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Dose-dependent change in tissue uptake of 17β-(16α-[^{125}I]iodo)-estradiol in female rats: Application to external imaging of mammary carcinoma

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Abstract. Recently, many studies have drawn attention to the possibility of imaging estrogen-receptor-positive breast cancer with a high-affinity ligand such as 17β-(16α-[^{125}I]iodo)-estradiol (I-E_2). We tried to determine the most suitable time and dose for imaging with this compound, using uteri of mature Sprague-Dawley rats. Although the uptake of I-E_2 in the target organ (uterus) reached its peak 1 h after subcutaneous injection, the ratio of uptake in the target organ to that in nontarget organs (lung and muscle) peaked at 4 h. We also found that this ratio decreased as the dose increased. The clearest image was available 4 h after the dose meeting the minimum requirement for imaging was administered. An imaging trial of dimethylbenzanthracene (DMBA)-induced mammary carcinoma is also demonstrated.

Recently, many studies have dealt with the synthesis and application of radiopharmaceuticals that bind with high affinity and specificity to estrogen receptors [1, 2, 12, 13]. Such compounds, if they are labeled by gamma-emitting radioisotopes with high specific activity, may be very useful for the external imaging of estrogen-receptor-positive breast cancer. In 1979, Hochberg [4, 5] introduced 17β-(16α-[^{125}I]iodo)-estradiol (I-E_2) as a very promising agent for affinity labeling of estrogen receptors. The ideal properties of this compound are that it binds to the estrogen receptor with almost the same affinity as estradiol, and it is selectively taken up by tissue that contains estrogen receptors.

A few investigators [7, 9] have reported successful imaging of demethylbenzanthracene (DMBA)-induced mammary carcinoma in female rats, using I-E_2. However, they have not mentioned the time and dose most suitable for imaging. In this report, these problems are studied.

Materials and methods

1. Biodistribution study of I-E_2

Unless stated otherwise, we used mature Sprague-Dawley rats[3] weighing 220–280 g. Each rat was ovariectomized under light ether anesthesia 24 h prior to the subcutaneous injection of 1.8 pmol I-E_2[4] (1,000 Ci/mmol) diluted in 0.5 ml 0.9% saline. At intervals of 15 min, 1, 2, 4, 8, and 16 h, three rats were killed by deep chloroform anesthesia, and several organs were removed and weighed. Samples from the removed organs were then assayed for radioactivity by an automatic gamma-well counter.

2. Dose-dependent change in the biodistribution of I-E_2

Fifteen rats were ovariectomized 24 h before the injection of I-E_2 in five groups. Each rat of the first group was subcutaneously injected with 1.3 pmol I-E_2, of the second group with 6.5 pmol, of the third group with 32 pmol, of the fourth group with 400 pmol, and of the fifth group with 2 nmol. Every injected solution was prepared by the dilution of I-E_2 with 0.5 ml 0.9% saline. The specific activity of I-E_2[5] was 1,300 Ci/mmol for the first, second, and third groups, and 2 Ci/mmol for the fourth and fifth groups. Every rat was killed 4 h after injection and processed as previously described.

3. Imaging trial of DMBA-induced mammary carcinoma

A Sprague-Dawley rat bearing mammary carcinoma induced by a single gastric injection of 20 mg DMBA[6] at the age of 50 days, was subjected to this trial. When the tumor grew to an adequate size (about 4 g) for imaging, the rat was injected with 68 μCi I-E_2 with a specific activity of 2,000 Ci/mmol. Four hours later, the rat was anesthetized by the intramuscular injection of 30 mg ketamine, and imaging was performed with a scinti-camera (Hitachi gamma-view H with low energy, high resolution, and parallel-hole collimeter, which can detect the radioactivity of I-E_2).

Results

1. Time course of tissue uptake

The tissue uptake of I-E_2 in several organs at various times is shown as the percentage of injected dose per gram of tissue in Table 1 and the ratio of tissue to blood in Table 2.

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Table 1. Time course of tissue uptake of 17β(16α-[^{125}I]iodo)-estradiol (% dose/g) in mature female rats (mean ± SD for three rats)

