CLINICAL EVALUATION OF RADIOLABELED BLEOMYCIN (BLEO) FOR TUMOR DETECTION

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Bleomycin (BLEO), a chemotherapeutic agent known to concentrate actively in certain types of neoplastic tissue, has been labeled in an effort to obtain a radiopharmaceutical of greater specificity and diagnostic accuracy for tumor localization. Studies have been completed on 15 patients using ^{57}Co and 9 patients using ^{111}In-BLEO and have been compared in each patient with ^{67}Ga-citrate. Semiquantitative analysis of the clinical scans with respect to visualization of the neoplastic lesions show that the use of ^{57}Co-BLEO did result in slightly higher sensitivity in the detection of tumors.

One approach in the search for tumor-localizing agents with greater specificity and diagnostic accuracy is to label currently available chemotherapeutic compounds with a radionuclide appropriate for scintillation scanning and then investigate the usefulness of the resultant radiopharmaceutical for tumor imaging. Bleomycin (BLEO) was chosen for investigation because of its chemical behavior as a metal chelate, because of distribution studies (using an antibacterial assay) suggesting that it had a propensity for localization in squamous epithelial tissue (*1*), and a preliminary report from France stating that BLEO could be labeled with ^{57}Co and effectively used for tumor detection (*2*).

Bleomycin is a group of water- and methanol-soluble basic glycopeptide antibiotics isolated from the fermentation products of Streptomyces verticillus by Umezawa (*3*). Using gradient Sephadex column chromatography, Umezawa and coworkers separated the crude BLEO extract into two classes of 16 different peptides which have a similar basic structure, bleomycinic acid, and differ only in one side chain (*4,5*). The crude extract contains copper which is subsequently removed during refinement for therapeutic use.

Since 1967 BLEO has undergone extensive clinical trials as an antineoplastic agent and has been reported to be of therapeutic value in man primarily in tumors of squamous cell origin, lymphomas, sarcomas, and some testicular neoplasms. An excellent review of the clinical use and toxicity of bleomycin recently has been published by Blum, et al (*6*).

Because of its long physical half-life, ^{57}Co is not a preferred label for human use. Therefore, BLEO was labeled with both ^{111}In and ^{67}Ga in addition to ^{57}Co. Tissue-distribution studies performed in our laboratory with these three labeled forms of BLEO in mice bearing a solid form of Ehrlich carcinoma suggested that neither ^{111}In nor ^{67}Ga were entirely suitable as substitutes for ^{57}Co when used for tumor localization (*7*). However, a report by Merrick (*8*) on the use of ^{111}In-bleomycin in animals and man indicated that this labeled form of bleomycin was useful in tumor localization. Because of this apparent contradiction, we undertook a comparative study of the tumor-imaging properties of ^{57}Co-bleomycin, ^{111}In-bleomycin, and ^{67}Ga-citrate in a variety of human malignancies.

METHODS

Labeling of BLEO was accomplished by adding 1 ml of ^{57}Co (0.5–1 mCi/ml) or 1 ml of ^{111}In (2 mCi/ml) in 0.5 *N* HCl to 2 units of BLEO* diluted in 2 ml of normal saline. The pH of each preparation was raised to 6.5 using NaOH. Total volume of each solution was adjusted to 4 ml by the addition of normal saline and then each was filtered through a 0.22-micron sterile Millipore filter into a sterile

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* Bristol Laboratories, Syracuse, N.Y. Supplied in individual vials of 15 units equal to 15 mg potency.

vial. The resultant radiopharmaceuticals contained a BLEO concentration of 0.5 units/ml and 0.125–0.25 mCi/ml of ^{57}Co or 0.5 mCi/ml of ^{111}In. The yield and uniformity of labeling of each preparation was checked by chromatography on Baker TLC silica plates in a 1:1 mixture of 10% ammonium acetate and methanol (7).

Patients received 3.5 ml of the labeled BLEO preparation containing 1.75 mCi ^{111}In intravenously. The dose of ^{67}Ga-citrate (New England Nuclear) was 3 mCi given intravenously. Cobalt-57-BLEO was given first, and scans performed using the 120-keV photopeak of ^{57}Co at 6 and 24 hr with either a gamma camera or dual 5-in. rectilinear scanner. Subsequently, ^{111}In-BLEO was administered and scans obtained at 6 and 24 hr using the 173- and 247-keV photopeaks of ^{111}In by centering an 80% window at 220 keV. Finally, ^{67}Ga-citrate was given and scans using the 296-keV photopeak of ^{67}Ga were performed at 48 hr. Pathologic confirmation of the nature and distribution of the neoplastic lesions was obtained in almost all cases. Plasma and urine clearance was determined in several patients for both ^{57}Co- and ^{111}In-BLEO. In addition to ^{111}In-BLEO prepared in our laboratory, a commercial preparation of ^{111}In-BLEO (Tumor Scintigraphin™, Medi+ Physics, Inc., Emeryville, Calif.) was also used in several patients (Patients 3, 7, and 15).

