Clinical Studies with In-111 BLEDTA, A Tumor-Imaging Conjugate of Bleomycin with a Bifunctional Chelating Agent

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Indium-111 BLEDTA, a bleomycin analog containing an EDTA group, was used for tumor imaging in 110 patients with cancer. Scans with In-111 BLEDTA agreed with biopsy results in 75 of 95 patients (79% accuracy). A positive scan was obtained in 71 of 88 patients with a positive biopsy (81% sensitivity). In 21 of 95 patients (22%), the scan revealed tumor sites that had not been detected. The main limitation to visualization was the size of the tumor (1.5–2.0 cm diameter was the smallest size seen). Background radioactivity in the liver, spleen, and bone marrow also made tumor detection in these areas more difficult. The cause of this background, and of false-positive uptake in sites of inflammation, is correlated with specific radiolabeling of polymorphonuclear leukocytes by In-111 BLEDTA. Means of eliminating this background are discussed.

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Since the introduction of Co-57-labeled bleomycin for tumor imaging in 1972 (*1*, *2*), a need has been recognized for an alternative radiolabel providing similar tumor affinity but improved physical characteristics. The clinical results with Co-57 bleomycin, involving 1,000 patient studies reported by the French group (*3*), showed high sensitivity and specificity. These workers suggested that the major clinical applications of Co-57 bleomycin were in screening for metastases (especially in lung cancer), although the studies were positive for many tumor types. Other studies have confirmed these findings; Poulose et al. (*4*) suggested that Co-57 bleomycin was superior to Ga-67 citrate in a comparative evaluation of 50 patients, and Kahn et al. (*5*) demonstrated its usefulness as a screening procedure for metastases in 140 patients. However, the long physical half-life of Co-57 (270 days) has posed serious contamination problems, and none of the other radioisotopes of cobalt has the desired physical properties. Other metal chelates of bleomycin—including In-111, Cu-64, Cu-67, Tc-99m, Zn-62, and Ga-67 (*6*, *7*)—do not have the stability of the cobalt chelate in vivo, and it appears that this unusual stability of Co-57 bleomycin is an important reason for its favorable tumor-imaging properties. In addition, Kono et al. (*8*) have demonstrated that the uptake of Co-57 bleomycin in Ehrlich solid mouse tumor was more than twice that of metal-free bleomycin or other metal chelates of bleomycin. Thus the binding of cobalt produces not only a stable product but also one with possibly enhanced tumor uptake.

Our approach to the problem of in vivo dissociation of metal-bleomycin complexes was to block the native metal-binding site with stable cobalt(III) and then to add a much more powerful metal-chelating group (EDTA) to a nonessential site in the "terminal amine" region of bleomycin (*9*). The resulting bleomycin conjugate, BLEDTA, when labeled with In-111, had excellent tumor imaging properties in tumor-bearing rabbits and mice (*7*) and in preliminary clinical trials (*10*). The present study reports the clinical use of In-111 BLEDTA in 110 patients referred to the Nuclear Medicine Laboratory for localization of their cancer sites.

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MATERIALS AND METHODS

The synthesis of BLEDTA is described in detail elsewhere (*9*). We chose a parasubstituted 1-phenylethylenedinitrilotetraacetic acid group that is known to form metal chelates that are unusually stable both thermodynamically and kinetically (*11*, *12*). In order to enhance tumor detection and to block the intrinsic metal-binding site of bleomycin, the nonradioactive cobalt(III) complex with bleomycin A_2 was first prepared. Then the EDTA-containing moiety was covalently attached to the sulfur atom in the "terminal amine" group of bleomycin by successive demethylation and alkylation, to yield BLEDTA (*9*).

