A Fluorine-18 Labeled Progestin as an Imaging Agent for Progestin Receptor Positive Tumors with Positron Emission Tomography

Aalt Verhagen, Philip H. Elsinga, Tijbbe J. de Groot, Anne M. J. Paans, Cornelis C. J. de Goeij, Mels Sluyser, and Willem Vaalburg

Department of Nuclear Medicine, University Hospital, Groningen [A. V., P. H. E., T. J. d. G., A. M. J. P., W. V.], and Division of Tumor Biology, The Netherlands Cancer Institute, Amsterdam [C. C. J. d. G., M. S.], The Netherlands

ABSTRACT

The potential of the fluorine-18 labeled progestin 21-[18F]fluoro-16α-ethyl-19-norpregn-4-ene-3,20-dione ([18F]FENP) as an imaging agent for the in vivo assessment of progestin receptor (PR) positive neoplasms with positron emission tomography has been investigated. Tissue distribution studies in immature estrogen primed female rats revealed high uptake of radioactivity, expressed as the differential absorption ratio, by uterine tissue. After simultaneous administration with unlabeled FENP, a significant decrease (83%) in uterine uptake was observed 60 min after injection. Uterine uptake was highly selective. The ratio of uptake of radioactivity by uterine tissue to that by blood was 39 at 180 min. In mice bearing transplanted Grunder strain mammary carcinomas tissue, distribution studies demonstrated a selective uptake of [18F]FENP by PR positive tumors. Pretreatment with unlabeled FENP caused a significant decrease (66%) in tumor uptake. Uptake by other tissues was not affected by the presence of unlabeled progestin. The ratio of uptake of radioactivity by tumor tissue to that by blood was 4.7 at 180 min. For FENP pretreated mice and mice bearing PR negative tumors, this ratio was 1.7 and 1.1, respectively. It is concluded that the uptake of [18F]FENP by uterine and by PR positive mammary tumor tissue in vivo is primarily receptor related, presumably to the PR. Furthermore, [18F]FENP appears to be suitable for imaging of PR positive human neoplasms with positron emission tomography.

INTRODUCTION

Receptor imaging with PET has focused primarily on receptor sites for specific neurotransmitters in the brain and the possible disturbance or dysfunction of these sites in neurological and psychiatric disorders (1). In addition, the principles of the in vivo measurement of neuroreceptors have been extended to oncology (2–4).

The presence and measurement of ER and PR are important for prognosis and therapy of breast cancer. It has been demonstrated that the concomitant presence of these receptors in breast cancer tissue indicates a high probability of responding to endocrine treatment (5, 6). Thus, in vivo imaging and quantification of ER and PR in human breast cancer with PET may be useful in the assessment of the potential hormone responsiveness of a primary tumor, and particularly of metastatic foci, and in monitoring the effect of various hormonal therapeutic regimens in individual patients. The ability of PET to evaluate ER in human breast cancer was demonstrated with the fluorine-18 labeled estrogen 16α-[18F]fluoro-estradiol (3).

Since steroid receptors occur at minute concentrations in tumor tissue, a very small quantity of radioactive steroid must be administered to achieve a ratio between receptor and non-receptor binding. This can be accomplished by using radio-pharmaceuticals with high pharmacological selectivity and high affinity for the particular steroid receptor and labeled with high specific radioactivity (GBq/μmol). For imaging PR with PET, the progestin FENP appears to be a very suitable ligand. FENP is an analogue of the synthetic progestin ORG 2058 in which the 21-hydroxy group has been replaced by a fluorine atom. Both ORG 2058 and FENP are high affinity progestins (7). We and others have labeled FENP isotopically with fluorine-18 (8, 9).

In this paper, we describe our investigations concerning [18F]FENP as a potential imaging agent for the in vivo assessment of PR positive neoplasms with PET. The uptake of [18F]FENP by uterine tissue is investigated in immature estrogen primed Wistar rats. GR mouse mammary carcinomas are used to examine [18F]FENP for tumor uptake. Mammary carcinomas can be induced in GR mice by treatment with estrone and progesterone (10, 11). Growth of these tumors is hormone (estrogen and progesterone) dependent. However, when the tumors are serially transplanted, a progressive decrease in hormone dependency is observed which eventually leads to complete hormone independency. This is accompanied by a decrease in tumor ER and PR densities (10, 11). Tumor uptake of [18F]FENP is investigated in these transplanted mammary carcinomas.

