Improved Sarcoma Imaging and Reduced Hepatic Activity with Indium-111-SCN-Bz-DTPA Linked to MoAb 19–24

M.J. Blend, J.A. Greager, R.W. Atcher, J.M. Brown, M.W. Brechbiel,*
O.A. Gansow, and T.K. Das Gupta

Michael Reese Hospital, Chicago, Illinois; University of Illinois at Chicago, Chicago, Illinois; Argonne National Laboratory, Argonne, Illinois; Radiation Oncology Branch, National Institutes of Health, Bethesda, Maryland

A murine monoclonal antibody (MoAb 19–24) directed against a human sarcoma antigen was prepared and labeled with ^{111}In by use of the linker 1-(p-isothiocyanatobenzyl)-diethylenetriaminepentaacetic acid (SCN-Bz-DTPA). Imaging and biodistribution of this radioimmunocomplex were evaluated. MoAb P3 was similarly labeled as a negative control. Of 24 athymic mice bearing s.c. human fibrosarcoma, 12 received 10 μCi of [^{111}In]MoAb 19–24 and 12 received 10 μCi of [^{111}In]MoAb P3. The mice were imaged at 24, 48, 68, or 168 hr, after i.p. injection. Region of interest and biodistribution analysis showed maximum localization of MoAb 19–24 in the tumor at 72 hr with maximum liver localization of 24 hr. Analysis of MoAb P3 showed maximum tumor and liver activity at 24 hr. Tumor specificity studies were also conducted in three nude mice bearing a sarcoma in the left flank and a Burkitt's lymphoma in the right flank. Selective uptake was seen in the sarcoma but not in the lymphoma. The excellent uptake of [^{111}In]SCN-Bz-DTPA-MoAb 19–24 in sarcoma without appreciable liver activity indicates that it may be a useful tumor imaging agent in man.

J Nucl Med 29:1810–1816, 1988

Monoclonal antibodies (MoAbs) have been raised against a wide range of antigens present on normal and malignant cells. Despite improvements in the control of local disease, the development of pulmonary metastases continues to be a therapeutic problem in sarcoma patients. The early identification of recurrent sarcoma, particularly in the lungs, by means of a noninvasive technique is essential for therapeutic efficacy. Two recent studies (*1,2*) have demonstrated that radioimmunoscintigraphy was moderately successful in patients with recurrent sarcoma. We have detected sarcoma deposits in athymic mice (*3*) and in patients (*2*) by using MoAb 19–24-labeled with iodine-125 (^{125}I) or with indium-111 (^{111}In) (unpublished data) linked via the cyclic anhydride DTPA method of Hnatowich et al. (*4*). However, problems such as poor spatial resolution and unacceptable sensitivity for the detection of deep lung lesions, coupled with increased background, remain unresolved issues. Our purpose in this study was to examine the effect of using a new bifunctional chelation system, that forms a stable radioimmunoconjugate with ^{111}In, on liver and tumor uptake in a nude-mouse model.

A murine monoclonal antibody directed against malignant fibrous histiocytoma (MFH), a human soft-tissue sarcoma, was produced in our laboratory (*5*) and used in this study. This antibody, known as MoAb 19–24, reacts with a cell surface membrane antigen (sarcoma associated antigen, known as p102) which has a molecular weight of 102,000 D; this protein is found in most human sarcomas at an average concentration of 300,000 sites per cell. Lower levels of p102 are found in some tumors other than sarcoma, and little or no antigen is present in normal tissues (*5*).

The isotope selected for imaging in this study was ^{111}In because of its favorable physical and chemical characteristics. The chelation method used for labeling the MoAbs was recently described by Brechbiel et al. (*6*). We used this technique in an effort to decrease nontumor associated radioactivity (i.e., in liver, spleen,

Received Feb. 1, 1988; revision accepted July 11, 1988.

For reprints contact: Michael J. Blend, PhD, DO, Associate Director, Div. of Nuclear Medicine, Michael Reese Hospital, Lake Shore Drive at 31st St., Chicago, IL 60616.

* Present address: M. W. Brechbiel, Centocor, 244 Great Valley Parkway, Malvern, PA 19355.

and bone marrow) by reducing cross-linking of MoAbs during preparation and increasing the stability of the resulting IgG complex. This effect has been reported to occur because a more stable ^{111}In complex is formed. An isotype matched control, P3, was also prepared in our laboratory and labeled by the same procedure (*6*).

MATERIALS AND METHODS

Cell Lines

Human tissues were obtained from biopsy, surgical, and autopsy specimens. Crude plasma membrane fractions were prepared by high speed homogenization and differential centrifugation as previously described (*5*). The human fibrosarcoma cell line HT-1080 (*7*) and Daudi Burkitt's lymphoma cell line were obtained from the American Type Culture Collection (American Type Culture Collection, Rockville, MD).