Tissue	15 min	1 h	2 h	4 h	8 h	16 h
Uterus	0.130 ± 0.099	0.539 ± 0.184	0.413 ± 0.205	0.442 ± 0.115	0.291 ± 0.112	0.047 ± 0.019
Blood	0.042 ± 0.021	0.064 ± 0.006	0.041 ± 0.009	0.067 ± 0.023	0.067 ± 0.006	0.028 ± 0.009
Lung	0.193 ± 0.099	0.273 ± 0.034	0.158 ± 0.033	0.086 ± 0.025	0.056 ± 0.04	0.023 ± 0.008
Liver	0.347 ± 0.194	0.389 ± 0.147	0.293 ± 0.080	0.351 ± 0.133	0.468 ± 0.145	0.231 ± 0.038
Kidney	0.192 ± 0.066	0.269 ± 0.024	0.156 ± 0.031	0.125 ± 0.037	0.096 ± 0.015	0.044 ± 0.015
Adrenal	0.554 ± 0.206	1.060 ± 0.272	0.432 ± 0.092	0.163 ± 0.055	0.049 ± 0.047	0.021 ± 0.008
Thyroid	0.162 ± 0.014	0.389 ± 0.160	0.373 ± 0.096	0.167 ± 0.054	0.353 ± 0.199	2.294 ± 0.196
Muscle	0.047 ± 0.023	0.102 ± 0.034	0.072 ± 0.019	0.059 ± 0.009	0.045 ± 0.017	0.019 ± 0.008
Pancreas	0.251 ± 0.086	0.429 ± 0.013	0.220 ± 0.024	0.109 ± 0.039	0.058 ± 0.004	0.018 ± 0.008

Table 2. Time course of tissue uptake of 17β(16α-[^{125}I]iodo)-estradiol (tissue-to-blood ratio) in mature female rats (mean ± SD for three rats)

Tissue	15 min	1 h	2 h	4 h	8 h	16 h
Uterus	3.08 ± 2.06	8.34 ± 2.40	8.25 ± 2.98	6.80 ± 0.71	3.29 ± 1.77	1.63 ± 0.37
Blood	1	1	1	1	1	1
Lung	4.99 ± 2.04	4.31 ± 0.99	3.36 ± 1.25	1.29 ± 0.10	0.86 ± 0.17	0.78 ± 0.06
Liver	8.04 ± 1.92	5.94 ± 1.88	5.92 ± 0.59	5.25 ± 0.15	6.99 ± 2.52	8.45 ± 1.88
Kidney	5.03 ± 1.39	4.18 ± 0.06	3.19 ± 0.32	1.90 ± 0.11	1.43 ± 0.30	1.55 ± 0.19
Adrenal	14.48 ± 4.39	16.40 ± 3.39	8.91 ± 1.75	2.46 ± 0.04	0.74 ± 0.70	0.70 ± 0.08
Thyroid	4.71 ± 2.61	5.92 ± 2.05	7.96 ± 3.36	2.25 ± 0.08	5.31 ± 3.31	48.68 ± 25.77
Muscle	1.15 ± 0.30	1.57 ± 0.41	1.55 ± 0.72	0.93 ± 0.18	0.68 ± 0.29	0.48 ± 0.11
Pancreas	6.61 ± 2.02	6.71 ± 0.66	4.83 ± 0.40	1.63 ± 0.01	0.86 ± 0.11	0.62 ± 0.11

Table 3. Time course of target- to nontarget-organ ratio[a] (mean ± SD for three rats)

	15 min	1 h	2 h	4 h	8 h	16 h
Target- to nontarget-organ ratio	1.06 ± 0.39	2.84 ± 0.75	3.70 ± 1.92	6.05 ± 0.14	4.91 ± 2.04	2.69 ± 0.56

[a] Calculated as percentage of dose per gram in uterus divided by mean of percentage of dose per gram in lung and muscle

Table 4. Dose-dependent change of tissue uptake (tissue-to-blood ratio) at various doses of 17β(16α-[^{125}I]iodo)-estradiol (mean ± SD for three rats)

Tissue	1.3 pmol	6.5 pmol	31 pmol	400 pmol	2 nmol
Uterus	8.86 ± 4.29	10.78 ± 1.06	7.00 ± 0.91	3.64 ± 1.89	3.56 ± 3.13
Lung	1.16 ± 0.25	2.14 ± 0.40	1.97 ± 0.57	1.41 ± 0.21	3.72 ± 2.20
Liver	7.32 ± 1.33	8.14 ± 0.63	4.24 ± 0.47	8.09 ± 1.76	6.32 ± 1.88
Kidney	2.17 ± 0.09	3.01 ± 0.48	2.31 ± 0.49	1.77 ± 0.26	2.04 ± 0.62
Muscle	0.89 ± 0.77	0.89 ± 0.09	0.80 ± 0.29	0.77 ± 0.25	1.15 ± 0.73
Pancreas	2.19 ± 1.01	4.35 ± 0.57	2.79 ± 0.48	2.54 ± 0.72	2.61 ± 1.42

There are many striking features about the tissue uptake and its time course. The uterus showed a high uptake, which appeared 1 h after injection and stayed fairly constant for 3 h. The uptake in the adrenal gland showed a sharp peak, the highest, at 1 h but rapidly decreased without holding a constant level as seen in the uterus. The liver showed a substantial uptake after 16 h, and the kidney also showed a slightly higher uptake than muscle, lung, and pancreas, especially after more than 4 h. The thyroid gland showed a low peak at 1 h and a gradually rising uptake after 4 h.

