16α-[^{77}Br]Bromoestradiol: Dosimetry and Preliminary Clinical Studies

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An estrogen-receptor-binding radiopharmaceutical, 16α-[^{77}Br]bromoestradiol-17β, has been used successfully at high specific activity to image carcinogen-induced mammary tumors in rats and in preliminary studies to image breast tumors in patients. The biodistribution of the labeled estrogen in rats and its clearance in a monkey were used to estimate the radiation absorbed doses to a human resulting from administration of the radiopharmaceutical. Preliminary imaging studies in patients with mammary carcinoma show promising results and warrant further development of radiolabeled estrogens, particularly those carrying positron emitters that could permit positron emission tomography—for example Br-75 or F-18.

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Radiopharmaceuticals that bind to estrogen receptors constitute a new class of compounds with great promise for both clinical and research applications. Estrogens are concentrated in specific target tissues and certain tumors derived from these tissues as a result of their binding with high affinity to the estrogen receptor (*1–7*). Accordingly, an estrogen labeled with a suitable radionuclide should provide a useful agent for the imaging of target tissues, such as estrogen-receptor-positive breast tumors (*8,9*). Such agents binding to estrogen receptors should enable the noninvasive detection of both primary and metastatic breast tumors containing estrogen receptors.

The required characteristics of a radiolabeled estrogen to be used for breast-tumor imaging have been outlined previously (*10*). We have recently described the synthesis of 16α-[^{77}Br]bromoestradiol-17β [BE(Br-77)], and have demonstrated its selective, receptor-mediated uptake by estrogen target tissues and mammary tumors induced by dimethylbenz(a)anthracene (DMBA) in rats (*11,12*). In this report, we describe additional studies carried out with this compound in DMBA-induced mammary tumors, as well as clearance data obtained in the monkey, dosimetry calculations, and preliminary studies of breast-tumor imaging in humans.

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MATERIALS AND METHODS

Synthesis and purification of BE(Br-77) was carried out as previously described (*12,13*) using spallation-produced bromine-77 (*14*). Ascorbic acid was added to the final purified sample of the labeled estrogen (50 μl of a saturated solution of ascorbic acid in ethanol to each 7 ml HPLC fraction) to retard oxidative degradation. After purification and solvent removal, the labeled estrogen was reconstituted in a small volume of absolute ethanol and stored at 0°C. Before injection, the BE(Br-77) was diluted with 0.9% NaCl to give a final ethanol concentration ≤1%. Radiochemical purity of the final BE(Br-77) preparation was ascertained by comparison with nonradioactive material by thin-layer chromatography (silica gel, 20% ethyl acetate-80% benzene). The specific activity of BE(Br-77) ranged from 900 to 1500 Ci/mmole at the time of synthesis, as determined by Scatchard analysis (*13,15*).

Animal studies. Mature female Sprague-Dawley rats (120 g), housed in metabolic cages, were injected by femoral vein with 20 μCi BE(Br-77) and killed 0.5, 1, 3, 6, 24, or 48 hr later. A total of 23 animals was studied in groups of three or more at each time of sacrifice. Tissues were removed, weighed, and assayed for radio-

activity in a sodium iodide well counter. Feces and urine were collected at 24 and 48 hr after injection and counted to determine clearance rates.

We also injected 200 μCi BE(Br-77) into mature (130 day) Sprague-Dawley female rats (n = 8) bearing mammary adenocarcinomas induced by i.v. injection of 5 mg DMBA at 50 days of age. Scintigrams were obtained with a gamma camera fitted with a high-energy pinhole collimator at 30 min to 1 hr after injection.

Clearance of BE(Br-77) was measured in a female pigtail monkey (*Macaca nemestrina*). The animal was injected intravenously with 1 mCi of the labeled estrogen and was maintained in a metabolic cage. Feces and urine were collected daily for 7 days and assayed for radioactivity.

