# In Vivo Comparison of 16α-[$^{77}$Br]Bromoestradiol-17β and 16α-[$^{125}$I]Iodoestradiol-17β

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**An in vivo comparison of two estrogen-receptor-binding radiopharmaceuticals of high specific activity, 16α-[$^{77}$Br]bromoestradiol-17β and 16α-[$^{125}$I]iodoestradiol-17β, has been carried out in immature female rats. The iodoestradiol has slightly higher uterine uptake at 1 hr after injection, whereas the brominated analog has significantly enhanced uptake at later times. The similar behavior of the two compounds in vivo suggests that estradiol labeled with I-123, Br-77, or Br-75 could be used interchangeably for the imaging of breast tumors containing estrogen receptors. In addition, coinjection of 16α-[$^{125}$I]iodoestradiol as an internal standard has been shown to be useful for comparison of different radiohalogenated estrogens.**

J Nucl Med 23: 420–424, 1982

In recent years there has been a great deal of interest in the development of estrogen-receptor-binding radiopharmaceuticals useful for the imaging of estrogen target tissues, such as breast tumors positive for estrogen receptors. The chemical and biochemical characteristics required of a radiolabeled estrogen suitable for use as a breast-tumor seeker have been outlined in earlier publications (*1*,*2*). We have recently described the synthesis of 16α-[$^{77}$Br]bromoestradiol-17β and its selective, receptor-mediated uptake by estrogen target tissues and carcinogen-induced mammary tumors in rats (*3*,*4*). We have also presented preliminary studies of breast-tumor imaging in humans (*5*). Hochberg has described the synthesis and receptor-mediated uptake of 16α-[$^{125}$I]-iodoestradiol (*6*,*7*), and others have confirmed uptake of this compound by estrogen target tissues in vivo (*8*).

In this report, we describe an in vivo comparison of two radiohalogenated estrogens of high specific activity—16α-[$^{77}$Br]bromoestradiol BE(Br-77) and 16α-[$^{125}$I]-iodoestradiol IE(I-125). The purpose of this study is twofold: (a) to compare the two labeled estrogens as potential agents for breast-tumor imaging, and (b) to investigate the use of 16α-[$^{125}$I]iodoestradiol as an internal standard for the evaluation of other radiolabeled estrogens.

Received Sep. 16, 1981; revision accepted Jan. 1, 1982.

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## MATERIALS AND METHODS

The synthesis of BE(Br-77) was carried out as previously described (*4*,*9*) using spallation-produced bromine-77 (*10*). The radiolabeled estrogen was separated from all other radioactive and UV-absorbing reaction products by high-pressure liquid chromatography on a Partisil M-9* magnum column eluted isocratically with hexane/methylene chloride/2-propanol (92:6.4:1.6) at 7 ml/min. The eluate was monitored with an ultraviolet detector at 254 nm and a sodium iodide scintillation detector. All UV and radioactivity peaks were identified by coinjection with authentic samples. Ascorbic acid was added to the final purified sample of BE(Br-77) (50 μl of a saturated solution of ascorbic acid to each 7-ml HPLC fraction) to retard oxidative degradation. IE(I-125) of high specific activity was obtained commercially.† Radioassay was carried out with a dose calibrator.

The specific activities of BE(Br-77) and IE(I-125) were measured using Scatchard analyses (cf. Fig. 1).