MATERIALS AND METHODS

Synthesis of [18F]FENP. [18F]FENP was synthesized by nucleophilic fluorination of the corresponding 21-tosylate (8). Briefly, the 21-tosylate was subjected to a substitution reaction with an aminopolyether/K⁺-complex containing [18F]fluoride. The [18F]fluoride was prepared from 18O enriched water by the 18O(p,n)18F nuclear reaction. Purification and measurement of specific radioactivity were performed according to the method described previously (8). A typical batch had a specific radioactivity of at least 185 GBq/μmol and a radiochemical purity of >99%. For animal studies [18F]FENP was dissolved in ethanol/propanol glycol/saline (1:2:2, v/v) and passed through a 0.2-μm membrane filter.

Tissue Distribution Studies in Normal Rats and Tumor Bearing Mice. Three groups of immature female Wistar rats (28–32 days) were used for tissue distribution studies. Each group consisted of 5 rats. Prior to the experiments all rats received 5 μg estradiol i.p. in 0.1 ml of 5% ethanol/sunflower oil for 3 successive days. Rats were used within 24 h after the last injection. The average animal body weight at the time of the experiment was 88 ± 10 (SD) g. Two groups were treated with 3.7 MBq [18F]FENP i.v. At 60 and 180 min after injection the animals were killed by cervical dislocation. To ascertain receptor related uptake by uterine tissue, the third group of rats was given [18F]FENP together with 20 μg unlabeled FENP. These rats were killed 60 min after injection. Blood and tissue samples were weighed and assayed for radioactivity in a calibrated well counter. Tissue uptake was expressed as the DAR and calculated from the formula:

1930
For each animal, the selectivity of uterine uptake was estimated by calculating the ratio of the uptake of radioactive by uterine tissue to that by blood or muscle tissue.

HD mammary carcinomas were induced in ovariectomized GR mice by treatment with estrone and progesterone. Serial transplantation and evaluation of the hormone dependence of the tumors were carried out as described previously (10, 11). HD and HI mammary tumor transplants from 4 different tumor lines were used. Experiments were carried out within 3 weeks after discontinuance of progesterone treatment. Two groups of mice bearing HD tumors and one group of mice bearing HI tumors were used for tissue distribution studies. Each group consisted of 4 mice. The average mouse body weight and tumor weight at the time of the experiment were 33 ± 4 and 3.8 ± 1.5 g, respectively. All mice received 3.7 MBq [18F]FENP i.p. and were killed by cervical dislocation after 180 min. One group of mice bearing HD tumors received 30 μg unlabeled FENP 30 min before the administration of the labeled progestin. Measurement of tissue uptake and tumor uptake selectivity was identical to that described for normal rats.

Positron Emission Tomography. A radiopharmaceutical labeled with a positron emitting radioisotope can be measured as a result of the annihilation of the positron in tissue. At the point of each annihilation, two photons are emitted in opposite directions. These coincidence photons, rather than the positron itself, are detected. We used a longitudinal positron camera system to image the distribution of [18F]FENP (12). The system consists of two uncollimated large field of view gamma cameras operating in a coincidence mode. The cameras are placed in a fixed colinear position. A computer system is used for data acquisition and reconstruction of the images.

The PET study was carried out with two tumor bearing mice. We used an HD and HI mammary tumor transplant. Both of the mice were given an i.p. injection of 1.9 MBq [18F]FENP. At 102 min after administration, data acquisition was started at a frame rate of 1 frame/3 min for 150 min. After 2 days the mouse bearing the HD tumor was imaged again according to the same procedure. However, in this experiment the animal was pretreated with 30 μg unlabeled FENP to demonstrate the blocking of tumor uptake. During data acquisition mice were under mild anesthesia with sodium pentobarbital. Tumor size was estimated by measuring length, width, and height of the tumor and calculating the volume assuming an ellipsoid shape. PET data were corrected for nonuniform sensitivity and physical decay (12). At 105, 165, and 225 min after injection the data were summed into 15-min images. The PET images were displayed and regions of interest were defined, representing the tumor and the total body. The amount of radioactivity within the assigned regions was retrieved. Subsequently, DAR values for tumor uptake were calculated. Because minced tumor tissue was transplanted by s.c. inoculation into the flank of the animals, the tumors were imaged free from surrounding tissue.