BLEDTA was stored in instant radiopharmaceutical kits, each containing only 50 μl of a 0.54 m*M* solution (54 μg BLEDTA). When the radiopharmaceutical was required for a patient, one of the kits was labeled with In-111. Fifty microliters of 0.01 *M* citric acid containing 1-2 mCi of carrier-free In-111 chloride were added and, after 5 min at room temperature, the resulting solution was diluted with normal saline for injection. Product purity was assessed by thin layer chromatography (*9*). The specific activity of a 2 mCi preparation was 70 Ci/millimol. One to two mCi In-111 BLEDTA was given intravenously, and whole-body scans were performed at 18-24 hr following injection. Computer digitization of the whole-body images was done in some cases, permitting quantitation of excretion, organ concentration, and tumor-to-background ratios with selected regions of interest. Blood samples and 24-hr urine collections were taken in seven patients for calculation of blood disappearance and renal excretion. Surgical samples of tumor and normal tissues were obtained from three patients 24 hr following injection. In one patient extensive tissue samples were obtained from autopsy 8 days following injection.

A total of 110 patients were injected with In-111 BLEDTA. Twelve of them had no tissue diagnosis established, two were technically inadequate, and one had kinetic studies only. The remaining 95 biopsy-proven cases fell into three clinical groups: squamous cell carcinoma of the head and neck (n = 61), lung cancer (n = 20), and miscellaneous (n = 14). Each scan result was correlated with the biopsy diagnosis on a patient-by-patient basis, and the true-positive (TP), true-negative (TN), false-positive (FP), and false-negative (FN) rates were calculated.

A similar analysis was carried out on a *site-by-site* basis using other diagnostic criteria, such as physical findings, x-ray, bone and liver scans, and tissue diagnosis. The results were expressed as the number of TP, TN, FP, and FN *sites*. In this analysis the patient groups are not mutually exclusive; for example, the same patient may have both true-positive and false-positive sites, as well as other combinations. Since tissue confirmation was not obtained for several of the sites, the true diagnosis in these cases is subject to clinical uncertainty.

RESULTS

Thin layer chromatography of urine collected up to 24 hr after injection showed that 87% of the radioactivity migrated with $R_f = 0.4$, the same as BLEDTA (*10*). The

TABLE 1. In-111 BLEDTA DOSIMETRY

Organ	% Uptake	$T_{1/2\ Eff}$	$\tilde{A}$ (μCi-hr)*	rads/mCi†
Whole body:				
White cells	2.0	67.4 hr	1940	
3-Component Excretion from plasma to organs:				
1	90.0	0.833 hr	104	
2	7.0	2.1833 hr	220	
3	1.0	13.0 hr	187	
	98.0			
Other organs (not listed below)	6.5	67.4 hr	6309	
			8760	0.37
bone marrow	27.0	67.4 hr	26205	2.13
Spleen	4.7	67.4 hr	4562	4.33
Liver	19.5	67.4 hr	18926	2.60
Bladder (void every 4.8 hr)	40.3	43.0 hr	72.5	0.17

* Cumulated activity.

† Calculated using $\tilde{A}$ and "S Values" (absorbed dose per unit cumulative activity) as tabulated in Medical Internal Dose Committee (MIRD) Pamphlet No. 11.

TABLE 2. OVERALL RESULTS, RELATING BIOPSY AND In-111 BLEDTA SCAN RESULTS IN 95 PATIENTS

	Biopsy positive	Biopsy negative	
Scan positive	71	3	74
Scan negative	17	4	21
	88	7	95

Overall sensitivity = 71/88 = 81%; positive predictive value = 71/74 = 96%; and accuracy = 75/95 = 79%.

urine collected within the first 24 hr (n = 7) contained 40.3% ± 7.5 s.d. of the injected dose. The amount of radioactivity in the blood (n = 7) declined rapidly; $t_{1/2}$ (from 60–480 min) = 136 min ± 26 s.d. The percent of injected dose in the total circulating cells was ≃2% ± 1 s.d. at 2 and 24 hr; this represented 27% ± 18 s.d. of the whole-blood radioactivity at 2 hr and 85% ± 7 s.d. at 24 hr. When circulating cells were fractionated on a Ficoll-Hypaque gradient (*13*), almost all of this cellular activity was associated specifically with the polymorphonuclear leukocytes, with less than 1% in erythrocytes, platelets, and lymphocytes.