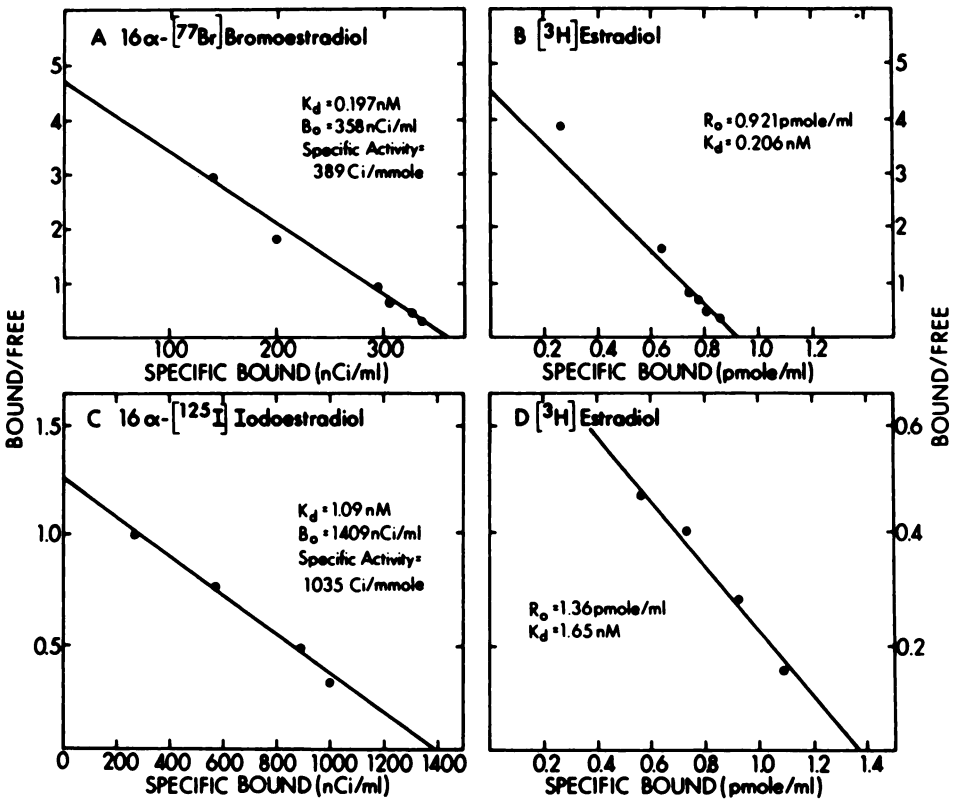


**FIG. 1.** Scatchard plots showing high-affinity binding of BE(Br-77) (Panel A) and estradiol(H-3) (Panel B) for use in determining specific activity of radiohalogenated estrogen. Analogous plots from a different set of experiments are shown in Panels C and D for IE(I-125) and estradiol(H-3), respectively. The lower binding affinities ($K_d$) noted in Panels C and D result from the shorter equilibration time (1 hr) in these experiments, compared with those in Panels A and B (18 hr). Estimates of binding-site concentration are not affected by the decrease in equilibration time.

Direct binding assays used lamb-uterus cytosol as the source of estrogen receptor (*11*); receptor-bound material was assayed by standard charcoal adsorption techniques (*11,12*). Parallel binding studies were done with estradiol (H-3)† of known specific activity to determine the molar concentration of receptor sites.

**In vivo studies.** Tissue uptake studies were performed in immature female Sprague-Dawley rats 21–25 days old. Under ether anesthesia the animals were injected by jugular vein with 0.1 ml of a solution containing 2 μCi each of BE(Br-77) (0.76 ng) and IE(I-125) (0.78 ng). The injection solutions were prepared by dilution of ethanol solutions of the agents with 0.9% sodium chloride (final ethanol concentration ≤5%). At various times after injection, the animals were killed by decapitation, and samples of blood and tissues were weighed and assayed for radioactivity in a sodium iodide well counter. Iodine-125 counts were corrected for crossover from bromine-77 present in the same sample. In some cases, rats were given a simultaneous injection of 18 μg unlabeled estradiol to block receptor-mediated uptake. In vivo washout experiments were carried out by giving the animals an intraperitoneal injection of 18 μg unlabeled estradiol at 1 hr after administration of the radiolabeled compounds.

## RESULTS

**Specific-activity determination.** High-affinity binding of both 16α-[$^{77}$Br]bromoestradiol and 16α-[$^{125}$I]iodoestradiol to the estrogen receptor can be demonstrated by direct binding assays; Scatchard plots are shown in Fig. 1, panels A and C, respectively. The effective specific activities of the two radiohalogenated estrogens are determined by comparing the maximum, high-affinity binding observed in this assay (Figs. 1A and 1C) with the receptor-site concentration measured in a parallel assay (Figs. 1B and 1D) using tritiated estradiol of known specific activity. The effective specific activity of BE(Br-77) was determined to be 389 Ci per mmole. This measurement was made two half-lives after the preparation of the compound; thus, this value corresponds to a specific activity of 1556 Ci per mmole at the time of synthesis BE(Br-77). Effective specific activities at the time of synthesis are typically 900–1500 Ci per mmole. The specific activity of IE(I-125) was found to be 1035 Ci per mmole, which extrapolates back to 1319 Ci per mmole at the time of synthesis. This value is in reasonable agreement with that reported by the manufacturer (1496 Ci per mmole). Similar comparisons of the specific activities of 16α-[$^{125}$I]iodoestradiol and