Monoclonal Antibodies

Mouse monoclonal antibody 19-24 (clone 6), an IgG-1 (with kappa light chains), was produced following fusion of immune BALB/c mouse splenocytes with the nonproducer myeloma X63-Ag8.653 (*5*). The antibody reacts with a cell surface membrane antigen quantitatively that is expressed to a much greater extent in many sarcomas than in other types of tumors or normal tissues that have been tested (*5*). Mouse monoclonal antibody P3, a nonspecific IgG-1 (also with kappa light chains) is secreted by the BALB/c mouse myeloma P3-X63-Ag8 cell line (*8*). This antibody (provided by Dr. Z. Steplewski, Wistar Institute, Philadelphia, PA) served as a nonspecific control, as it was nonreactive in our standard binding assays.

Antibody Purification

MoAb 19-24 was purified from ascitic fluid by ammonium sulfate precipitation (*5*) followed by affinity chromatography (*9*) on protein A-Sepharose (Pharmacia, Piscataway, NJ). Sterile, pyrogen free reagents were used throughout. Protein which eluted from the affinity column at pH 6 was dialyzed against PBS, centrifuged at 100,000 *g* for 1 hr, sterilized with a 0.22-μm Millex-GV filter (Millipore Corporation, Arlington Heights, IL) and stored at −70 C. MoAb P3 was purified from ascitic fluid directly on protein A-Sepharose as described above. SDS-PAGE analysis of the purified MoAbs (19-24 and P3) showed no contaminating proteins (*5*).

MoAb Conjugation

MoAbs 19-24 and P3 were conjugated to 1-[p-isothiocyanatobenzyl]-DTPA (SCN-Bz-DTPA) according to the technique of Brechbiel et al. (*6*). Briefly, the SCN-Bz-DTPA was reacted with the MoAbs at an initial 3:1 ratio of chelate to antibody in 50 m*M* HEPES buffer, pH 8.5. After 24 hr of reaction, MoAbs were separated from unreacted chelate by dialysis.

MoAb Radiolabeling

Sixty microliters of ^{111}InCl (Amersham Corporation, Arlington Heights, IL) (2 mCi, 0.05*M* HCl) were added to 26.4 μl of 2*M* HCl. After 5 min, the solution was adjusted to pH 4.2 with 1*M* sodium acetate. Next, 200 μl of conjugated MoAb 19-24 or P3 (3 mg/ml, pH 6.0) were immediately added and allowed to react for 30 min before purification. Labeled complex was separated from free ^{111}InCl with the use of a 1 × 10 cm column of Sephadex G-25 (medium grade) equilibrated overnight in PBS buffer. Collection of 0.5-ml aliquots showed that labeled complex consistently appeared in tubes 5 and 6 with ~80% labeling efficiency.

Testing for Pyrogenicity and Sterility

Immediately after production, random preparations were tested for pyrogens with the LAL test (M.A. Bioproducts, Walkerville, MD), and for microbial contamination with the Mycotrim culture system (Hana Media, Berkeley, CA). No contaminated preparations were detected.

Tumors in Athymic Mice

One million human fibrosarcoma HT-1080 cells were injected s.c. into the right flanks of each of 24 male athymic (nude) mice. When the tumors had grown to ~1 cm in diameter (after 10 to 14 days of growth), animals were entered into the study. An additional study of three mice bearing fibrosarcoma HT-1080 (antigen positive tumor) in the right flank also received an injection of Duadi Burkitt's lymphoma cells (10^7 cells) in the left flank. This antigen-negative tumor was allowed to grow to the same size as the sarcoma xenografts (after 20 to 21 days of growth), and these xenografts, in these animals were used for the specificity study described below.

Experimental Design

The 24 male athymic NCr-nu/nu mice (N.C.I. Frederick Cancer Research Facility, Frederick, MD) (average weight of 22 g) bearing s.c. implanted human fibrosarcoma xenografts in the right flank were randomly assigned to one of two groups. All animals were injected i.p. either with 10 μCi of [^{111}In] SCN-Bz-diethylenetriaminepentaacetic acid (DTPA) 19-24 (2.8 μCi/μg) or with 10 μCi of [^{111}In]SCN-Bz-DTPA-P3 (1.33 μCi/μg). Six mice (three with radiolabeled MoAb 19-24 and three with radiolabeled MoAb P3) were killed by an overdose of pentabarbital and imaged with a gamma camera at 24 hr postinjection. The same procedure was performed with similar groups of six mice at 48, 68, and 168 hr. When imaging was completed, the animals were dissected, and tumors as well as solid organs were removed and washed before being counted with a NaI gamma well counter. The results are expressed as percent injected dose (i.d.) of antibody complex per gram wet weight of tissue.