Patient's tissue samples (n = 3) contained from 2.3 × 10^{-3} to 6.0 × 10^{-3} percent of injected dose per gram of tumor at 24 hr, with tumor to normal tissue ratios of 3:1–11:1. Tissue samples from autopsy of a patient injected 8 days previously with 1.2 mCi In-111 BLEDTA all had very low In-111 BLEDTA concentrations: Approximately 12% of the dose remained in the body, with 3.0% in the liver, 1.0% in the spleen, and 5.0% in the bone marrow. In-111 BLEDTA dosimetry based on the blood and urine kinetics and tissue and whole-body scan data (unpublished information) is shown in Table 1.

An analysis of the overall scan results is shown in Table 2. The sensitivity, accuracy, and positive predictive value were high (≃80%). The lack of biopsy-proven negative cases made estimates of the specificity and negative predictive value unreliable.

Seventeen patients had false-negative (FN) scans. Ten of these were small head and neck or lung tumors approximately 2 cm in diameter or less; one patient was on bleomycin therapy; and one had liver metastases not visualized because of liver background. The cause of a negative scan in the remaining five is not clear, but may be related to cell type (prostatic cancer, pancreatic cancer, and an oligodendroglioma).

A *site-by-site* analysis of each clinical subgroup was undertaken in an attempt to show the cause and frequency of visualization or nonvisualization. In the head and neck cancer group (61 patients), 124 sites showed TP = 83, FN = 17, FP = 21, and TN = 3. The site sensitivity was 83%. A summary of these results is shown in Table 3. The scan was helpful in revealing new metastases in 29 sites (23%), especially in lymph nodes. False negatives occurred in tumors approximately 2 cm or less in diameter, although some tumors in this size range showed intense uptake (Fig. 1). Most of the false-positive sites were readily explainable on a clinical basis, such as in healing tracheostomy sites, surgical wounds, or radioactivity excreted in the bowel. One patient had a true-negative neck nodule confirmed by showing it to be normal thyroid tissue on I-123 scan, and one patient with suspected brain metastases had a negative scan confirmed at autopsy.

In the lung cancer group (20 patients), 30 sites showed TP = 22, FN = 5, FP = 2, and TN = 1. The site sensitivity was 81%. A summary of these results is shown in Table 4. Three previously unidentified bone metastases and one lymph node metastasis were visualized. Small primary lesions and mediastinal and liver metastases were missed. False-positive uptake was seen in a recently biopsied supraclavicular area.

In the miscellaneous group (14 patients), 28 sites showed TP = 11, FN = 13, FP = 1, and TN = 3. The site sensitivity was only 46% in this group. A summary of these results is shown in Table 5. Visualization of a primary adenocarcinoma of the colon in a 55-year-old male is seen in Fig. 2. Two patients with metastatic thyroid carcinoma and negative I-131 scans had intense uptake of In-111 BLEDTA. One patient had metastasis to the cervical spine with cord compression (Fig. 3) and

TABLE 3. 124 HEAD AND NECK CANCER SITES (61 PATIENTS)

A. True positive (83)	
I. Previously diagnosed (54)	
a. Primary sites	41
b. Metastatic sites	13
II. Previously undiagnosed (29) (all new metastatic sites)	
Lymph node	24
Bone	3
Retropharyngeal	1
Lung	1
B. False negative (17)	
a. Primary sites	
Small < 2 cm	4
Parotid tumors	2
Unknown cause	2
b. Metastatic sites	
Palate and esophagus	2
Lymph node	3
Lung	4
C. False positive (21)	
Tracheostomy and surgical incisions	8
Bone	3
Bowel	2
Lung	4
Various	4
D. True negative (3)	

