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Biodistribution and breast tumor uptake of 16α-[^{18}F]-fluoro-17β-estradiol in rat

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To evaluate the usefulness of 16α-[^{18}F]-fluoro-17β-estradiol (FES) for the assessment of estrogen receptor (ER), we examined the tissue distribution and kinetics of FES in immature female Sprague-Dawley rats and then examined FES uptake in rat breast tumors induced by 7,12-dimethylbenz(a) anthracene (DMBA). The FES uptake by the uterus, an ER-rich tissue, was highly selective and it was 3.34 ± 0.79%ID/g at 60 minutes and 1.57 ± 0.57%ID/g at 120 minutes after injection. The FES uptakes in ER-negative tissues were 0.12 ± 0.05%ID/g or less and 0.05 ± 0.03%ID/g or less, respectively. Coadministration of unlabeled β-estradiol showed marked depression of uterine FES uptake. The FES uptake by rat breast tumors was 0.14 ± 0.06%ID/g at 60 min and 0.12 ± 0.09%ID/g at 120 min. The FES uptake by rat breast tumors correlated with the ER concentration ($r = 0.45$, $p < 0.05$). In conclusion, these results suggest that the FES uptake by tissue is mainly ER mediated and FES is thus useful for detecting ER positive breast tumors.

Key words: breast cancer, estrogen receptor, ^{18}F-estradiol

INTRODUCTION

AN ASSESSMENT of estrogen receptor (ER) is thought to be useful for predicting the response to hormonal therapy in patients with breast cancer.[1] Because *in vitro* ER assays are routinely used to measure the ER concentration, *in vivo* non-invasive measurement has been required to determine the functional status of the ER, especially in patients with multiple metastases. 16α-[^{18}F]-fluoro-17β-estradiol (FES) is an *in vivo* imaging agent for ER developed by Kiesewetter et al.[2] In rats, the FES uptake by target tissue has been demonstrated to be highly selective[2] and it was suppressed by pretreatment with anti-estrogen tamoxifen.[3] The FES uptake was also demonstrated by ER-positive rat mammary tumors.[4] In human studies, the FES uptake in both primary breast carcinoma and metastatic lesions and a good correlation between FES uptake and ER concentrations have been demonstrated.[5–7] A decrease in the FES uptake by tumor after the initiation of anti-estrogen therapy was also demonstrated.[6] Based on these considerations, FES appears to be a promising radiopharmaceutical for the assessment of ER, though FES examinations can as yet only be performed at a limited number of institutions.

The aim of this study is to synthesize FES and to investigate its effectiveness in our institution before clinical use. We synthesized FES first and then examined its tissue distribution and kinetics in rat. We next evaluated the usefulness of FES for assessing estrogen receptors in rat breast tumors.

METHODS

Synthesis of 16α-[^{18}F]fluoro-17β-estradiol

The FES was synthesized according to the method established by Lim JL et al.[8] An in-house cyclotron (BC1710; JSW Corp., Muroran, Japan) was used for the production of ^{18}F from [^{18}O]H_2O by the reaction of ^{18}O (p, n) ^{18}F. 16-epiestriol was reacted with chloromethyl methyl ether to protect phenolic hydroxyl at 3-position. The resulting

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diol was then reacted with sulfuryl diimidazole to produce cyclic sulfate. The FES was prepared by reacting the cyclic sulfate with stoichiometric anhydrous [^{18}F]fluoride, followed by hydrolysis. High-performance liquid chromatography (HPLC) was performed. The eluant was monitored with an UV detector at 280 nm and a sodium iodide scintillation detector. The specific activity of FES was more than 150 Ci/mmol. The FES solution for intravenous injection was prepared by dissolving the dried FES in 3.2% polyoxyethylenesorbitan monooleate (Tween-80) and 1.6% ethanol in saline solution.

