TUMOR IMAGING AFTER ADMINISTRATION OF ^{99m}Tc-LABELED BLEOMYCIN

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Bleomycin, an anticancer drug, was labeled with ^{99m}Tc using stannous chloride and ascorbic acid and specific activities of 1–3 mCi/mg-eq with labeling efficiencies of 50–75% were achieved. Very rapid excretion of ^{99m}Tc-bleomycin through the kidney and concomitant rapid decrease of radioactivity in blood, various tissues and organs, and whole body were observed after intravenous administration of the radiopharmaceutical into tumor-bearing mice. In such animals, approximately 1% of the label was found in a transplanted fibrosarcoma within 30 min while 0.58% was recovered in such lesions even after 24 hr. In patients positive tumor images were obtained by scintigraphy as early as 1 hr after intravenous administration of 3–5 mCi of ^{99m}Tc-bleomycin. A total of 142 cases were examined by scintigraphy after administration of ^{99m}Tc-bleomycin and/or ^{67}Ga-citrate. In 93 cases with various malignant tumors, tumor was detected in 80% using ^{99m}Tc-bleomycin and in 63% using ^{67}Ga-citrate. Technetium-99m-bleomycin scintigraphy successfully detected tumors of the thyroid, lung, face, breast, extremity, and digestive tract and was also useful in finding metastatic lesions and brain tumors. However, ^{67}Ga scintigraphy gave superior results in detecting lesions in patients with malignant lymphomas. In patients with inflammatory diseases, accumulation in lesions was detected in 13% using ^{99m}Tc-bleomycin and in 48% using ^{67}Ga-citrate. The further use of ^{99m}Tc-bleomycin scintigraphy for tumor detection in patients appears to be warranted.

Physicians have long desired a sensitive and simple test to detect and localize malignant neoplasms. In 1969 Edwards and Hayes observed accumulation of ^{67}Ga in the involved neck lymph nodes in a patient with Hodgkin's disease who had been given ^{67}Ga-citrate intravenously (*1*). Gallium-67 has since been shown to have an affinity not only for malignant lymphomas but also for a variety of cancers and sarcomas (*2–4*). In addition to ^{67}Ga, ^{111}In, ^{169}Yb, and certain other radioactive metals may also concentrate in some tumors (*5–7*) but all unfortunately also concentrate in inflammatory lesions (*3–7*). Materials having greater specificity for malignant tissue are needed.

Bleomycin, an anticancer antibiotic (*8*), has been known to have an inhibitory effect on cancers of epithelial cell origin (*9*). Recently the clinical usefulness of ^{57}Co-labeled bleomycin for tumor imaging was reported (*10,11*). However, the long life of ^{57}Co is an undesirable attribute that limits wide use of this radiopharmaceutical.

We have prepared ^{99m}Tc-labeled bleomycin. This paper describes the method of preparing the compound and the results of experimental and clinical studies. The radiopharmaceutical localizes relatively quickly into a number of different types of tumors while uptake in nonmalignant inflammatory lesions is relatively infrequent. Its use as a diagnostic agent is associated with a very small body radiation dose.

MATERIALS AND METHODS

Preparation of ^{99m}Tc-bleomycin. Bleomycin (*8*) was a gift from Nihon Kayaku Pharmaceutical Co. Ltd. Its chemical structure is shown in Fig. 1. Several derivatives having different terminal amines exist; the materials used throughout this study were the copper-dechelated sulfate compounds of both A_2 and B_2 [Lot No. F26AS 2N(-Cu)].

Technetium-99m-labeling was performed by using stannous chloride according to our previously described procedures (*12*). Fifteen milligram-equiva-

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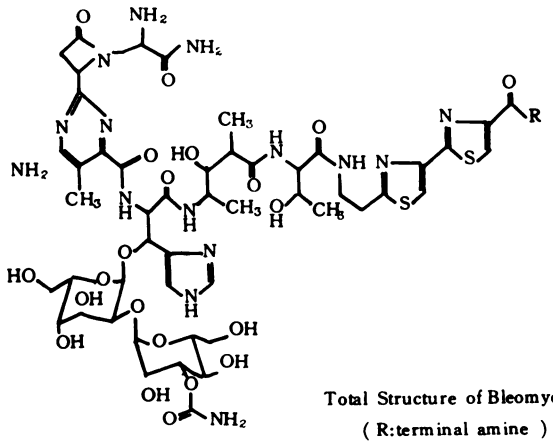

Total Structure of Bleomycin
(R:terminal amine)

