Effects of Pegfilgrastim on Normal Biodistribution of $^{18}$F-FDG: Preclinical and Clinical Studies

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The purpose of this study was to evaluate the effects of pegfilgrastim, a long-acting granulocyte colony-stimulating factor, on the normal biodistribution of $^{18}$F-FDG in an animal model and in humans. Methods: Two groups of 12 rats received a single subcutaneous injection of either normal saline or pegfilgrastim. One, 7, 14, and 21 d after injection, biodistribution studies were performed 1 h after $^{18}$F-FDG injection. Sixteen breast cancer patients underwent baseline $^{18}$F-FDG PET/CT and, approximately 1 wk after receiving 1 dose of docetaxel and adjunctive pegfilgrastim, follow-up $^{18}$F-FDG PET/CT (scan 2). Standardized uptake values corrected for lean body mass (SUL) were determined for several normal organs before and after therapy. Results: In rats, bone marrow $^{18}$F-FDG uptake (standardized uptake value) was higher in the pegfilgrastim group 1 d after injection (mean ± SD, 8.3 ± 4.1 vs. 2.5 ± 0.2, $P < 0.05$), whereas $^{18}$F-FDG uptake in blood was lower (0.41 ± 0.06 vs. 0.49 ± 0.01, $P < 0.05$). In patients, mean SUL was higher in bone marrow (4.49 ± 1.50 vs. 1.33 ± 0.22, $P < 0.0001$), spleen (3.29 ± 0.83 vs. 1.23 ± 0.23, $P < 0.0001$), and liver (1.45 ± 0.25 vs. 1.31 ± 0.23, $P = 0.01$) but lower in brain (4.18 ± 0.76 vs. 5.14 ± 1.44, $P < 0.01$) on scan 2 than on the baseline scan. Conclusion: In both the animal model and humans, pegfilgrastim markedly increased bone marrow uptake of $^{18}$F-FDG and reduced $^{18}$F-FDG uptake in some normal tissues. These profound alterations in $^{18}$F-FDG biodistribution induced by pegfilgrastim must be considered when one is evaluating quantitative $^{18}$F-FDG PET scans for tumor response to therapy. Key Words: $^{18}$F-FDG; G-CSF; pegfilgrastim; bone marrow; positron

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PET with $^{18}$F-FDG is a useful noninvasive imaging tool for evaluating tumor response to therapy (1–3). Typically, a baseline scan and then a follow-up scan after treatment are obtained. In the interval between scans, patients with nonmyeloid malignancies may receive adjunctive hematopoietic cytokines to counteract the myelosuppressive side effects of the administered chemotherapeutic agents. Pegfilgrastim (Neupogen; Amgen Inc.) and pegfilgrastim (Neulasta; Amgen Inc.) are granulocyte colony-stimulating factors (G-CSFs) that act on the monocyte–macrophage hematopoietic cell lineage to stimulate progenitor proliferation, differentiation, and functional activation of the mature hematopoietic cells (4–6). The 2 agents have been shown to have similar efficacy in decreasing the incidence of chemotherapy-induced neutropenia and the resulting complicated infections (7–9).

Pegfilgrastim, a covalent conjugate of filgrastim and monomethoxy polyethylene glycol (10), was approved by the Food and Drug Administration in 2002. Pegfilgrastim and filgrastim have the same mechanism of action, but the addition of the glycol moiety reduces renal clearance of pegfilgrastim, compared with that of filgrastim, prolonging the serum half-life of the drug in vivo (10,11). In humans, clearance of pegfilgrastim depends on neutrophil receptor binding and correlates directly with serum neutrophil count. Consequently, pegfilgrastim can be administered as a single dose rather than the daily doses required for filgrastim (10), and this advantage has led to its increased and preferred use.

Clinical studies have demonstrated that the administration of short-acting hematopoietic cytokines, such as filgrastim, to patients shortly before imaging evaluation with $^{18}$F-FDG PET markedly increases $^{18}$F-FDG accumulation in bone marrow (12–16) and spleen (16). Bone marrow is a frequent site of metastatic disease in several tumor types, and this altered biodistribution should not be interpreted as diffuse metastases to bone marrow or bone.

