RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

[I-125] 17α-Iodovinyl 11β-Methoxyestradiol: In Vivo and In Vitro Properties of a High-Affinity Estrogen-Receptor Radiopharmaceutical

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17α-[^{125}I]Iodovinyl 11β-methoxyestradiol ([I-125]MIVE$_2$) has been prepared with high specific activity (1500–2000 Ci/mmol) and a high affinity for the estrogen receptor ($K_A = 6.8 \times 10^9$ M/Ml). In vivo distribution studies using immature rats result in high levels of activity in the uterus (20–30% dose/g) with uterus-to-plasma ratios on the order of 68 to 100. Peak activity in the uterus is obtained between 2 and 4 hr, and by 6 hr 50% of the activity has washed out. The [I-125]MIVE$_2$ exhibits a slower rate of washout relative to the washout of H-3 estradiol. By in vivo competition studies with nonradioactive estradiol, we found that 95% of the [I-125]MIVE$_2$ bound in the uterus is specifically bound to estrogen receptors. The radioactive labeling of MIVE$_2$ is sufficiently rapid so that [I-123]MIVE$_2$ has been synthesized and is currently in clinical trials. These results suggest that MIVE$_2$ would be an excellent agent for the study of estrogen receptors in vivo and in vitro.

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Since the detection of specific estrogen receptors in breast carcinoma, much effort has been expended to find a suitable gamma- or positron-emitting tracer that would provide for rapid, noninvasive detection of these receptors (*1*). The presence or absence of estrogen receptors determines which therapeutic regimen, hormonal or chemotherapeutic, is likely to be most effective in treatment. In addition, the continued presence of estrogen-positive tumors has been associated with a disease-free interval that is independent of variables such as age, menopausal stage, tumor size, or nodal status (*2*). A diagnostic agent capable of monitoring the estrogen-receptor content of breast carcinoma would not only indicate the appropriate therapeutic regimen but would also identify various stages in the time course of the disease. A suitable radiotracer developed for such diagnostic purposes would have to have a high affinity for the estrogen receptor and high in vivo stability. The halogenated estradiols 16 α-[I-125]iodoestradiol and 16α-[Br-77]bromoestradiol have been shown to localize in uterus from an immature rat and in induced mammary tumors in rats (*3–5*). By contrast, a 6-iodo derivative of estradiol that exhibits an affinity for the estrogen receptor in vitro similar to that of estradiol, did not provide tumor localization (*6*). 17α-[Br-77]Bromoethynyl estradiol, a derivative of the metabolically more stable ethynylestradiol (*7*), localized in immature rat uterus to the same extent as the 16α-halogenated analogs but exhibited a lower target-to-blood ratio due to a high affinity for serum proteins (*8*). Hanson et al. (*9*) have reported a rapid synthesis for I-125-labeled 17α-iodovinylestradiol. This derivative exhibits high affinity for the receptor and provides the desired localization in target tissues. Nonsteroidal estrogen deriva-

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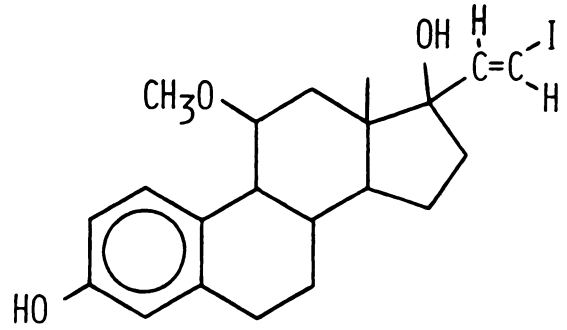

FIG. 1. 17α-(E)-2-iodovinyl estra-1,3,5(10)-triene-3,17β-diol ($MIVE_2$).

tives—which include halogenated derivatives of hexestrol (*10*), particularly [F-18]fluoropentestrol (*11*)—provide reasonable localization in the immature rat uterus. However, the compound that has been reported to exhibit the lowest extent of metabolism in vivo and the least amount of nonreceptor binding is the 11β-methoxy derivative of ethynylestradiol, moxestrol (*7*). We have, therefore, synthesized a derivative of moxestrol (Fig. 1) in which the radioiodine is incorporated on carbon 21 as a stable iodovinyl derivative: 17α-(E)-2-[I-125]iodovinyl estra-1,3,5,(10)-triene-3,17β-diol ([I-125]$MIVE_2$), and have determined its in vivo and in vitro properties.

