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Comparison of Radiolabeled Bleomycins and Gallium Citrate in Tumor-Bearing Mice

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Radioiodinated bleomycin is a chemically stable radiopharmaceutical that can be prepared with high specific activity using ^{123}I. Its pharmacokinetics were compared with those of ^{99m}Tc-, ^{111}In-, and ^{57}Co-bleomycin, and ^{67}Ga citrate in mice bearing a transplanted KHJJ tumor. The in vivo kinetics and stability of ^{123}I- and ^{57}Co-bleomycin were similar: both were acceptable, although not equivalent, tags for bleomycin and, along with ^{67}Ga citrate, both had biologic properties suitable for tumor detection. Both ^{99m}Tc- and ^{111}In-bleomycin dissociated rapidly in vivo and hence do not represent legitimate tags for bleomycin. However, ^{111}In-bleomycin may have tumor-localizing properties related to its biochemical properties after the indium and chelate separate in vivo. Iodine-123 is superior to either ^{57}Co or ^{55}Co. Tumor-to-blood and tumor-to-liver ratios were higher for I-bleomycin than for ^{67}Ga or Co-bleomycin. The nearly ideal nuclear properties of ^{123}I should complement the biologic properties of bleomycin and lead to a useful tumor radiodiagnostic agent.

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Bleomycin, a mixture of chemically similar glycopeptide antibiotics, has been investigated as a tumor-localizing radiodiagnostic agent (*1–5*). It exists naturally as a Cu^{2+} ligand (*6*), and this chelating ability has been exploited in most of the bleomycin tracers reported so far. Both divalent and trivalent ions attach to bleomycin, but divalent cations, especially Co^{2+}, form more stable bonds than such higher-valence cations as In^{3+} or $SnCl_2$-reduced technetium (*1*). Because of the suboptimal nuclear properties of the radionuclides available from divalent ions (e.g., ^{64}Cu, ^{57}Co, ^{62}Zn) and the instability of higher-valence complexes of bleomycin (e.g., with ^{111}In, ^{67}Ga, $^{99m}TcO_4^-/SnCl_2$), we have investigated the labeling of bleomycin with radioiodine. Bleomycin contains an imidazole ring in the histidine residue, and this has been successfully iodinated by both the iodine monochloride (ICl) and chloramine-T methods to provide covalently labeled bleomycin (*7–9*). We concentrated our investigations on the ICl product, which we found more stable in vitro (*8*). The comparative pharmacokinetics of ^{67}Ga citrate and radioiodine-, ^{99m}Tc-, ^{111}In-, and ^{57}Co-labeled bleomycin in mice bearing a transplanted KHJJ adenocarcinoma (*10*) were studied to determine the best radioactive label to use with bleomycin as an oncophilic radiodiagnostic agent.

MATERIALS AND METHODS

Radiopharmaceuticals. Bleomycin was obtained as the sterile copper-free sulfate and dissolved in distilled water to a concentration of 1 mg/ml. The ^{125}I and ^{131}I were obtained as sodium iodide in 0.1 *N* NaOH. Labeling was performed by the ICl method and in-

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volved the reaction of 140 μg of bleomycin (0.1 μmoles) in 0.2 ml of saline citrate buffer (0.15 *N* NaCl, 0.02 *N* Na citrate, pH 7.0). The radioiodine was added in a volume of 100 μl, after which 30 μl of 0.0033 *M* ICl, prepared by McFarlane's method (*11*), was added. The reaction mixture was shaken for 5 min and then allowed to react for at least 1 hr. The I-bleomycin was purified to greater than 98% by Dowex 1X-8 anion-exchange resin or Sephadex G-10 column chromatography.

Technetium-99m was obtained as pertechnetate from a generator, and labeling was accomplished by the method of Odori et al. (*12*) under a nitrogen atmosphere. Pertechnetate (≤200 μl) in saline was added to 200 μl of bleomycin solution, after which 100 μl of 0.013 *M* $SnCl_2$, freshly prepared in 1 *N* HCl, was added. The mixture was agitated slightly to ensure mixing, after which 10 μl of ascorbic acid (0.045 *M*) was added as a stabilizer.

Cobalt-57 was obtained as carrier-free $^{57}CoCl_2$ in 0.5 *N* HCl. Labeling followed the procedure of Grove et al. (*13*). Under a nitrogen atmosphere, 0.5 ml of $^{57}CoCl_2$ was added to 0.5 ml of bleomycin solution and the mixture was agitated. The acid solution was then titrated to pH 6.5 using 0.1 *N* NaOH. The labeled bleomycin preparation was analyzed for free radiocobalt by cellulose acetate electrophoresis. The chelate-labeled bleomycin consistently had a radiochemical purity of nearly 100%, and these products were not purified further. The ^{111}In-bleomycin and ^{67}Ga citrate were obtained commercially.