Similar to the findings reported after the administration of short-acting G-CSF, we have observed increased $^{18}$F-FDG accumulation in the bone marrow and spleen of patients who have received a long-acting G-CSF, pegfilgrastim, shortly before $^{18}$F-FDG PET. The effects of pegfilgrastim on normal $^{18}$F-FDG biodistribution have not, however, been formally evaluated. At the time of the initial publications evaluating the effects of hematopoietic growth factors on $^{18}$F-FDG biodistribution, pegfilgrastim was not yet approved by the Food and Drug Administration for routine use in the care of oncology patients.

We hypothesized that the expected preferential accumulation of $^{18}$F-FDG in bone marrow and spleen after
were obtained just before the start of therapy and then at about day 8, after the first cycle of chemotherapy. Patients received single doses of docetaxel and pegfilgrastim in the interval between PET/CT scans.

**18F-FDG PET/CT**

The patients fasted for a minimum of 4 h and had blood glucose levels no greater than 200 mg/dL just before injection of the 18F-FDG. A weight-based dose of 18F-FDG (8.14 MBq/kg [0.22 mCi/kg]) was injected intravenously in the arm contralateral to the primary breast carcinoma. The mean 18F-FDG radioactivity injected for all scans was 618 ± 167 MBq (16.7 ± 4.5 mCi). Oral, but not intravenous, contrast material was administered for the CT portion of the study.

After an approximately 60-min uptake phase, a combined PET/CT scan (Discovery LS; GE Healthcare) was obtained from the mid-skull level to the mid-femur level. Whole-body CT was performed first, with a 4-slice multidetector helical scanner and the following parameters: 140 kV, weight-based-amperage range (80–160 mA), 0.8 s per CT rotation, a pitch of 6, a table speed of 22.5 mm/s, 722.5-mm coverage, and a 31.9-s acquisition time. A CT transmission map was generated for image fusion. Emission data were acquired for 5 min at each bed position. PET images were reconstructed using the ordered-subset expectation maximization algorithm (2 iterations, 28 subsets), an 8-mm gaussian filter with a 128 ×128 matrix, and CT attenuation correction.

**Quantitative Image Analysis**

Regions of interest (ROIs) of nearly constant size and location were manually drawn on sections of spleen (number of pixels, 109 ± 19), liver (427 ± 28), lung (95 ± 20), and brain (70 ± 10) on 3 consecutive transaxial PET slices. The mean number of pixels did not significantly differ between the baseline scan and scan 2 for the ROIs of any organ. ROIs were drawn in the inferior right hepatic lobe (to avoid large veins or the biliary tree), the posterior right upper lobe of the lung, and the left cerebellum. The mean standardized uptake value corrected for lean body mass (SULmean) was determined for the ROI on each transaxial PET slice, and the average SULmean of the 3 transaxial slices was used for further analysis. The SULmean in brain could not be determined for 4 patients because only a small portion of the cerebellum was included in the images.

SULmean in bone marrow was determined by placing a 1.5-cm circular ROI in the middle of T10, T11, and T12 and taking the average SULmean of bone marrow in these 3 vertebral bodies. To determine the SULmean of blood, we placed 1.2-cm circular ROIs in the center of the ascending aorta, from the level of the carina cranially, on 6 consecutive transaxial PET slices and used the average of these. The maximum standardized uptake value corrected for lean body mass (SULmaximum) was determined for the primary tumors. All ROIs were placed using CT guidance.

**Statistical Analysis**

For the animal studies, differences in tissue uptake of 18F-FDG between the pegfilgrastim and saline groups were compared by the t test. ANOVA was used to evaluate changes in 18F-FDG uptake in bone marrow over time in the animal studies. For patient studies, paired t tests were used to compare the average SULmean values of normal organs on the baseline and follow-up PET/CT scans and to compare patient and technical parameters that can affect the standardized uptake value corrected for lean body mass (SUL). A P value of less than 0.05 was considered significant, using a 1-tailed t test.
RESULTS

Animal Studies

$^{18}$F-FDG activity in normal tissues at 1 h after radiotracer injection is presented in Figure 1. Twenty-four hours after pegfilgrastim or saline administration, $^{18}$F-FDG activity (standardized uptake value) in bone marrow was much higher in the pegfilgrastim group than in the saline group ($8.3 \pm 4.1$ vs. $2.5 \pm 0.2$, $P < 0.05$), whereas activity in blood ($0.41 \pm 0.06$ vs. $0.49 \pm 0.01$) and kidneys ($1.3 \pm 0.2$ vs. $1.7 \pm 0.2$) was lower ($P < 0.05$). Activity in brain tended to be lower in the pegfilgrastim group ($6.8 \pm 1.2$ vs. $8.2 \pm 0.7$, $P = 0.07$).