The resultant images were compared by several observers and a semiquantitative grading of the degree of visualization of each lesion in the three studies was made using a 0–4+ scale. The scan that best demonstrated the lesion was used for grading analysis. For ^{57}Co-BLEO and ^{111}In-BLEO, 6 and 24 hr scans were taken and for ^{67}Ga-citrate only 48 hr scans were obtained.

RESULTS

Fifteen patients with a variety of neoplastic diseases have been studied. None had received therapeutic doses of BLEO. Plasma clearance data in two patients for the two forms of labeled BLEO revealed that ^{57}Co-BLEO was cleared more rapidly, falling to an average of 7% of the administered dose per liter of plasma at 15 min as compared with 15% for ^{111}In-BLEO. At 24 hr the level of ^{57}Co had fallen to 0.5%/liter of plasma whereas eight times this amount of ^{111}In remained in circulation (4%/liter plasma).

Urine excretion of ^{57}Co averaged 70% from 0 to 24 hr and 10% from 24 to 48 hr, a total of 80% of the administered dose being excreted in the first 48 hr. Sixty percent of the dose of ^{111}In appeared in the urine from 0 to 24 hr, and 4% from 24 to 48 hr, an average of 64% of the total dose being excreted in the first 48 hr. This more rapid clearance pattern of ^{57}Co than ^{111}In was confirmed in the serial images obtained in all of the patients.

The results of the semiquantitative analysis of the images obtained in each patient are presented in Table 1. Of the 15 patients, 9 had studies with ^{111}In-BLEO, 15 with ^{57}Co-BLEO, and all had studies with ^{67}Ga-citrate. Positive scans were obtained in 2 of the 9 patients given ^{111}In-BLEO, in 7 of the 15 patients receiving ^{67}Ga-citrate, and in 11 of the 15 patients given ^{57}Co-BLEO. Semiquantitative comparison of the degree of uptake of each nuclide and visualization of the lesions revealed that ^{67}Ga-citrate was the superior agent in four cases, ^{111}In-BLEO was superior in none of the cases, and ^{57}Co-BLEO was superior in seven. There were four cases in which the ^{57}Co-BLEO and ^{67}Ga-citrate studies were considered to be equivalent.

CASE REPORTS

Patient 1. A 52-year-old man had a small malignant gastric ulcer. This lesion was not visualized but the normal appearance of a total-body ^{57}Co-bleomycin scan at 6 hr is shown (Fig. 1A and B) and compared with a normal 48-hr total-body scan with ^{67}Ga-citrate (Fig. 1C).

Patient 2. A 68-year-old man had squamous carcinoma of the lung located in the left hilum that was felt to be unresectable at thoracotomy. Postoperatively, suppurative drainage developed from the surgical incision. The tumor was not visualized with any of the three agents but all three showed uptake in the infected thoracotomy wound (Fig. 2). No x-ray therapy had been given at the time of the scans.

Patient 3. A 45-year-old man had squamous carcinoma of the lung involving the left hilum. This

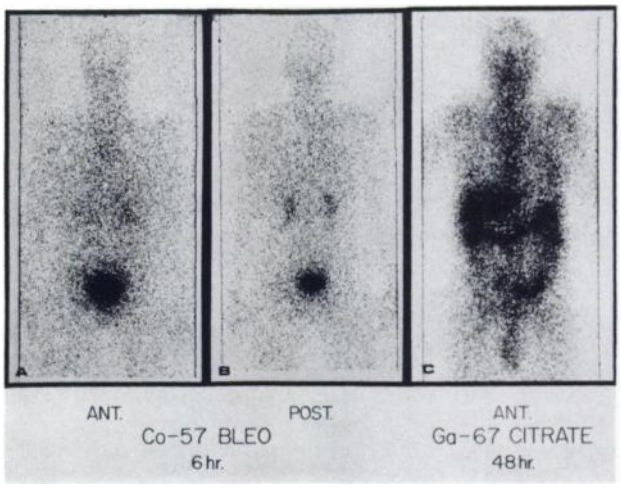

FIG. 1. Patient 1: (A) and (B) are normal ^{57}Co-BLEO scans at 6 hr; (C) is ^{67}Ga-citrate scan at 48 hr without patient preparation.