**TABLE 1. UTERINE UPTAKE OF 16α-[$^{77}$Br]BROMOESTRADIOL-17β AND 16α-[$^{125}$I]IODOESTRADIOL-17β IN IMMATURE FEMALE RATS (MEAN ± s.d. FOR ≥8 RATS)**

| | Uterus-to-blood ratio | | Uterus-to-nontarget ratio | | % I.D. per gram uterus | |
|---|---|---|---|---|---|---|
| | Br-77 | I-125 | Br-77 | I-125 | Br-77 | I-125 |
| 1 hr | 11.4 ± 5.6 | 16.5 ± 7.4 | 16.4 ± 5.0 | 16.0 ± 7.5 | 8.41 ± 1.78 | 10.9 ± 2.4 |
| 1 hr* | 1.44 ± 0.83 | 1.34 ± 0.43 | 1.72 ± 0.86 | 1.72 ± 0.59 | 0.48 ± 0.07 | 1.25 ± 0.70 |
| 3 hr | 15.8 ± 9.5 | 13.8 ± 6.6 | 22.8 ± 9.0 | 23.6 ± 8.6 | 7.93 ± 2.15 | 6.22 ± 1.79 |
| 6 hr | 8.95 ± 5.71 | 7.18 ± 3.40 | 14.9 ± 5.5 | 13.7 ± 5.2 | 5.39 ± 0.98 | 1.78 ± 0.64 |

* With 18 μg unlabeled estradiol added.

[$^3$H]estradiol have been carried out by others (*13*,*14*).

**In vivo studies.** Tissue uptake studies gave results similar to those previously reported for both BE(Br-77) (*3*,*4*) and IE(I-125) (*8*). Selective uptake of both compounds was observed in the uterus and to a lesser extent in the ovaries; this uptake was suppressed by coinjection of unlabeled estradiol. Other tissues did not show enhanced uptake of the compounds, and uptake was not altered by the presence of excess estradiol.

The mean values for uterine uptake of BE(Br-77) and IE(I-125) for the total animal population are summarized in Table 1. The data are expressed as the uterus-to-blood ratio, percent injected dose per gram of uterus, and ratio of uterus to nontarget tissue, the latter being defined as the mean of muscle, spleen, esophagus, and lung. The large standard deviations in Table 1 show that the uterine uptake values vary widely from animal to animal, but the ratio of the uptake of brominated estradiol to that of the iodinated derivative remains fairly constant for each sacrifice time.

The means of the ratios of uptake of the radiobrominated estradiol to that of the iodinated compound for each animal are listed in Table 2. One hour after injection, the uterus-to-blood ratio and percent injected dose per gram of uterus are significantly higher for IE(I-125); the brominated estradiol shows a significantly higher percent injected dose per gram of uterus and uterus-to-nontarget ratio at 3 hr; and all of the uterine uptake values are significantly higher for BE(Br-77) at 6 hr after injection.

Uptake of radioactivity in the thyroid was also determined after injection of BE(Br-77) and IE(I-125) (Table 3). These data clearly show that some iodide is being released from IE(I-125) and is accumulated by the thyroid, whose activity increases with time and can reach high levels. On the other hand, radiobromide does not accumulate in the thyroid, this being consistent with the known absence of a bromide-concentrating mechanism in this organ (*15*).

In vivo washout experiments were carried out to observe the displacement of the labeled estrogens from the target tissues (Table 4). The injection of an excess of unlabeled estradiol 1 hr after the initial dose of BE(Br-77) and IE(I-125) results in a rapid decrease in uterine activity. The uterine content for both compounds reaches the levels observed in animals that were pretreated with unlabeled estradiol.