Patient studies. Eight patients with primary or metastatic mammary carcinoma underwent intravenous administration of 4.1–5.2 mCi BE(Br-77). Images were obtained with a standard-field scintillation camera fitted with a high-energy diverging collimator at 1, 4, and 24 hr after injection. In some cases, urine was collected for 24 hr and assayed for radioactivity. These studies were approved by the Radioactive Drug Research Committee and the Human Studies Committee at Washington University School of Medicine; each patient gave her written informed consent before participating in the study.

RESULTS

Dosimetry. Table 1 summarizes the biodistribution of BE(Br-77) in mature female rats at various times after injection; the results are expressed as percent injected dose per organ. The percentages of the injected doses found in the urine and feces of the rats killed at 24 and 48 hr were used to determine the activity remaining in the whole body. Ninety-two percent of the activity was cleared from the rats in 24 hr, and an additional 6% was excreted by 48 hr. Urinary excretion was ~1/10 of that in the feces. The largest amounts of the labeled estrogen are found in the liver and intestines, organs involved in its metabolism and clearance. Microcuries present in each organ per millicurie injected were plotted against time, and the areas under these curves (μCi-hr/mCi) were determined. These values were used in conjunction with the MIRD tables (*16*) to yield the estimated radiation absorbed doses to a human (Table 2). The primary critical organ is the lower large intestine, which

TABLE 1. TISSUE DISTRIBUTION OF 16α-[^{77}Br]BROMOESTRADIOL IN MATURE FEMALE RATS (MEAN AND RANGE FOR ≥3 RATS)

	% Injected dose per organ					
	0.5 hr	1 hr	3 hr	6 hr	24 hr	48 hr
Blood*	4.52	4.24	3.18	1.85	0.93	0.38
	(3.08–6.64)	(3.92–4.56)	(3.11–3.24)	(1.24–3.00)	(0.81–1.02)	(0.17–0.57)
Uterus	1.15	1.08	0.71	0.48	0.09	0.04
	(0.75–1.54)	(0.94–1.21)	(0.50–0.83)	(0.46–0.50)	(0.08–0.12)	(0.03–0.05)
Ovaries	0.10	0.17	0.04	0.02	0.01	0.00
	(0.06–0.14)	(0.09–0.24)	(0.03–0.04)	(0.01–0.02)	(0.01–0.01)	(0.00–0.00)
Spleen	0.19	0.22	0.03	0.08	0.04	0.02
	(0.02–0.27)	(0.20–0.24)	(0.01–0.05)	(0.04–0.15)	(0.02–0.05)	(0.01–0.03)
Kidneys	1.04	0.88	0.20	0.28	0.10	0.08
	(0.96–1.14)	(0.84–0.92)	(0.12–0.28)	(0.14–0.54)	(0.08–0.12)	(0.02–0.16)
Liver	10.2	7.82	3.02	2.69	1.51	0.50
	(9.8–11.0)	(7.49–8.14)	(1.84–4.19)	(1.66–4.70)	(0.93–2.07)	(0.23–0.67)
Lungs	0.52	0.42	0.19	0.11	0.07	0.04
	(0.44–0.58)	(0.42–0.42)	(0.18–0.20)	(0.07–0.18)	(0.05–0.11)	(0.02–0.05)
Esophagus	0.03	0.05	0.01	0.01	0.01	0.00
	(0.02–0.03)	(0.02–0.07)	(0.00–0.01)	(0.01–0.01)	(0.00–0.01)	(0.00–0.00)
Stomach†	0.60	0.80	0.35	0.23	0.40	0.10
	(0.48–0.80)	(0.50–1.10)	(0.21–0.49)	(0.20–0.26)	(0.28–0.54)	(0.05–0.13)
Small intestine†	55.4	47.4	4.91	2.19	0.76	0.14
	(33.7–79.3)	(40.3–54.5)	(4.39–5.42)	(0.75–3.81)	(0.12–1.99)	(0.12–0.15)
Upper large intestine†	7.26	49.1	12.5	8.22	2.05	0.18
	(0.35–14.3)	(36.9–61.3)	(7.4–17.6)	(0.81–16.5)	(1.10–2.59)	(0.13–0.23)
Lower large intestine†	0.48	0.31	4.15	15.1	1.77	0.20
	(0.32–0.76)	(0.29–0.32)	(0.42–7.88)	(6.4–22.4)	(0.50–3.45)	(0.04–0.32)

* Blood volume calculated as 7% total body weight.