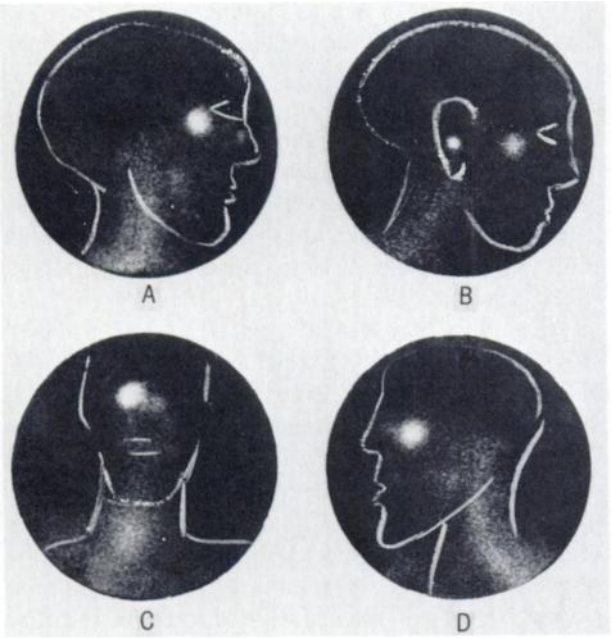

FIG. 1. White male, age 71, with squamous carcinoma of soft palate approximately 2.5 cm in diameter, shows intense uptake of In-111 BLEDTA (A) R lat, (B) R lat with radioactive markers over nose and external auditory meatus, (C) ant., (D) L lat.

the other had metastases to the lung and soft tissue in the neck and face (Fig. 4).

This group of miscellaneous tumors had the largest number of false negatives, possibly due to either tumor cell type or competitive inhibition of BLEDTA by bleomycin therapy. These included four primary sites—two pancreas, one oligodendroglioma, one testis (on bleomycin therapy)—and nine metastatic sites—one testis (from pancreas), one lung (from thyroid), one liver (from bladder), one bone (from prostate), a liver and lung (from testis, on bleomycin), one liver (from colon), and two lymph nodes (from lymphoma).

Overall, in 21 patients (22%) the scan revealed 36 sites that were not previously identified by other techniques.

TABLE 4. 30 LUNG CANCER SITES (20 PATIENTS)

A. True Positive (22)	
I. Previously diagnosed (18)	
a. Primary	9
b. Metastatic	
Bone	1
Liver	2
Lymph nodes	6
II. Previously undiagnosed (4)	
B. False negative (5)	
C. False positive (2)	
D. True negative (1)	

TABLE 5. 28 MISCELLANEOUS CANCER SITES (14 PATIENTS)

A. True positive (11)	
I. Previously diagnosed (8)	
a. Primary	
bladder, colon, & hemangiosarcoma	3
b. Metastatic	
2 lymph node, 1 bone, 1 brain, 1 eye	5
II. Previously undiagnosed (3)	
Metastatic: thyroid	3
B. False negative (13)	
a. Primary: (4)	
b. Metastatic (9)	
C. False positive (1)	
D. True negative (3)	
Spindle cell ca., bladder; benign prostatic hypertrophy.	

DISCUSSION AND SUMMARY

In addition to location and extent of the primary tumor, determination of metastatic spread is critical for patient management. Ideally, the presence of secondary cancer sites would be revealed, regardless of their location, by whole-body scanning with tumor-seeking radiopharmaceuticals. An idea of the effectiveness of In-111 BLEDTA for this purpose is obtained both from the number of patients with known cancer sites that failed to visualize (17 out of 95) and from the number of patients that had previously unidentified sites revealed on the scan (21 out of 95). The most frequent cause of nonvisualization was small size of the tumor (2 cm or less), although background radioactivity in the liver, spleen, and bone marrow made detection in these areas more difficult. In this respect Co-57 bleomycin is a superior agent, since body background other than in kid-