## DISCUSSION

Both BE(Br-77) and IE(I-125) show selective receptor-mediated uptake by estrogen target tissues in vivo; simultaneous comparison of the labeled estrogens in vivo has shown that the two compounds behave quite similarly. While the iodinated compound has slightly higher uterine uptake at 1 hr, the bromoestradiol has significantly enhanced uptake at later times. This change in relative uptake values of the two compounds with time is most likely the result of more rapid clearance of the I-125-labeled compound from the uterus (cf. Table 1).

These results suggest that either radioiodinated or radiobrominated estradiol could be used with about equal success in imaging estrogen-receptor-positive breast tumors. Iodine-123-labeled estradiol would have

**TABLE 2. IN VIVO COMPARISON OF 16α-[$^{77}$Br]BROMOESTRADIOL-17β AND 16α-[$^{125}$I]IODOESTRADIOL-17β IN IMMATURE FEMALE RATS (MEAN ± s.d. FOR ≥8 RATS)**

| | Ratio of Br-77 to I-125 | | |
|---|---|---|---|
| | Uterus-to-blood ratio | Uterus-to-nontarget ratio | % I.D. per gram uterus |
| 1 hr | 0.88 ± 0.17 | 1.11 ± 0.28 | 0.73 ± 0.08 |
| | (p = 0.015)* | (p = 0.496) | (p < 0.001) |
| 1 hr† | 1.23 ± 0.24 | 1.46 ± 0.50 | 1.08 ± 0.25 |
| | (p = 0.059) | (p = 0.350) | (p = 0.500) |
| 3 hr | 1.07 ± 0.16 | 1.31 ± 0.17 | 1.36 ± 0.22 |
| | (p = 0.496) | (p = 0.001) | (p = 0.008) |
| 6 hr | 1.35 ± 0.28 | 1.26 ± 0.17 | 1.80 ± 0.19 |
| | (p = 0.013) | (p = 0.001) | (p = 0.002) |

* p values (one-tailed) determined from paired-sample t-test.

† With 18 μg unlabeled estradiol added.

**TABLE 3. THYROID UPTAKE OF 16α-[$^{77}$Br]BROMOESTRADIOL-17β AND 16α-[$^{125}$I]IODOESTRADIOL-17β IN IMMATURE FEMALE RATS* (MEAN ± s.d. FOR ≥8 RATS)**

| | Thyroid-to-blood ratio | | % I.D. per gram thyroid | |
|---|---|---|---|---|
| | Br-77 | I-125 | Br-77 | I-125 |
| 1 hr | 1.13 ± 0.23 | 18.6 ± 13.5 | 0.83 ± 0.40 | 14.5 ± 4.0 |
| 1 hr† | 1.38 ± 0.36 | 42.6 ± 9.7 | 0.33 ± 0.08 | 10.8 ± 2.3 |
| 3 hr | 0.96 ± 0.20 | 80.9 ± 30.7 | 0.37 ± 0.07 | 24.9 ± 10.3 |
| 6 hr | 0.82 ± 0.15 | 124.9 ± 32.7 | 0.33 ± 0.09 | 40.9 ± 11.2 |

* Thyroid weight is taken to be 5 mg per 100 g body weight (*17*).
† With 18 μg unlabeled estradiol added.

some advantages for human breast-tumor imaging as compared with the Br-77-labeled analog: the radiation from I-123 makes it more suitable for imaging on standard gamma cameras, and the shorter half-life of the iodine would allow the administration of a larger amount to a patient for an equivalent radiation absorbed dose. However, the synthesis of IE(I-125) reported by Hochberg (*6,7*) requires a 24-hr reaction time and so would not be readily applicable to use with iodine-123. The method that we have used to synthesize BE(Br-77) would also be unsuitable in its present form to the preparation of an I-123-labeled compound, since the iodine is lost during the reduction step. For these reasons, continued work with Br-77-labeled estrogens seems warranted, especially when one considers the possible extension of these syntheses to prepare compounds labeled with the positron emitter bromine-75. The combination of an estrogen labeled with a short-lived positron emitter and positron emission tomography would provide three-dimensional images of breast tumors with increased resolution.

