Comparison of the Distribution of Radioiodinated-E-17α-Iodovinyl-11β-Methoxyestradiol and 2-Iodo-1,1-bis(4-Hydroxyphenyl)-Phenylethylene Estrogens in the Immature Female Rat

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Current therapies for estrogen receptor-positive (ER+) cancers rely heavily on growth prevention by cytostatic agents rather than destruction of the cancer cells. In these studies, we compared the tissue distribution of two radioiodinated estrogens, one a triphenylethylene estrogen, IBHPE, the other steroidal, IVME2. The radioiodoestrogens were prepared using the halodestannylation reaction from the respective tributyltin precursors. The specificity of binding of these radioiodinated estrogens to the estrogen receptor (ER) was established by sucrose-density gradient analyses and their specific activities by comparison with the ER binding of ^{3}H-estradiol of known specific activity. The time-dependent tissue distribution of the two radioiodoestrogens in immature female rats was studied to compare the relative uptake and specific retention of the two estrogens in ER target organs and assess their possible use for imaging ER positive tissues or for Auger electron-mediated ER-directed therapy. While the uterus showed only slightly poorer retention of the nonsteroidal estrogen (IBHPE) than the steroidal estrogen (IVME2), those target tissues that required blood-supplied ligand (e.g., vagina, pituitary) showed substantially higher uptake of the steroidal estrogen. IBHPE showed significantly higher blood levels at all time points. While the tissue-to-muscle ratios for IBHPE in the uterus and ovary were higher initially, the IVME2 showed higher tissue-to-muscle ratios, suggesting that the steroidal estrogen may be the more promising ligand for imaging purposes. However, IBHPE showed excellent uptake by peritoneal target tissues, with much lower concentrations in more distant target tissues (e.g., pituitary) so it might have distinct potential for therapy of intraperitoneal ER-containing cancers.

J Nucl Med 1993; 34:272-280

Received May 21, 1992; revision accepted Sept. 30, 1992.

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The discovery of highly specific receptors for estradiol (ER) in a proportion of breast, ovarian and endometrial cancers led to the hope that ER could be used to identify those cancers that would respond to endocrine therapy (*1–3*). However, while essentially all breast cancers that respond to endocrine therapy contain ER, it has become clear that the presence of ER in these tumors does not necessarily ensure response to endocrine therapy, only about half of ER-positive breast cancer patients benefit from such therapy (*4*), and even fewer patients with ER-positive endometrial and ovarian cancers (*5*). Thus, it would be advantageous to develop an endocrine therapy which relies simply on the presence of the specific steroid receptors in the cancer via specific endocrine cytotoxic agents to supplement the cytostatic (antihormone) agents currently in use.

We and others have suggested that estrogens containing Auger electron-emitters [e.g., ^{80m}Br (*6,7*) or ^{123}I (*7–9*)] could be the basis for such a new therapeutic approach to ER-positive cancers. Cell culture studies with radiolabeled estrogens have demonstrated the feasibility of specific cytotoxicity (*8–12*) of Auger electron-emitting estrogens for ER-positive cells in culture. In addition, several studies have indicated that radiohalogen-labeled estrogens could be useful for imaging ER-positive breast cancers (*13–16*).

Our main interest in these studies was the evaluation of ligands suitable for treatment of ovarian cancers. Clinically, ovarian cancer spreads initially within the peritoneal cavity and is thus amenable to direct infusion of ligand, hence the use of i.p. injections in these experiments. Clearly, for other ER-positive cancers such as breast carcinomas, which spread via the blood and would require intravenous administration, the choice of route of administration and possibly also ligand could be different. Furthermore, in the area of detection and diagnosis of ER-positive lesions the use of estrogens containing positron-emitting nuclides like ^{18}F and PET imaging is an important

alternative to ^{123}I and SPECT imaging. The current study, however, compares in the same animals, the specific uptake, retention and distribution of a nonsteroidal estrogen (^{123}IBHPE) and a steroidal estrogen (^{125}IVME2) in the tissues of immature rats to assess their potential for therapy of ER-containing cancers in patients. By using the two different isotopes of radioiodine, it was possible to compare the two ligands in the same animals, thus reducing some of the animal variation (*15*).

MATERIALS AND METHODS

All chemicals were of reagent grade or better. Acetonitrile (HPLC grade) was purchased from Baxter (McGraw Park, IL). Iodine-125 (as Na^{125}I) was purchased from Amersham (Arlington Heights, IL) and ^{123}I (as Na^{123}I), free of ^{125}I, was obtained from Nordion International Inc, (Kanata, Ontario).

Synthesis and Specific Activities of Labeled Compounds

17α-E[^{125}I]iodovinyl-11β-methoxyestradiol (IVME2) and 2-[^{123}I]-iodo-1,1-bis(4-hydroxy-phenyl)-2-phenylethylene (IBHPE) (Fig. 1) were prepared by the halodestannylation reaction using the appropriate tributylstannyl precursors which were previously described in detail (*9,10,17*).

Preparation of 17α-E-[^{125}I]iodovinyl-11β-methoxyestradiol (^{125}IVME2)

Sodium iodide, 148 MBq (4 mCi), in 50 μl 0.1 *M* NaOH, was placed in a conical 1-ml capacity Wheaton vial. To this was added 100 μg of 17α-E-tri-n-butylstannylvinyl-11β-methoxyestradiol in 100 μl ethanol, followed by 100 μl of 30% hydrogen peroxide:glacial acetic acid mixture (2:1 v:v). The reactants were allowed to react for 30 min at room temperature, then injected directly onto a HPLC column (Alltech, C18, 0.46 × 25 cm, 10 μ, reverse phase) and the ^{125}IVME2 eluted with a gradient (10%–100%) of acetonitrile:water (flow rate 5 ml/min). The ^{125}IVME2 eluted at 40 min. The radioactivity was monitored using a modified Ludlum model 2 probe. The fraction(s) containing ^{125}IVME2 were transferred to a round bottomed flask and the acetonitrile was removed under vacuum (Rotovap, Büche). The aqueous phase was extracted at least three times with ether, the ether extracts combined and evaporated and the radioactive product was dissolved in ethanol. Total radioactivity was 92.5 MBq (2.5 mCi) as measured in a dose calibrator (Capintec), giving a radiochemical yield of about 63%.