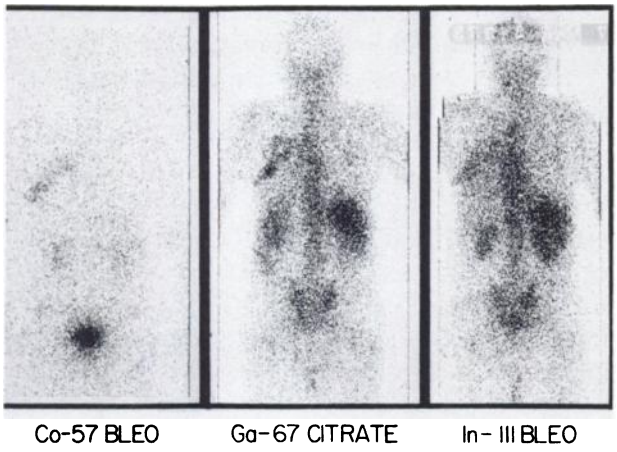

FIG. 2. Patient 2: squamous carcinoma of lung BLEO scans at 6 hr, ^{67}Ga-citrate scan at 48 hr. Note localization in thoracotomy wound in all scans.

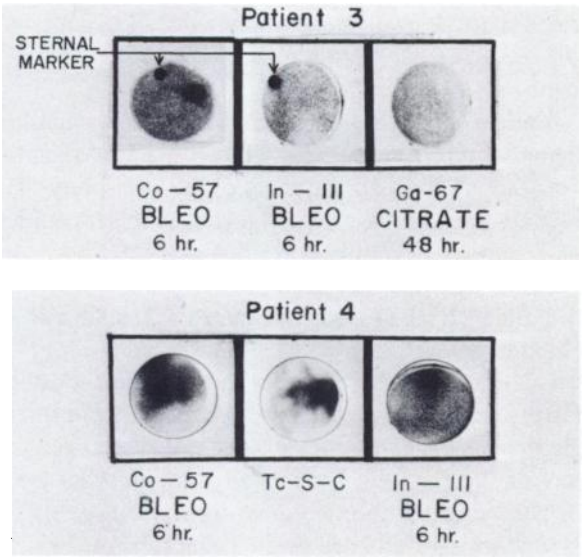

FIG. 3. Patient 3: squamous carcinoma of lung involving left hilum. Patient 4: adenocarcinoma of colon metastatic to liver. Patient 5: adenocarcinoma of colon metastatic to liver.

lesion (Fig. 3) showed a marked uptake of ^{57}Co-BLEO but was negative with both ^{111}In-BLEO and ^{67}Ga-citrate.

Patient 4. A 65-year-old woman had adenocarcinoma of the colon metastatic to the liver. A ^{99m}Tc-sulfur colloid liver scan (Fig. 3) demonstrated multiple round areas of decreased activity. An ^{111}In-BLEO study was negative but the ^{57}Co-BLEO scan revealed areas of increased uptake corresponding to the metastatic lesions. A rectilinear ^{67}Ga-citrate liver scan (not shown) was negative.

Patient 5. A 54-year-old woman had a left hemicolectomy for adenocarcinoma of the colon several years before. Her ^{99m}Tc-sulfur colloid study (Fig. 3) showed a large area of decreased activity in the porta hepatis. Indium-111-BLEO did not visualize this tumor but ^{57}Co-BLEO and ^{67}Ga-citrate showed marked uptake in this metastatic liver lesion.

Patient 6. A 69-year-old man had a small malignant melanoma removed from his left foot in 1969. Left inguinal node dissection was negative at that time. He had a grand mal seizure in April 1973 and an EEG showed a right-sided focus. A ^{99m}Tc-pertechnetate brain scan (Fig. 4) was negative as was a ^{67}Ga-citrate brain scan. However, a brain scan using ^{57}Co-BLEO revealed a small lesion in the right frontoparietal region. Subsequently, an arteriogram confirmed the presence of a small metastatic lesion presumed to be malignant melanoma.

Patient 7. A 79-year-old woman had a carcinoma of the right breast and a radical mastectomy 15 years previously. At the time of this admission she had a mass in the left breast which was excised and was reported as an infiltrating ductal carcinoma. There was a 7-cm soft-tissue mass in the posterior aspect of the right lower lobe of the lung which was biopsied by needle; pathology was squamous cell carcinoma of the right lung. Surgery confirmed the diagnosis; hilar lymph nodes were negative. The ^{57}Co-BLEO (Fig. 5) clearly concentrated within the soft-tissue mass as did the ^{67}Ga-citrate. The ^{111}In-BLEO scan did not visualize the abnormality.