We also conducted specificity studies by selecting three additional athymic mice with bilateral s.c. tumors and injecting 10 μCi of [^{111}In]SCN-Bz-DTPA-MoAb 19-24. The right flank tumor was derived from human fibrosarcoma cells (HT-1080) and the left flank tumor was a Daudi Burkitt's lymphoma. Gamma camera imaging was performed on the third day after injection. Following imaging, biodistribution studies were carried out as described above.

Imaging

Conventional posterior views of recently killed mice were obtained with a standard large field of view (LFOV) gamma camera fitted with a pinhole collimator of 4 mm aperature. Symmetric energy windows (20%) were placed over the 172 and 247 keV photopeaks of ^{111}In. Imaging times were typically 10 min per view, although a preset protocol of 50,000 counts per image was followed. In addition to analog images, static views were acquired on a computer system (Digital Equipment

TABLE 1
Tissue Uptake of Indium-111-Labeled MoAbs 19-24 and P3 in Tumor-Bearing Mice (Percent Injected Dose per Gram of Tissue)*

Day	MoAb	Blood	Tumor	Liver	Lungs	Kidneys	Stomach	Small intestine	Muscle
1	19–24	13.9	24.9	7.2	8.3	10.1	1.1	2.8	1.3
		(11.3–17.5)	(19.0–29.8)	(6.5–8.0)	(5.7–9.9)	(7.9–13.3)	(0.6–1.5)	(2.6–3.1)	(1.0–1.5)
	P3	14.9	4.9	7.8	8.7	9.4	0.9	2.6	1.3
		(13.8–17.0)	(4.4–5.3)	(7.7–8.0)	(7.8–9.5)	(8.9–10.0)	(0.7–1.0)	(2.3–3.0)	(1.0–1.5)
2	19–24	9.1	35.2	6.2	5.7	7.9	1.0	2.0	1.1
		(8.1–11.1)	(31.0–38.4)	(6.0–6.5)	(3.7–6.7)	(7.0–8.9)	(0.6–1.3)	(1.5–2.6)	(0.8–1.3)
	P3	12.4	5.8	7.8	7.0	8.8	1.2	2.8	1.2
		(11.6–13.3)	(4.9–6.6)	(7.5–8.2)	(4.4–8.6)	(7.7–10.9)	(1.0–1.3)	(2.6–3.1)	(1.0–1.3)
3	19–24	8.2	52.0	6.8	6.6	7.6	1.1	2.1	1.0
		(7.2–10.2)	(45.4–57.6)	(6.7–7.0)	(5.7–7.4)	(6.1–10.1)	(0.8–1.3)	(1.9–2.4)	(0.8–1.1)
	P3	11.9	6.2	7.4	9.3	7.9	1.6	2.5	1.2
		(11.6–12.3)	(5.8–6.5)	(6.8–8.1)	(8.6–9.9)	(7.2–8.7)	(1.4–1.7)	(2.3–2.8)	(1.0–1.3)
7	19–24	3.3	36.9	4.7	2.5	5.1	0.5	0.7	0.5
		(2.1–5.5)	(31.3–41.5)	(4.2–5.3)	(1.7–3.2)	(3.7–7.5)	(0.3–0.6)	(0.5–1.0)	(0.3–0.6)
	P3	7.9	5.5	5.6	5.3	5.7	0.6	1.1	1.0
		(7.0–8.9)	(4.7–6.2)	(4.5–7.7)	(4.8–5.7)	(5.3–6.2)	(0.4–0.7)	(0.9–1.4)	(0.8–1.1)

* Mean value from three animals and the range (in parentheses).

Corporation; Marlboro, MA) in a 128 × 128 matrix and stored on disk for later analysis and display. Images were compressed to a 64 × 64 matrix for region of interest (ROI) analysis.

Scintigraphic Analysis

We applied computer-assisted ROI techniques to each image in order to correlate the counts present in the total body, tumor, liver, and background at selected times after injection. When it was judged necessary, ROIs were drawn manually over the liver area, the tumor area(s), and the whole body. Count densities (cpm/pixel) were also obtained and compared. Total counts in each ROI as a function of the total-body count were calculated and correlated with biodistribution data.

RESULTS

Biodistribution Studies

Tissue counts of animals injected with ^{111}In-labeled MoAbs 19–24 and P3 correlated well with the results of gamma imaging. The percent injected dose per gram of tissue increased with time, demonstrating specific uptake of MoAb 19–24 in the tumor without appreciable "nonspecific" uptake in the liver (Table 1). Figure 1 displays the rise in percent i.d./g of tumor over time in relation to other tissues. A maximum uptake of 52% i.d. of specific MoAb per gram of tumor tissue was seen on Day 3, compared to 6% for the nonspecific MoAb.