† Organ walls plus contents.

TABLE 2. HUMAN RADIATION ABSORBED DOSES FOR 16α-[^{77}Br]BROMOESTRADIOL

Target organ	rads/mCi
Stomach	0.08
Small intestine	0.25
Upper large intestine	0.34
Lower large intestine	0.43
Kidneys	0.05
Liver	0.07
Lungs	0.01
Ovaries	0.02
Spleen	0.03
Uterus	0.17
Whole body	0.03

receives 0.43 rad/mCi, followed by the upper large intestine with 0.34 rad/mCi.

Clearance of BE(Br-77) was measured in a female pigtail monkey. As shown in Fig. 1, the labeled estrogen clears with a half-time of ~36 hr, which is within the range (26–40 hr) reported for clearance of estradiol(C-14) in humans (*17*). Twenty-four-hour urinary clearance was measured in several patients and found to be similar to that shown in Fig. 1 for the monkey. Study of literature data on the metabolism and excretion of estrogens in rats and humans suggests that the biologic half-time of estrogens in humans is roughly twice that in rats (*12,17–19*). Because of this difference in clearance rates, several approximate calculations were made assuming a longer clearance half-time to improve the validity of the absorbed doses in Table 2. The whole-body radiation dose, assuming an excretion rate equal to that for estradiol(C-14) in humans (*17*), was calculated for the case of uniform distribution of the labeled estradiol, yielding 0.07 rad/mCi. The worst possible case, that corresponding to no excretion of the BE(Br-77), yields a whole-body dose of 0.24 rad/mCi.

Owing to the increased urinary clearance of estrogens in humans compared with rats (*17–19*), it seems likely that the human bladder may receive a relatively high radiation dose from administration of the labeled estradiol. An estimate of the radiation dose to the bladder wall, due to bladder contents, was calculated based on the following assumptions: (a) urinary clearance rate of BE(Br-77) equal to that reported for estradiol(C-14) in humans (*17*); (b) bladder voided every 6 hr; (c) approximately 10% of the urine remaining in the bladder after voiding; and (d) linear growth of activity in the bladder during each 6-hr period. The estimated absorbed radiation dose to the bladder determined in this manner is 0.44 rad/mCi.

Animal imaging studies. The left panel in Fig. 2 shows the scintillation image obtained 30 min after injection of 200 μCi BE(Br-77) in a rat with a DMBA-induced mammary tumor. The area of increased uptake in the right flank corresponded to the location of a tumor, as confirmed by the image on the right obtained after surgical removal of the tumor. Similar tracer uptake in tumors was observed in all 8 rats studied.

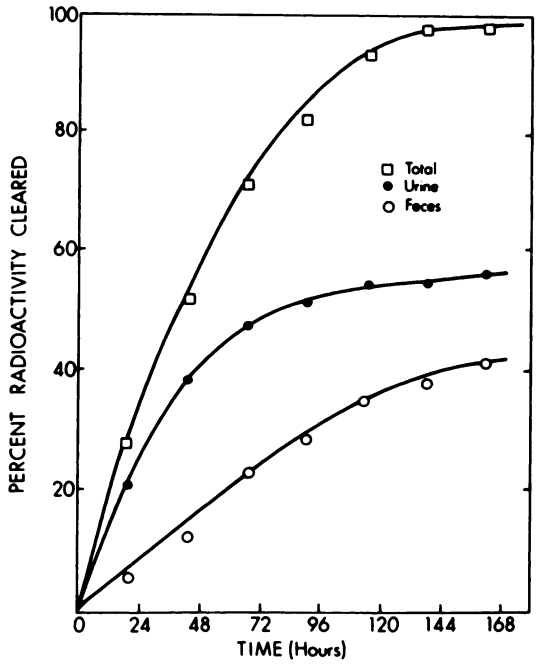

FIG. 1. Clearance of BE(Br-77) in a female pigtail monkey.