Terminal Amines of Bleomycins

BLM	Structure
A_1	$NH_2-CH_2-CH_2-CH_2-SO-CH_3$
demethyl-A_2	$NH_2-CH_2-CH_2-CH_2-S-CH_3$
A_2	$NH_2-CH_2-CH_2-CH_2-\overset{+}{S}(CH_3)_2\ X^-$
A'_2- a	$NH_2-CH_2-CH_2-CH_2-CH_2-NH_2$
A'_2- b	$NH_2-CH_2-CH_2-CH_2-NH_2$
A_5	$NH_2-(CH_2)_3-NH-(CH_2)_4-NH_2$
A_6	$NH_2-(CH_2)_3-NH-(CH_2)_4-NH-(CH_2)_3-NH_2$
B_2	$NH_2-(CH_2)_4-NH-\overset{\overset{NH}{\|}}{C}-NH_2$
B_4	$NH_2-(CH_2)_4-NH-\overset{\overset{NH}{\|}}{C}-NH-(CH_2)_4NH-\overset{\overset{NH}{\|}}{C}-NH_2$

FIG. 1. Chemical structure of bleomycin. Various derivatives having different amine groups at position R are known.

lents* (mg-eq) of bleomycin were dissolved in 5–10 ml of ^{99m}Tc-pertechnetate solution. To the solution, 150–250 μg of freshly prepared $SnCl_2 \cdot 2H_2O$ in 1 *N* HCl was added and the pH was adjusted between 2.5 and 3.0. After 5 min of constant stirring, the reaction was terminated by the addition of 1–3 mg of ascorbic acid (vitamin C, Takeda) and then the pH was brought back to neutral (not exceeding 7.5). For this pH adjustment, 7% $NaHCO_3$ solution was used. The possible appearance of slight cloudiness indicates the formation of an insoluble chelated compound of ^{99m}Tc and Sn (*12,13*) but filtration of the mixture through a 0.22-micron Millipore filter eliminated such side products almost completely and the filtrate was used for intravenous administration. The purity of the labeled compound was monitored by Sephadex G-25 gel filtration, paper chromatography using 75% methanol, and silica gel plate thin-layer chromatography using 1:1 mixture of 75% methanol and 10% ammonium acetate. The effect of labeling procedures on the antimicrobial activity of bleomycin (*14*) was also studied in two preparations. One of them was labeled by using a 1/20 concentration of ^{99m}Tc-pertechnetate. The tests were performed by Nihon Kayaku Pharmaceutical Co. Ltd.

Animal studies. The metabolism, toxic effects, and affinity to malignant tumors of ^{99m}Tc-bleomycin were studied in normal and tumor-bearing A-Jackson-strain mice weighing 20 gm.

As an experimental tumor, a transplantable fibrosarcoma (MCS strain) was used. Five million tumor cells were transplanted subcutaneously into the left shoulder region, and the mice were studied when the tumor had grown about 1 cm in diameter. Three mice each were sacrificed at 30 min and 1, 2, 3, 6, 12, and 24 hr after the intravenous injection of 300 μCi/0.15 mg-eq dose.

Whole-body scintigraphy was performed using a scintillation camera (Pho/Gamma III, Searle Radiographics) with pinhole collimator at 140 keV ± 10% spectrometer setting. After whole-body counting and scintigraphy were performed, the animals were dissected and radioactivities in blood (0.1 ml), tumor, kidney, lung, stomach, liver, intestine, and spleen were measured in a well scintillation counter.

Clinical studies. To determine the appropriate timing for scintigraphy and to study stability of the labeled compounds, serial measurements of plasma radioactivity were made on four patients with different malignant tumors, using four different separately labeled batches of ^{99m}Tc-bleomycin. Renal function was normal in these patients. Plasma and urine samples obtained at 2 hr after injection were counted, chromatographed, and measured.

A larger series of 142 patients was then examined. Table 1 shows the location of their main lesions.

TABLE 1. CLASSIFICATION OF EXAMINED CLINICAL CASES ACCORDING TO LOCATION OF THEIR MAIN LESION

Location	No. of cases	
	Overall	Malignant*
Brain	17	—
Face	20	13
Neck	21	13
Chest	43	32
Abdomen	23	18
Pelvic	2	2
Extremity	9	8
Malignant lymphoma	7	7
Total	142	93

* When metastatic lesions were found together with primary lesions, they were classified according to latter.

* Amount of bleomycin is not shown by weight basis but shown by antimicrobial biologic potency equivalent to standard bleomycin A.