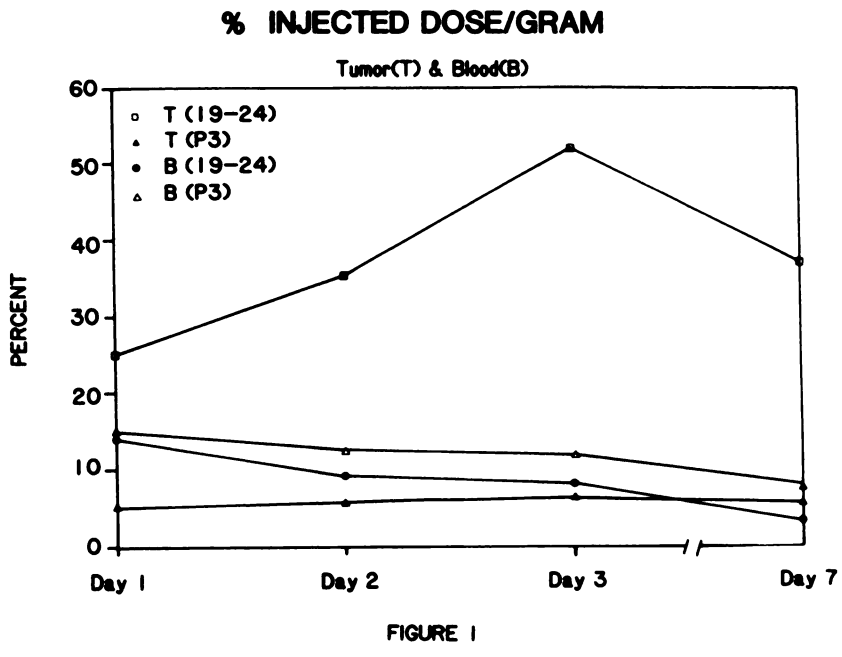

FIGURE 1

FIGURE 1
Percent of injected dose per gram of tumor (T) and blood (B) over 7 days in nude mice bearing sarcoma xenografts and injected with [^{111}In]MoAb 19–24 or [^{111}In]MoAb P3.

TABLE 2
Tissue Localization Index of Indium-111-Labeled MoAb 19-24 in Nude Mice Bearing Sarcoma Xenografts*

Day	MoAb	Tumor	Liver	Lungs	Kidneys	Stomach	Small Intestine	Muscle
1	19-24/P3	5.48†	1.00	1.02	1.14	1.27	1.15	1.09
2	19-24/P3	8.35	1.09	1.11	1.23	1.13	0.95	1.26
3	19-24/P3	12.38	1.33	1.02	1.39	1.04	1.23	1.27
7	19-24/P3	17.26	2.21	1.16	2.26	2.22	1.68	1.37

* Localization index = 19-24 (tissue:blood ratio)/P3 (tissue:blood ratio).
† Mean value from three animals.

Blood demonstrated a maximum activity on Day 1 which then slowly decreased. The liver showed a maximum uptake of MoAb 19-24, on Day 1, of 7.2% (i.d./g), which slowly decreased over time. All other tissues demonstrated clearance curves similar to that for blood. The clearance half-time ($T_{½}$) for ^{111}In-labeled MoAb 19-24 in blood was 71.9 hr (3 days).

In contrast to these data, the biodistribution results for the nonspecific MoAb, ^{111}In-SCN-Bz-DTPA-P3, failed to demonstrate any appreciable uptake of antibody in tumor tissues (Table 1). Intravascular activity was increased and prolonged, with the highest counts noted at 24 hr and then a gradual clearing with a $T_{½}$ of 163.3 hr (6.8 days). Slightly increased activity was seen in the liver and lungs when compared to that of the specific MoAb 19-24.

The localization index defined as MoAb 19-24 tumor/blood ratio divided by P3 tumor/blood ratio, was calculated for several major tissues (Table 2). The maximum tumor localization index was noted on Day 7, with a value of 17.3.

As a control for tumor specificity, ^{111}In-labeled 19-24 was administered to three athymic mice bearing both HT-1080 human fibrosarcoma and Daudi Burkitt's lymphoma as 1 cm xenografts on contralateral flanks. As shown in Table 3, labeled antibody 19-24 was localized in the sarcoma, but not in the Burkitt's lymphoma in the same animals. The percent i.d./g of radiolabeled MoAb 19-24 for the antigen positive tumor on the third day after injection was 50% (range 41.2-59.8%) compared to 1.5% (1.2-1.9%) in the antigen negative tumor. Table 3 lists % i.d./g and tumor to tissue ratios for the specific MoAb 19-24 in both sarcoma and lymphoma on Day 3.