Patient imaging studies. Eight patients, four with primary breast carcinoma and four with metastatic lesions, underwent imaging with BE(Br-77). The results of these clinical studies are summarized in Table 3. Both of the patients who had metastatic lesions and were on antiestrogen therapy had negative scintigraphic results; one abnormal scan was obtained in a patient with chest-wall metastases from an estrogen-receptor-positive primary tumor; and three abnormal scintigrams were observed in patients with primary breast carcinomas, two of which were biopsied and found to be estrogen receptor positive. No uptake of the tracer was seen in the single estrogen-receptor-negative primary tumor.

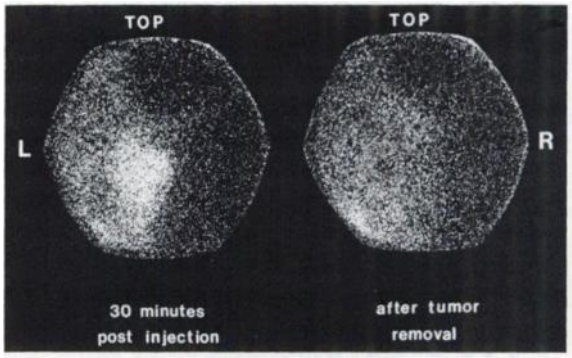

FIG. 2. Scintillation images at 30 minutes after injection of 200 μCi BE(Br-77) (left) and following surgical removal of tumor (right) of mature female Sprague-Dawley rat bearing DMBA-induced mammary adenocarcinoma.

TABLE 3. SUMMARY OF STUDIES IN PATIENTS WITH MAMMARY CARCINOMA

Patient	Age	Diagnosis	Estrogen receptor assay*	Scan	Comment
1	65	Bone metastases	+	−	Tamoxifen therapy
2	54	Primary tumor	−	−	
3	74	Bone and lung metastases	n.d.†	−	Tamoxifen therapy
4	68	Primary tumor	n.d.†	+	
5	81	Axillary lymph node metastases	+	−	
6	65	Chest wall and bone metastases	+	+	Uptake in chest wall lesion, but not in bone lesions
7	73	Primary tumor	+	+	
8	66	Primary tumor	+	+	

* Estrogen-receptor assay carried out by Surgical Pathology, Jewish Hospital of St. Louis; specimens showing saturable binding of ≥ 10 *fM* (10^{-15}) per mg cytosol protein are defined as estrogen-receptor positive.

† n.d., not determined.

Figure 3 shows a scintillation image obtained from Patient 6 one hour after injection of 4.3 mCi BE(Br-77). This patient had metastatic carcinoma in an axillary lymph node, which was removed for biopsy, and local recurrence in the left chest wall. The area of increased uptake indicated by the arrow most likely corresponds to these metastatic lesions. The activity in the lower portion of the image is in the liver and gallbladder.

Figure 4 shows an image obtained 1 hr after injection of 4.1 mCi BE(Br-77) in Patient 7, who had estrogen-receptor-positive carcinoma of the right breast. The activity at the bottom of the image is in the liver and gallbladder, and the area of increased uptake indicated by the arrow corresponds to the primary tumor.

DISCUSSION

We have shown that 16α-[^{77}Br]bromoestradiol can be synthesized in high specific activity and that this agent binds selectively to estrogen target tissues and DMBA-induced mammary tumors in rats. Tissue distribution of the labeled estradiol in rats was used in conjunction with the MIRD tables (*16*) to estimate radiation absorbed doses to a human. These calculations suggest that the intestines, which serve as the primary conduit for excretion of estrogens, are the critical organs.