DISCUSSION

Gallium-67 citrate, currently in widespread use as a tumor imaging agent, has several distinct dis-

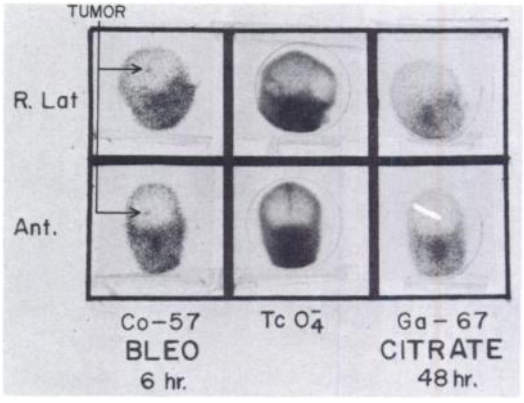

FIG. 4. Patient 6: melanoma metastatic to brain.

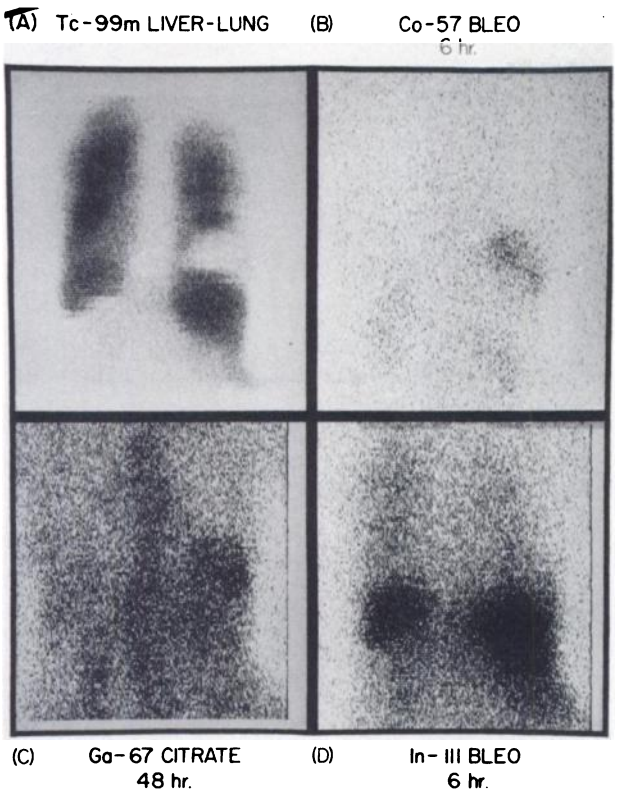

FIG. 5. Patient 7: squamous carcinoma of lung.

advantages. Prominent among these are (A) excretion into the gastrointestinal tract which makes interpretation in this area difficult, (B) a delay of 48–72 hr necessary between administration and imaging because of the relatively slow blood clearance and gastrointestinal excretion of ^{67}Ga-citrate, (C) a low degree of sensitivity in certain tumor types such as adenocarcinoma, and (D) a lack of specificity.

Labeled BLEO, in particular ^{57}Co-BLEO, would appear to have distinct advantages over ^{67}Ga-citrate in several respects. As noted in Patient 1, normally ^{57}Co-BLEO (Fig. 1A and 1B) is seen only in the kidneys and bladder with no localization in other body organs*. This is in marked distinction to ^{67}Ga-citrate (Fig. 1C) which normally accumulates in the liver, spleen, bowel, and bone marrow. This lack of localization of ^{57}Co-BLEO, particularly in the abdomen, represents a definite advantage over ^{67}Ga-citrate.

Blood levels of ^{57}Co-BLEO are significantly lower at all times after injection than are blood levels of ^{67}Ga-citrate, permitting enhanced lesion-to-blood ratios and lesion visualization as early as 6 hr after injection. This fact allows diagnostic information to be more rapidly obtained and considerably reduces patient inconvenience.

As noted in Table 1, positive studies were obtained with ^{57}Co-BLEO in 11 out of 15, or 73% of the patients whereas positive ^{67}Ga-citrate scans were obtained in only 7 out of 15, or 47% of the patients. These data are not sufficient to allow firm, statistically valid conclusions regarding the sensitivity of ^{57}Co-BLEO in particular tumor types but they do show that the tumor spectrum for this compound encompasses a wide variety of malignancies and is not limited to those specific malignancies for which BLEO is used therapeutically. In particular, the data suggest that ^{57}Co-BLEO may be a very good agent for the localization of adenocarcinoma for which ^{67}Ga-citrate is quite poor. Further clinical investigation is necessary to confirm the suggestion of significantly greater sensitivity of ^{57}Co-BLEO compared with ^{67}Ga-citrate.