In order to decide the most suitable time for imaging, the ratio of uptake in the target organ (uterus) to that in nontarget organs (lung and muscle) was calculated. The time course is shown in Table 3. Among the nontarget organs, only lung and muscle were selected because they are the main constituents of the background of mammary carcinoma in the chest. The peak of this ratio appeared 4 h after injection, although the peaks of the percentage of dose per gram and tissue-to-blood ratio appeared at 1 h.

2. Dose-dependent change of tissue uptake

The tissue uptake in several organs at various doses is summarized in Table 4. The figures in this table are tissue-to-blood ratios 4 h after injection, when the difference of tissue uptake between the target organ and nontarget organs became most prominent (as shown in Table 3). The tissue-to-blood ratio in the uterus appears to decline as the dose

Table 5. Dose-dependent change of target- to nontarget-organ ratio at various doses (mean ± SD for three rats)

	1.3 pmol	6.5 pmol	31 pmol	400 pmol	2 nmol
Target- to nontarget-organ ratio	8.65 ± 0.15	7.19 ± 1.14	5.24 ± 0.91	3.35 ± 1.65	2.35 ± 0.92

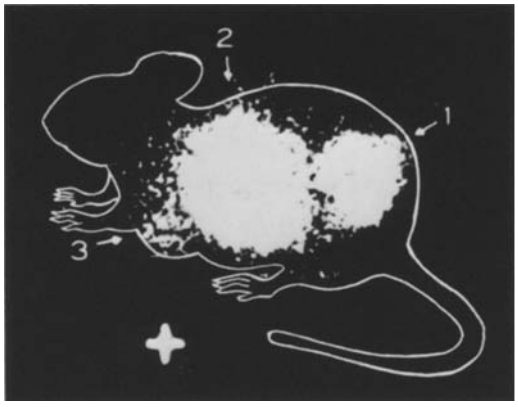

Fig. 1. A scinti-camera image of a Sprague-Dawley rat bearing DMBA-induced mammary carcinoma in the chest. Images were obtained 4 h after the subcutaneous injection of 68 μCi I-E_2 (2,000 Ci/mmol). Increased uptake can be identified in three different regions. The first (*arrow 1*) represents the residual radioactivity at the injected portion. The second (*arrow 2*) represents radioactivity mainly accumulated in the liver and intestine. The third (*arrow 3*) corresponds to the mammary carcinoma

increases, except for a low peak at a dose of 6.5 pmol. Other organs showed no pronounced dose-dependent changes. The dose-dependent change of the target- to nontarget-organ ratio is shown in Table 5. It is clear from this table that the ratio declines as the dose increases. The highest ratio is seen at the lowest dose.

3. Imaging trial of DMBA-induced mammary carcinoma

A scinti-camera image of the Sprague-Dawley rat bearing DMBA-induced mammary carcinoma is shown in Fig. 1. Increased uptake can be identified in three different regions. The first (arrow 1) represents the residual radioactivity at the injected portion. The second (arrow 2) represents radioactivity mainly accumulated in the liver and intestine. The third (arrow 3), located in the chest, corresponds to the mammary carcinoma.

Discussion

In the treatment of breast cancer, knowledge of the estrogen-receptor status is essential for predicting responses to hormone therapy and the prognosis of the patient [8, 11]. The measurement of the estrogen receptors by the in vitro assay of biopsy samples has currently come into general use. This method, however, is difficult to use for the analysis of metastatic lesions and is not practical for repeated measurements.

More recently, to overcome these drawbacks, intense interest has been concentrated on the development of affinity-labeling reagents for the in vivo assay of estrogen receptors. With this method, it will be possible to detect the presence of estrogen receptors in metastatic lesions without taking biopsy samples, and at the same time, to show them scintigraphically in estrogen-receptor-positive cases. Thus far, many radiopharmaceuticals – halogenated estrogens and their derivatives – have been synthesized and applied for this purpose. However, most have been abandoned, since they do not have the properties required of potent affinity-labeling reagents for estrogen receptors. The requisites which have been emphasized by Katzenellenbogen et al. [6] include that the reagents should bind to estrogen receptors with high affinity and selectivity, and should be both chemically and metabolically stable. In 1979, Hochberg introduced I-E_2 as a very promising reagent for this purpose. This potent affinity-labeling reagent seems to meet all the previously mentioned requisites. The tissue distribution of I-E_2 has been studied to demonstrate high and selective uptake in the uterus and estrogen-receptor-positive mammary carcinoma in rats. Our results in tissue-uptake studies are comparable to those which have been obtained by other investigators [3, 7, 10]. I-E_2 has been applied to the external imaging of DMBA-induced mammary carcinoma in female rats, and appreciable results have been obtained. However, little has been mentioned concerning the time and dose appropriate for imaging, although these two factors play important roles. We studied these problems, using the uteri of rats as a model for estrogen-receptor-positive mammary carcinoma. As shown in Tables 1 and 2, the tissue uptake in the uterus as a percentage of dose per gram and tissue-to-blood ratio reached their peaks 1 h after injection. However, the target- to nontarget-organ ratio peaked 3 h later. Consequently, the most suitable time for imaging should be 4 h after injection, when the target- and nontarget-organ tissue-uptake differential is most extreme, and the radioactivity in the target organ itself still remains high enough for external imaging. The clearest image of the target organ is obtained if imaging is done at this time.