Animal studies. Pieces of KHJJ adenocarcinoma were transplanted by subcutaneous trocar injection into the flanks of adult Balb C mice weighing 20–25 gm (*10*). After 14 days the tumor weighed about 0.7 gm and was not grossly necrotic. At this time approximately 10 μCi of radiotracer (≤4 μg bleomycin in 50 μl) was injected into the dorsal tail vein. The total amount of radioactivity injected into each mouse was measured by counting the animal in a fixed geometry immediately after injection. A 20-ml standard of equivalent activity was counted in the same geometry. The mice were killed at selected times after injection, and samples of blood, skin, and flank muscle, as well as the entire bladder, lungs, liver, brain, intestines, kidneys, and tumor, were excised, weighed wet, and counted in a NaI(Tl) well. A 1-ml aliquot of the standard was counted in the same geometry so that absolute tissue concentrations could be expressed as a percentage of the injected dose per gram of wet tissue. The tails were counted and any mouse with more than 10% of the injected dose remaining in the tail was excluded from the study. Each group of distribution experiments contained three or more mice.

RESULTS

Tissue pharmacokinetic data are reported in Table 1 as percent injected dose per gram. In addition, the range is given as an indication of the spread in data.

The blood clearance of ^{67}Ga citrate was slowest and followed a single phase (Fig. 1). The clearances of ^{99m}Tc- and ^{111}In-bleomycin (not plotted) were biphasic, with only one-fourth clearing at a rate comparable to that of ^{67}Ga citrate; the remainder cleared much faster. The most rapid blood clearances were exhibited by I- and Co-bleomycin; the curves for both were biphasic but showed less than 1% of the injected dose remaining in the total blood volume at 2 hr after injection. Although these two clearances were similar (Fig. 1), the KHJJ tumor uptake of I-bleomycin was greater than that of Co-bleomycin (Fig. 2). The concentration of ^{67}Ga citrate in the tumor was highest at all times and increased gradually for 24 hr, whereas the labeled bleomycins reached peak tumor concentrations within 6 hr and decreased slightly for the remainder of the first day. The average tumor concentrations during the first day after injection for I-, In-, Co-, and Tc-bleomycin were 2.3, 2.4, 1.1, and 1.1, respectively, whereas the Ga citrate concentration increased continuously from 4.7 to 10.5.

Tumor-to-blood ratios for all five radiopharmaceuticals (Fig. 3) were calculated from the mean of the tumor-to-blood ratios of individual animals rather than the ratio of mean tumor concentration to mean blood concentration. Only the tumor-to-blood ratios for I- and Co-bleomycin were greater than unity during the first 6 hr. At later times the Ga citrate and In-bleomycin ratios increased significantly and in parallel; however, those for Tc-bleomycin never exceeded unity. The radiobleomycins showed much lower accumulation in the intestines, brain, liver, lung, and muscle than did gallium citrate (Table 1). There was a distinct separation, however, between the tumor-to-liver ratios for the metal-labeled bleomycins and I-bleomycin (Fig. 4). For the metal chelates the tumor-to-liver concentration ratio never reached 2, whereas for I-bleomycin it rose as high as 20.

DISCUSSION

In selecting the best radionuclide for labeling bleomycin as an oncophilic radiopharmaceutical, the physical, chemical, and biologic properties must all be considered. The nuclear properties of ^{99m}Tc, ^{111}In, and ^{123}I are nearly ideal for gamma imaging. The long physical half-life of ^{57}Co, on the other hand, may result in an excessive radiation dose to the patient and requires special radiation safety procedures.