Clearance of BE(Br-77) was measured in a female pigtail monkey; the clearance half-time was slightly longer in the monkey than in rats. In addition, a significant fraction of the labeled estradiol was excreted in the urine in the monkey; this was not observed in rats. The routes of metabolism and clearance rates of estrogens in humans, as measured with estradiol(C-14) (*17*), are similar to those obtained with BE(Br-77) in the monkey. Because of the slower clearance half-time and increased urinary excretion of estrogens in humans, several approximate calculations were performed to estimate the whole-body radiation absorbed dose to a human under

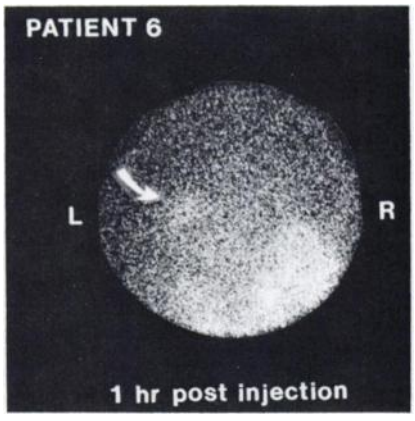

FIG. 3. Posterior scintigram of chest and upper abdomen of a patient with metastatic mammary carcinoma of the left chest wall. Arrow indicates uptake of tracer in tumor.

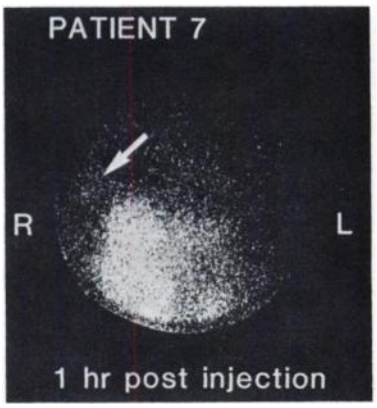

FIG. 4. Anterior scintigram of chest and upper abdomen of a patient with primary adenocarcinoma of the right breast. Activity in tumor is noted in region of right breast (arrow); uptake in liver and gallbladder is normal.

assumed conditions of slower clearance and no clearance of the tracer. In addition, the absorbed dose to the bladder was estimated. These dosimetry calculations, and those directly based on the tissue distribution data obtained in rats, yield radiation absorbed doses for a patient receiving 5 mCi BE(Br-77) that are well within acceptable limits.

Preliminary studies have been carried out using BE(Br-77) as an agent to image estrogen-receptor-positive cancer lesions in humans. Other groups have reported the use of I-131-labeled estrogens (*20*), Tc-99m diethylene triamine penta-acetic acid (*21*), and Tc-99m-labeled bleomycin (*22*) for imaging human breast tumors. In patients the quality of the Br-77 scintillation images obtained with a high-energy diverging collimator (see Figs. 3 and 4) is poor in comparison with those obtained with a high-energy pinhole collimator in rats (Fig. 2). These imaging studies in rats, as well as phantom studies reported by Eckelman et al. (*23*), suggest the superiority of a high-energy pinhole collimator for use with bromine-77, but our attempts to use such a collimator for patient imaging were unsuccessful because of scatter from the high activity present in the liver, gallbladder, and intestines. By increasing the shielding in a high-energy pinhole collimator and designing a shield for the patient's abdominal region, one might improve the quality of BE(Br-77) images.

Despite the limitations in imaging techniques, the results obtained from these preliminary studies with BE(Br-77) in our patients are sufficiently promising to encourage the continued development of this and other radiolabeled estrogens for use as imaging agents for breast tumors containing estrogen receptors. The possible extension of these synthetic methods to prepare estrogens labeled with the positron emitter bromine-75, or other estrogen-receptor-binding agents labeled with fluorine-18, is even more promising, since three-dimensional imaging by positron emission tomography would provide greater contrast and spatial resolution, and would minimize the problem of scatter from abdominal activity.

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