Seven days after pegfilgrastim or saline injection, the level of $^{18}$F-FDG activity in most tissues was similar between the pegfilgrastim and saline groups. $^{18}$F-FDG activity in bone marrow ($2.0 \pm 0.3$ vs. $2.5 \pm 0.2$, $P = 0.05$) and liver ($0.53 \pm 0.03$ vs. $0.66 \pm 0.04$, $P < 0.01$) was significantly lower in the pegfilgrastim group than in the saline group, whereas significantly higher activity was found in muscle ($1.2 \pm 0.2$ vs. $0.55 \pm 0.2$, $P = 0.01$). Almost all tissues showed no significant differences in $^{18}$F-FDG uptake between the pegfilgrastim and saline groups at 14 and 21 d (data not shown). The only exceptions were colon and bone, which showed decreased activity (respectively, $3.7 \pm 0.5$ vs. $3.0 \pm 0.2$, $P = 0.04$, and $0.25 \pm 0.05$ vs. $0.20 \pm 0.2$, $P = 0.05$) in the saline group at 21 d. Bone marrow uptake of $^{18}$F-FDG was significantly higher ($P < 0.05$) in the rats receiving the dose of pegfilgrastim 24 h before the $^{18}$F-FDG study ($8.3 \pm 4.1$) than in the rats receiving the dose 7, 14, or 21 d before the $^{18}$F-FDG study ($2.0 \pm 0.3$, $1.8 \pm 0.8$, and $2.2 \pm 0.1$, respectively; Fig. 2). This was not the case in the control group.

Patient Studies

All 16 patients received the prescribed single doses of docetaxel and pegfilgrastim during the interval between the baseline PET/CT scan and scan 2. All patients received pegfilgrastim 1 d after receiving docetaxel. The mean interval was $14 \pm 11$ d (range, 8–52 d) between the baseline PET/CT scan and scan 2; $7 \pm 0.7$ d between docetaxel administration and scan 2; and $6 \pm 0.7$ d between pegfilgrastim administration and scan 2. Patient and technical parameters that can affect SUL determination are shown in Table 1.

$^{18}$F-FDG uptake in the bone marrow and spleen of individual patients is shown in Figure 3. The average SULmean was significantly higher in bone marrow ($4.49 \pm 1.50$ vs. $1.33 \pm 0.22$, $P < 0.0001$) and spleen ($3.29 \pm 0.83$ vs. $1.23 \pm 0.23$, $P < 0.0001$) on scan 2 than on the baseline scan. SULmean in blood, brain, liver, and lung on the baseline scan and scan 2 is presented in Table 2. Mean $^{18}$F-FDG uptake in brain was significantly less on scan 2 than on the baseline scan ($4.18 \pm 0.76$ vs. $5.14 \pm 1.44$, $P < 0.01$), and $^{18}$F-FDG uptake was lower in blood on scan 2;
however, this difference did not reach statistical signifi-
cance (0.87 ± 0.18 vs. 0.93 ± 0.16, P = 0.08). 18F-FDG uptake in liver was higher on scan 2 than on the baseline scan (1.45 ± 0.25 vs. 1.31 ± 0.23, P = 0.01). Images from a representative case are shown in Figure 4. Tumor SUL maximum decreased significantly between the baseline scan and scan 2 (6.25 ± 5.07 vs. 3.74 ± 2.10, P = 0.02); however, the full results for tumor response will be reported elsewhere.

**DISCUSSION**

Our rat study showed that pegfilgrastim induces a rapid rise in 18F-FDG uptake in bone marrow 24 h after administration of a single dose—a result that can be explained by increased cellular proliferation and metabolism in bone marrow because of pegfilgrastim-induced stimulation of neutrophil progenitor cells (4–6). This same finding for bone marrow was previously observed in different strains of rats after administration of a shorter-acting G-CSF, filgrastim (15), and was expected given that both drugs have the same mechanism of action. Unlike what happens in humans, and for unclear reasons, neither of the G-CSF agents seems to increase 18F-FDG uptake in rat spleen. One consideration is that the effects of pegfilgrastim on 18F-FDG uptake in spleen occur at time points different from those evaluated in this and previous studies.