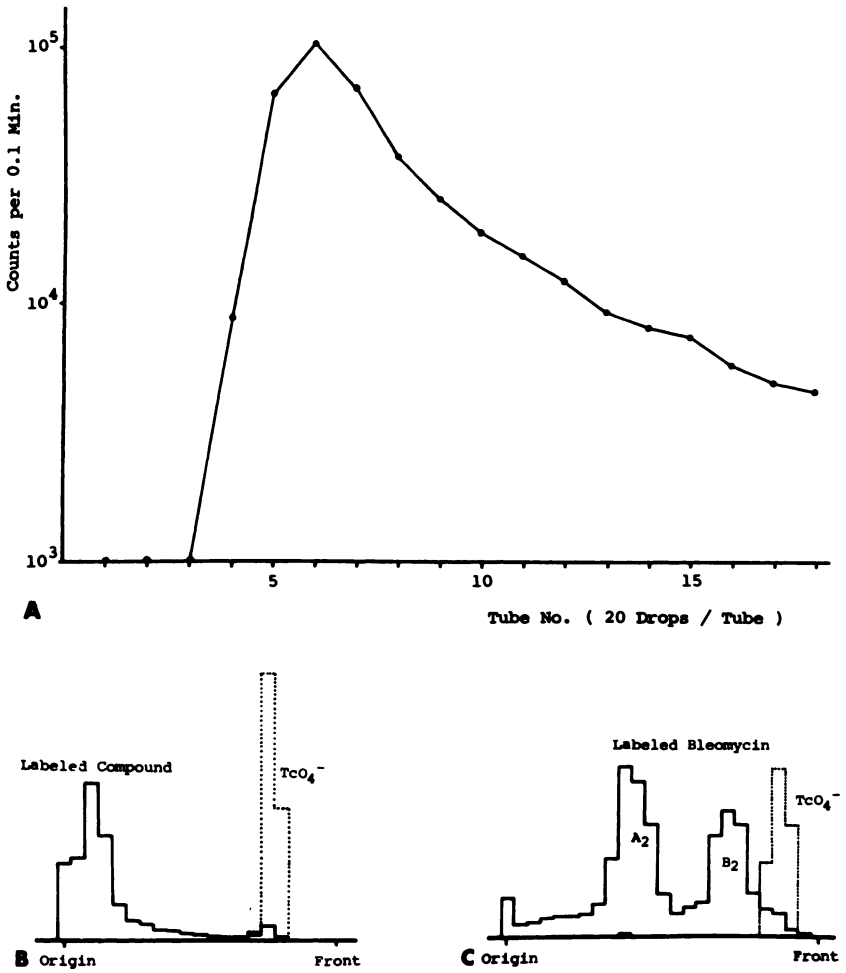

FIG. 2. Radiochemical purity of labeled compound. (A) Sephadex G-25 gel filtration pattern. Labeled compound was subjected to Sephadex G-25 (1 × 22 cm) column chromatography using physiologic saline as effluent. Twenty-drop aliquots were collected and counted. Free pertechnetate should be eluted at tubes later than the 13th. (B) Paper chromatography pattern. Solvent: 75% methanol. (C) Silica gel plate thin-layer chromatography pattern. Solvent: 1:1 mixture of 75% methanol and 10% ammonium acetate. As comparison, mixture of bleomycin and ^{99m}Tc-pertechnetate was also chromatographed simultaneously (dotted line).

The clinical diagnoses were established by histologic and/or clinical findings; 93 of them were proved to bear a malignancy. After intravenous administration of 3–5 mCi of ^{99m}Tc-bleomycin, radioactivity (140 keV ± 10%) distributions in the body were monitored by the images built up on the persistence scope connected to the scintillation camera using a 4,000-hole collimator. Scintiphotos of regions of interest were generally taken within 2 hr. Early studies at 15–30 min often gave sufficient results.

In most instances, scintigraphy at 72 hr after intravenous administration of 1–2 mCi of ^{67}Ga-citrate was also performed by the scintillation camera using a 1,000-hole collimator and a spectrometer setting of 190 keV ± 12.5%. Regularly in our laboratory, ^{67}Ga-citrate is injected on Tuesday and scintigraphy is performed on Friday. Technetium-99m-bleomycin studies were performed either prior to the injection of ^{67}Ga-citrate or immediately after ^{67}Ga scintigraphy. Even when there was significant accumulation of ^{67}Ga, the differences of administered radioactivities and energy distributions minimized the contribution of ^{67}Ga in the ^{99m}Tc window and ^{99m}Tc-bleomycin scintigraphy could be performed satisfactorily. In patients with brain tumors, scintigraphic results after ^{99m}Tc-bleomycin were compared with those obtained using ^{99m}Tc-pertechnetate.

Scintiphotos were read by three persons independently and results were considered positive only when all three judges agreed.

RESULTS

Quality of the radiopharmaceutical. As shown in Fig. 2, ^{99m}Tc-bleomycin was eluted as a fast-moving single component and negligible amounts of contamination of $^{99m}TcO_4^-$ were observed (Fig. 2A). Paper chromatography also revealed that free pertechnetate was less than 5% (Fig. 2B). Thin-layer chromatography was found quite suitable for the quality check of the material (Fig. 2C). Twelve-centimeter chromatography was achieved within 2.5 hr, and bleomycin A_2, B_2, and free pertechnetate showed R_f values of 0.35, 0.68, and 0.88, respectively. Radioactivity distribution at R_f 0.35 and 0.68 was always close to the ratio of 2:1 which was the ratio of original biochemical potency. Technetium-99m-labeling of bleomycin could be performed without addition of ascorbic acid and the labeled ma-