Preparation of 2-[^{123}I]iodo-1,1-bis(4-hydroxyphenyl)-2-phenylethylene (^{123}IBHPE)

The reaction mixture and conditions were essentially as described above, however, the reaction was conducted in the shipping vial for the Na^{123}I. To this vial containing ca. 185 MBq (5 mCi) Na^{123}I in 0.1 *M* NaOH, 100 μl of an ethanol solution of 100 μg of 2-tributylstannyl-1,1-bis(4-hydroxyphenyl)-2-phenylethylene were added, followed by the 100 μl of the 2:1 v:v of 30% hydrogen peroxide:glacial acetic acid mixture. The vial was then sealed, agitated to mix and allowed to react for 30 min at room temperature with occasional mixing. Isolation of the product was carried out on the HPLC system, described above, but an isocratic elution with 40% acetonitrile:water mixture (flow rate 9 ml/min) for the elution of ^{123}IBHPE (elution time 40 min) was used. After evaporation of the ether extracts from the ether/water partition as described above, the product was dissolved in ethanol, giving 47.4 MBq (1.28 mCi), 25.8% radiochemical yield, of the purified ^{123}IBHPE.

Specific Activities of ^{125}IVME2 and ^{123}IBHPE

Specific activities were determined by sedimentation analysis with sucrose-density gradients, comparing the specific binding (i.e., the unlabeled estradiol-inhibitable binding) to the rat uterine estrogen receptor (ER) of the two radioiodinated estrogens with that of [^{3}H]estradiol (specific activity 2220 TBq [60 Ci] per mmol) under low salt conditions (10 m*M* tris, 10 m*M* KCl, 1 m*M* EDTA, pH 7.2). Sedimentation analysis is particularly useful to quantitate specific binding when substantial nonspecific binding is present, and in addition, by use of specific antibodies against ER, can be used to further substantiate that the radioiodinated estrogen is indeed binding to ER. For both ^{125}IVME2 and ^{123}IBHPE, an excess of the labeled compound (calculated to be in excess of 5 n*M* final concentration) was placed in 1.5 ml chilled Eppendorf tubes, either alone or with a 500 n*M* (final) concentration of unlabeled DES. Rat uterine cytosol (100,000 × g, 1 hr supernatant of a 1+4 homogenate of immature rat uteri in 10 m*M* tris, 10 m*M* KCl, 1 m*M* EDTA pH 7.4) was added to each tube and, after mixing, the tubes were incubated for 1 hr at 2°C. In addition to the tubes described above, another tube in each set also contained 0.5 μg of the specific monoclonal antibody, H222 (*6*), against the estrogen receptor. After 1 hr of incubation in the cold, the unbound estrogen was removed by delivering the incubation mixtures onto a pellet of dextran-coated charcoal, DCC, (1% Norit A, 0.1% dextran T40), resuspending the charcoal by vortexing, incubating at 2° for 10 min, and then pelleting the charcoal by centrifugation (Beckman Microfuge B, 13000 rpm, 1 min in the cold). For sedimentation analyses, the DCC superna-

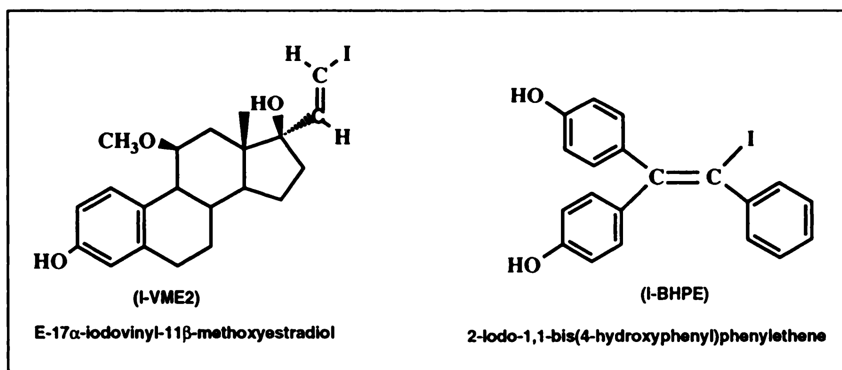

FIGURE 1. Structural formulae for iodoestrogens.

tants were layered either on 10%–30% sucrose gradients in low salt (10 m*M* tris, 1 m*M* KCl, 1 m*M* EDTA pH 7.4), or high salt (10 m*M* Tris, 4000 m*M* KCl, 1 m*M* EDTA, pH 8.2) and centrifuged for 15 hr at 4°C, 208000 × g, (Beckman L8-70). One hundred-microliter fractions were collected from the bottom of the centrifuge tubes and counted for radioactivity.

Experiments in Immature Female Rats

To directly compare the distribution of both ^{125}IVME2 and ^{123}IBHPE, the two radioiodinated estrogens were mixed together in the same injectant solutions, so that both iodoestrogens were administered to each animal and animal variation affecting differences in distribution of the ligands were eliminated. The labeled iodoestrogens were prepared for injection in 1.0 ml isotonic saline containing a final concentration of ~3% ethanol, alone or with unlabeled DES to partially (0.1 μg DES) or completely (1.0 μg DES) inhibit the specific (i.e., ER-mediated) uptake of radioiodinated estrogen. Three 22-day-old Sprague-Dawley female rats (~40–50 g body wt) were used for each group at each time point and the injectants were administered intraperitoneally. Aliquots of each of the injectants were assayed in a gamma counter (model 5130 Auto-gamma Scintillation Spectrometer, Packard Instruments, Downers Grove, IL) so that the results could be related to the injected dose (ID), which varied from about 440–630 kBq (12 to 17 μCi) for each of the two radioiodoestrogens. Counting efficiency was determined by counting aliquots of standard solutions of the two radioiodine preparations (calibrated in the Capintec dose calibrator) in the gamma counter at appropriate settings used for ^{125}I or ^{123}I. The ^{123}I assays were carried out immediately after obtaining the tissues using a window setting in the gamma counter which excluded counts from the low-energy region containing ^{125}I emissions. The ^{125}I radioactivity of samples containing both nuclides was assayed after 2 wk when the ^{123}I counts had decreased to background level. All ^{123}I radioactivity was corrected for decay back to the time of the injections for comparison of relative concentrations in the tissues taken at several time points and counted at different times.

At 0.5, 2.5 and 20 hr after injection, various groups of rats were killed by decapitation, blood was collected directly into heparin-containing vials and 100-μl aliquots of each blood sample were taken for assay of radioiodine. The entire uterus, vagina, ovaries, adrenals and one kidney were removed intact, dissected free of connective tissue and fat, blotted on filter paper, weighed and transferred to 12 × 75 mm tubes for counting in the gamma counter. Portions (100 mg–200 mg) of the larger tissues (liver, muscle, brain) were similarly assayed. The pituitary was excised and transferred directly, without weighing, to the assay tube and the %ID/g of this tissue was based on the average weight of ~2 mg per pituitary found from weighing pooled pituitaries of 22-day-old rats. Results are expressed as the mean and standard deviations of the %ID/g wet weight.