Biodistribution and Kinetics of FES

Immature female Sprague-Dawley rats (Kyudo Corp., Kumamoto, Japan), from 24 to 28 days old, were used for this study to exclude the effect of endogenous estadiol, because the estrus cycle in rat begins around 30 days after birth.[4] The rats were housed on a regular 12:12 light/dark cycle in a specific pathogen free temperature controlled room. Their body weight was 74.5 ± 8.56 grams (mean ± standard deviation, ranges from 53.2 to 91.0 grams). Each rat was anesthetized by inhaling ethyl ether and then was injected intravenously with 1.75 ± 1.22 (ranging from 0.20 to 4.19) MBq of FES via the tail vein. At 7, 15, 30, 60, 120, 180 and 240 minutes after injection, the rats were sacrificed by cervical dislocation under anesthesia and tissue samples were removed and assayed for radioactivity in a gamma counter (n = 5 at each time). The tissue uptake of FES was evaluated as percent injected dose per a weight (%ID/g). In experiments to show blocking of the FES uptake by ER positive tissue, 15 μg of unlabeled β-estradiol (Sigma, St Louis, MO, USA) was coinjected with the FES.[3]

FES Uptake in Rat Breast Tumor

Breast tumors were induced in female Sprague-Dawley rats at 6 weeks by the oral administration of 5 mg of 7,12-dimethylbenz(a) anthracene (DMBA) in an oil emulsion.[9] The rats were housed on a regular 12:12 light/dark cycle in a specific pathogen free temperature controlled room. After 60–90 days, breast tumors appeared and the rats were then subjected to the experiment within 180 days. Sixteen rats developed breast tumors including 3 carcinomas, 2 fibroadenomas and 11 papillomas. The average body weight was 317 ± 42 grams (ranging from 261 to 411 grams). Each rat was anesthetized by the inhalation of the ethyl ether and then was injected intravenously with 4.37 ± 2.65 (ranging from 2.57 to 10.9) MBq of FES via the tail vein. At 60 or 120 minutes after administration, the rat was sacrificed by decapitation under anesthesia and tissue samples were then removed and assayed for radioactivity in a gamma counter (n = 13 and n = 3, respectively). The FES uptake in a tumor was then evaluated as %ID/g. A part of the tissue was immediately frozen in liquid nitrogen and stored for *in vitro* receptor assay evaluation.

The *in vitro* receptor assay was a radioreceptor assay (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan) for a quantitative measurement of the ER content of the frozen tumor specimens.[10] Briefly, tumor tissue was homogenized and then incubated with 16α-^{125}I-estradiol-17β for 18 hours at 4°C with or without diethylstilbestrol. After removing free 16α-^{125}I-estradiol-17β by the dextran-coated charcoal (DCC) method, the radioactivity was counted in a gamma counter. The Scatchard analysis determined the ER concentration (Bmax, fmol/mg protein) and the dissociation constant (Kd, × 10^{-10} M). The binding potential (BP) was calculated by Bmax over Kd.

These experiments were reviewed by the Committee of the Ethics on Animal Experiments in the Graduate School of Medical Sciences, Kyushu University and carried out under the control of the Guidelines for Animal Experiments in the Graduate School of Medical Sciences in Kyushu University and The Law (No. 105) and Notification (No. 6) of the Government.

Differences in the mean values were assessed by one-way factorial analysis of variance (ANOVA) for statistical analysis. A linear regression analysis was made for the correlation study. Probability values < 0.05 were considered to be significant.

RESULTS

Biodistribution and Kinetics of FES in Immature Female Rats

The FES uptake and kinetics in the tissue specimens are shown in Figure 1. FES exhibits highly selective uptake by the uterus, an ER-rich target tissue of FES. FES uptake by the uterus reached a maximum at 60 minutes and gradually decreased thereafter. It was 3.34 ± 0.79%ID/g at 60 minutes and 1.57 ± 0.57%ID/g at 120 minutes. FES uptake in ER-negative tissues, including the lungs and muscle, reached initial peaks of 1.69%ID/g or less at 7 minutes after administration and gradually decreased