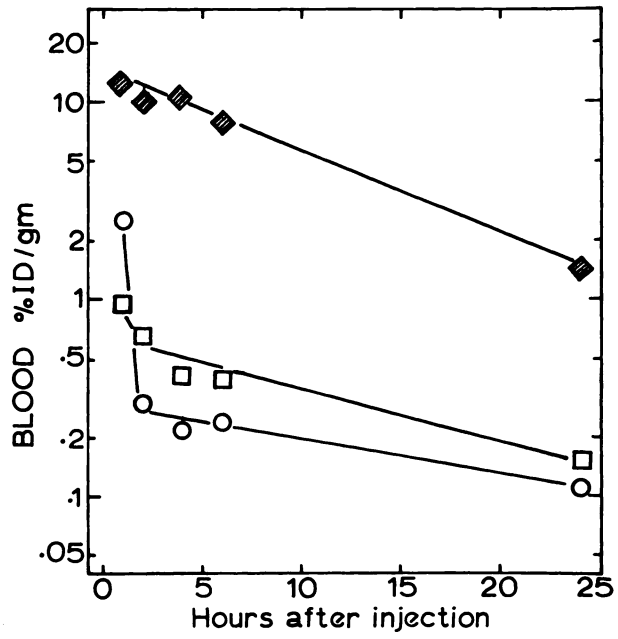

FIG. 1. Blood clearance of radiopharmaceuticals from KHJJ-tumor-bearing mice: I-bleomycin (○), Co-bleomycin (□), and Ga citrate (◆). Clearances of Tc-bleomycin and In-bleomycin were intermediate between those for Ga citrate and Co-bleomycin.

While these factors represent serious disadvantages, the shorter-lived ^{55}Co ($t_{1/2}$-18 hr) may be a useful label for bleomycin.

With the exception of cobalt, all of the chelates of bleomycin dissociate extensively in vivo (*1,14,15*), and this prompted us to look for a stable label for bleomycin. Radioiodination of bleomycin by the chloramine-T method was described in 1972 but was abandoned because the product deiodinated quickly (*14*); we encountered the same problem. In contrast, bleomycin labeled with ICl hydrolyzed at a rate of only 1.2% per day (*7*), and we therefore selected this product for animal investigation.

Bleomycin is a small molecule (MW 1,400) whose biologic activity and distribution may be significantly altered by added atoms, thereby making the labeled material an unsuitable tracer. If hydrolysis occurs, the distribution of the radioactive label is even less indicative of the pharmacokinetics of authentic bleomycin. We felt it was necessary to compare the time-dependent tissue distribution of each labeled bleomycin in the same tumor model. Three bleomycin chelates (Co, Tc, In) and covalently labeled bleomycin (I) were compared with gallium citrate in mice bearing a transplanted KHJJ adenocarcinoma. The absolute tumor concentration for ^{67}Ga citrate was much higher than that for any of the labeled bleomycins. The I- and In-bleomycin had equivalent tumor concentrations during the first day, but during the next day the radioiodine cleared from the tumor while ^{111}In radioactivity remained constant. Tumor accumulation of Co- and Tc-bleomycin was only half that of In- and I-bleomycin. Blood clearance of labeled bleomycins was much faster than for gallium citrate. The blood concentrations of

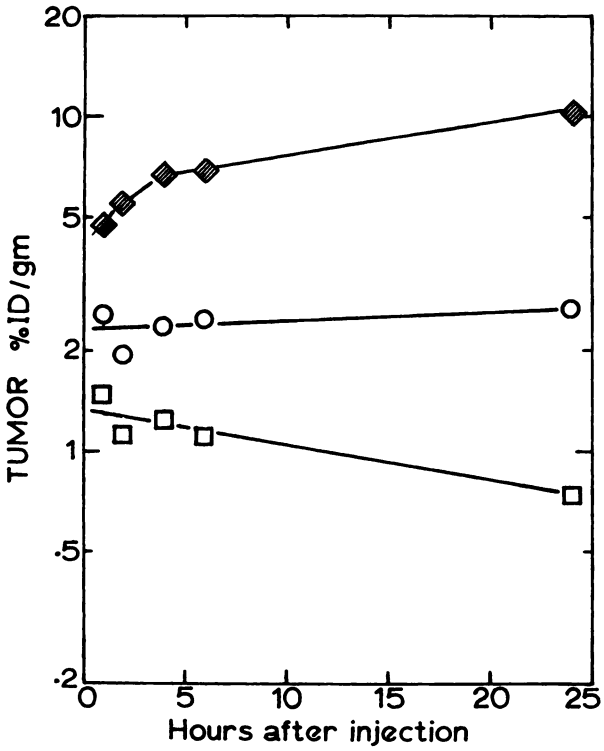

FIG. 2. Absolute tumor concentration of radiopharmaceutical in KHJJ tumors of mice: I-bleomycin (○), Co-bleomycin (□), and Ga citrate (◆). Tumor concentration of In-bleomycin was comparable with that of I-bleomycin during initial 24 hr after injection.