RESULTS

The binding of ^{123}IBHPE and ^{125}IVME2 to rat uterine ER, as compared with that of [^{3}H]E2, are illustrated in sucrose-gradient analyses (Fig. 2). Both radioiodoestrogens show clear E2 inhibitable binding in the 8S region (fractions 8–15), similar to that seen for [^{3}H]E2. While neither E2 (Fig. 2A) nor IVME2 (Fig. 2C) show appreciable nonspecific binding, it can be seen that there is appreciable binding of IBHPE in the 4S region (Fig. 2B, fractions 20–26), which is not prevented by incubation with a large excess of unlabeled estrogen. The profiles of the sedimentation analyses of ^{123}IBHPE and ^{125}IVME2 in the presence of the anti-ER antibody in high salt gradients (Fig. 2D, E) show the characteristic down-field shift due to the association of the specific antibody with these radioiodinated estrogen-receptor complexes, showing that these iodoestrogens are indeed binding to ER. Based on the relative amounts of radioactivity specifically bound to ER for ^{125}IVME2 and ^{123}IBHPE, relative to that of [^{3}H]E2 whose

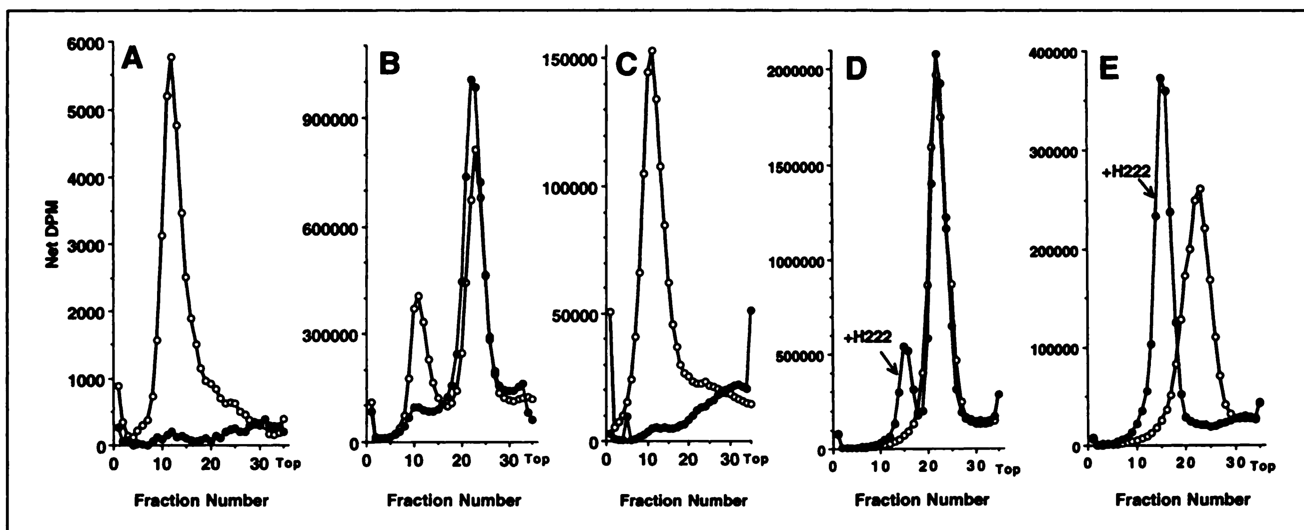

FIGURE 2. Sedimentation analysis of [^{3}H]E2, ^{125}IVME2 and ^{123}IBHPE. Following incubation of the radioactive estrogens with rat uterine cytosol and DCC treatment as described in the Methods section, the mixtures were analyzed on low salt, $T_{10}K_{10}E_1$, (A–C), or high salt, $T_{10}K_{400}E_1$ (D, E), 10%–30% sucrose gradients. For the low salt gradients, incubations were carried out in the absence (O, —) or presence (●, ···) of 100-fold excess DES (A = ^{3}H-estradiol, B = ^{123}IBHPE, C = ^{125}IVME2); for the high salt gradients in the absence (O, —) or presence (●, ···) of anti-ER antibody H222 (D = ^{123}IBHPE, E = ^{125}IVME2).

specific activity is known to be 2.22 TBq (60 mCi) per μmole, specific calculated activities of 69.2 and 103 TBq/μmole (~1.87 and 2.79 Ci/μmole), respectively, were obtained for these iodoestrogens. In addition to providing a reliable method to assay for specific activity of the radioiodinated estrogens, the gradients also demonstrate a major difference in binding characteristics between IVME2 and IBHPE, the former showing little significant nonspecific binding, while IBHPE has considerable nonspecific 4S binding.

The tissue concentrations of ^{123}IBHPE and ^{125}IVME2 at 0.5, 2.5 and 20 hr after injection are shown in Figures 3 and 4 when administered alone or together with either 0.1 or 1.0 μg unlabeled DES. It is clear that for both compounds the highest uptake and longest retention is in the uterus. DES coadministration reduces the uptake of both ^{123}IBHPE (Fig. 3) and ^{125}IVME2 (Fig. 4) in the ER target tissues (i.e., uterus, vagina, ovary and pituitary). The uptake of IVME2 by the pituitary appears faster and more substantial than is seen with IBHPE. The other tissues studied, namely liver, adrenal, kidney, muscle and brain, did not show DES-inhibitable uptake. Of these tissues, the liver showed the highest concentration of each iodoestrogen at all time points. One clear difference between the distribution patterns for these two iodoestrogens was the relative effectiveness of the low dose of DES to inhibit specific uptake. While with ^{123}IBHPE, the triphenylethylene estrogen, the low dose of DES was nearly as compet-