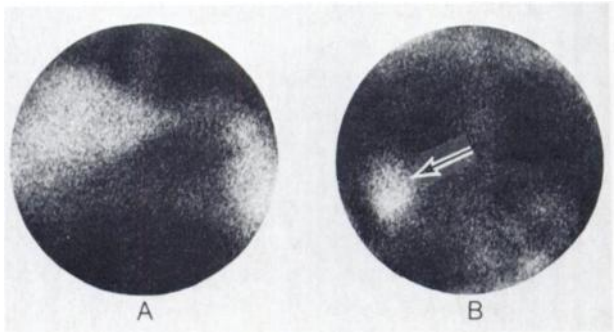

FIG. 2. White male, age 55, with well-differentiated adenocarcinoma measuring 7 cm in length at surgery, involving circumference of cecum and proximal ascending colon. Pathological exam showed extension through the serosa as well as lymph node metastases. In-111 BLEDTA image (A) shows anterior upper abdomen with normal liver and spleen, and (B) shows lower anterior abdomen with intense tumor activity in right lower quadrant (arrow). This activity remained unchanged at 24, 48, and 72 hr. Two barium enemas were difficult to interpret because of stool, but no large lesions were reported.

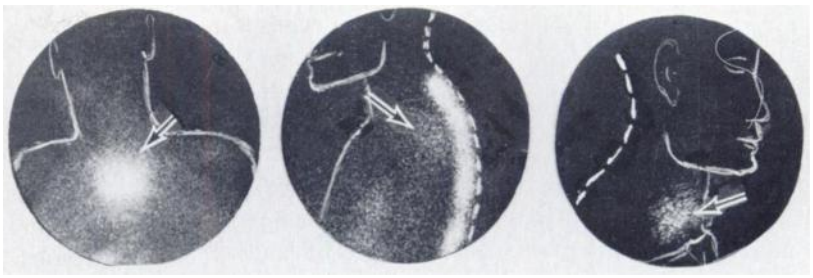

FIG. 3. White male, age 63, with papillary and follicular thyroid carcinoma metastatic to thoracic spine at T3 and T4. (A) Posterior thoracic spine shows intense In-111 BLEDTA uptake (arrow). (B) Left lateral thoracic spine with radioactive marker over spine shows posterior location of In-111 BLEDTA activity in spine (arrow). (C) Right lateral of cervical spine (dotted line) 24 hr after 1 mCi I-131 shows I-131 activity in remaining thyroid tissue anteriorly (arrow) but no activity in the thoracic spine metastasis.

neys and bladder is almost negligible (*14*).

The clinical results we obtained with In-111 BLEDTA are similar to those reported by other investigators using Co-57 bleomycin (*3–5*). In particular our results in head and neck cancers were comparable to recently published data in a similar group of patients with Co-57 bleomycin (*14*). The site sensitivity of In-111 BLEDTA in 61 patients with head and neck cancer was 83%, an improvement over the sensitivity of 56% recently reported for Ga-67 citrate in 65 head and neck cancer patients (*15*). However, the two series are not strictly comparable, as rectilinear scanning was used for the Ga-67 studies and the sensitivity may improve with the use of gamma camera and multiple views.

The In-111 BLEDTA plasma disappearance half-time was 2.17 hr, compared with 3.4 hours for Co-57 bleomycin; the 24-hr urinary excretion was 40.3% for In-111 BLEDTA, compared with 82% for Co-57 bleomycin (*16*). Unlike Co-57 bleomycin, the 24-hr whole-body scan with In-111 BLEDTA showed radioactivity in normal liver, spleen, and bone marrow; this pattern was also seen at earlier times (3–4 hr after injection). This rapid plasma disappearance and early marrow visualization are not characteristic of free ionic In^{3+} (*17*); and the absence of any contaminating In^{3+} was proven by chromatography of the injected In-111 BLEDTA, the plasma, and the urine. The majority of the urinary radioactivity (87%) had an R_f (0.4) identical to that of In-111 BLEDTA. These findings are consistent with our earlier observations that, once labeled, In-111 does not dissociate from the chelate under physiologic conditions and transferrin binding of In-111 does not occur (*12*).

