DISTRIBUTION OF LABELED BLEOMYCIN IN NORMAL AND TUMOR-BEARING MICE

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The chemotherapeutic drug bleomycin has been labeled with ^{57}Co, ^{111}In, ^{67}Ga, and ^{59}Fe. Chromatographic analysis indicates that ^{57}Co, ^{111}In, and ^{67}Ga label various bleomycin fractions. Distribution studies in Ehrlich carcinoma-bearing mice indicate that ^{67}Ga- and ^{111}In-bleomycin do not clear as rapidly from the blood and liver as ^{57}Co-bleomycin and should be used with caution as a substitute for the ^{57}Co compound.

One approach in the search for tumor-localizing radiopharmaceuticals of greater specificity and diagnostic accuracy is to investigate available chemotherapeutic drugs. This paper deals with one such therapeutic compound, bleomycin, and the attempts to radiolabel this compound with an appropriate nuclide and evaluate the usefulness of the resultant radiopharmaceutical for the diagnosis and management of malignant tumors.

Bleomycin is a group of water and methanol soluble basic glycopeptide antibiotics isolated from the fermentation products of streptomyces verticillus in 1966 by Umezawa in Japan (*1*). The original material has been separated into 2 classes of 13 different water soluble peptides (A_1–A_6, A_2' and B_1–B_6) by column chromatography using Sephadex.

Distribution studies in normal and tumor-bearing mice have been performed using a biological assay method (*2,3*). The data derived from the biological method are not relevant to diagnostic applications because these results are affected by the ability of organs to reduce the antibacterial activity or inactivate the bleomycin. The distribution of ^3H-bleomycin in normal mice shows the highest concentration of radioactivity in skin followed by small intestine, muscle, liver, peritoneum, and lung (*4*). Bleomycin has exhibited specific antineoplastic activity against squamous cell carcinoma (*3*) and diagnostically should be most useful in this type of tumor.

A preliminary report from France has suggested that bleomycin can be labeled with ^{57}Co and used for the detection of various types of malignancy with considerable accuracy (*5*).

MATERIALS AND METHODS

Radioisotopes. Cobalt-57† was obtained as $CoCl_2$ in 0.5 *M* HCl at 50 mCi/ml. Gallium-67† was supplied as $GaCl_3$ in 0.05 *M* HCl at 1.0 mCi/ml. Iron-59† was obtained as the $FeCl_3$ in 0.5 *M* HCl. Indium-111 ‡ was supplied as $InCl_3$ in 0.05 *M* HCl. A radiopharmaceutical grade of ^{67}Ga-citrate was used†.

Bleomycin. Lyophilized bleomycin¶ was supplied in individual vials of 15 mg potency equal to 15 units. A typical batch contained 50–70% A_2, 24–33% B_2 and $<10\%$ A_1 and B_1.

Standard labeling procedure. All isotopes were diluted with 0.1 *N* HCl to obtain required specific concentration for counting purposes. Two solutions were prepared to obtain 0.5 units/ml bleomycin and 3.0 units/ml bleomycin:

1. 0.5 units/ml bleomycin: five milliliters of the radioisotope in 0.1 *N* HCl are added directly to a vial of 15-units lyophilized bleomycin. This solution is transferred to a 50-ml beaker with a syringe and stainless steel needle. To this solution is added 20 ml of the labeling radionuclide in 0.1 *N* HCl and 4.9-ml stock solu-

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tion of 0.5 *N* NaOH. This mixture is immediately taken to pH 6.5 using 0.5 *N* NaOH, 0.05 *N* NaOH, and 0.01 *N* NaOH. The final volume is 30 ml.

2. 3.0 units/ml bleomycin: four milliliters of the radioisotope in 0.1 *N* HCl is added directly to the vial of lyophilized 15-units bleomycin. This solution is transferred to a 10-ml beaker with syringe and stainless steel needle. To this solution is added 0.68 ml of 0.5 *N* NaOH. The solution is immediately taken to a final pH of 6.5 using 0.5 *N* NaOH, 0.05 *N* NaOH, and 0.01 *N* NaOH. The final volume is 5 ml.