Uterine uptake of BE(Br-77) varies widely from animal to animal, but the ratios of uptake of the bromoestradiol to that of the iodinated analog are quite consistent. Thus, coinjection of 16α-iodoestradiol(I-125) may provide a useful internal standard for the comparison of various estrogen-receptor-binding radiopharmaceuticals. For example, comparison of the uterine uptake of the Br-77- and I-125-labeled compounds (cf. Table 1) using Student's t-test does not demonstrate a statistically significant difference in the uterus-to-nontarget ratios for the two compounds at 3 or 6 hr after injection (p values of 0.423 and 0.314, respectively), whereas comparison of the two radiolabeled compounds in the same animal using a paired-sample t-test gives the significant differences shown in Table 2 (p values of 0.001).

Both BE(Br-77) and IE(I-125) are rapidly displaced from estrogen-target tissues in vivo following injection of an excess of unlabeled estradiol. These results suggest that administration of a loading dose of estradiol following imaging of estrogen-target tissues using these radiopharmaceuticals might provide a rapid and reliable method for confirming receptor-mediated uptake of the labeled compounds. Comar and co-workers (*16*) have successfully visualized washout of flunitrazepam(C-11) from the monkey brain. Since the data presented here (cf. Table 4) show faster and more efficient displacement of these radiohalogenated estrogens from target tissues than that reported by Comar for the labeled flunitrazepam, similar in vivo washout studies with these es-

**TABLE 4. IN VIVO WASHOUT OF 16α-[$^{77}$Br]BROMOESTRADIOL-17β AND 16α-[$^{125}$I]IODOESTRADIOL-17β IN IMMATURE FEMALE RATS MEAN (RANGE) FOR ≥3 RATS**

| | Uterus-to-blood ratio | | Uterus-to-nontarget ratio | | % I.D. per gram uterus | |
|---|---|---|---|---|---|---|
| | Br-77 | I-125 | Br-77 | I-125 | Br-77 | I-125 |
| 3 hr | 10.4 | 16.2 | 20.6 | 28.7 | 11.0 | 6.83 |
| | (8.5–12.3) | (7.62–29.1) | (16.2–24.9) | (13.0–36.9) | (10.9–11.2) | (3.98–8.96) |
| 3 hr* | 1.39 | 1.52 | 3.01 | 2.55 | 1.13 | 0.68 |
| | (1.22–1.50) | (1.14–1.97) | (2.07–4.06) | (1.97–3.05) | (0.97–1.27) | (0.47–0.90) |
| 6 hr | 4.47 | 7.4 | 11.7 | 15.5 | 5.38 | 1.57 |
| | (3.66–5.05) | (4.79–10.2) | (8.58–17.5) | (12.3–20.9) | (4.60–5.84) | (1.04–2.50) |
| 6 hr* | 1.12 | 1.53 | 2.20 | 2.36 | 1.01 | 0.29 |
| | (0.74–1.37) | (0.67–2.86) | (1.51–2.94) | (1.38–4.27) | (0.81–1.32) | (0.13–0.54) |

* 18 μg unlabeled estradiol administered 1 hr after injection of radiolabeled compound.

trogen-receptor seekers should provide results even more dramatic.

In summary, we have described an in vivo comparison of two estrogen-receptor-binding radiopharmaceuticals of high specific activity. The results indicate similar behavior of the two radiolabeled estrogens and suggest that estradiol could be labeled with I-123, Br-77, or Br-75 and used interchangeably for image human breast tumors. Although I-123 has some advantages with respect to imaging and radiation dose, limitations of currently available syntheses preclude its use. Bromine-75, which can be used in conjunction with positron emission tomography, has the most desirable characteristics as a heavy radiohalogen label for estrogens. The utility of IE(I-125) as an internal standard for comparison of different estrogen-receptor-binding radiopharmaceuticals has been demonstrated.

## FOOTNOTES

* Whatman Inc., Clifton, NJ.

† New England Nuclear Corp., Boston, MA.

## ACKNOWLEDGMENTS

This work was supported by grants from the U.S. Department of Energy (DE-AC02-81EV10650) and the National Institutes of Health (PHS HHS CA-25836). The authors thank Drs. J. W. Barnes, G. E. Bentley, P. M. Grant, and H. A. O'Brien, Jr. of Los Alamos National Laboratory for their assistance in providing bromine-77 for use in these experiments.

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