MATERIALS AND METHODS

Radioligands. [I-125]$MIVE_2$ was prepared as previously described (*12*). The product was isolated by high performance liquid chromotography (column: Lichrosorb RP-18, 10 mm × 250 mm; flow rate 2 ml/min) using 70:30 EtOH/H_2O, and radiochemical purity was determined using thin layer chromatography (TLC) (silica gel) in toluene/ethyl acetate (8:2). The radiotracer is coincident with the uv spot (R_f = 0.18) of carrier $MIVE_2$, which analyzed within 0.4% of the theoretical values for carbon and hydrogen. The product was quickly frozen and stored at −70°C. Dehalogenation did not occur on storage. The H-3 estradiol ([H-3]E_2) is commercially available.

IN VIVO DISTRIBUTION STUDIES

Three to five μCi of the radioligand (either [H-3]E_2 and/or [I-125]$MIVE_2$) were injected through the femoral vein of female Sprague-Dawley rats, 20–25 days old, and the animals were killed at various times by cervical dislocation. Samples of uterus, liver, muscle, and blood were removed. Tissues were blotted free of excess blood and 10- to 30-mg samples were solubilized for 2–3 days for the determination of H-3. Heparinized blood samples were centrifuged to separate the plasma, and 20-μl samples were removed and solubilized as previously described (*8*). All samples were prepared further for scintillation counting by addition of 0.1 ml glacial acetic acid to neutralize the excess base, followed by 15 ml of ACS*. Samples were dark-adapted and counted until no chemiluminescence was indicated. Samples that contained I-125 were counted on an auto-gamma counter, with cpm determined by the absolute counting method (*13*).

Samples that contained both H-3 and I-125 were counted on a system that can count dual-labeled samples when the absolute activities of the I-125 to H-3 are not greater than −5 to 1, respectively.

In vitro equilibrium association constants (K_A) and specific-activity determinations. Binding studies using H-3 and I-125 used the cytosol from uteri of immature rats prepared as described previously (*14*). Incubation with the radioligand proceeded for 30 min at room temperature, with and without $10^{-6}M$ unlabeled estradiol. The extent of ligand binding was determined by the dextran-coated charcoal (DCC) assay (*14*), and the results plotted by the method of Scatchard (*15*). The R_0 and K_A values were substantiated by computer analysis of the data using the LIGAND program (15) modified for use on the IBM PC. Assuming that [I-125]$MIVE_2$ and [H-3]E_2 are binding to the same population of receptors, we determined the specific activity of the [I-125]$MIVE_2$ by matching the R_0 (receptor concentration) of [I-125]$MIVE_2$ with that obtained using [H-3]E_2 of known specific activity.

RESULTS

Equilibrium association constant (K_A) and specific activity. Both the specific activity and the K_A of [I-125]$MIVE_2$ were determined using estrogen receptors from the uterus of immature rats as described in Methods. Figure 2 presents a Scatchard plot of [I-125]$MIVE_2$ and [H-3]E_2 binding to the estrogen receptor. The specific activity of the preparation was 1890 Ci/mmol and the affinity constant was $6.8 \times 10^9\ M^{-1}$—half that of [H-3]E_2 ($K_A = 1.65 \times 10^{10}\ M^{-1}$). The K_A values for other [I-125]$MIVE_2$ preparations ranged from 50% to 150% of that of [H-3] E_2. The two- to threefold difference in the affinity constants was within the range encountered from daily experimental variability. Nonreceptor binding was no more than 5% of the total radiotracer bound at the highest concentration assayed. Five other preparations of $MIVE_2$, which used I-123 as the radionuclide, exhibited specific activities that ranged from 500–1400 Ci/mmol. These activities were determined by a one-point binding assay to facilitate a rapid determination for clinical use; therefore the K_A could not be determined.