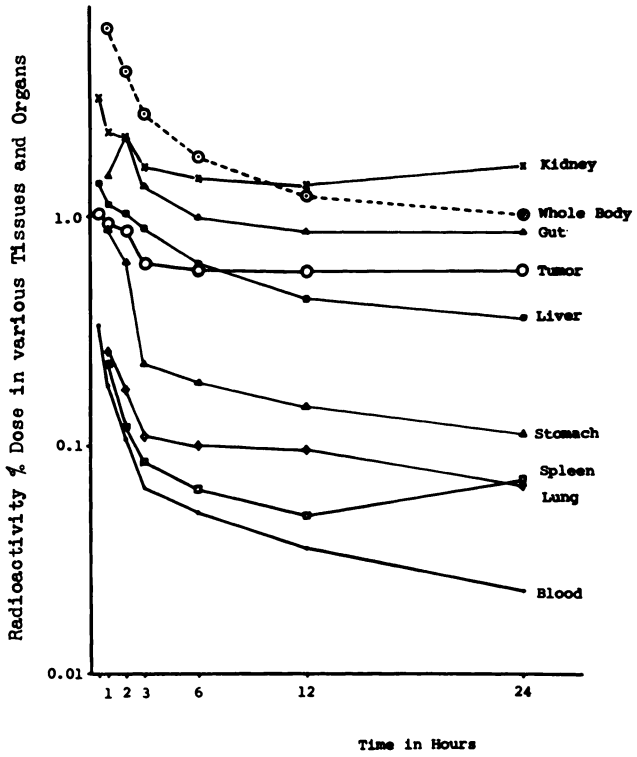

FIG. 3. Sequential changes in tissue and organ radioactivity in fibrosarcoma-bearing mice. Tumors were transplanted on left shoulder region and weighed approximately 1 gm at time of experiment. Each point represents mean value of three mice each and is expressed as percent radioactivity in whole organs except for blood (0.1 ml) and whole-body retention (× 1/10).

terials did not show significant changes in the thin-layer chromatography pattern. However, lack of in vivo stability of later material was considered unapplicable for clinical study. Usually 50–75% of labeling efficiencies with specific activities of 1–3 mCi/mg-eq were achieved. Loss of radioactivity during the procedure resulted from insoluble colloidal components that were effectively eliminated by Millipore filtration.

Bioassayed antimicrobial activities in two specimens (originally 1.25 mg-eq/ml) were reduced to 0.033 and 0.031 mg-eq/ml, respectively. Thus, only about 2.5% of the original biologic activities were recovered indifferent to the amounts of radiotechnetium applied.

When relatively small amounts (50 μCi/0.02 mg-eq) of ^{99m}Tc-bleomycin were injected intravenously into normal mice, significant accumulations were demonstrable in the kidneys within 3 min. At 7 min, increased activity in the kidneys and urinary bladder was observed and at 30 min very high activity was seen in the bladder with very little seen elsewhere in the body.

Plasma and urinary samples obtained from clinical subjects 2 hr after injection were found not to contain more than 5% of free pertechnetate by paper chromatography and thin-layer chromatography.

As to toxicity, intravenous dose of 3 mg-eq of the radiopharmaceutical which contained half of the equivalent of an LD_{50} dose of unlabeled bleomycin did not kill any of four mice during an observation period of 2 weeks.

Studies in tumor-bearing experimental animals. Figure 3 shows sequential changes in tissue and organ radioactivity in tumor-bearing mice. Very rapid declines of radioactivity were observed in blood, lung, stomach, spleen, and liver. In the whole

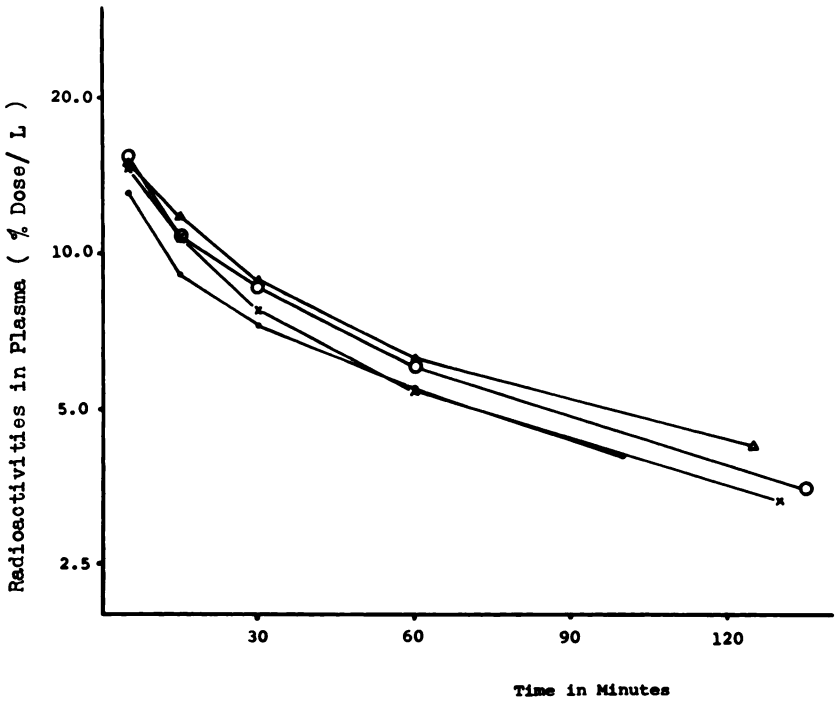

FIG. 4. Plasma radioactivity disappearance curves after administration of ^{99m}Tc-bleomycin. Plasma samples were taken from four patients with different malignant tumors. Each patient was injected with separately labeled batches of ^{99m}Tc-bleomycin.