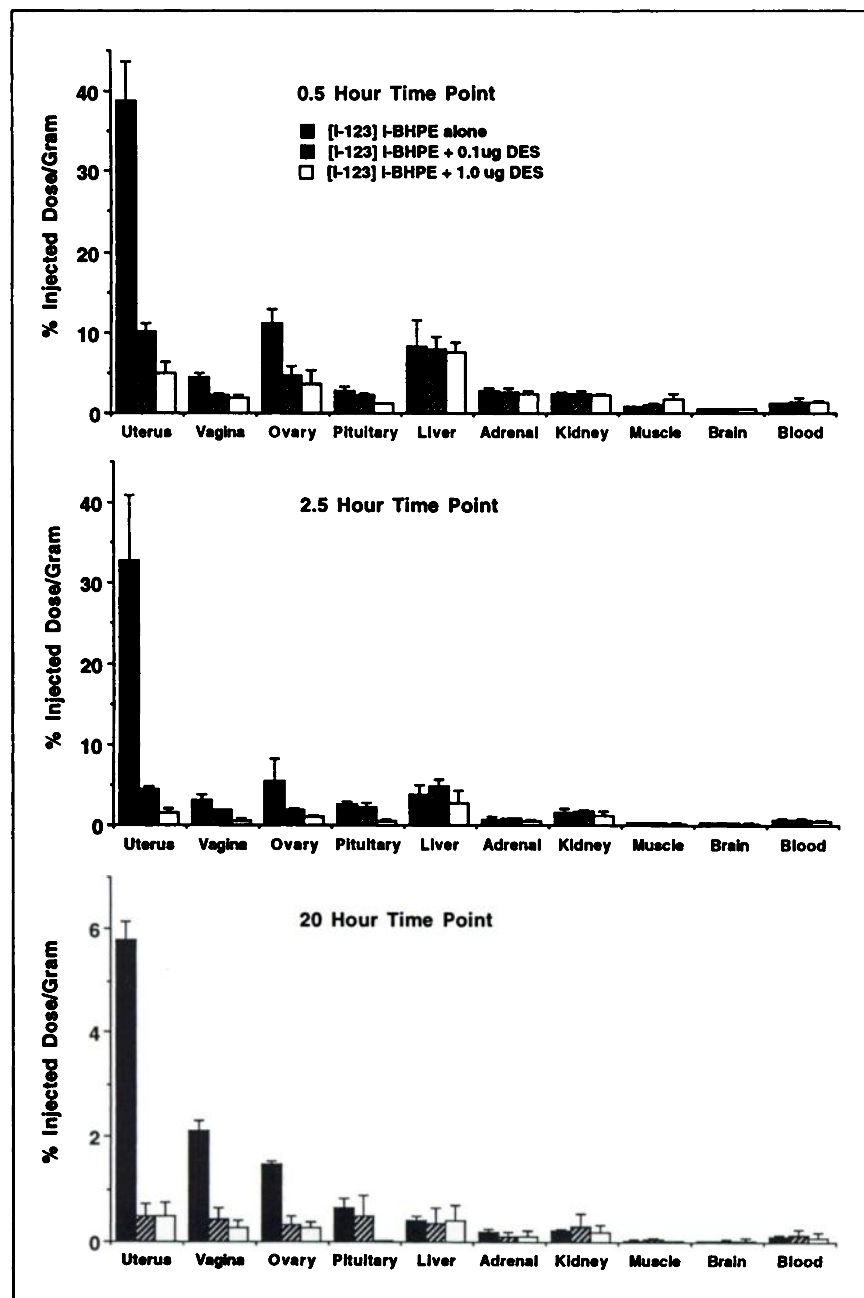

FIGURE 3. Distribution of ^{123}IBHPE in rat tissues following i.p. administration. Results are plotted as mean %ID/g ± s.d. at the three time points. For each tissue, the three bars represent results of administration of the ^{123}IBHPE alone (solid bars), with 0.1 μg DES (cross-hatched bars) or 1.0 μg DES (clear bars).

itive as the high dose. When administered with the steroidal estrogen [125]IVME2, the low dose was significantly less competitive than the high dose of DES. With [123]IBHPE, the clearest DES inhibitable uptake at the early time points is in the uterus and ovary. By the 20 hr time point, however, DES inhibitability is also impressive for the pituitary and vagina. On the other hand, at the early time points, for [125]IVME2, the low dose of DES is unable to significantly inhibit its uptake in the vagina and pituitary, while the high DES dose does effectively prevent its uptake by all of the ER target tissues. With IVME2, the apparent nonspecific uptake observed in nontarget tissues at the early time points is greater than that seen with IBHPE.

A comparison of the tissue concentrations of radioiodine as a function of time after administration for the four ER target tissues is shown in Figure 5. Two types of time-dependent radioactivity concentration curves can be seen, one for uterus and ovary, another for vagina and pituitary. For the uterus and ovary, the highest concentrations of both radioiodoestrogens occur at the earliest time point, 30 min, at which time the relative concentration of the two iodoestrogens, when administered alone, are quite similar. In both of these tissues, the retention of the steroidal estrogen IVME2 is more prolonged than that of IBHPE and this is especially evident in the ovary. On the other hand, for both the vagina and pituitary the concen-

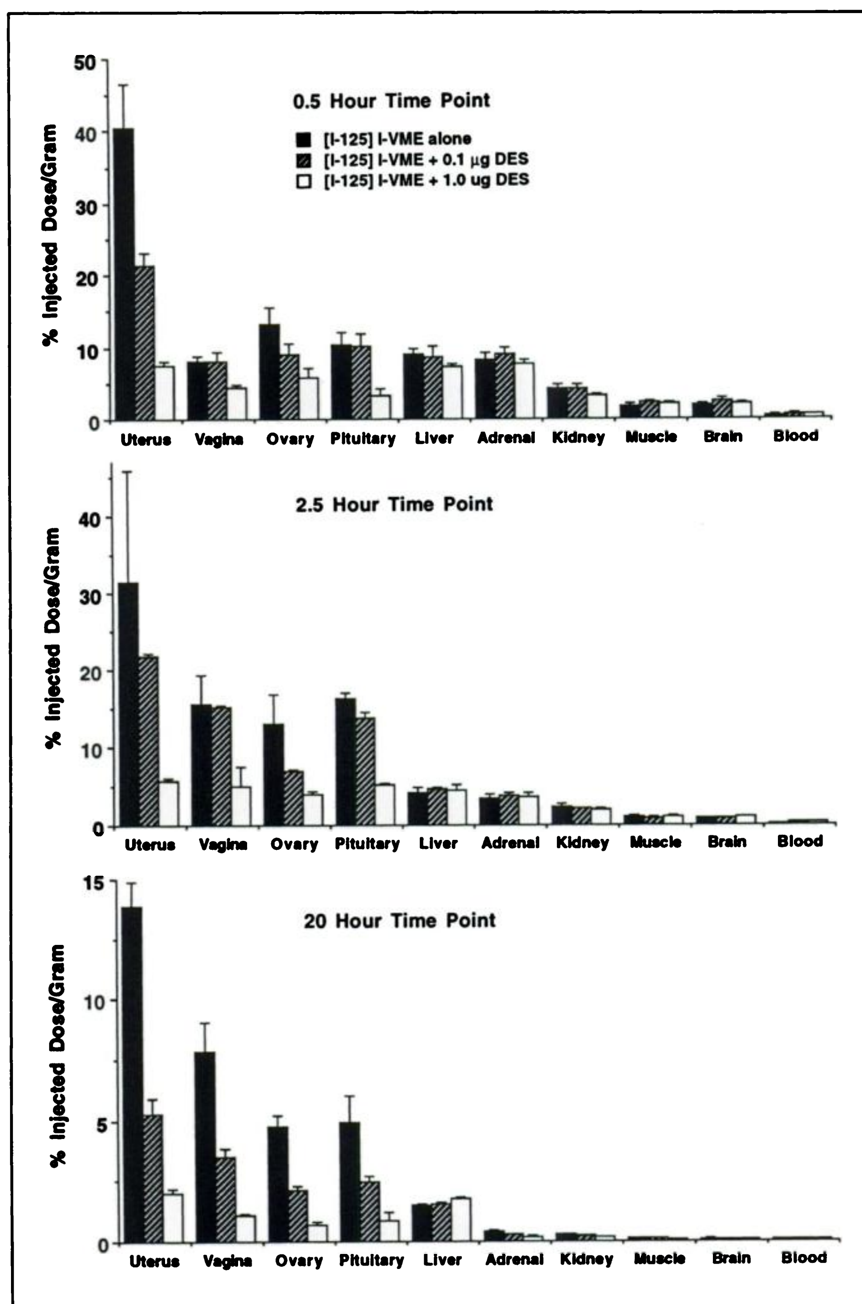

FIGURE 4. Distribution of [125]IVME2 in rat tissues. Details as in Figure 3.