In vivo distribution. The distribution of [I-125]$MIVE_2$ in immature rats is summarized in Table 1. Peak activity in the uterus is obtained between 2 and 4 hr after injection (Fig. 3). With some groups of animals we observed localization in the uterus as high as 27% of the injected

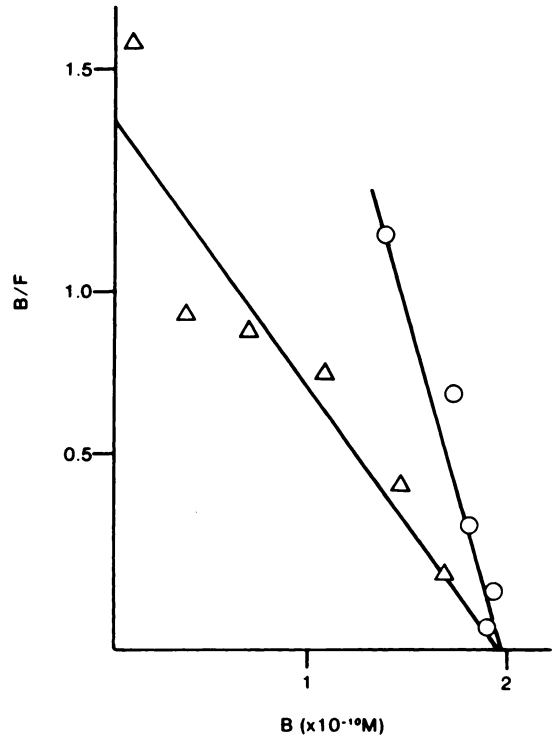

FIG. 2. Scatchard analysis of [I-125]MIVE$_2$ (Δ) and [H-3]E$_2$ (O) specifically binding to estradiol receptor from immature rat uterus. $K_A = 1.65 \times 10^{10} M^{-1}$ for [H-3]E$_2$ and $K_A = 6.8 \times 10^9 M^{-1}$ for [I-125]MIVE$_2$ (specific activity = 1890 Ci/mmol).

dose/g (CV < 20%). By 6 hr, 54% of the activity had washed out of the uterus. The activity in the plasma was low at 15 min (0.48% dose/g) and fell at a rate sufficient to maintain a high uterus-to-plasma ratio at all times (Fig. 4); at 2 hr it was 68, and by 6 hr it was as high as 97. Samples of muscle were also examined, since it had been suggested that muscle provides a more consistent measure of nontarget localization of activity; variations in the amount of alpha-fetoprotein in the blood can raise blood levels artificially (*17,18*). Activity in the liver remained relatively constant over the period examined; the slight washout was not statistically significant. Dehalogenation of [I-125]MIVE$_2$ was not detected in vivo, as was indicated by low uptake of activity by the thyroid (0.1% injected dose/g).

The pharmacokinetics of [I-125]MIVE$_2$ and [H-3]E$_2$ are compared in Fig. 3. All the time-points represented were determined from a co-injection of 5 μCi of [H-3]E$_2$ and 5 μCi of [I-125]MIVE$_2$. [H-3]E$_2$ reached peak levels from 1 hr to 1 hr 15 min, as in previous reports (*19,20*). By contrast, [I-125]MIVE$_2$ peaked between 2 and 4 hr, attaining levels 3 to 4 times those seen with [H-3]E$_2$. Moreover, from 15 min to 2 hr the uterus-to-plasma ratios were three times those for [H-3]E$_2$, indicating both lower activity in the plasma and higher levels in the uterus (Fig. 4). At later times (2 to 6 hr) the uterus-to-plasma ratio was as much as ten times greater than [H-3]E$_2$, reflecting the slower washout of the [I-125]MIVE$_2$ from the uterus.

