^{18}F-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 ^{18}F-fluorodeoxythymidine (^{18}F-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 ^{18}F-FLT for response evaluation. **Results:** Before HILP, all tumors were clearly visible on ^{18}F-FLT PET; for the maximum standardized uptake value (SUV_{max}), the mean was 3.5 (range, 1.0–6.7), and for the mean standardized uptake value (SUV_{mean}), the mean was 1.9 (range, 0.7–2.7). After HILP, all but 1 tumor showed necrosis ranging from 10% to 95%. ^{18}F-FLT PET after HILP revealed significantly decreased uptake of the tracer. The mean SUV_{max} decreased to 1.7 ($P = 0.008$), and the mean SUV_{mean} decreased to 0.8 ($P = 0.002$). One small axillary lymph node metastasis was not visible on ^{18}F-FLT PET. **Conclusion:** ^{18}F-FLT PET revealed high uptake in soft-tissue sarcomas. ^{18}F-FLT uptake was correlated with the mitotic index of the tumors ($r = 0.82$ and $P = 0.004$ for SUV_{max}; $r = 0.87$ and $P = 0.001$ for SUV_{mean}). After HILP, the uptake of ^{18}F-FLT decreased significantly ($P = 0.008$ and $P = 0.002$ for SUV_{max} and SUV_{mean}, respectively). Tumors with initially high ^{18}F-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: ^{18}F-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 limb-saving 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 non-invasive way. ^{18}F-FDG is the most widely used PET tracer in oncology. ^{18}F-FDG uses the increased glycolytic activity of cancer cells for PET visualization. ^{18}F-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, ^{18}F-fluorodeoxythymidine (^{18}F-FLT), was developed (*10,11*). ^{18}F-FLT is a pyrimidine analog that uses the salvage pathway of DNA synthesis for PET visualization. ^{18}F-FLT is taken up through facilitated transport and diffusion and is phosphorylated by thymidine kinase 1 (TK_1) into ^{18}F-FLT-monophosphate. TK_1 is a cell cycle–regulated enzyme, and its activity is high in the stationary phase of normal cells. TK_1 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 activity of tissues. 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-α 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°C–40°C). Perfusion is performed with a roller pump, a DIDECO 902 (DIDECO) 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-α (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,

TABLE 1
Patient and Tumor Characteristics

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

*Established by MRI.
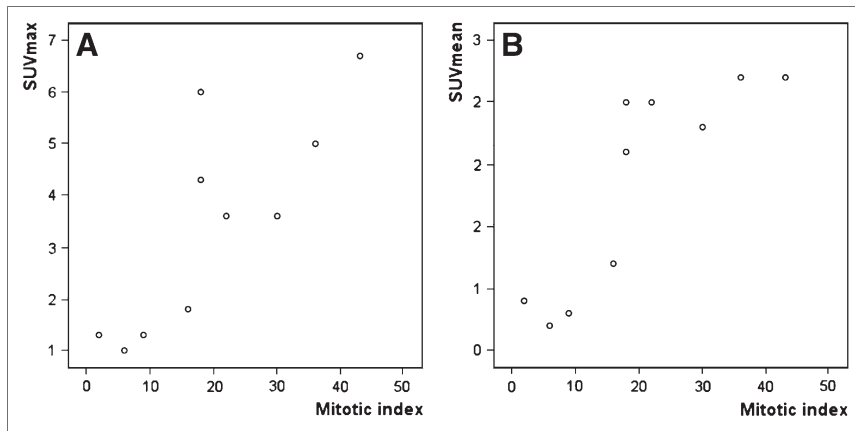
FIGURE 1. (A) Correlation between SUV_{max} and mitotic index before HILP ($r = 0.87$, $P = 0.001$). (B) Correlation between SUV_{mean} and mitotic index before HILP ($r = 0.82$, $P = 0.004$).

grade 2, or grade 3 tumors. The mitotic index (number of mitotic figures per 2 mm^2) 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

^{18}F-FLT was synthesized by the method of Machulla et al. (*19*). ^{18}F-FLT was produced by fluorination with ^{18}F-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. ^{18}F-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 ^{18}F-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 (Syngo 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 ^{18}F-FLT uptake, taking into account the pattern of physiologic uptake of ^{18}F-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