Tumor-bearing mice. An ascites form of Ehrlich carcinoma* was used to produce a solid tumor in the subcutaneous tissue of the right lower quadrant of the abdomen of 20–25 gm Swiss mice. The tumor is allowed to develop at least 10 days before injection with the radioisotopic bleomycin. The mice were injected with 0.1 ml solution into the tail vein. The average percent dose per gram of tissue for four mice and the standard deviation is reported.

Chromatography. Two chromatography systems suggested by Umezawa, et al (*6*) were used: (A) Whatman No. 1 paper in 10% ammonium chloride, and (B) Baker TLC Silica plates in 1:1 mixture of 10% ammonium acetate and methanol. The major component A_2 was identified in the 3 units/ml bleomycin solutions by I_2 stain.

RESULTS

Paper chromatography of ^{57}Co-bleomycin indicates that the A_2 fraction ($R_f = 0.85$) is being labeled and that a small percentage of the activity is contained in the shoulder of the peak at the R_f of B_2. This is illustrated more clearly in the TLC system which clearly separates A_2 ($R_f = 0.40$) and B_2 ($R_f = 0.67$) and shows about 75% in the A_2 fraction. Both chromatographic systems gave reproducible results for ^{57}Co-bleomycin throughout the course of the study. Control studies with $CoCl_2$ demonstrated a fast-moving component on TLC which does not coincide with A_2 or B_2.

Chromatographic analysis of ^{67}Ga-bleomycin gave similar labeling distribution on the TLC system; that is, about 75% labeling of the A_2 fraction and the remainder bound to B_2. Paper chromatography showed a higher percentage of A_2 labeling and a reciprocal decrease of B_2 labeling, apparently due to an interaction of B_2 with the paper or impurities in the ^{67}Ga-chloride solution. Control studies with $^{67}GaCl_3$ at pH 7 showed origin material in both systems indicating gallium oxide.

The ^{111}In-bleomycin chromatography was nonreproducible on the paper system. Radioactive peaks were found with R_f values varying between 0.51 and 0.93 with multiple peaks appearing in some determinations. The distribution from the TLC system was more reproducible. Again two peaks were obtained with the majority of the ^{111}In coinciding with the A_2 peak ($R_f = 0.40$) as determined by I_2 stain. The B_2 peak varied in percentage ^{111}In from 0 to 20%. This variation is apparently due to impurities in the $InCl_3$ solution or the weak nature of the chelate (*7*) because ^{57}Co-bleomycin preparations of the same lot have not showed this variation. Chromatography of the $InCl_3$ solution at pH 7 in both

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TABLE 1. DISTRIBUTION OF RADIOACTIVE LABELED BLEOMYCIN IN EHRLICH CARCINOMA-BEARING MICE

Isotope	Injected bleo (mg/kg)	Time (hr)	% dose gm^{-1}* (±s.d.)					
			Lung	Tumor	Blood	Liver	Skin	Muscle
^{57}Co	2.5	1	1.03 (0.28)	3.73 (1.67)	0.54 (0.04)	1.23 (0.34)	0.91 (1.02)	2.31 (3.41)
	2.5	4	0.14 (0.08)	1.34 (0.55)	0.02 (0.01)	0.40 (0.20)	0.14 (0.06)	0.06 (0.01)
	2.5	24	0.08 (0.03)	0.72 (0.23)	0.01 (0.00)	0.25 (0.06)	0.11 (0.05)	0.03 (0.00)
	15.0	1	1.22 (0.28)	3.16 (0.41)	0.55 (0.04)	1.54 (0.16)	0.78 (0.19)	0.69 (0.72)
	15.0	4	0.18 (0.08)	2.66 (0.42)	0.02 (0.00)	0.40 (0.07)	0.14 (0.01)	0.08 (0.04)
	15.0	24†	0.19 (0.09)	1.78 (0.49)	0.04 (0.04)	0.56 (0.19)	0.22 (0.06)	0.15 (0.05)
^{111}In	2.5	1	6.47 (6.02)	1.12 (0.32)	1.95 (0.03)	1.12 (0.11)	1.17 (0.35)	0.36 (0.07)
	2.5	4†	0.85 (0.19)	0.94 (0.34)	1.13 (0.10)	1.05 (0.15)	0.83 (0.39)	0.32 (0.09)
	2.5	24	1.10 (0.18)	1.20 (0.14)	0.35 (0.11)	2.70 (0.23)	1.19 (0.25)	0.43 (0.19)
	15.0	1	1.11 (0.31)	1.26 (0.32)	1.89 (0.42)	1.30 (0.27)	1.13 (0.20)	0.40 (0.12)
	15.0	4†	0.93 (0.10)	1.09 (0.46)	1.11 (0.18)	1.12 (0.30)	0.95 (0.12)	0.41 (0.10)
	15.0	24	0.97 (0.56)	1.95 (0.35)	0.43 (0.15)	2.41 (0.47)	2.91 (2.81)	0.68 (0.24)
^{67}Ga	2.5	1	1.62 (0.35)	1.41 (0.27)	2.67 (0.28)	3.57 (0.84)	1.40 (0.64)	0.44 (0.14)
	2.5	24	0.84 (0.13)	1.18 (0.14)	0.64 (0.08)	4.80 (0.98)	0.51 (0.09)	0.14 (0.02)
	15.0	1	1.45 (0.22)	1.31 (0.18)	2.04 (0.31)	3.07 (0.34)	1.30 (0.25)	0.36 (0.02)
	15.0	24	1.45 (0.47)	2.05 (0.95)	1.02 (0.33)	4.90 (1.04)	0.83 (0.11)	0.32 (0.07)