Our patient study also clearly demonstrated a rise in splenic uptake of 18F-FDG after pegfilgrastim administration, in accord with the effect of filgrastim on 18F-FDG uptake in spleen (16). In none of our patients did the pretherapy PET/CT studies performed just 2 wk before scan 2 show splenic disease. It is unlikely that such disease developed in the short interval between the scans. The diffuse increase in splenic 18F-FDG activity on the follow-up scan most likely represented G-CSF–induced extramedullary hematopoiesis, a phenomenon that has been reported to occur after administration of high-dose G-CSF (17,18). Increased hematopoiesis in spleen has been observed after shorter, intermittent therapy with G-CSF (19), and in the initial study reporting G-CSF–induced 18F-FDG uptake in spleen, patients had also received relatively few doses of short-acting G-CSF (16).

One limitation of this study was that the pegfilgrastim was administered as an adjunct to chemotherapy. Because we did not evaluate any patients who received chemotherapy alone, we cannot definitively state that the increased 18F-FDG activity in bone marrow was due to pegfilgrastim alone. That possibility is likely, however, because previous studies have demonstrated no significant rise in bone marrow SUL on serial 18F-FDG PET scans of patients who received chemotherapy, of several types, alone (15).

The results of our study also demonstrated that long-acting G-CSF affected 18F-FDG biodistribution in several normal organs. We observed significant declines in 18F-FDG uptake in brain on 18F-FDG PET patient scans obtained 6–8 d after pegfilgrastim administration, and 18F-FDG uptake in blood tended (P = 0.08) to be slightly lower at this time point. Sugawara et al. did not observe significant declines in blood SUL during or after G-CSF treatment (15); however, in their subgroup of patients receiving G-CSF, blood SUL was lower after G-CSF treatment than at baseline. The small number of patients likely limited the statistical power of that study. To our knowledge, the present report is the first on the effect of filgrastim or pegfilgrastim on 18F-FDG uptake in brain.

18F-FDG uptake is a measure of 18F activity in tissues and correlates somewhat with tumor blood flow and inversely with the serum concentration of glucose (20). Metabolic glucose rate can be calculated using a 3-compartment model. Free 18F-FDG in blood serves as the source of the precursor intracellular pool of 18F-FDG that is phosphorylated and retained within tumor cells (21). This model assumes the availability of sufficient radiotracer in the blood to enter
the cell. Our study suggested that less 18F-FDG activity is in the blood, and thus in the brain, of patients who recently received G-CSF therapy, probably because of preferential 18F-FDG uptake by bone marrow and spleen.

The more rapid neutrophil response and mobilization of the peripheral blood progenitor cells induced by pegfilgrastim (10) may increase the glycolytic requirements of these organs. This possibility has potentially significant

**FIGURE 3.** 18F-FDG uptake in bone marrow (A) and spleen (B) in patients (n = 16) at baseline and after single doses of adjuvant pegfilgrastim (scan 2). Average SULmean in bone marrow and spleen is significantly higher on scan 2 (P < 0.0001).

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**TABLE 2**

18F-FDG Uptake (SULmean) in Normal Organs of Patients Before and After Therapy

<table>
<thead>
<tr>
<th>Patient no.</th>
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<th>SUL marrow</th>
<th>SUL spleen</th>
<th>SUL brain</th>
<th>SUL liver</th>
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<td>Scans 2</td>
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<td>Scans 2</td>
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is metabolized in liver, predominantly by the CYP3A4 isoenzyme (22). Induction of CYP3A4 transcription by hepatic nuclear factor 4 has been suggested (23,24), and hepatic nuclear factor 4 has also been shown to regulate transcription of genes involved in glucose entry into hepatocytes and glucose metabolism (25). It is possible that upregulation of hepatic nuclear factor 4 in the presence of docetaxel leads to increased glucose metabolism in liver.

CONCLUSION

The results of this study demonstrate that long-acting G-CSF has substantial effects on the normal biodistribution of \[^{18}F\]-FDG in rats and humans. These effects have significant implications for the interpretation of \[^{18}F\]-FDG PET scans obtained shortly after G-CSF therapy. Increased bone marrow and splenic \[^{18}F\]-FDG uptake should not be misinterpreted as metastases but could mask metastases in these organs. The decreased radiotracer activity in blood may lower the bioavailability of \[^{18}F\]-FDG to tumors, resulting in lower tumor SUL, similar to what was seen with the lower brain \[^{18}F\]-FDG uptake. Future criteria for PET evaluation of the metabolic response of tumors to therapy should take into account the changes in normal \[^{18}F\]-FDG biodistribution caused by both antineoplastic agents and adjunctive therapies.

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