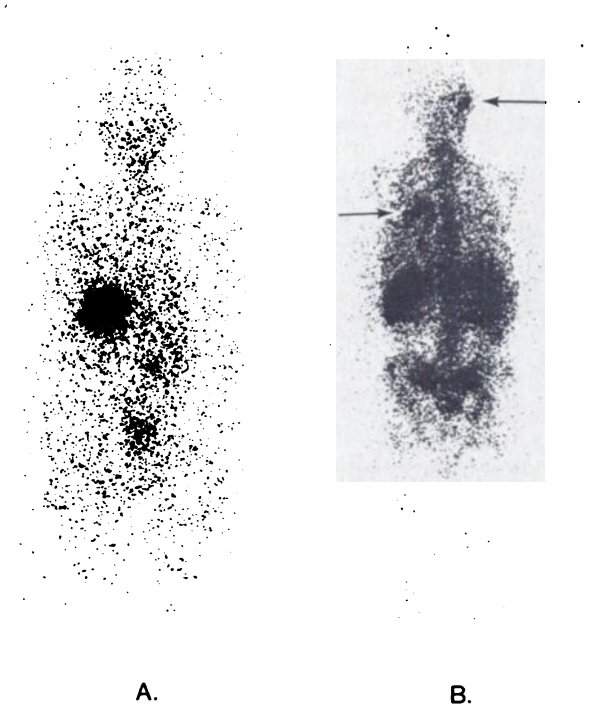

FIG. 4. White male, age 36, with rapidly growing anaplastic carcinoma. Posterior whole-body scans 24 hr after (A) I-131 and (B) In-111 BLEDTA. Arrows in (B) show metastases in right neck and face and left lung, not seen with I-131. Note normal background activity in stomach, gut, and bladder with I-131, and in the liver, spleen, and bone marrow with In-111 BLEDTA.

The evidence obtained from pharmacokinetic data in these patients as well as from whole-blood and white-cell labeling studies in vitro (unpublished data) suggests that the background radioactivity seen with In-111 BLEDTA is due to specific labeling of polymorphonuclear leukocytes. Eighty-five percent of the total blood radioactivity at 24 hr was associated with the polymorphonuclear leukocytes, with less than 1% in the lymphocyte, erythrocyte, and platelet fractions. Cobalt-57 bleomycin and In-111 transferrin did not label leukocytes in vitro. We have shown in preliminary studies using double-labeled (Co-57 and In-111) BLEDTA that WBC radiolabeling is associated with cleavage of the bond between bleomycin and the chelator; presumably this occurs at the sulfonium group, which can react with sulfhydryl groups and similar nucleophiles (*18*). Elimination of this white-cell labeling and its resultant background radioactivity in spleen, liver, and bone marrow should therefore be possible by use of a different (nonreactive) linkage between the chelate and the terminal amine group of bleomycin.

False-positive uptake of BLEDTA was usually due to inflammation or to recent surgical wounds or tracheostomies, which were often clinically evident. For this reason it was important to ascertain whether biopsy or some other surgical procedure had been performed within 2 wk of the scan. Other causes of false-positive

uptake were bowel radioactivity (usually associated with an upper respiratory infection) and osteomyelitis. Labeled white cells may partly explain these false-positive findings.

In summary, the results of these clinical studies support the usefulness of In-111 BLEDTA for tumor imaging and show promise for the development of other bleomycin conjugates with higher sensitivity and specificity. The new conjugates should have biological properties close to those of Co-57 bleomycin, but will have the added advantage of rapid labeling with In-111 and possibly Tc-99m for ease of clinical preparation.

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