Imaging Results

Injection of ^{111}In-SCN-Bz-DTPA-MoAb 19-24 into 12 of the 24 athymic mice bearing s.c. human fibrosarcoma xenografts showed preferential localization of the radiolabel in the tumor, with excellent visualization by 72 hr as demonstrated in Figure 2 A and C. Tumor-to-target ratios and image quality improved over time, as demonstrated in Figure 3 B and D. The injection of ^{111}In-111-SCN-Bz-DTPA-MoAb P3 into the remaining 12 athymic mice bearing s.c. human fibrosarcoma xenografts did not result in increased localization in tumor tissues that was seen for MoAb 19-24 at 72 hr (Fig. 2B). Tumor-to-target ratios and image quality did not improve over time, as demonstrated in Figure 3 A and C.

TABLE 3
Percent Uptake of ^{111}In-Labeled MoAb 19-24 and Tumor/Tissue Ratios in Animals Bearing Two Different Tumors at 3 Days Postinjection*

Tissue	%Injected dose/g.	HT-1080/tissue	Daudi/tissue	Tissue/blood
Blood	3.8	13.6	0.4	1.0
	(2.4-6.2)	(10.1-16.1)	(0.3-0.6)	(1.0-1.0)
Human sarcoma	50.0	1.0	0.1	13.6
	(41.2-59.8)	(1.0-1.0)	(0.1-0.3)	(10.1-16.1)
Burkitt's lymphoma	1.5	32.8	1.0	0.4
	(1.2-1.9)	(27.6-37.0)	(1.0-1.0)	(0.2-0.5)
Liver	3.1	16.1	0.5	0.9
	(2.6-3.7)	(12.6-18.6)	(0.4-0.7)	(0.6-1.1)
Lungs	3.3	15.7	0.5	0.9
	(2.3-5.3)	(12.8-17.6)	(0.4-0.7)	(0.7-1.0)
Kidneys	5.3	9.5	0.3	1.4
	(4.1-7.5)	(8.9-10.0)	(0.2-0.5)	(1.1-1.6)

* Mean value from three animals and the range (in parentheses).