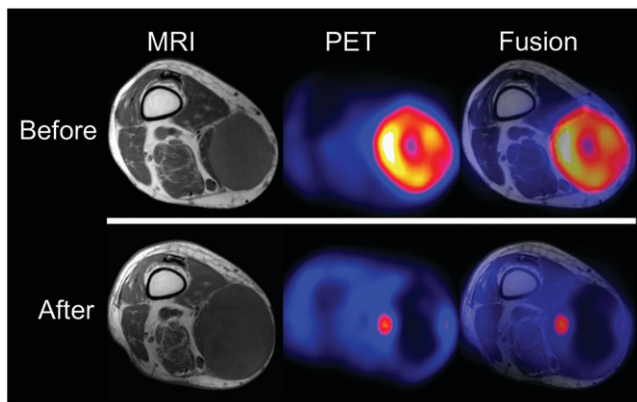|	Before HILP			After HILP				
Patient	SUV_{max}	SUV_{mean}	Interval (d)	SUV_{max}	SUV_{mean}	Change in SUV_{max} (%)	Change in SUV_{mean} (%)	Necrosis (%)
1	6.7	2.7	28	6.4	2.0	5	24	75
2	3.6	2.5	34	1.2	1.0	67	61	70
3	5.0	2.7	30	1.2	0.7	77	73	90
4	3.6	2.3	43	0.9	0.6	77	76	80
5	4.3	2.1	49	2.4	0.5	44	77	80
6	1.0	0.7	34	1.0	0.7	2	3	95
7	6.0	2.5	41	1.2	0.7	79	71	95
8	1.8	1.2	45	1.2	0.9	34	25	10
9	1.3	0.9	47	0.7	0.5	45	45	10
10	1.3	0.8	43	0.8	0.7	39	21	0

FIGURE 2. Examples of ^{18}F-FLT PET, MRI, and software fusion images of high-grade myxofibrosarcoma before and after HILP. After HILP, this tumor showed 80% necrosis. ^{18}F-FLT PET revealed heterogeneity of tumor before and after HILP.

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 ^{18}F-FLT PET, with a mean SUV_{max} of 3.5 (range, 1.0–6.7) and a mean SUV_{mean} of 1.9 (range, 0.7–2.7). Both SUV_{max} and SUV_{mean} correlated with the mitotic index of the tumors ($r = 0.82$ and $P = 0.004$ for SUV_{max}; $r = 0.87$ and $P = 0.001$ for SUV_{mean}) (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. ^{18}F-FLT PET after HILP revealed significantly decreased uptake of the tracer. The SUV_{max} decreased to 1.7 ($P = 0.008$), and the mean SUV_{mean} decreased to 0.8 ($P = 0.002$). Most tumors showed a center of very little or no ^{18}F-FLT uptake and a rim of moderate ^{18}F-FLT uptake. For some tumors, ^{18}F-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 ^{18}F-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 SUV_{mean} could be demonstrated. A weak but significant correlation ($r = 0.642$, $P < 0.05$) between the initial SUV_{mean} and the percentage of necrosis after HILP was demonstrated (Fig. 3).