The results of the dose-dependent change of tissue uptake, described as tissue-to-blood ratio in Table 4 and target- to nontarget-organ ratio in Table 5, reveal that both ratios, interestingly, decrease as the dose increases. This phenomenon is probably due to the dose-dependent increase in nonspecific uptake, both in the target organ and nontarget organs. Therefore it is inconvenient for external imaging, since, to obtain an image, considerable radioactivity must be accumulated in the target organ. To achieve this, a relatively high dose must be administered, even though higher doses are apt to decrease the target- and nontarget-organ tissue-uptake differential.

All these results indicate that the best image of the target organ is obtained when a dose meeting the minimum requirement for imaging is administered, and the image is taken 4 h later.

Here, we used a DMBA-induced mammary carcinoma weighing about 4 g in an imaging trial. To obtain a clear image of such a tumor, at least 1 μCi I-E_2 must be accumulated in it. The dose of I-E_2 was calculated as follows:

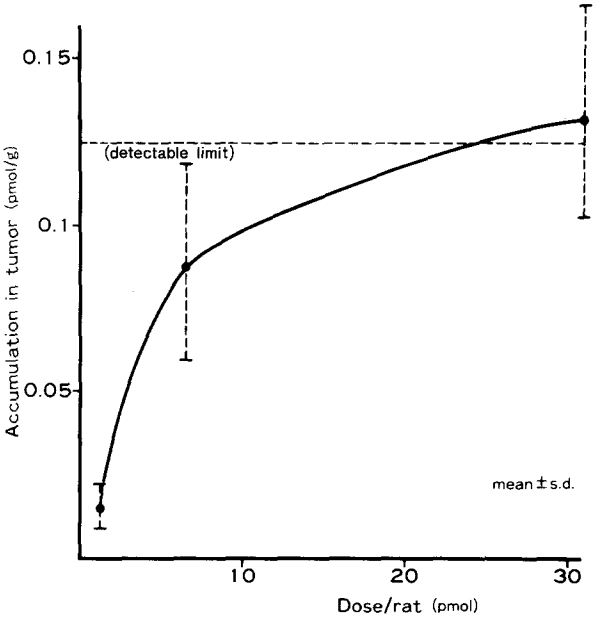

Fig. 2. Accumulation of I-E_2 in 1 g of tumor at various doses. Accumulation in the tumor was calculated using the hypothesis that uptake in the tumor is one-half of that in the uterus. The *horizontal broken line* shows the minimum requirement for a tumor weighing 4 g to accumulate at least 1 μCi I-E_2

for a tumor weighing 4 g to accumulate 1 μCi I-E_2, 0.25 μCi must be accumulated per gram of tumor, and such an accumulation is equal to 0.125 pmol I-E_2 with a specific activity of 2,000 Ci/mmol. To investigate what dose of I-E_2 is needed, the dose-dependent change of uptake in a gram of tumor was plotted as in Fig. 2. The uptake in the tumor was calculated from Table 4, following the hypothesis that the uptake in the tumor is about one-half of that in the uterus [7]. According to Fig. 2, no less than 0.125 pmol I-E_2 is estimated to be accumulated per gram of tumor, when a dose above 25 pmol is administered.

To verify this calculated value, 34 pmol I-E_2 with a specific activity of 2,000 Ci/mmol was administered to a Sprague-Dawley rat bearing a mammary carcinoma weighing about 4 g, and imaging was performed 4 h later. As shown in Fig. 1, increased uptake in the tumor, located in the chest, can be identified. However, if the tumor were located in the abdomen, imaging would be unsuccessful, because high uptake in the liver and intestine would overlap with the image of the tumor.

In conclusion, DMBA-induced mammary carcinoma of the rat, located anywhere except in the abdomen, can be scintigraphically visualized with I-E_2 if the proper dose is administered and imaging is performed at the correct time.

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