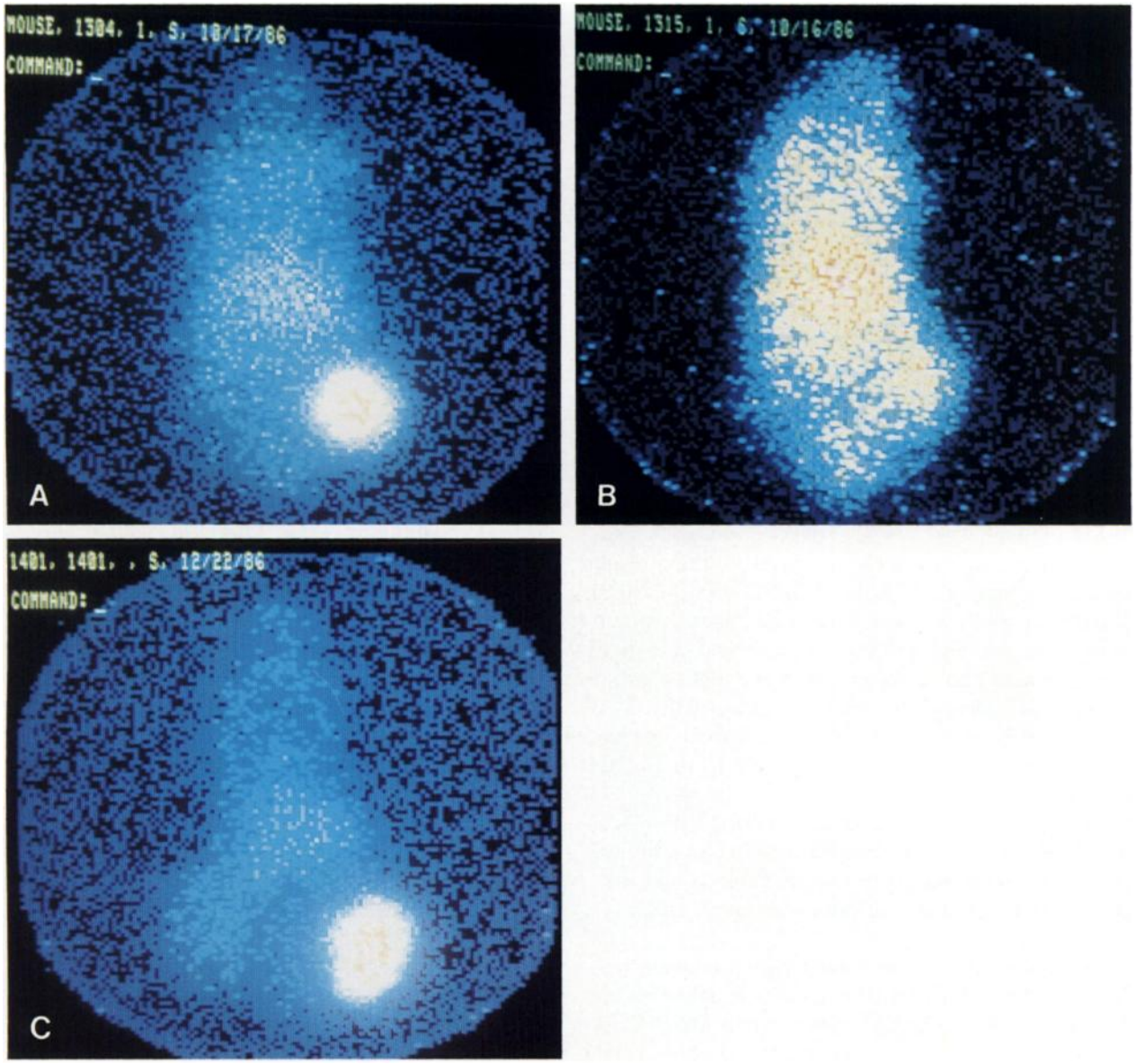

FIGURE 2
A: Posterior view of an athymic mouse bearing a human fibrosarcoma xenograft in the right thigh, at 72 hr after i.p. injection of 10 μCi of [^{111}In]MoAb 19–24. B: Posterior view of an another mouse bearing a human fibrosarcoma xenograft in the right thigh, at 72 hr after i.p. injection of 10 μCi of [^{111}In]MoAb P3. C: Posterior view of a mouse bearing a human fibrosarcoma xenograft in the right thigh area and a Burkitt's lymphoma in the left flank, at 72 hr after i.p. injection of 10 μCi of [^{111}In]MoAb 19–24.

Figure 2C dramatically illustrates the specificity of the ^{111}In-labeled MoAb 19–24 for the human fibrosarcoma, in mice bearing a Burkitt's lymphoma, at 3 days after injection.

Scintigraphic Analysis

Table 4 lists computer-integrated counts per min from ROIs of total-body, tumor, and liver in athymic mice injected with radiolabeled MoAbs 19–24 and P3. The localization of ^{111}In-SCN-Bz-DTPA-19–24 in the tumor was maximal (20% of the total-body activity) at 48 hr, whereas maximum liver uptake (12% of total-body activity) occurred at 24 hr. Indium-111-SCN-Bz-DTPA-P3 images demonstrated a maximum tumor activity (6%) on Day 3 and maximum liver activity (22%) on Day 1. It is clear that more counts were seen in the liver region with MoAb P3 than with MoAb 19–24 over time and this may be explained. In part, by the prolonged blood-pool activity seen with P3, which cannot be easily subtracted out during scintigraphic analysis. However, the ROI values were essentially in agreement with the biodistribution data.

DISCUSSION

Soft-tissue sarcomas are a class of malignant tumors arising largely from mesenchymal connective tissues.

