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**IN-VITRO AND IN-VIVO CHARACTERIZATION OF RUTHENIUM-BLEOMYCIN
COMPARED TO COBALT- AND COPPER-BLEOMYCIN**

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INTRODUCTION

Bleomycin (BLM) has undergone extensive investigation both as a cancer chemotherapeutic agent, and as a carrier for radionuclides for tumor imaging. The available methods for the radionuclides used, however, have had limited effectiveness. Although labeling of BLM with ^{103}Ru has been reported earlier (1), we carried out a study to develop a more reproducible method of labeling particularly for use with BLIP (Brookhaven Linac Isotope Producer)-produced ^{97}Ru . Ruthenium-97 has favorable physical properties that make it ideal for imaging applications: decay by electron capture; γ 216 keV, 85%; $t_{1/2}$ 2.9 d. A novel method based on the reduction of Ru^{3+} to Ru^{2+} using stannous chloride was investigated for labeling BLM with ^{97}Ru and/or ^{103}Ru . In-vitro and in-vivo comparison of the product(s) with ^{57}Co and ^{67}Cu -labeled BLM was also carried out.

MATERIALS AND METHODS

Radiolabeling.

The optimized method for labeling BLM with ruthenium was as follows. To an aqueous solution (9 mg/ml) of BLM, under slow argon bubbling, was added an aliquot (1-5 μ g tin) of a freshly prepared stannous chloride solution (1 mg/ml in 0.1N HCl). Carrier-free ^{97}Ru or ^{103}Ru trichloride in 3N HCl was added next, and the pH then adjusted to between 6 and 8 using dilute NaOH. The molar ratio of BLM to tin was kept between 27 and 135. The mixture was incubated at 37°C for 3 hours. Labeling with ^{57}Co and ^{67}Cu was carried out using a literature procedure (2).

Purification and quality control.

Radiolabeled BLM preparations were purified by column chromatography on CM-Sephadex C-25 using ammonium formate gradient elution (3). Purification on alumina column eluted with 0.9% NaCl was also effective. Thin-layer chromatography was performed on Merck silica gel plates using 10% (w/v) ammonium acetate/methanol (1:1). High-performance liquid chromatography (HPLC) was carried out on a μ porasil column using 0.3% ammonium formate in 50% methanol as the eluting buffer (4).

Animal biodistribution studies.

Normal BNL mice bearing melanoma were injected i.v. with the various preparations. The injectate (0.2 ml) contained 50 μ g BLM. The mice were sacrificed 24 h after injection and the radioactivity of collected tissue samples was measured and compared with appropriate standards.

RESULTS

The following labeling parameters for ruthenium-bleomycin were studied in detail: (1) effect of BLM concentration; (2) effect of stannous chloride concentration; and (3) effect of pH. The relative in-vitro stability of ^{103}Ru , ^{57}Co , and ^{67}Cu -labeled BLM was also evaluated. All preparations based on TLC analysis showed no evidence of label breakdown following storage at 4°C for 4 d, and after 24 h storage in air at room temperature.

The results are summarized in the following tables.

TABLE 1

TLC and HPLC Analyses of Labeled Bleomycin¹

Radionuclide	R _f values (TLC)				Retention times (HPLC), min		
	A ₂	B ₂	A ₁	?	A ₂	B ₂	A ₁
⁵⁷ Co	0.38	0.68	0.80	-	7.2,10.8	4.2	2.0
⁶⁷ Cu	0.38	0.68	0.80	-	7.0	3.0	-
¹⁰³ Ru	0.38	0.63	0.73	0.22	-	-	-

¹TLC on silica gel using 10% ammonium acetate/methanol (1:1); HPLC on μ -porasil column with 0.3% ammonium formate in 50% methanol as the eluting buffer. Activity associated with various BLM fractions was separated.

TABLE 2

Effect of Variables on the Labeling Yield of ^{103}Ru -Bleomycin

BLM (mg)	$\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (μg)	pH	Percent labeling yield
0.22	5	6	59
0.90	5	6	78
4.50	5	6	83
0.9	1	6	78
0.9	5	6	78
0.9	10	6	47
0.9	50	6	31
0.9	5	4	30
0.9	5	6	78
0.9	5	8	78

TABLE 3

Tumor to Organ Ratios of Labeled Bleomycin Preparations
in Melanoma Mice

Radiolabel	Blood	Muscle	Liver	Lung	% ID/g in tumor	%ID whole body
Cobalt-57	37	25	3	11	1.20	4.7
Copper-67	1.7	7.6	0.6	0.7	0.54	8.8
Ruthenium-97	3.4	5.4	0.7	1.0	0.23	6.0

24 h circulation time (n=5)

DISCUSSION

Results from this study demonstrate that bleomycin can be labeled with ^{97}Ru , ^{103}Ru , ^{67}Cu , and ^{57}Co with good labeling yields. An investigation of the various parameters has led to optimized labeling procedures for these radionuclides. Higher than 75% labeling yields for Ru-BLM are obtained using the following conditions: 1.8 mg BLM, 1-5 μg tin(II), pH 6-8, 3 h incubation at 37°C . Purification by CM Sephadex using an ammonium formate gradient (0.05-1.0 M) elution gave three u.v. peaks: BLM A_1 , B_2 , and A_2 , all containing a proportionate amount of radoruthenium. Purification on alumina columns eluted with 0.9% NaCl was also effective. TLC (silica gel) using 10% (w/v) ammonium acetate/methanol (1:1) gave good separations with R_f values of 0.73, 0.63, and 0.38 corresponding to BLM A_1 , B_2 , and A_2 , respectively. An unknown radioactivity peak at $R_f = 0.22$ was also present. HPLC analysis of Ru-BLM was not effective due to poor recovery from the column; however, ^{67}Cu -BLM and ^{57}Co -BLM were separated effectively into their component fractions.

Preliminary biodistribution data in melanoma-bearing mice indicate the following relative effectiveness of the three labeled BLM preparations for tumor imaging: $^{57}\text{Co} > ^{67}\text{Cu} > ^{97}\text{Ru}$. The tumor to tissue ratios for ^{67}Cu -BLM and ^{97}Ru -BLM were comparable; however, ^{57}Co -BLM provided much higher ratios and the greatest total uptake in tumor. Even though these results indicate Ru-BLM and Cu-BLM to be only modestly effective for imaging, other tumor models must be evaluated in order to more precisely determine the ultimate clinical potential of these compounds as tumor imaging agents.

These results also suggest that ^{55}Co and ^{67}Cu -labeled BLM may warrant evaluation for adjuvant radiation-chemotherapy applications.

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