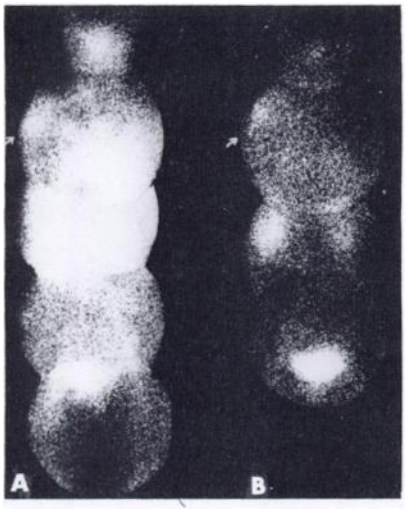

FIG. 5. Whole-body radioactivity distribution in woman with recurrent breast cancer. Scintiphotos for 20 sec for each frame were taken at 15 (A) and 120 (B) min after administration of ^{99m}Tc-bleomycin. Arrows indicate accumulation of ^{99m}Tc-bleomycin on skin lesion of right chest wall near axilla.

body only 15% of the initial dose was recovered at 24 hr. In the tumor, approximately 1% of the dose was localized at 30 min and 0.58% was retained even after 24 hr. The slow loss of activity in the tumor was associated with a ninefold rise in tumor-to-blood activity ratios over the time period between 30 min and 24 hr. Activities in the kidney and lower gastrointestinal tract also remained relatively high over the 24-hr period.

Optimal timing for clinical scintigraphy. Figure 4 shows plasma radioactivity disappearance curves obtained from four subjects. Approximately 15%/liter was recovered at 5 min after injection; thereafter, disappearance was rapid in all cases so that at 120 min only about 4%/liter was recoverable.

Figure 5 shows the body distribution of ^{99m}Tc at two intervals after injection of ^{99m}Tc-bleomycin in a woman with recurrent breast cancer. At 15 min after injection, relatively high radioactivity was seen over the kidneys, urinary bladder, cardiac blood pool, and facial area, and at the same time some accumulation of activity was also seen at the site of a skin lesion of the right chest wall near the axilla. After 120 min, radioactivity in the tumor region was relatively high compared with other thoracic activity.

Because of rapid blood clearance and urinary excretion of the radiopharmaceutical and relatively good retention in tumors during the first hour or two, scintigraphy was usually carried out within 2 hr after administration of the drug. When the region of interest was distant from the cardiac area or big blood vessels, scintigraphy as early as 15–30 min yielded satisfactory images.

Clinical results: *malignant diseases.* Table 2 shows the scintigraphic results obtained by both ^{99m}Tc-bleomycin and ^{67}Ga-citrate in patients bearing malignancies. Technetium-99m-bleomycin scintigraphy gave positive results in 74 of 93 cases (80%) compared with 63% positive results obtained by ^{67}Ga-citrate. Except for malignant lymphomas, ^{99m}Tc-bleomycin scintigraphy appeared to be superior to that of ^{67}Ga-citrate in all body sites. Technetium-99m-bleomycin scintigraphy gave particularly good results in patients with cancers of the face, thyroid, lung, breast, and gastrointestinal tract. However, results were poor in patients with cancers of pelvic organs and with malignant lymphomas.

By contrast, ^{67}Ga-citrate scintigraphic results were excellent in malignant lymphomas but the detection rate was low in lesions of the orbit, thyroid, breast, gastrointestinal tract, and pelvic organs.

TABLE 2. SCINTIGRAPHIC RESULTS OF ^{99m}Tc-BLEOMYCIN AND ^{67}Ga-CITRATE IN MALIGNANT CASES WITH RELATION TO THEIR TUMOR LOCALIZATION

		^{99m}Tc-bleomycin			^{67}Ga-citrate		
Face	Orbital	3/3	11/13	(85%)	0/3	4/9	(44%)
	Paranasal, mouth, and mandibular	8/10			4/6		
Neck	Epipharynx and larynx	3/5	11/13	(85%)	5/5	6/10	(60%)
	Thyroid	8/8			1/5		
Chest	Lung	20/22	28/32	(88%)	16/20	19/28	(68%)
	Breast	5/5			1/4		
	Esophagus	3/5			2/4		
Abdomen	Liver and pancreas	6/9	14/18	(78%)	7/9	10/18	(56%)
	Gastrointestinal	8/9			3/9		
Pelvic organ			0/2	(0%)		0/2	(0%)
Extremity cancer and sarcoma			7/8	(88%)		4/6	(67%)
Malignant lymphoma			3/7	(43%)		7/7	(100%)
Total		74/93		(80%)	50/80		(63%)