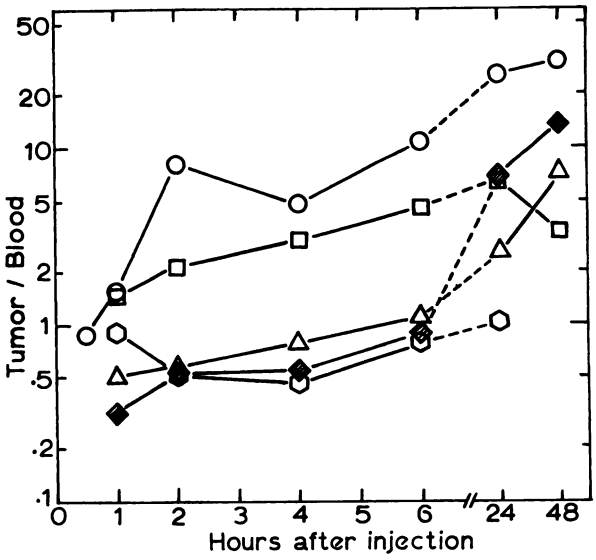

FIG. 3. Tumor-to-blood ratios for oncophilic radiopharmaceuticals in KHJJ-tumor-bearing mice: I-bleomycin (○), Co-bleomycin (□), Tc-bleomycin (⬡), In-bleomycin (△), and Ga citrate (◆).

TABLE 1. DISTRIBUTION OF ONCOPHILIC RADIODIAGNOSTIC AGENTS IN KHJJ-TUMOR-BEARING MICE*

DISTRIBUTION AT 2 HOURS

Organ	^{131}I-bleomycin (n = 6)	^{67}Ga citrate (n = 3)	^{99m}Tc-bleomycin (n = 3)	^{57}Co-bleomycin (n = 3)	^{111}In-bleomycin (n = 3)
Tumor	2.05 (2.64)	5.76 (2.88)	1.24 (1.25)	1.19 (0.54)	2.89 (0.81)
Blood	0.30 (0.33)	10.13 (4.07)	2.14 (0.89)	0.66 (0.58)	5.06 (0.37)
Liver	0.36 (0.44)	7.56 (3.77)	2.18 (0.52)	1.05 (0.55)	2.19 (0.57)
Lung	0.39 (0.24)	7.15 (2.93)	1.05 (0.14)	0.60 (0.17)	3.58 (1.64)
Muscle	0.15 (0.23)	1.65 (0.16)	0.25 (0.16)	0.39 (0.39)	0.71 (0.17)
Skin	2.10 (3.48)	4.25 (3.61)	1.97 (2.04)	23.11 (37.7)	7.57 (7.07)
Intestines	0.65 (0.78)	2.61 (0.90)	2.22 (0.84)	1.41 (1.37)	1.43 (0.27)
Kidneys	3.79 (6.43)	9.09 (3.65)	8.45 (0.97)	3.55 (0.35)	11.00 (2.89)
Brain	0.06 (0.00)	0.56 (0.27)	0.07 (0.01)	0.06 (0.06)	0.24 (0.14)
Bladder	2.93 (5.05)	5.05 (1.98)	1.84 (3.51)	3.87 (3.67)	37.68 (105)

DISTRIBUTION AT 6 HOURS

Organ	^{131}I-bleomycin (n = 5)	^{67}Ga citrate (n = 5)	^{99m}Tc-bleomycin (n = 3)	^{57}Co-bleomycin (n = 3)	^{111}In-bleomycin (n = 3)
Tumor	2.47 (3.34)	6.94 (3.87)	1.04 (1.23)	1.12 (0.27)	2.22 (0.29)
Blood	0.24 (0.15)	8.16 (3.79)	1.36 (0.28)	0.40 (0.63)	2.05 (0.70)
Liver	0.20 (0.16)	4.53 (0.36)	1.87 (0.42)	1.04 (0.50)	1.84 (0.22)
Lung	0.17 (0.15)	6.57 (1.14)	0.82 (0.35)	0.37 (0.10)	1.57 (0.15)
Muscle	0.08 (0.10)	1.30 (1.33)	0.16 (0.14)	0.08 (0.02)	0.34 (0.05)
Skin	0.34 (0.23)	2.40 (2.42)	1.38 (1.56)	0.60 (0.37)	1.28 (0.31)
Intestines	0.68 (1.19)	3.13 (1.56)	2.81 (1.82)	1.08 (1.46)	1.29 (0.67)
Kidneys	2.43 (0.85)	8.54 (3.53)	7.22 (3.68)	3.74 (0.07)	9.03 (1.11)
Brain	0.02 (0.01)	0.43 (0.25)	0.06 (0.02)	0.03 (0.02)	0.10 (0.08)
Bladder	0.35 (0.48)	2.71 (32.3)	4.23 (11.3)	2.72 (4.37)	1.50 (1.56)