RESULTS AND DISCUSSION

In immature estrogen primed female rats the uterus demonstrated a pronounced uptake of [18F]FENP (Table 1). In addition, uterine uptake remained relatively high even after 180 min. A statistically significant decrease in uptake by the uterus and the ovaries was observed when rats were cotreated with an excess of unlabeled FENP. Moreover, the decrease in uptake of radioactivity by the uterus, and to some extent the ovaries, appeared to be selective, whereas all the other tissues showed equivalent uptake in the absence and presence of the blocking dose of unlabeled progesterin. The selectivity of uterine uptake was measured by the ratios of radioactivity localized in uterine tissue to that localized in blood or in muscle tissue. Although uterine uptake decreased during the specified period of time, the ratios continued to increase, as [18F]FENP was cleared more rapidly from tissues with known undetectable PR. A consider-

### Table 1. Tissue distribution of [18F]FENP after i.v. injection of [18F]FENP into immature estrogen primed female rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>DAR*</th>
<th>60 min</th>
<th>60 minb</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus</td>
<td>2.08 ± 0.38</td>
<td>0.36 ± 0.05</td>
<td>1.69 ± 0.44</td>
<td></td>
</tr>
<tr>
<td>Ovaries</td>
<td>1.17 ± 0.27</td>
<td>0.70 ± 0.20</td>
<td>0.65 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>0.15 ± 0.03</td>
<td>0.15 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0.29 ± 0.05</td>
<td>0.27 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.59 ± 0.28</td>
<td>1.63 ± 0.20</td>
<td>0.76 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.55 ± 0.06</td>
<td>0.52 ± 0.08</td>
<td>0.23 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>0.32 ± 0.08</td>
<td>0.36 ± 0.10</td>
<td>0.09 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0.36 ± 0.06</td>
<td>0.36 ± 0.05</td>
<td>0.09 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>1.14 ± 0.21</td>
<td>1.24 ± 0.21</td>
<td>1.48 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>1.37 ± 0.30</td>
<td>1.28 ± 0.33</td>
<td>1.57 ± 0.33</td>
<td></td>
</tr>
<tr>
<td>Uterus/blood</td>
<td>13.78 ± 2.56</td>
<td>2.49 ± 0.45</td>
<td>39.42 ± 10.87</td>
<td></td>
</tr>
<tr>
<td>Uterus/muscle</td>
<td>7.34 ± 1.45</td>
<td>1.35 ± 0.19</td>
<td>15.89 ± 5.50</td>
<td></td>
</tr>
</tbody>
</table>

* N = 5.
* In order to block receptor related uterine uptake, [18F]FENP was coinjected with 20 μg FENP.
* Mean ± SD.
* The uptake by the uterus and the ovaries was significantly decreased after coinjection of 20 μg FENP (two sided t test, P < 0.001 and P < 0.02, respectively).

able uptake of the lipophilic progestin by fat tissue was noticed. Furthermore, radioactivity appeared to accumulate markedly in the bones. Most likely, this is attributable to metabolic defluorination of the labeled progesterin and subsequent uptake of [18F]fluoride by bone tissue. Our results concerning the tissue distribution of [18F]FENP in normal rats are comparable with those reported previously (9). However, when we consider the relatively high PR density in uterine tissue, the potential of [18F]FENP is perhaps overestimated. This prompted us to investigate whether selective PR related mammary tumor uptake is measurable. By using different transplant generations of GR mouse mammary carcinomas, we can compare tumor uptake of [18F]FENP by HD tumors with that of HI tumors. HD mammary tumors are ER and PR positive, whereas HI mammary tumors are practically devoid of these receptors (11, 12).

Tissue distribution data in mice bearing GR mammary tumor transplants showed that the uptake of [18F]FENP by HD tumor tissue was selective, as was evident by the uptake of this tissue and by the tumor to blood ratio (Table 2). Tumor uptake was considerably less than the observed uterine uptake in normal rats. Receptor related tumor uptake was indicated by a statistically significant decrease in uptake when mice were pretreated with an excess of unlabeled FENP. Uptake in other tissues was not affected by the presence of unlabeled progesterin. In contrast, to demonstrate the blocking of tumor uptake. During data acquisition mice were under mild anesthesia with sodium pentobarbital. Tumor size was estimated by measuring length, width, and height of the tumor and calculating the volume assuming an ellipsoid shape. PET data were corrected for nonuniform sensitivity and physical decay (12). At 105, 165, and 225 min after injection the data were summed into 15-min images. The PET images were displayed and regions of interest were defined, representing the tumor and the total body. The amount of radioactivity within the assigned regions was retrieved. Subsequently, DAR values for tumor uptake were calculated. Because minced tumor tissue was transplanted by s.c. inoculation into the flank of the animals, the tumors were imaged free from surrounding tissue.