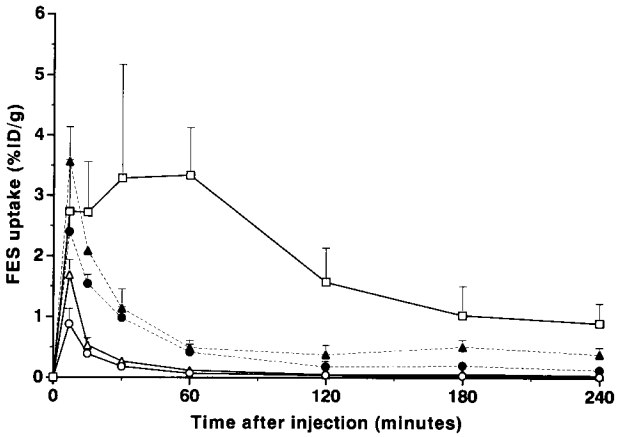

Fig. 1 The time course of the FES uptake in tissues in immature female rat. (square: uterus; open triangle: lung; open circle: muscle; closed triangle: liver; closed circle: kidney)

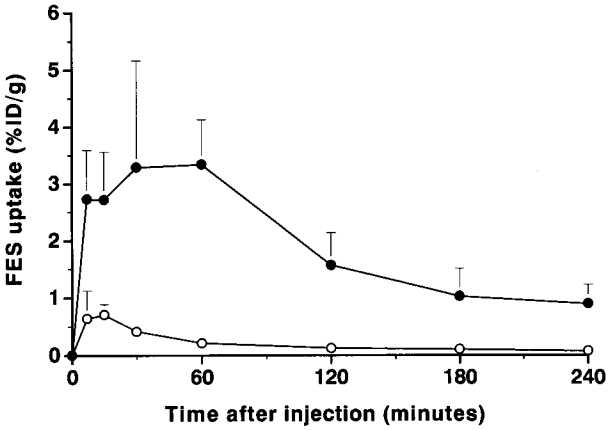

Fig. 2 The time course of uterine uptake of FES with or without unlabeled β-estradiol in immature female rat. (closed circle: FES only; open circle: coinjection of FES with 15 μg of unlabeled β-estradiol)

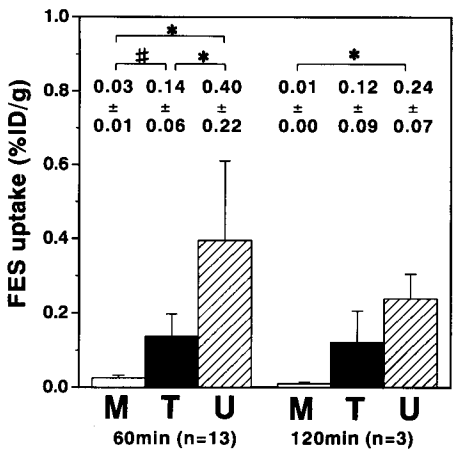

Fig. 3 FES uptake in rat breast tumors. (open bar, M: muscle; closed bar, T: breast tumor; dashed bar, U: uterus; *: $p < 0.01$, #: $p < 0.05$)

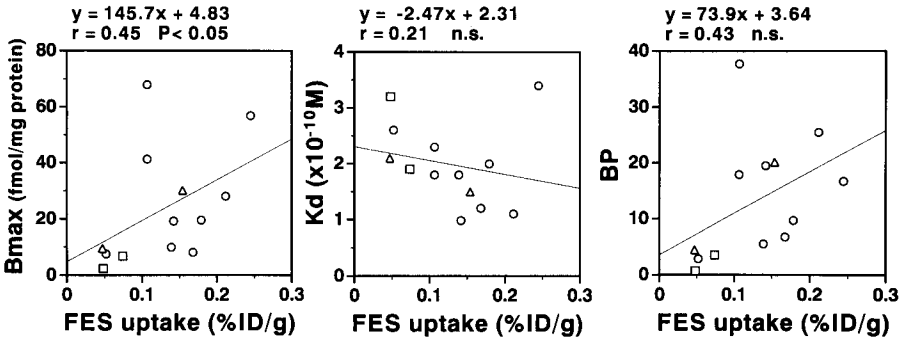

Fig. 4 Relationship between the FES uptake and the results of *in vitro* ER assays. (square: carcinoma; triangle: fibroadenoma; circle: papilloma) A significant correlation was observed between the FES uptake and the Bmax of ER ($p < 0.05$).

