1).

In all but 1 tumor, necrosis ranging from 10% to 95% was found after HILP (Table 2). The 2 grade 1 tumors (patients 9 and 10) showed little or no necrosis, and the grade 2 epithelioid sarcoma showed only 10% necrosis. 18F-FLT PET after HILP revealed significantly decreased uptake of the tracer. The SUVmax decreased to 1.7 ($P = 0.008$), and the mean SUVmean decreased to 0.8 ($P = 0.002$). Most tumors showed a center of very little or no 18F-FLT uptake and a rim of moderate 18F-FLT uptake. For some tumors, 18F-FLT PET images were fused with the corresponding MR images by use of dedicated computer software. For these tumors, we were able to show that 18F-FLT PET can identify areas of necrosis and viable tumor after HILP (Fig. 2).

No significant correlation between the percentage of necrosis and the decrease in the SUVmean could be demonstrated. A weak but significant correlation ($r = 0.642$, $P < 0.05$) between the initial SUVmean and the percentage of necrosis after HILP was demonstrated (Fig. 3).

DISCUSSION

In this study, the value of 18F-FLT PET for evaluating the response of locally advanced soft-tissue sarcomas to HILP...
was investigated. 18F-FLT is a relatively new tracer that uses 1 of the DNA synthesis pathways of tumor cells for PET visualization. There is considerable evidence that 18F-FLT has the potential to visualize and measure the viability of tumor cells during or early after (chemo)therapy because it does not accumulate in inflammatory cells (13).

18F-FLT PET after HILP was scheduled shortly before the resection of the tumor, together with MRI for therapy evaluation. There is some evidence in the literature that 18F-FLT PET can monitor the response to therapy at an early stage (21). However, in this study, we wished to compare the results of 18F-FLT PET after HILP with histopathologic examinations of the resected tumors and decided to perform 18F-FLT PET shortly before the resection.

For our group of 10 patients with locally advanced soft-tissue sarcomas, the baseline uptake of 18F-FLT correlated with the mitotic index, which is a derivative of tumor aggressiveness. Cobben et al. previously showed that 18F-FLT PET could differentiate between high-grade tumors (Coindre grades 2 and 3) and low-grade tumors (grade 1) (12). For other tumor types, similar correlations between pathologic proliferation markers and 18F-FLT uptake have been reported (22–24).

Furthermore, tumors with high initial 18F-FLT uptake seemed to show a good pathologic response after HILP, although the studied number of patients is too small to draw strong conclusions. Three tumors in our study showed little or no necrosis after HILP. These tumors had a low initial SUVmax, ranging from 1.3 to 1.8, and an SUVmean ranging from 0.7 to 1.2. In general, high-grade or aggressive soft-tissue sarcomas respond better to HILP than low-grade tumors (25). 18F-FLT PET may therefore be able to identify patients who will benefit the most from HILP.

Cobben et al. previously investigated the value of 18F-FLT PET for the staging of soft-tissue sarcomas (12). In the present study, besides the uptake in the area of the primary tumor, no additional areas of increased 18F-FLT uptake were found on whole-body 18F-FLT PET; 1 patient had a proven lymph node metastasis at the time of the PET scan. For other types of cancer, the sensitivity of 18F-FLT PET for detecting metastases has been investigated by several research groups, and 18F-FLT PET has been shown to be probably not superior to 18F-FDG PET (11). For different reasons, 18F-FLT uptake is lower than 18F-FDG uptake in almost all types of cancer. Bastiaanet et al. recently reviewed the literature regarding 18F-FDG PET for patients with soft-tissue sarcomas and concluded that there is no indication to use 18F-FDG PET routinely (26).

We believe that the true value of 18F-FLT is not its ability to detect tumors but its capacity to evaluate the response to therapy. van Ginkel et al. previously investigated 18F-FDG PET and 11C-tyrosine-PET for patients undergoing HILP for soft-tissue sarcomas and skin cancers (27,28). They concluded that 18F-FDG PET was able to measure the response to HILP; however, 18F-FDG could not discriminate between viable tumor cells and inflammatory tissues because 18F-FDG uptake was observed in both. 11C-Tyrosine PET was not observed in inflammatory cells, but the use of this tracer is limited because of its short half-life, 20.4 min. We believe that 18F-FLT, like 11C-tyrosine, also will not show uptake in inflammatory tissues, although this notion was not investigated in the present study. Recently, van Waarde et al. investigated uptake in an acute inflammation model and showed that 18F-FLT did not demonstrate uptake in inflammatory tissues (13).

CONCLUSION

Although 18F-FLT PET after HILP did not directly influence our decision regarding whether to perform a local resection or an amputation, we observed that the heterogeneity of 18F-FLT uptake for a few tumors seemed to correspond well to areas of necrosis and viable tumor tissue in the resected specimens. This finding supports our opinion that 18F-FLT PET could be valuable in the future for guiding surgeons in planning resections or radiotherapists in planning conformal radiotherapy.

REFERENCES