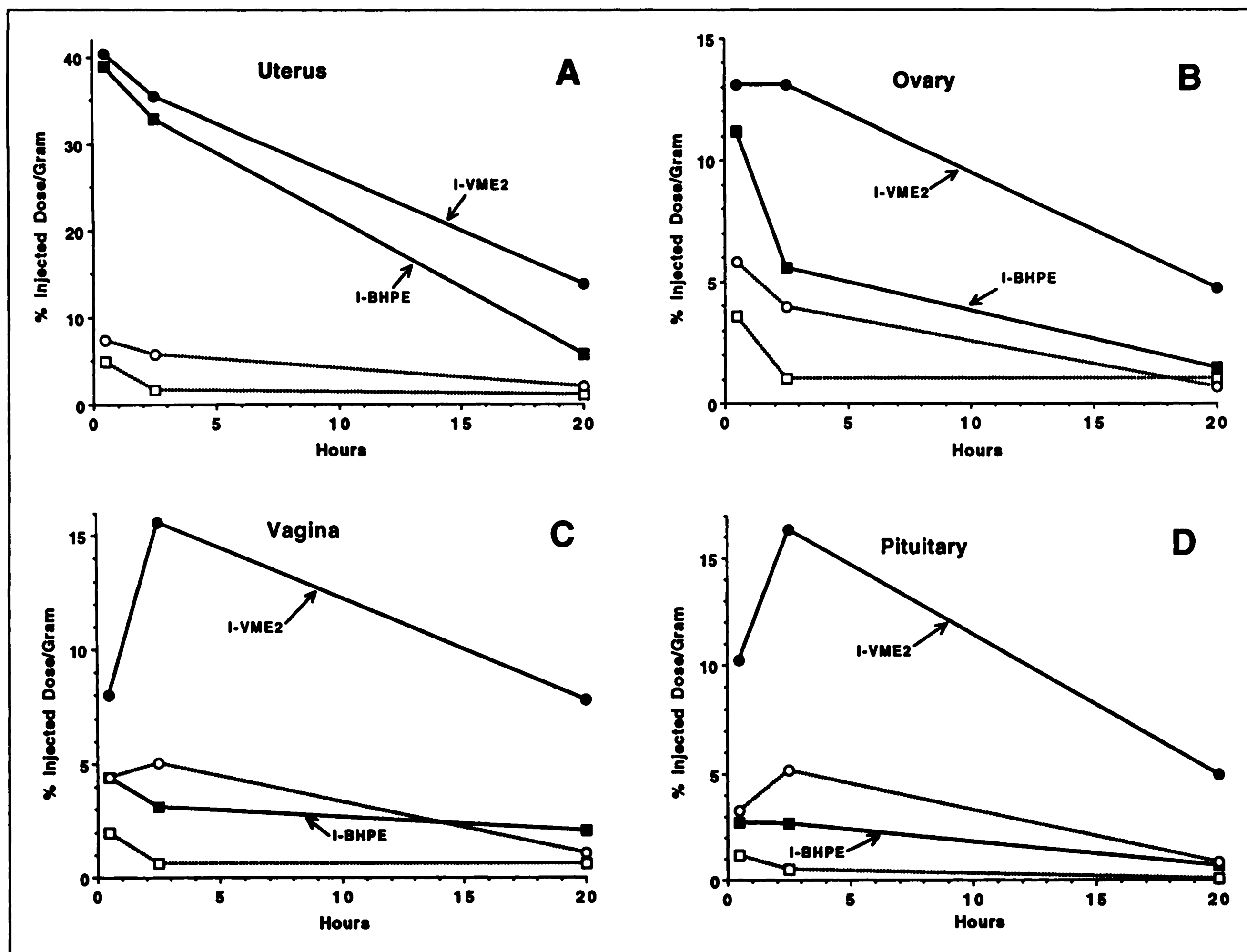

FIGURE 5. Time course of retention of [^{123}I]IBHPE and [^{125}I]IVME2 in estrogen target tissues. Iodoestrogens were administered alone (solid symbols) or with 1.0 μg DES (open symbols, dotted lines) and the mean %ID/g determined for each tissue. (A = uterus, B = ovary, C = vagina, D = pituitary). Circles = [^{125}I]IVME2 and squares = [^{123}I]IBHPE.

tration of IVME2 is significantly higher at 2.5 hr than at 30 min. For IBHPE, the uptake at both these times appears to be substantially less in the vagina and pituitary than for the uterus and ovary, although it can be seen that in each case the uptake is specific, as is evident from the significantly lower radioactivity when IBHPE was administered along with the high dose of DES. It would appear that the IBHPE is comparable to IVME2 in initial uptake for those tissues that it can reach directly from i.p. administration (uterus, ovary), whereas when the ligand must depend on the circulation to reach the tissues (pituitary and vagina), the steroidal estrogen is much more effective at reaching those target tissues. Interestingly, in each of these tissues the apparent nonspecific uptake (i.e., uptake in the presence of the high dose of DES) is significantly greater with the steroidal estrogen. When the results are expressed as tissue-to-muscle ratios (Table 1), it is apparent that the ratio for IBHPE is higher than that for IVME2 for the uterus and ovary at the early time points. By 20 hr, however, the ratios for the two ligands are similar. In contrast, for target tissues requiring blood supplied ligand, i.e., vagina and pituitary, the tissue-to-muscle ratios are greater for IVME2 rather than IBHPE and increase over the time studied.