thereafter. It was 0.12 ± 0.05%ID/g or less at 60 minutes and 0.05 ± 0.03%ID/g or less at 120 minutes. FES uptake in other ER-negative tissues, including the spleen, esophagus, heart, muscle, skin, brain and bone, had a similar pattern to that of muscle (data not shown). The liver and kidneys, the primary organs of metabolism and excretion of estrogen, take up a great deal of FES particularly soon after the injection. After a gradual decrease in FES uptake in both the liver and kidneys, it increased slightly after 180 minutes.

Coadministration of 15 μg of unlabeled β-estradiol resulted in noticeable depression of uterine FES uptake. It was 0.21 ± 0.04 at 60 minutes and 0.12 ± 0.03 at 120 minutes (Fig. 2). Coadministration of unlabeled β-estradiol did not cause a depression of FES uptake in liver, kidneys, muscle or other ER-negative tissues (data not shown).

FES Uptake in Rat Breast Tumors

FES uptake in the rat breast tumors, uterus and muscle in mature female rats is shown in Figure 3. Both at 60 minutes and 120 minutes after administration, FES uptake in breast tumors was higher than in muscle, but lower than in the uterus. A significant difference among tissues was observed at 60 minutes.

FES uptake in rat breast tumor was compared with the estrogen receptor concentration measured by radioreceptor assay (Fig. 4). The FES uptake significantly correlated with the Bmax ($r = 0.45$, $p < 0.05$) but not with either Kd or BP.

DISCUSSION

In this study we observed high FES uptake by the uterus which is an ER-rich tissue and observed low FES uptake by ER-negative tissues. Coadministration of unlabeled β-estradiol showed marked depression of uterine FES uptake but did not change the FES uptake by ER-negative tissues. These results are consistent with a previous report[2] and therefore suggest that FES uptake by the uterus

is ER mediated while that by ER-negative tissues is not. The uterine FES uptake in mature female rats, which we examined in a breast tumor analysis, was lower than that in immature rats. This suggests that the endogenous estrogen may affect FES uptake by ER-positive tissue. The presence of steroid binding protein in the blood may also be involved in this process.[11] In rats, the fetal alphafetoprotein binds to estradiol and affects the blood FES levels.[12] Regarding the clinical use of FES in young female patients, we should pay attention to both the estrous cycle and the steroid binding protein which may affect FES uptake.

The FES uptake in rat breast tumor was higher than that in ER-negative tissue. We found a weak but significant correlation between FES uptake and the Bmax of tumor ER, although tumors in this study included various histological types. A significant correlation between FES uptake and the ER content in breast tumor was also observed in human studies,[5,7] whereas an aminal study demonstrated that there was no correlation between them.[4] These contradictory reports suggests that the FES uptake in breast tumors mainly depends on the ER concentration in tumors, but various factors are considered to play a role in tumor FES uptake. Mathias et al. examined the effect of some factors such as blood flow, blood volume in tumors and tracer metabolism, but found no correlation between FES uptake and these factors.[4] FES would be therefore useful for detecting ER positive tumors, but simple FES uptake is not considered to be a parameter for estimating ER content *in vivo*. Further examinations are still called for to establish the appropriate parameters for estimating ER concentrations by using a simple histological type of tumor.

CONCLUSIONS

In conclusion, we examined the tissue distribution and the kinetics of FES in immature female rats and observed highly selective uptake by uterus which is an ER rich tissue. Furthermore, the high FES uptake by the uterus was depressed after the coadministration of unlabeled β-estradiol. We also observed a high FES uptake in rat breast tumors induced by DMBA and found a significant correlation between FES uptake and the Bmax of ER in breast tumors. FES is therefore considered to be useful for detecting ER positive breast tumors.

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