* Average value for four mice unless noted.
† Average value for three mice.

TABLE 2. DISTRIBUTION OF ^{67}Ga-CITRATE IN EHRLICH CARCINOMA-BEARING MICE

Time (hr)	% dose gm^{-1}* (±s.d.)					
	Lung	Tumor	Blood	Liver	Skin	Muscle
1	4.92 (0.70)	2.52 (0.52)	11.11 (2.45)	3.26 (0.80)	2.27 (0.27)	1.30 (0.19)
24	2.55 (0.54)	3.70 (1.03)	2.32 (0.94)	6.70 (1.43)	2.54 (0.65)	0.71 (0.23)

* Average value for four mice.

systems showed origin material indicating indium hydroxide.

Ferric chloride bound bleomycin at the 3 units/ml level but formed ferric oxide at the 0.5-units/ml level and was not pursued further.

The animal data are presented in Table 1; Table 2 contains ^{67}Ga-citrate data in the same tumor model. The ^{57}Co-bleomycin clears the blood and liver more rapidly than does the ^{111}In- and ^{67}Ga-bleomycin ($p < 0.05$ for all three times). The blood clearance is not significantly dose dependent. The absolute tumor uptake of the three labeled bleomycins is about equal for this tumor model. Therefore, tumor-to-blood and tumor-to-liver ratios are superior for ^{57}Co-bleomycin at 24 hr compared with ^{111}In- and ^{67}Ga-bleomycin. Tumor-to-nontumor ratios for the labeled bleomycins as a group were superior at 24 hr compared with ^{67}Ga-citrate.

DISCUSSION

To date, only the Hammersmith group have published distribution data, and neither of their reports included ^{57}Co-bleomycin. The early work (*8*) compared various indium compounds to gallium citrate. These data were obtained in Wistar rats at 72 hr and cannot be compared with our values. In later work (*9*) the same group published a comparison between ^{67}Ga-, ^{99m}Tc, and ^{111}In-bleomycin. The authors concluded that ^{111}In-bleomycin is superior because of the high tumor uptake and rapid clearance. Again ^{57}Co-bleomycin was not included and comparison is difficult. However, our data agree that labeled bleomycin may be a useful agent for tumor localization.

Although rapid excretion of the cobalt complex minimizes radiation dose to the body, it can create a significant contamination problem in the hospital. Therefore, an isotope with chemical properties similar to cobalt but with physical properties more suited for widespread clinical use must be found. The ^{111}In- and ^{67}Ga-bleomycin do not have the same biological characteristics as ^{57}Co-bleomycin and should be used with caution as a replacement for the cobalt compound. In this work and in a limited series of patients, ^{57}Co-bleomycin has been far superior to ^{111}In-bleomycin (*10*). We, therefore, suggest that other alternatives to ^{57}Co-bleomycin be sought.

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