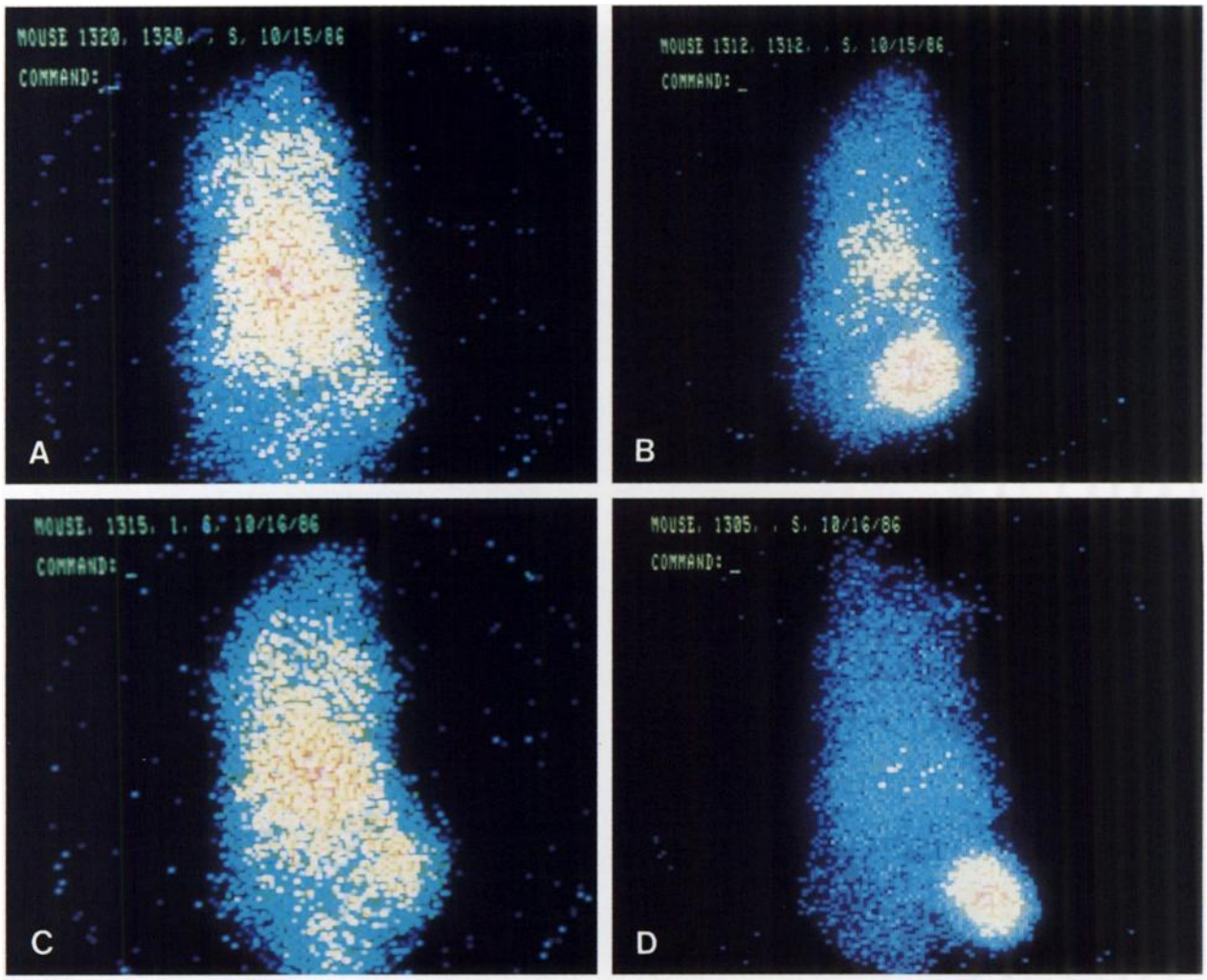

FIGURE 3
A and C: Posterior views of an athymic mouse bearing a human fibrosarcoma xenograft in the right flank, at 24 hr (A) and 48 hr (C) after injection of 10 μCi of [^{111}In]SCN-Bz-DTPA-MoAb P3. B and D: Posterior views of an athymic mouse bearing a human fibrosarcoma xenograft in the right flank, at 24 hr (B) and 48 hr (C) after injection of 10 μCi of [^{111}In] SCN-Bz-DTPA-MoAb 19–24.

Although soft tissue sarcomas constitute only 1–2% of all adult malignancies reported in the United States, they rank fifth as a killer in the age group below 15 years (*10*). Early detection of recurrent sarcoma, particularly of pulmonary metastases, is essential for early and effective therapy. Although the survival rate for patients who develop pulmonary lesions has improved over the years, pulmonary involvement is still the main cause of death in these patients.

The ability to produce MoAbs from crude cell preparations has lent itself readily to the study of tumor cell surface antigens from many tissues. Equally important is the processing and labeling of MoAbs such that stable radioimmunocomplexes are formed; that inter MoAb cross-linking does not occur, and that nonspecific uptake is minimal. We developed a murine monoclonal antibody, MoAb 19–24, that reacts with human sarcoma tissues in man (*2*) and in a mouse model (*3*). In the present study, we labeled MoAbs 19–24 and P3 with ^{111}In by using an isothiocyanate (SCN-Bz-DTPA) method which demonstrated excellent tumor uptake and specificity, with minimum background, on Day 3 after injection. Despite the fact that radioimmunocomplexes were not purified by HPLC before injection, low hepatic activity similar to that reported previously (*7*) was seen. This suggests that formation of immune or aggregate complexes (cross-linking) were minimal in the final preparation.

An excellent tumor localization index of 17.3 was achieved on Day 7 after injection which also suggests that the ^{111}In-SCN-Bz-DTPA-MoAb 19–24 retained adequate immunological activity. This tumor localization index also represents a considerable improvement over the previously reported index of 13.1 seen with ^{125}I-labeled MoAb 19–24 on Day 7 in the same mouse tumor model (*3*).

We compared tissue biodistribution data with results of a previous study (*3*) when ^{125}I and ^{111}In immunocon-

TABLE 4
Scintigraphic Analysis of ^{111}In-Labeled MoAbs 19–24 and P3 in Tumor-Bearing Mice*

Day	MoAb	Total body cpm	Tumor cpm	Liver cpm	%Tumor cpm	%Liver cpm
1	19–24	43,785† ± 410	6,788 ± 90	5,439 ± 1,198	16	12
	P3	41,754 ± 758	1,482 ± 100	9,249 ± 158	4	22
2	19–24	34,707 ± 205	6,798 ± 390	3,978 ± 331	20	11
	P3	33,617 ± 430	1,116 ± 126	6,321 ± 255	3	19
3	19–24	30,983 ± 552	5,334 ± 127	2,758 ± 254	17	9
	P3	30,399 ± 50	1,831 ± 148	5,075 ± 188	6	17
7	19–24	10,912 ± 84	1,804 ± 82	1,155 ± 43	17	11
	P3	11,571 ± 177	499 ± 22	1,938 ± 28	4	17