One area in which there is little if any advantage of ^{57}Co-BLEO over ^{67}Ga-citrate is that of tumor specificity. The scans obtained in Patient 2 (Fig. 2) show localization of both labeled forms of bleomycin and ^{67}Ga-citrate in the pyogenic inflammation of his thoracotomy wound.

A distinct disadvantage of ^{57}Co-BLEO is the long physical half-life of ^{57}Co. Although rapid excretion of the ^{57}Co results in a relatively low patient radiation dose, it does present significant problems of contamination and waste disposal in the hospital. It

TABLE 1. COMPARATIVE STUDIES* OF ^{57}Co-BLEO, ^{111}In-BLEO, AND ^{67}Ga-CITRATE

Patient	Diagnosis	^{57}Co-BLEO	^{111}In-BLEO	^{67}Ga-Citrate
1. HY	CA, stomach	—	ND	—
2. DL	Squamous CA, lung	—	—	—
3. EF	Squamous CA, lung	4+	—	—
4. MH	Liver mets, adeno CA	3+	—	±
5. JF	Liver mets, adeno CA	3+	—	4+
6. EW	Brain mets, melanoma	2+	ND	—
7. MP	Squamous CA, lung	3+	—	3+
8. LB	Melanoma, chest wall	4+	ND	3+
9. TS	Lymphosarcoma	3+	1+	4+
10. HR	Undifferentiated CA	2+	—	4+
11. OJ	Liver mets, adeno CA	—	ND	2+
12. JP	Liver mets, adeno CA	2+	ND	±
13. JM	Hepatoma	4+	ND	±
14. DH	Liver mets, adeno CA	1+	±	—
15. MF	Liver mets, adeno CA	±	1+	1+
Studies performed		15	9	15
Positive studies:		11	2	7
Superior studies:		7	0	4

* Semiquantitative evaluation of activity concentration in tumor with a scale of 0–4 (ND = not done).

* In contradistinction to the normal where little or no ^{57}Co activity is seen in the liver, in some patients who have an abnormal liver, such as Patient 4 (Fig. 3) and Patient 5 (Fig. 3), there is significantly more localization of ^{57}Co-BLEO in the uninvolved portions of the liver.

was this problem which prompted the investigation of ^{111}In, a nuclide with many desirable physical properties, as a substitute for ^{57}Co in the labeling of BLEO.

Clinical results with ^{111}In-BLEO were quite poor as noted in Table 1. Of the nine patients in which this agent was used, only two minimally positive studies were obtained. In addition to its failure to localize in the neoplastic lesions, ^{111}In, unlike ^{57}Co-BLEO, was noted to accumulate in the liver, spleen, and bone marrow and to have significantly higher blood levels (Fig. 2). The data strongly suggest that ^{111}In in some way either damages the bleomycin molecule or that it forms a much weaker chelate with BLEO than does ^{57}Co, and is broken down in vivo with the ^{111}In binding to transferrin. A recent study by Thakur (*9*) describing chromatography of human serum at various intervals after administration of ^{111}In-BLEO shows that this latter possibility does occur. This would account for the bone marrow localization and for the slower clearance from blood.

In addition to ^{111}In-BLEO prepared in our laboratory with ^{111}In we have also used a commercially available preparation of ^{111}In-BLEO in some of the patients presented in this study (Patients 3, 7, and 15) as well as in other patients in whom no comparative studies were performed. No improvement in tumor-localizing capability was noted with the commercial preparation although it is reported to be free of contaminating metals such as zinc. [Although the presence of stable metal contamination in ^{111}In obtained from various scans is thought to vary the binding properties of ^{111}In (*8*), we have observed little difference in diagnositic sensitivity among preparations with varying concentrations of metal contamination.] It is apparent that ^{111}In is a poor substitute for ^{57}Co as a label for BLEO.

The maximum whole-body dose for ^{57}Co and ^{111}In-BLEO, respectively, was calculated to be 0.03 and 0.15 rad as compared with 0.9 rad for ^{67}Ga-citrate (*10–12*). The critical organ in the case of ^{57}Co- and ^{111}In-BLEO is the bladder wall with a dose of 0.45 and 1.4 rad as compared with a ^{67}Ga-citrate dose of 1.2 rad to the kidney.

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