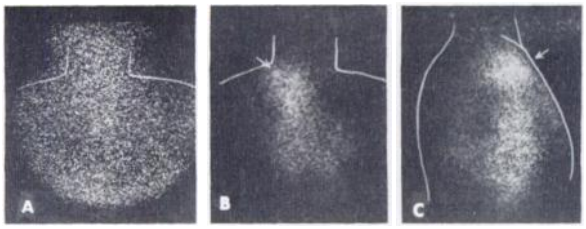

FIG. 6. Scintiphotos of (A) frontal view obtained with ^{67}Ga-citrate 72 hr after injection, (B) frontal, and (C) right lateral views obtained with ^{99m}Tc-bleomycin 60 min after injection in patient with squamous cell cancer on right lower neck. Arrows in B and C indicate accumulation of ^{99m}Tc-bleomycin on tumor area.

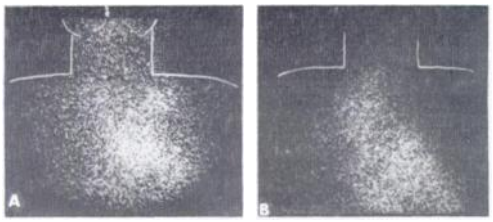

FIG. 7. Scintiphotos obtained with (A) ^{67}Ga-citrate 72 hr after injection and (B) ^{99m}Tc-bleomycin 30 min after injection in patient with undifferentiated cell cancer of lung. Primary lesion originated from superior segment of left lower lobe and massive mediastinal involvement was associated. Gallium-citrate accumulations were observed at site of primary lesion, mediastinum, and left supraclavicular region; ^{99m}Tc-bleomycin also demonstrated mediastinal involvement. However, primary lesion could not be differentiated clearly from cardiac blood pool.

Figure 6 shows scintiphotos obtained using ^{67}Ga-citrate and ^{99m}Tc-bleomycin in a patient with a small (3 × 3.5 cm) squamous cell cancer on the right lower neck. Gallium-67-citrate failed to show the existence of the tumor (Fig. 6A) but significant accumulation of ^{99m}Tc was observed in the site corresponding to the tumor (Fig. 6B) and a tumorous extension was well demonstrated in the right lateral view (Fig. 6C).

Somewhat surprising in view of the selective clinical effects of unlabeled bleomycin, accumulations of ^{99m}Tc-bleomycin were observed in a wide variety of types of malignant tumors. Figure 7 shows the scintigraphic findings in a patient with an undifferentiated small cell cancer of lung originating in the superior segment of left lower lobe. A chest roentogenogram showed a widened mediastinum. Gallium-67 accumulated significantly in the mediastinal and left supraclavicular region and at the site of the primary lesion (Fig. 7A). The primary lesion was not clearly visualized with ^{99m}Tc-bleomycin because of interference with the cardiac blood pool activity. However, the mediastinal lesion was detected (Fig. 1B).

When lesions were located in the peripheral region of the body, scintigraphy using ^{99m}Tc-bleomycin usually gave better results than that after ^{67}Ga-citrate. Figure 8 shows a case with fibrosarcoma on the left upper arm with involvement of the surface of humerus. Gallium-67 (Fig. 8B) and ^{87m}Sr (Fig. 8C) showed significant accumulation but with scintigraphy using ^{99m}Tc-bleomycin (Fig. 8A) the lesion was more clearly detected.

Table 3 shows a comparison of the scintigraphic results obtained using ^{99m}Tc-bleomycin and ^{67}Ga-citrate in patients with malignant diseases with relation to their cell types. Of 82 primary lesions, 64 (78%) were positive by ^{99m}Tc-bleomycin scintigraphy and, except for the low figure (43%) in malignant lymphomas, no significant differences in the detection rate were observed in various types of malignancy.

On the other hand, scintigraphic results obtained by ^{67}Ga-citrate appeared to vary more with the tumor cell type. Undifferentiated cell cancers and malignant lymphomas were well demonstrated while 61% of

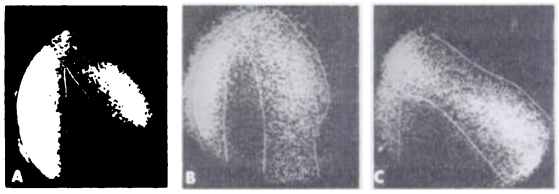

FIG. 8. Scintiphotos obtained with (A) ^{99m}Tc-bleomycin 30 min after injection, (B) ^{67}Ga-citrate 72 hr after injection, and (C) ^{87m}Sr 3 hr after injection in patient with fibrosarcoma on left upper arm with involvement of surface of humerus.