The uterine radioactivity depended upon the weight of the organ (Fig. 5), which introduced considerable variability in our results. Animals with uterine weights of 20–40 mg localized as much as 40% of the injected dose/g in the uterus, while animals having uterine weights >160 mg localized significantly less activity,

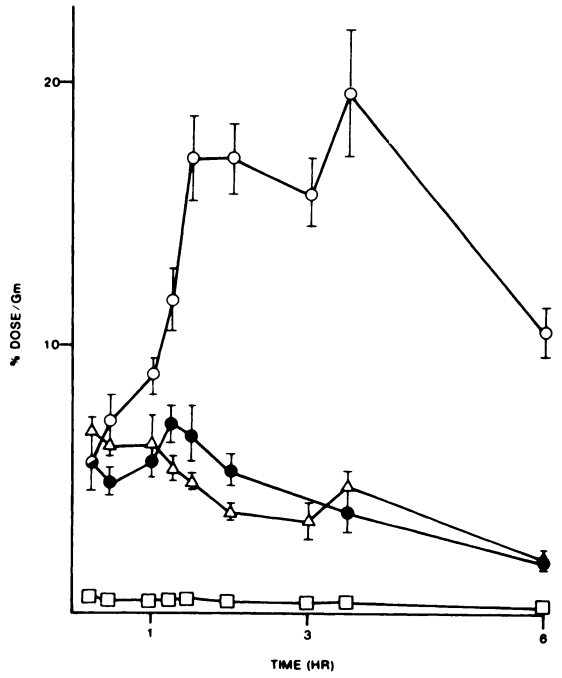

FIG. 3. In vivo uptake of [I-125]MIVE$_2$ in uterus of immature rats from 15 min to 6 hr: uterus (O), plasma (□), liver (Δ). For comparison, uptake of [H-3]E$_2$ in uterus (●) is plotted, as obtained from i.v. co-injection of 5 μCi of [H-3]E$_2$ and 5 μCi of [I-125]MIVE$_2$ (refer to Materials and Methods).

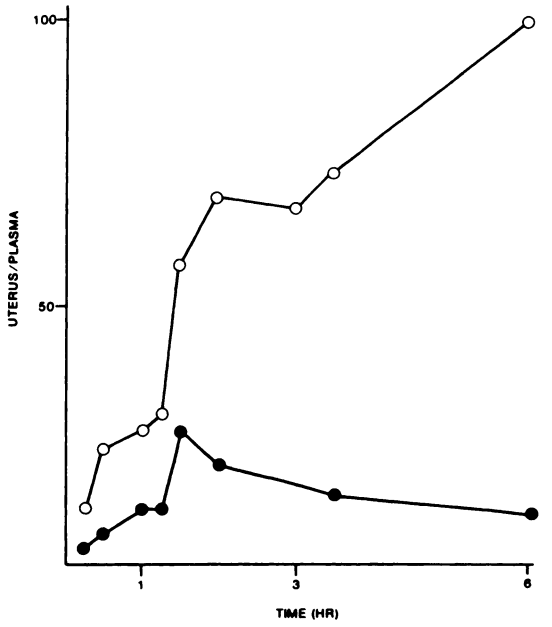

FIG. 4. Effect of time on uterus-to-plasma ratios of immature rats after i.v. injection of 5 μCi each of [H-3]E$_2$ (●) and [I-125]MIVE$_2$ (O).