In Figure 6, the concentration curves as a function of time are shown for blood and some nontarget tissues. In Figure 6A, it can be seen, especially at the early time points, that the blood level of radioiodine is significantly higher after IBHPE than after IVME2, and as expected,

TABLE 1
Tissue-to-Muscle Ratio

	0.5 hr		2.5 hr		20 hr	
	IBHPE	IVME2	IBHPE	IVME2	IBHPE	IVME2
1 Uterus	45.0	23.5	101.0	28.4	105.4	121.6
2 Vagina	5.2	4.8	9.4	13.1	34.7	68.3
3 Ovary	13.1	7.6	17.3	11.0	28.3	43.0
4 Pituitary	3.4	5.9	9.0	14.6	16.8	43.1
5 Liver	10.1	5.3	10.3	3.6	9.9	13.0
6 Adrenal	3.4	4.8	2.0	2.9	5.4	3.5
7 Kidney	3.0	2.5	4.0	2.1	5.5	2.5
8 Brain	0.6	1.2	0.8	0.7	0.5	0.7

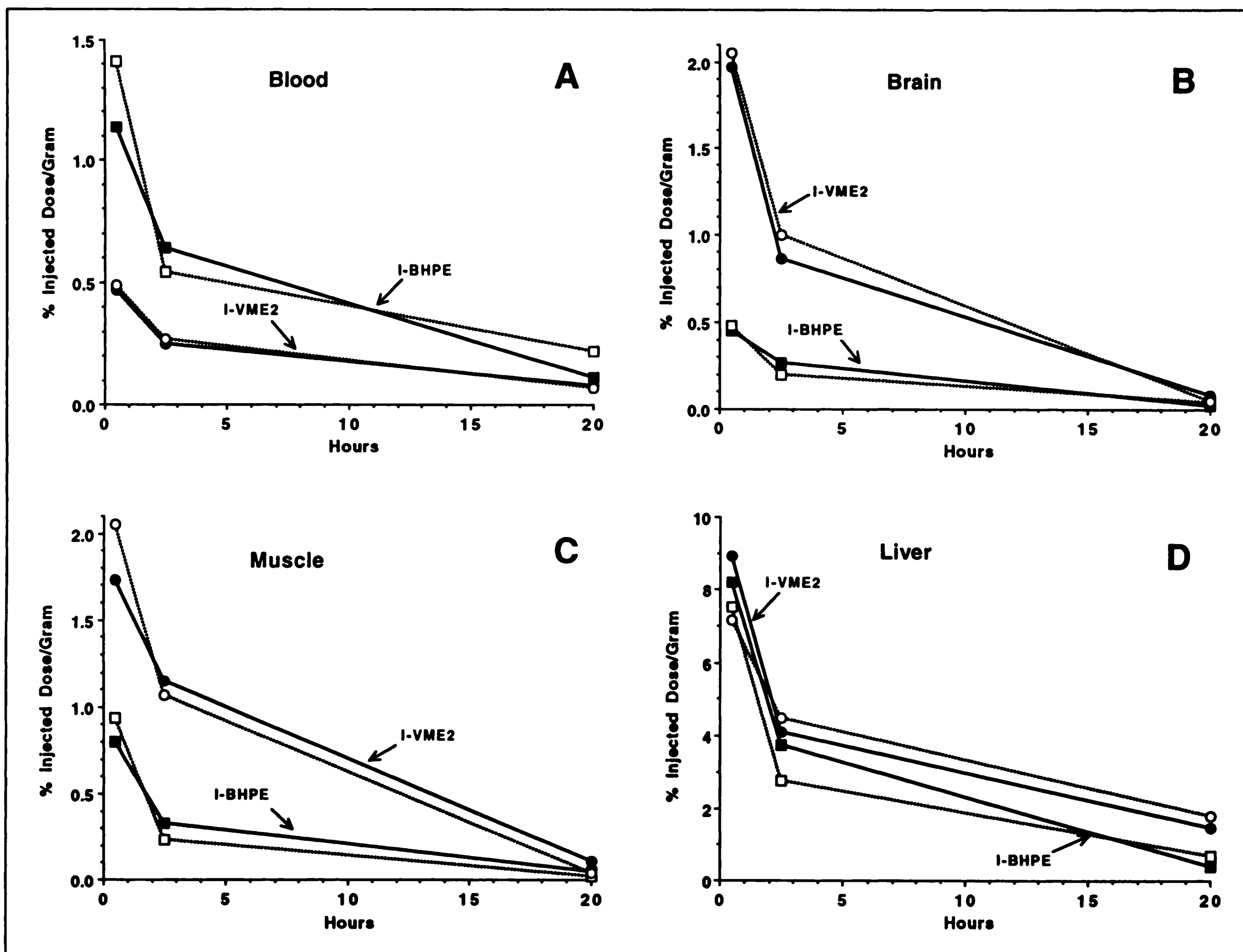

FIGURE 6. Time course of retention of ^{123}IBHPE and ^{125}IVME2 in nontarget tissues. Symbols as in Figure 5.

there is no significant difference in blood levels whether either of these ligands were administered in the absence or presence of DES. On the other hand, in the nontarget tissues, brain and muscle, the concentration of IVME2 is significantly higher than that of IBHPE, again both in the absence and presence of DES. However, in the liver, where both of these ligands are most likely metabolized, the levels of radioiodine from the two iodoestrogens are much more comparable and also not affected by coadministration of DES.

DISCUSSION

There is now evidence for the potential of radioiodinated estrogens for both imaging (i.e., diagnosis) (*13–16*) and also possibly therapy (*8–12*) of ER-positive cancers. To accomplish either useful imaging or effective therapy, it will be important to choose the best iodoestrogens for each purpose (*15–18*), and also label the estrogens at high specific activities. For this study, it was advantageous to have the iodoestrogens at approximately equal specific activities so that similar numbers of molecules of the estrogens would be injected. Recently, we have been able to prepare both ^{123}I-labeled I-BHPE and I-VME2 at near the theoretical specific activity of ^{123}I (~8.9 MBq/pmole; 240 μCi/pmole) (*8,9*). It is likely that high specific activities will be required for therapy and will be useful for imaging with ^{123}I-labeled estrogens as well. For therapy each estrogen, at such high specific activity, taken up into the nucleus of a target cell would have the potential to deliver radiotherapy to the cells. For imaging, it is clearly important to have not only high concentrations in the ER-containing tissues that one wishes to detect, but also low concentrations in the nontarget tissues. This latter characteristic is frequently related to blood levels, since the major vessels and capillary beds constitute a significant component of the background, over which the signal (ER-bound ligand) is to be detected. In this respect, the rapid loss of the radioiodine from the blood when IVME2 is administered would appear to be attractive (Fig. 6). Also, there are several reports that the 11β-methoxy substituent increased target tissue-to-blood ratios (*13,18–21*). And, as seen in the sedimentation analyses (Fig. 2), IVME2, characteristic of many 11β substituted estrogens, shows little nonspecific

binding in tissue extracts. However, the other significant difference between the nontarget tissue distribution of IVME2 and IBHPE is the significantly higher levels of tissue radioiodine from IVME2, especially at the 0.5 and 2.5 hr time points, when the highest levels are present in the target tissues (Table 1). It appears that when the estrogen does not bind very well to blood proteins or carriers, it can more readily diffuse into tissues. Since estrogens in general are somewhat lipophilic and thus readily associate with the lipid-rich cell membranes, with IVME2 there is a higher nonspecific concentration throughout the body than with IBHPE, which binds avidly to nonspecific proteins in the blood and may require higher affinity binding proteins in tissues to effect release from serum proteins. This interaction in the blood probably prevents unrestricted diffusion into all tissues and results in lower concentrations of IBHPE in most nontarget tissues. Interestingly, the uptake of the two estrogens by the liver, the site of extensive metabolism of estrogens, is comparable for those two estrogens, supporting the conclusion that the aforementioned differences are characteristic of the ligands and not of the method of study. The ability to draw such conclusions emphasizes the inherent advantages in the use of dual isotopes in the same animals, whereby the precision of the comparison is increased by eliminating differences due to animal to animal variation or injection differences. Furthermore, we had also noted high nonspecific tissue levels of the bromine analog of this estrogen (*6*). As seen in Figure 6, the blood levels of radioiodine after injection of IBHPE were significantly higher at both 0.5 and 2.5 hr than those of animals given IVME2.