### Table 2. Tissue distribution of [18F]FENP into mice bearing GR mammary tumor transplants

<table>
<thead>
<tr>
<th>Tissue</th>
<th>DAR*</th>
<th>HD tumor</th>
<th>HD tumorb</th>
<th>HI tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.02</td>
<td>0.06 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.62 ± 0.09</td>
<td>0.66 ± 0.16</td>
<td>0.59 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.26 ± 0.09</td>
<td>0.27 ± 0.09</td>
<td>0.21 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>0.10 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>0.10 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>2.02 ± 0.48</td>
<td>2.00 ± 0.60</td>
<td>1.67 ± 0.32</td>
<td></td>
</tr>
<tr>
<td>Tumorb</td>
<td>0.29 ± 0.13</td>
<td>0.10 ± 0.02</td>
<td>0.06 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

* N = 4.
* In order to block receptor related tumor uptake mice were pretreated with 30 μg FENP.
* Mean ± SD.
* Of each tumor 2 samples were weighed and assayed for radioactivity.

The uptake by tumor tissue was significantly decreased after pretreatment with 30 μg FENP (two sided t test, P < 0.001).
HI mammary tumors hardly showed any uptake of radioactivity. Moreover, the low uptake by HI mammary tumors emphasized the observed selective tumor uptake of \([^{18}F]\)FENP by HD mammary tumors. Again a considerable accumulation of radioactivity in the bones was noticed.

The most commonly affected distant sites of metastatic disease in breast cancer are lung, bone, and liver. With regard to imaging of metastases, especially the nonspecific uptake by bone and liver tissue appears to reduce selective uptake of \([^{18}F]\)FENP by PR positive tumors. However, to arrive at definite conclusions clinical investigation is required.

PET images revealed a receptor related uptake of \([^{18}F]\)FENP by an HD mammary tumor transplant (Fig. 1). These images were used to calculate the differences in uptake of radioactivity before and after pretreatment with an excess of unlabeled FENP and were compared with similar images of a mouse bearing an HI mammary tumor transplant (Fig. 2). Initially, the uptake by the HD mammary tumor declined slowly and remained constant thereafter. The difference with tumor uptake after pretreatment with unlabeled FENP increased during the specified period of time. The ratios of tumor uptake before and after pretreatment were 1.3 and 2.1 at the second and third time interval after injection, respectively. In addition, during the specified time intervals the tumor region of the mouse bearing the HD mammary tumor hardly showed any visible uptake of radioactivity (images not shown). At 165 to 180 min after injection the DAR values for HD and HI tumor uptake, resulting in a ratio of about 7.4, correlated well with the results obtained with the tissue distribution studies (Table 2). However, in the PET images the HD tumor uptake after pretreatment with unlabeled FENP appeared to be higher.

Whether \([^{18}F]\)FENP and PET can be used clinically for the in vivo assessment of PR in neoplasms is currently under investigation. We are studying patients with breast cancer and patients with meningiomas. The latter neoplasms have also been shown to contain high PR densities (13).

In summary, our present results obtained with two animal models indicate a selective uptake of \([^{18}F]\)FENP by uterine tissue and by PR positive mammary tumor tissue. Unlabeled FENP suppresses the uptake of radioactivity to the level observed in tissues with known undetectable PR. Therefore, we conclude that uterine and HD mammary tumor uptake in vivo is primarily receptor related, presumably to the PR. This suggests the potential applicability of \([^{18}F]\)FENP and PET for imaging PR positive neoplasms.

ACKNOWLEDGMENTS

The authors are grateful to Dr. M. B. Groen of Organon International BV, The Netherlands, for providing samples of ORG 2058 and ORG OH-06 (FENP), and to the operating team of the cyclotron of the Kernfysisch Versneller Instituut, Groningen, The Netherlands, for expert technical assistance.

REFERENCES


Fig. 1. Anterior whole body PET images of a mouse bearing an HD mammary tumor. The images were made 225-240 min after i.p. injection. Tumor uptake was visible after administration of \([^{18}F]\)FENP with high specific radioactivity (A). After pretreatment with excess unlabeled FENP, hardly any tumor uptake was observed (B). Organs involved in steroid metabolism and excretion showed high uptake in the images. Arrows, location of the tumor.
ASSESSMENT OF PR POSITIVE TUMORS WITH PET


A Fluorine-18 Labeled Progestin as an Imaging Agent for Progestin Receptor Positive Tumors with Positron Emission Tomography


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/51/7/1930

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.