* Three animals/study/day.
† Mean ± s.e.m.

jugates were used in the same nude-mouse tumor model. We found that there was greater tumor activity with ^{111}In-SCN-Bz-DPTA-MoAb 19–24 than with ^{125}I MoAb 19–24 at 24 hr after injection (52.1% vs. 12% i.d./g), suggesting improved tumor uptake. This difference in the tumor activity noted at 24 hr may be due, in part, to the high degree of dehalogenation that occurs with ^{125}I-labeled immunocomplexes in various tissues, which decreases the tissue residence time of the label (*11*). Hepatic activity at 24 hr was similar for both ^{125}I- and ^{111}In-labeled MoAb 19–24 (6–7%). This finding was encouraging in that it indicated a decrease in the amount of hepatic activity at 24 hr for this ^{111}In-labeled immunocomplex which was not seen when another MoAb was labeled with ^{111}In using the cyclic anhydride conjugation procedure (*4*), or when we labeled MoAb 19–24 by the same method (7.2% vs. 24%, unpublished data).

The data presented in this study agree with the observations reported by Esteban et al. (*12*) who used a similar conjugation technique (SCN-Bz-DTPA) and an anti-colon antibody (B72.3) in athymic mice bearing human colon carcinoma xenografts. Our preliminary results are promising because they demonstrate that labeling of MoAb 19–24 with a covalently bound radiotracer complex such as SCN-Bz-DTPA-^{111}In yields excellent imaging and biodistribution data. We are also encouraged by the decreased amount of hepatic activity at 24 hr compared to that in previous studies with ^{111}In-labeled 19–24 in which we used a cyclic anhydride conjugation procedure (unpublished data). The new data merit future studies with the isothiocyanate conjugation technique and other MoAbs, as well as with MoAb 19–24 for the development of a new diagnostic and therapeutic tool in humans.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service Grant CA 35354 from the National Cancer Institute, Office of Health & Environmental Research of the Department of Energy, and the Evelyn Steinberg Cancer Foundation.

REFERENCES

1. Pimm MV, Embleton MJ, Perkins AC, et al. In vivo localization of anti-osteogenic sarcoma 791T monoclonal antibody in osteogenic sarcoma xenografts. *Int J Cancer* 1982; 30:75–85.
2. Greager JA, Brown JM, Pavel DG, Garcia JL, Blend MJ, Das Gupta TK. Localization of human sarcoma with radiolabeled monoclonal antibody: a preliminary study. *Cancer Immunol Immunother* 1986; 23:148–154.
3. Brown JM, Greager JA, Pavel DG, Das Gupta TK. Localization of radiolabeled monoclonal antibody in a human soft tissue sarcoma xenograft. *JNCI* 1985; 75:637–644.
4. Hnatowich DJ, Griffin TW, Kosciuczyk C, et al. Pharmacokinetics of an Indium-111-labeled monoclonal antibody in cancer patients. *J Nucl Med* 1985; 26:849–858.
5. Brown JM. Detection of a human sarcoma-associated antigen with monoclonal antibodies. *Cancer Res* 1983; 43:2113–2120.
6. Brechbiel MW, Gansow OA, Atcher RW, et al. Synthesis of 1-(p-Isothiocyanatobenzyl) derivatives of DTPA and EDTA. Antibody labeling and tumor-imaging studies. *Inorgan Chem* 1986; 25:2772–2781.
7. Rasheed S, Nelson-Rees WA, Toth EM, Arnstein P, Gardner MB. Characterization of a newly derived human sarcoma cell line (HT-1080). *Cancer* 1974; 33:1027–1033.
8. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; 256:495–497.
9. Ey PL, Prowse SJ, Jenkin CR. Isolation of pure IgG-1, IgG-2a and IgG-2b immunoglobulins from mouse serum using protein A-sepharose. *Immunochemistry* 1978; 15:429–436.
10. Silverberg E. Cancer statistics: 83 CA 1983; 33:9–25.
11. Sullivan DC, Silva JS, Cox CE, et al. Localization of I-131 labeled goat and primate anti-carcinoembryonic antigen (CEA) antibodies in patients with cancer. *Invest Radiol* 1982; 17:350–355.
12. Esteban JM, Schlom J, Gansow OA, et al. New method for the chelation of indium-111 to monoclonal antibodies: biodistribution and imaging of athymic mice bearing human colon carcinoma xenografts. *J Nucl Med* 1987; 28:861–869.