Fluorine-18-labeled estrogens also appear promising for imaging, especially in that PET imaging can provide better three-dimensional localization. Keisewetter et al. (*22*) compared four different ^{18}F-labeled estrogens in the immature rat and found that while all four showed selective uptake by the uterus after i.v. administration, 16α-fluoro-17β-estradiol gave the highest %ID/g level, uterus-to-blood ratio and uterus-to-nontarget tissue ratios. While none of these values were as high as those of the iodoestrogens reported here in the uterus after i.p. administration, a subsequent study of 6α-[^{18}F]fluoro-17β-estradiol in patients showed this fluoroestrogen to have high sensitivity and specificity for detecting metastatic breast carcinoma (*23*).

For therapy via emission of Auger electrons, it is important to be able to reach the highest ligand concentration in the cell nucleus, since it is well-documented that the short range of Auger electrons essentially limits their radiotoxic effectiveness to the nucleus of the cell in which the decay occurs (*24*). The basis for the proposed use for steroid-receptor containing cancers is that the steroid receptors in general, and ER in particular (*25*), reside in the cell nucleus of target tissues and the ER complex with estrogen binds to specific regions in the DNA (*26*) of the target cells. We have focused on the i.p. route of administration of the iodoestrogens because of our interest in approaching ovarian cancer which spreads in the peritoneal cavity. It is one of the greatest therapeutic challenges of ER-containing cancers (*5*) because, while nearly half of all ovarian cancers contain ER, conventional endocrine therapies cause few remissions and conventional chemotherapies are of limited effectiveness as well. For therapy with Auger electron-emitting estrogens to be effective, one would want to use the estrogen with the best localization in the peritoneal ER-positive cancer tissue, as well as the most prolonged retention. Even though ^{123}I has a relatively short half-life (i.e., 13.2 hr), the biologic retention of estrogen in its target tissues is also rather short. Since normal tissues also contain estrogen receptors, one would ideally like to find a ligand that would localize preferentially in the target cancer in the peritoneal cavity with minimal systemic redistribution via the circulation to other normal target tissues, such as the pituitary and hypothalamus. For this purpose the IBHPE looks promising. It is clear that the normal ER target tissues in the peritoneal cavity, uterus and ovary, which it reached directly from the i.p. route, showed substantially higher concentrations than those tissues, like the vagina and pituitary, which depended on supply via the circulation (Fig. 5). It is also likely that the substantial nonspecific binding to various proteins present in the peritoneal fluid enhanced the retention in the peritoneal cavity allowing prolonged access to the peritoneal target tissues. One might be concerned about uptake to the normal tissues of the uterus and ovary. However, because of the nature of the disease and treatment, these tissues, if present, are generally removed at the time of first surgery for ovarian cancer. While IVME2 shows excellent, and probably superior, uptake and retention by the ER-positive peritoneal tissues, it also is concentrated to much higher levels in the distant ER target tissues such as the vagina and pituitary, which depend upon systemic blood supply for uptake. Gatley et al. (*27,28*) have shown similar patterns between IBHPE and IVME2 in both immature and mature rats, albeit with a reduced target tissue uptake in the mature animals. In the same study, they also showed that both iodoestrogens were stable in the target tissues but were rapidly deiodinated in the liver and that IBHPE was stably transported in the blood. Thus, it would appear for the purpose of preferentially concentrating an iodoestrogen in peritoneal ER-containing tissue relative to other estrogen target organs, the triphenylethylene iodoestrogen, IBHPE, would have the advantage. On the other hand, the higher peripheral ER-target tissue uptake and the more rapid clearance of I-VME2 from the blood would suggest that the steroidal iodoestrogen would be more attractive for imaging of ER-positive lesions, especially at a number of hours after administration. With a view to establishing the dosimetry of ^{123}IBHPE for its therapeutic use in humans, a preliminary study of the biodistribution in women has been under-

taken (*29*). In that study, also via the intraperitoneal route, it was found that injection of the IBHPE with albumin significantly prolonged the intraperitoneal residence time, thus reducing liver radiation exposure. These results are clinically desirable for either diagnosis or therapy.

SUMMARY

We have compared the total and specific uptake and retention of two radioiodoestrogens, a triphenylethylene estrogen ^{123}IBHPE and a steroidal estrogen ^{125}IVME2, after intraperitoneal administration to immature female rats. The steroidal estrogen gave the highest uptake and longest retention in both peritoneal and distant estrogen target tissues and was rapidly cleared from the blood. However, it showed unexpectedly high nonspecific diffusion into all tissues, especially at the earlier time points. The triphenylethylene iodoestrogen also showed excellent uptake and extended retention in peritoneal estrogen target tissues, especially the uterus, but substantially decreased concentrations in estrogen target tissues that depended upon the circulation for the delivery of ligand, such as the pituitary and vagina. These distant tissues thus might be spared radiation dose from the therapeutic use of ^{123}IBHPE relative to their exposure from ^{123}IVME2.

ACKNOWLEDGMENTS

This research was supported by the National Cancer Institute (CA27476, CA49906). Technical assistance was provided by R. Dizon, P.V. Harper, P.C. Kuivanen, R. Mease and M.-L. Pan.