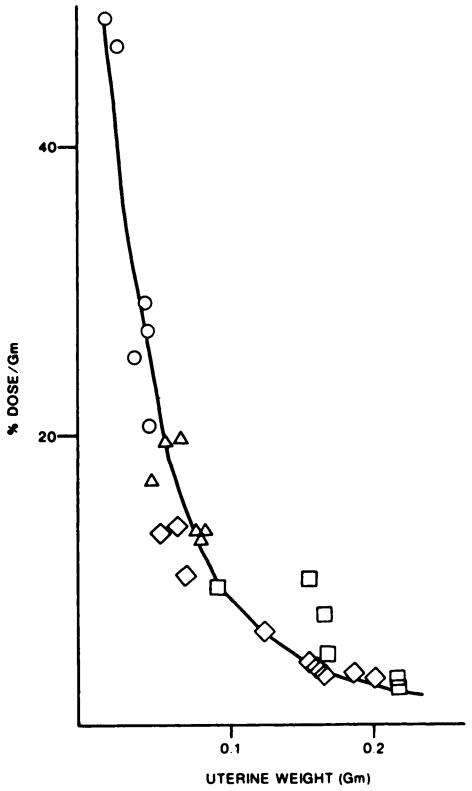

FIG. 5. Uterine weight of immature rats is related to fraction of [I-125]MIVE$_2$ that is localized. Each point represents weight of individual uterus, which was removed from the animal at 3 hr (Δ), 3½ hr (O), and 6 hr (□) after i.v. injection of 5 μCi of [I-125]MIVE$_2$; (◇) represents group of animals at 6 hr on different day.

indicating that the animals were possibly in estrus. The relationship was observed at all times examined. The total weight of the animals in most cases was not indicative of the uterine weight.

Competition studies. When estradiol (50 μg) was co-injected with 5 μCi of [I-125]MIVE$_2$, the radioactivity concentrating in the uterus was only 5% of that detected in control animals at 3 hr (Table 1). The co-injection had no effect on the levels of radioactivity in nontarget tissues. Since estradiol can influence blood flow (*21*), other methods of demonstrating receptor-specific localization have been suggested. McElvany et al. (*22*) found increased washout of activity when 18 μg of estradiol was administered as a chaser 1 hr after administering the radiotracer. We similarly were able to reduce the level of activity by 60% in the uterus upon administration of 50 μg of estradiol 1 hr after injection of [I-125]MIVE$_2$ (Table 2). Similiar results were obtained when the chaser was administered 3 hr after injection of the radiotracer. Moreover, the reduction of activity was not significantly different whether 30 min or 1 hr elapsed following the E_2 chaser. We observed considerable variability in the effectiveness of the chaser in various studies, which can be attributed to the differences in ages of the animals or the uterine weights, as discussed previously.

TABLE 1. DISTRIBUTION OF I-125-LABELED MIVE$_2$ IN UTERUS, PLASMA, LIVER, AND MUSCLE

Tissue	%-Dose/g Tissue							
	15 min	30 min	1 hr	1 hr 15 min	1 hr 30 min	2 hr	3 hr	3 hr 30 min	6 hr
Plasma	0.48 (0.24)*	0.32 (0.10)	0.34 ± 0.03†	0.40 ± 0.03	0.30 ± 0.02	0.25 ± 0.03	0.24 ± 0.02	0.28 ± 0.04	0.11 ± 0.006
Uterus	5.64 (3.49)	7.14 (3.91)	8.87 ± 0.68	11.8 ± 1.19	17.1 ± 1.63	17.2 ± 1.37	15.9 ± 1.31	19.7 ± 2.35	10.6 ± 0.94
Liver	6.70 (2.25)	6.29 (1.74)	6.24 ± 1.05	5.37 ± 0.43	4.92 ± 0.27	3.78 ± 0.19	3.43 ± 0.67	4.79 ± 0.52	2.19 ± 0.18
Muscle	0.91 (0.37)	1.09 (0.55)	1.12 ± 0.11	1.57 ± 0.09	1.29 ± 0.11	0.82 ± 0.06	0.86 ± 0.20	1.11 ± 0.13	0.31 ± 0.05

* Values in parentheses are the range for four animals.
† Standard error for the mean of six animals.