DISCUSSION

In this study, the value of ^{18}F-FLT PET for evaluating the response of locally advanced soft-tissue sarcomas to HILP
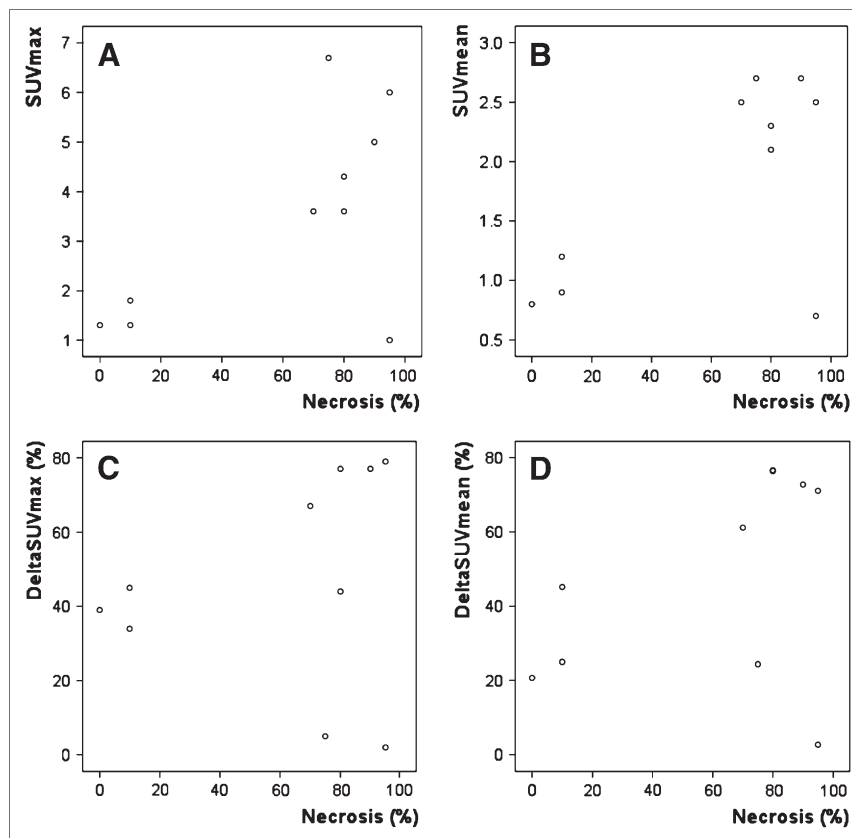

FIGURE 3. (A) No significant correlation between SUV_{max} before HILP and percentage of necrosis after HILP ($r = 0.622$, $P = 0.055$). (B) Correlation between SUV_{mean} before HILP and percentage of necrosis after HILP ($r = 0.642$, $P < 0.05$). These data indicated that tumors with high initial ^{18}F-FLT uptake showed better response to HILP. (C) No significant correlation between decrease in SUV_{max} and percentage of necrosis after HILP ($r = 0.190$, $P = 0.60$). (D) No significant correlation between decrease in SUV_{mean} and percentage of necrosis after HILP ($r = 0.404$, $P = 0.25$).

was investigated. ^{18}F-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 ^{18}F-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).

^{18}F-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 ^{18}F-FLT PET can monitor the response to therapy at an early stage (21). However, in this study, we wished to compare the results of ^{18}F-FLT PET after HILP with histopathologic examinations of the resected tumors and decided to perform ^{18}F-FLT PET shortly before the resection.

For our group of 10 patients with locally advanced soft-tissue sarcomas, the baseline uptake of ^{18}F-FLT correlated with the mitotic index, which is a derivative of tumor aggressiveness. Cobben et al. previously showed that ^{18}F-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 ^{18}F-FLT uptake have been reported (22–24).

Furthermore, tumors with high initial ^{18}F-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 SUV_{max}, ranging from 1.3 to 1.8, and an SUV_{mean} 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). ^{18}F-FLT PET may therefore be able to identify patients who will benefit the most from HILP.

Cobben et al. previously investigated the value of ^{18}F-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 ^{18}F-FLT uptake were found on whole-body ^{18}F-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 ^{18}F-FLT PET for detecting metastases has been investigated by several research groups, and ^{18}F-FLT PET has been shown to be probably not superior to ^{18}F-FDG PET (11). For different reasons, ^{18}F-FLT uptake is lower than ^{18}F-FDG uptake in almost all types of cancer. Bastiaannet et al. recently reviewed the literature regarding ^{18}F-FDG PET for patients with soft-tissue sarcomas and concluded that there is no indication to use ^{18}F-FDG PET routinely (26).

We believe that the true value of ^{18}F-FLT is not its capacity to detect tumors but its capacity to evaluate the response to therapy. van Ginkel et al. previously investigated ^{18}F-FDG PET and ^{11}C-tyrosine-PET for patients undergoing HILP for soft-tissue sarcomas and skin cancers (27,28). They concluded that ^{18}F-FDG PET was able to measure the response to HILP; however, ^{18}F-FDG could not discriminate between viable tumor cells and inflamma-

tory tissues because ^{18}F-FDG uptake was observed in both. ^{11}C-Tyrosine uptake 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 ^{18}F-FLT, like ^{11}C-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 ^{18}F-FLT did not demonstrate uptake in inflammatory tissues (13).

CONCLUSION

Although ^{18}F-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 ^{18}F-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 ^{18}F-FLT PET could be valuable in the future for guiding surgeons in planning resections or radiotherapists in planning conformal radiotherapy.

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