TABLE 3. SCINTIGRAPHIC RESULTS OF ^{99m}Tc-BLEOMYCIN AND ^{67}Ga-CITRATE IN MALIGNANT CASES WITH RELATION TO THEIR CELL TYPES

Lesions	^{99m}Tc-bleomycin		^{67}Ga-citrate	
Primary				
Undifferentiated cell carcinoma	9/11	(82%)	11/11	(100%)
Squamous cell carcinoma	20/25	(80%)	11/18	(61%)
Adenocarcinoma	26/33	(79%)	13/28	(45%)
Malignant lymphoma	3/7	(43%)	7/7	(100%)
Fibro- and myosarcoma	3/3	(100%)	3/3	(100%)
Malignant melanoma	1/1	(100%)	0/1	(0%)
Retinoblastoma	1/1	(100%)	0/1	(0%)
Teratoma	1/1	(100%)	1/1	(100%)
Total	64/82	(78%)	46/70	(66%)
Metastatic				
Undifferentiated cell carcinoma	1/1		—	
Squamous cell carcinoma	7/7		2/4	
Adenocarcinoma	13/15		5/10	
	21/23	(91%)	7/14	(50%)

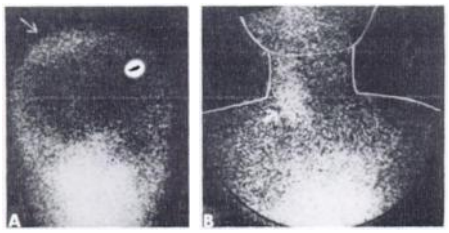

FIG. 9. Scintiphotos of head (A) 60 min after injection and neck (B) 30 min after injection obtained with ^{99m}Tc-bleomycin in patient with follicular adenocarcinoma of thyroid with skull metastasis. Arrows indicate metastatic lesion in A and primary lesion in B.

squamous cell cancers and 45% of adenocarcinomas were detected.

Technetium-99m-bleomycin scintigraphy was also successful in detecting metastatic lesions. Figure 9 shows ^{99m}Tc-bleomycin scintiphotos in a patient with a follicular adenocarcinoma of right lobe of the thyroid. Together with accumulation on the right neck region (Fig. 9B) significant accumulation was seen on the parietal region (Fig. 9A). This site of cranial localization detected by ^{99m}Tc-bleomycin scintigraphy was later histologically confirmed as a skull metastasis. Twenty-one of 23 metastatic lesions were demonstrable by ^{99m}Tc-bleomycin scintigraphy but only 7 of 14 were positive using ^{67}Ga-citrate.

Benign diseases. Table 4 shows the scintigraphic results using ^{99m}Tc-bleomycin and ^{67}Ga-citrate in patients with benign diseases. In 32 patients ^{99m}Tc-bleomycin scintigraphy gave a false-positive rate of 13% but this was much lower than the comparable 48% rate using ^{67}Ga-citrate.

None of five benign tumors was detected by ^{67}Ga-citrate scintigraphy but 2 of 13 were detected using ^{99m}Tc-bleomycin. These were an adenoma of the left main bronchus and Hurthle cell adenoma of thyroid. On the other hand, in only 2 of 16 patients were inflammatory and granulomatous lesions detected using ^{99m}Tc-bleomycin. These were in a patient with a lung abscess and a patient with aspergillosis of the right upper lung lobe. Gallium-67 scintigraphy was positive in 67% of patients with inflammatory lesions. Most patients with active pulmonary tuberculosis, sarcoidosis, and parasinusitis were found positive by ^{67}Ga-citrate. The thyroid gland in two patients with Graves' disease showed no accumulation of ^{99m}Tc-bleomycin. The abdomen in a patient with malabsorption syndrome was also negative on scintigraphy.

Technetium-99m-bleomycin accumulates to some extent at the site of brain lesion. Table 5 shows a comparison of results of scintigraphy using ^{99m}Tc-bleomycin and $^{99m}TcO_4^-$. All patients with glioma, meningioma, and neurinoma who were examined had their lesions detected by ^{99m}Tc-bleomycin. All but one were also detected by $^{99m}TcO_4^-$; the exception was a small acoustic neuroma. Other tumors encountered included craniopharyngioma, pituitary adenoma, ectopic pinealoma, and other small tumors of undetermined histology located in the brain stem. In spite of its rapid clearance from blood, ^{99m}Tc-bleomycin scintigraphy was not superior to that using $^{99m}TcO_4^-$. However, in 4 of 93 patients with malignant disease metastatic brain lesions were first detected by whole-body monitoring after the administration of ^{99m}Tc-bleomycin.

Radiation dose and side effects. From the results of animal studies and assuming a uniform ellipsoid 60-kg standard man, the radiation dose from 5 mCi ^{99m}Tc-bleomycin to the whole body, kidney, and bladder were estimated by the MIRD formula (*15*) at only 2, 100, and 100 mrads, respectively.

No untoward side effects were encountered during the performance of these clinical studies.