TABLE 2. EFFECT OF CO-INJECTION OR POSTINJECTION CHASE WITH E_2 ON THE DISTRIBUTION OF [I-125]$MIVE_2$ %-DOSE/g TISSUE

	Time after injection of I-125 $MIVE_2$									
	1 hr									
	Time of sacrifice after chase with E_2									
			30 min		1 hr		3 hr			
							30 min		1 hr	
Tissue	Control*	Co-injected†	Control	Chase‡	Control	Chase	Control	Chase§	Control	Chase
Plasma	0.34 ± 0.03¶	0.34 ± 0.02	0.30 ± 0.02	0.28 ± 0.01	0.25 ± 0.03	0.26 ± 0.02	0.28 ± 0.04	0.22 ± 0.09	0.22 ± 0.01	0.17 ± 0.01
Uterus	8.87 ± 0.68	1.25 ± 0.10	17.1 ± 1.63	9.46 ± 0.75	17.2 ± 1.37	6.93 ± 0.65	19.7 ± 2.35	10.7 ± 3.77	27.7 ± 1.89	14.9 ± 0.93
Liver	6.42 ± 1.05	4.52 ± 0.28	4.92 ± 0.27	5.30 ± 0.32	3.78 ± 0.19	4.00 ± 0.18	4.78 ± 9.52	3.68 ± 0.42	4.69 ± 0.49	3.50 ± 0.35
Muscle	1.12 ± 0.11	1.29 ± 0.12	1.29 ± 0.11	1.12 ± 0.04	0.82 ± 0.06	1.04 ± 0.11	1.11 ± 0.13	1.03 ± 0.15	0.89 ± 0.04	0.90 ± 0.09

* Controls were determined on the same day as the experimental study, either co-injected or chased.
† 50 μg nonradioactive E_2 and 5 μCi of I-125-labeled $MIVE_2$ were administered simultaneously.
‡ 50 μg nonradioactive E_2 was injected intravenously 1 hr after [I-125]$MIVE_2$ and allowed to chase for 30 min or 1 hr before sacrifice.
§ 50 μg nonradioactive E_2 was injected intravenously 3 hr after [I-125]$MIVE_2$ and allowed to chase for 30 min or 1 hr before sacrifice.
¶ All values represent the mean ± standard error of at least six animals.

DISCUSSION

Iodine 125 $MIVE_2$ provides high levels of accumulation in immature rat uterus, with the highest target-to-plasma ratios seen to date for any radiohalogenated derivative of estradiol. The compound exhibits excellent stability in vitro and in vivo. The synthesis of [I-125]-$MIVE_2$ is sufficiently rapid so that [I-123]$MIVE_2$ has been prepared and is currently in clinical trials. Images of estrogen-dependent breast tumors have been obtained using 16α-[Br-77]bromoestradiol (*23*). Although I-123 has a better energy for imaging purposes, the procedure for the synthesis of the 16-iododerivative is prohibitively long for the preparation of I-123 derivatives (*22*). The F-18 derivative of pentestrol (*11*) provides the advantage of a positron emitter, but $MIVE_2$ provides better localization and target-to-plasma ratios.

Receptor-specific localization of a radiotracer is validated by the administration of a saturating dose of a competing compound that prevents the localization of the radiotracer. The competitive compound can be administered before, with, or after injection of the radiotracer. In the case of the estrogen receptor, preinjection of the unlabeled agent results in the depletion of receptor, and therefore reduces the specific localization. Co-injection effectively reduces the specific activity to levels where the specific binding of the radioactive ligand cannot be observed. In the case of postinjection with an unlabeled agent, increased washout of the radioligand is achieved by competition. The percentage of radioactivity that washed out after 1 hr of chaser did not differ from that washed out after 30 min of chaser, regardless of whether the dose of [I-125]$MIVE_2$ was administered 1 hr or 3 hr previously. This indicates that washout of approximately 50% of the activity occurs rather rapidily in vivo and probably represents activity bound to those receptors involved in uptake but not necessarily retention (*24*). It has been suggested that as much as 50% of [H-3]E_2 present in the uterus is bound to "secondary sites" that have lower affinities for estrogens and may serve as an estrogen-concentrating agent for the uterus (*25*). But this has not been demonstrated in vivo and this observation may be due to the in vitro environment of the nuclear and cytosol receptor preparations. We do not know whether $MIVE_2$ translocates into the nucleus or whether the complex of $MIVE_2$ and estrogen receptor binds to chromatin. Because of its structural similarity to moxestrol—an established estrogen agonist with many of the same properties as $MIVE_2$—our results suggest that $MIVE_2$ is a promising tracer for the study of estrogen receptors in vivo and in vitro.

FOOTNOTE

* Aqueous Counting Scintillant, Amersham Corp, Arlington Heights, IL.

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