REFERENCES

1. Huggins C, Bergenstal DM. Inhibition of human mammary and prostatic cancer by adrenalectomy. *Cancer Res* 1952;12:134–141.
2. Jensen EV, Block GE, Smith S, et al. Estrogen receptors and breast cancer response to adrenalectomy. In: Hall TC, ed. *Prediction of response in cancer therapy.* National Cancer Institute Monograph, No. 34. Washington, DC: U.S. Government Printing Office; 1971:55–70.
3. McGuire WL, Carbone PP, Vollmer EP, eds. *Estrogen receptors in human breast cancer.* New York: Raven Press; 1975.
4. DeSombre ER. Steroid receptors in breast cancer. In: McDivitt RW, Oberman HA, Ozzello L, Kaufman N, eds. *The breast.* Baltimore: Williams and Wilkins; 1984:149–184.
5. DeSombre ER, Holt JA, Herbst AL. Steroid receptors in breast, uterine and ovarian malignancy and consideration of their diagnostic and therapeutic usefulness. In: Josimovich JB, Gold J, eds. *Gynecologic endocrinology*, 4th edition. New York: Plenum Press; 1987:511–528.
6. DeSombre ER, Mease RC, Hughes A, Harper PV, DeJesus OT, Friedman AM. Bromine-80m-labeled estrogens: Auger electron-emitting, estrogen receptor-directed ligands with potential for therapy of estrogen receptor-positive cancers. *Cancer Res* 1988;48:899–906.
7. Seevers RH, Mease RC, Friedman AM, DeSombre ER. The synthesis of non-steroidal estrogen receptor binding compounds labeled with ^{80m}Br. *Nucl Med Biol* 1986;13:483–495.
8. DeSombre ER, Shafii B, Hanson RN, Kuivanen PC, Hughes A. Estrogen receptor-directed radiotoxicity with Auger Electrons: specificity and mean lethal dose. *Cancer Res* 1992;52:5752–5758.
9. DeSombre ER, Hughes A, Shafii B, et al. Estrogen receptor-directed radiotoxicity with Auger-electron-emitting nuclides: E-17α-(^{123}I)-11β-methoxyestradiol and CHO-ER cells. In: Howell RW, Nara VR, Sastry KSR, Rao DV, eds. *Biophysical aspects of Auger processes.* New York: AIP Press; 1992:352–371.
10. Lippman ME, Do HMT, Hochberg RB. Specific estrogen receptor binding and biological effects of 16α-iodoestradiol on human breast cancer cells. *Cancer Res* 1991;41:3150–3154.
11. McLaughlin WH, Milius RA, Pillai KMR, Edasery JP, Blumenthal RD, Bloomer WD. Cytotoxicity of receptor-mediated 16α-(^{125}I)iodoestradiol in cultured MCF-7 human breast cancer cells. *J Natl Cancer Inst* 1989;81: 437–440.
12. Scharl A, Beckmann MW, Schreiber JR, Holt JA. Zytotoxizität von 16-alpha-(^{125}Jod)-17-beta-Östradiol für östrogenrezeptor-positive mammakarzinomzellen in vitro. *Onkologie* 1991;14:433–436.
13. Zielinski JE, Larner JM, Hoffer PB, Hochberg RB. The synthesis of 11β-methoxy-(16α-^{123}I)iodoestradiol and its interaction with the estrogen receptor in vivo and in vitro. *J Nucl Med* 1989;30:209–215.
14. Scheidhauer K, Muller S, Smolarz K, Brautigan P, Briele, B. Tumorszintigraphie mit ^{123}J-markiertem östradiol beim mammakarzinom-rezeptorszintigraphie. *Nuklearmedizin* 1991;30:84–99.
15. McElvany KD, Carlson KE, Welch MJ, Senderoff SG, Katzenellenbogen JA. In vivo comparison of 16α-(^{77}Br)bromoestradiol-17β and 16α-(^{125}I)iodoestradiol-17β. *J Nucl Med* 1982;23:420–424.
16. Pavlik EJ, Nelson K, Gallion HH, et al. Characterization of high specific activity (16α-^{123}I)iodo-17β-estradiol as an estrogen receptor-specific radioligand capable of imaging estrogen receptor-positive tumors. *Cancer Res* 1990;50:7799–7805.
17. DeSombre ER, Hughes A, Mease RC, Harper PV. Comparison of the distribution of bromine-77-bromovinyl steroidal and triphenylethylene estrogens in the immature rat. *J Nucl Med* 1990;31:1534–1542.
18. McElvany KD, Carlson KE, Katzenellenbogen JA, Welch MJ. Factors affecting the target site uptake selectivity of estrogen radiopharmaceuticals: serum binding and endogenous estrogens. *J Steroid Biochem* 1983;18: 635–641.
19. Jagoda EM, Gibson RE, Goodgold H, Ferreira N, et al. (I-125)17α-iodovinyl-11β-methoxyestradiol. In vivo and in vitro properties of a high affinity estrogen receptor pharmaceutical. *J Nucl Med* 1984;25:472–477.
20. Hanson RH, Franke LA. Preparation and evaluation of 17α-(^{125}I)iodovinyl-11β-methoxyestradiol as a highly selective radioligand for tissues containing estrogen receptors: concise communication. *J Nucl Med* 1984;25: 998–1002.
21. Hanson RH, Franke LA, Kaplan M. Radioiodinated ligands for the estrogen:tissue distribution of 17α-(^{125}I)iodovinylestradiol derivatives in normal and tumor bearing adult female rats. *Nucl Med Biol* 1990;17:239–245.
22. Kiesewetter DO, Kilbourn MR, Landvatter SW, et al. Preparation of four fluorine-18-labeled estrogens and their selective uptakes in target tissues of immature rats. *J Nucl Med* 1984;25:1212–1221.
23. McGuire AH, Dehdashti F, Siegel BA, et al. Positron tomographic assessment of 16α-[^{18}F] fluoro-17β-estradiol uptake in metastatic breast carcinoma. *J Nucl Med* 1991;32:1526–1531.
24. Kassis AL, Adelstein SJ, Bloomer WD. Therapeutic implications of Auger-emitting radionuclides. In: Spencer RP, Seavers R, Friedman AM, eds. *Radionuclides in therapy.* Boca Raton, FL: CRC Press; 1987:119–134.
25. King WJ, Greene GL. Monoclonal antibodies localize estrogen receptor in the nucleus of target cells. *Nature* 1984;307:745–747.
26. Carson-Jurica MA, Schrader WL, O'Malley BW. Steroid receptor family: structure and function. *Endo Rev* 1990;11:201–220.
27. Gatley SJ, DeSombre ER, Hughes A, Pan M-L, Teaster B. Comparison of steroidal and triphenylethylene-based iodovinylestrogens with regard to biodistribution and metabolism [Abstract]. *J Nucl Med* 1989;30:924.
28. Gatley SJ, DeSombre ER, Mease RC, et al. Synthesis, purification and stability of no carrier added radioiodinated 1,1-bis(4-hydroxyphenyl)-2-iodo-2-phenylethylene (IBHPE), a prototype triphenylethylene estrogen-receptor binding radiopharmaceutical. *Nucl Med Biol* 1991;18:769–775.
29. Ryan JW, Rotmensch J, Pan M-L, et al. Human biodistribution studies of a novel non-steroidal estrogen (I-123-IBHPE) [Abstract]. *J Nucl Med* 1989; 30:883.