DISCUSSION

The results of the present investigation show (A) the anticancer antibiotic bleomycin can be labeled with ^{99m}Tc by using stannous chloride; (B) in spite of losing almost all its original antimicrobial biologic activity, the labeled compound maintains the ability to concentrate in various malignant tissues; and (C) moreover, scintigraphy after intravenous administration of the radiopharmaceutical yielded evidence of

TABLE 4. SCINTIGRAPHIC RESULTS OF ^{99m}Tc-BLEOMYCIN AND ^{67}Ga-CITRATE IN BENIGN DISEASES

Disease	^{99m}Tc-bleomycin		^{67}Ga-citrate	
Benign tumors	2/13	(15%)	0/5	(0%)
Inflammatory and granulomatous changes	2/16	(13%)	10/15	(67%)
Others	0/3	(0%)	0/1	(0%)
Total	4/32	(13%)	10/21	(48%)

TABLE 5. RESULTS OF SCINTIGRAPHY USING ^{99m}Tc-BLEOMYCIN AND ^{99m}Tc-PERTECHNETATE IN VARIOUS BRAIN TUMORS

Tumor	^{99m}Tc-bleomycin		^{99m}Tc-pertechnetate	
Glioma	5/5		4/4	
Meningioma	2/2		2/2	
Neurinoma	2/2		1/2	
Metastatic brain tumor	(4/4)		—	
Others	2/8		2/7	
Total	11/17	(65%)	9/15	(60%)

tumor localization in many different kinds of malignant tumors. Distribution studies of ^{3}H-bleomycin show that it diffuses rapidly into various tissues including tumors but only in tumors sensitive to bleomycin is the drug protected against enzymatic cleavage (*16*). The present results are at variance with this interpretation. The therapeutic usefulness of bleomycin is rather limited to cancers of epithelial cell origin. Yet, localization of ^{99m}Tc-bleomycin to tumors is not similarly confined. We do not have a completely satisfactory explanation for this discrepancy. The drug is believed to have an affinity for the malignant tumor cell surface; its therapeutic effect may be derived from the ability of the drug to penetrate into the cell and therein react with DNA. This latter characteristic may vary in different types of tumor cells. This may account for the apparent dissociation between drug localization and therapeutic effect.

Recently, the use of ^{57}Co-labeled bleomycin for tumor scintigraphy has also been reported by two groups of investigators (*10,11*). Scintigraphy using ^{57}Co-bleomycin was performed 15–24 hr after intravenous administration of the drug. Kono observed that at 24 hr the intracellular distribution of ^{57}Co-bleomycin was significantly different from that of ^{14}C-bleomycin; around 75% of the former but only 30% of the latter was recovered bound with DNA (*17*). This observation suggests that labeling procedures might alter the nature of bleomycin differently. Although the number of reported cases was small and limited as to type and location of tumors, the clinical results of ^{57}Co-bleomycin appear to be good and seem comparable to ours. The long half-life of ^{57}Co (270 days) offers the possibility that a better resolution than ours might be attained if the drug were retained in tumor and eliminated from other sites during renal excretion of the drug. However, since the highest radioactivity in tumor could be achieved within 30 min after its intravenous administration, the rapid clearance from blood plasma would appear to reduce the necessity of a prolonged examination time. Moreover, the in vivo administration of too long-lived a radionuclide is certainly undesirable.

Quite recently the clinical use of ^{111}In-bleomycin has also been reported (*18*). From the point of physical decay, the shorter life of ^{111}In is more favorable for scintigraphy than is ^{57}Co-bleomycin. However, significant amounts of ^{111}In were released from the bleomycin by 24 hr after its intravenous administration. Indium-111 itself has some affinity to tumor tissue as well as to inflammatory lesions plus normal liver and bone marrow (*5,6*). Release of ^{111}In, therefore, might prove to represent a significant disadvantage for that radiopharmaceutical. Indeed, the recommended optimal scanning time of 24–72 hr suggests that ^{111}In-labeling does not improve the affinity of bleomycin for tumor. Gallium-67-bleomycin was also found to be unfavorable for scintigraphy (*17*).

Following the introduction of ^{67}Ga-citrate by Edwards, et al (*1*), favorable as well as unfavorable results of clinical ^{67}Ga scintigraphy have been reported (*2–4*). The results of the present study confirmed the fact that malignant lymphomas and undifferentiated cell cancers can often be detected by this agent but poor results were obtained in detecting adenocarcinomas.

Gallium-67 was demonstrated to have a significant affinity for inflammatory lesions; thus, a positive accumulation of ^{67}Ga could not reliably be interpreted as indicating the presence of a malignancy nor could a negative result be used reliably to exclude a malignancy.

The high tumor detection rate associated with ^{99m}Tc-bleomycin and its relative infrequent accumulation in inflammatory lesions represent favorable characteristics with respect to its potential use as a screening agent for tumor detection. Other advantages are (A) the favorable physical characteristics of ^{99m}Tc for scintigraphy, (B) ease and rapidity of preparation, (C) low radiation dose, and (D) apparent absent clinical toxicity. However, the method has one disadvantage: high radioactivity distributions in the kidney, urinary bladder, nasal area, and circulating blood pool. These activities cause difficulty in interpreting scintigraphs in some instances. This might be responsible for the lower detectability rate in patients with cancer of the esophagus, and abdominal and pelvic organs.

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