DISTRIBUTION AT 24 HOURS

Organ	^{131}I-bleomycin (n = 3)	^{67}Ga citrate (n = 3)	^{99m}Tc-bleomycin (n = 3)	^{57}Co-bleomycin (n = 3)	^{111}In-bleomycin (n = 3)
Tumor	2.71 (1.70)	10.50 (10.6)	1.34 (1.90)	0.74 (0.05)	2.58 (0.75)
Blood	0.11 (0.07)	1.47 (0.91)	3.94 (9.04)	0.15 (0.21)	0.99 (0.11)
Liver	0.08 (0.08)	5.27 (3.49)	2.49 (3.20)	0.72 (0.27)	2.16 (0.83)
Lung	0.08 (0.03)	2.66 (2.48)	0.91 (1.38)	0.19 (0.07)	1.40 (0.05)
Muscle	0.02 (0.00)	0.31 (0.32)	0.13 (0.28)	0.05 (0.01)	0.41 (0.46)
Skin	0.17 (0.21)	1.10 (1.09)	0.88 (0.81)	0.40 (0.21)	1.93 (1.00)
Intestines	0.09 (0.02)	5.54 (4.39)	1.48 (1.70)	0.27 (0.24)	1.77 (1.92)
Kidneys	1.71 (0.38)	9.20 (9.61)	11.51 (18.5)	2.26 (0.12)	9.14 (4.38)
Brain	0.02 (0.01)	0.18 (0.13)	0.11 (0.18)	0.02 (0.01)	0.11 (0.03)
Bladder	0.43 (0.66)	1.39 (1.59)	1.28 (2.51)	0.49 (0.58)	1.90 (0.72)

DISTRIBUTION AT 48 HOURS

Organ	^{131}I-bleomycin (n = 3)	^{67}Ga citrate (n = 3)	^{57}Co-bleomycin (n = 3)	^{111}In-bleomycin (n = 3)
Tumor	0.60 (0.62)	6.28 (2.35)	1.35 (1.30)	2.83 (1.33)
Blood	0.02 (0.02)	0.50 (0.45)	0.38 (0.11)	0.48 (0.34)
Liver	0.07 (0.01)	4.44 (2.37)	2.03 (1.01)	2.40 (1.15)
Lung	0.03 (0.01)	3.96 (6.20)	0.53 (0.13)	1.86 (0.96)
Muscle	0.02 (0.01)	0.21 (0.08)	0.18 (0.07)	0.53 (0.28)
Skin	0.12 (0.08)	0.82 (0.56)	1.17 (0.81)	1.82 (2.20)
Intestines	0.02 (0.03)	2.70 (1.02)	1.32 (0.33)	1.07 (0.21)
Kidneys	0.65 (0.16)	8.07 (1.19)	7.48 (2.40)	8.53 (3.57)
Brain	0.01 (0.02)	0.14 (0.05)	0.06 (0.03)	0.12 (0.05)
Bladder	0.12 (0.15)	0.88 (0.28)	1.02 (0.29)	1.78 (1.11)

* Values are percentages of injected dose per gram of tissue: mean and width of range.

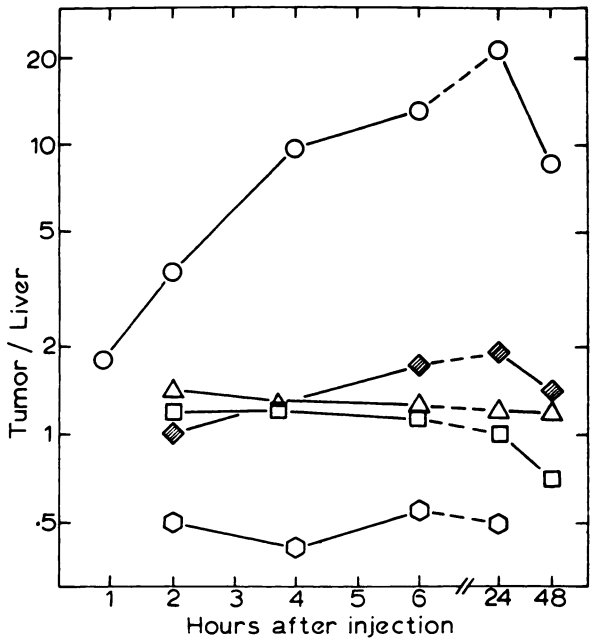

FIG. 4. Tumor-to-liver ratios for oncophilic radiopharmaceuticals in KHJJ-tumor-bearing mice: I-bleomycin (○), Co-bleomycin (□), Tc-bleomycin (⬡), In-bleomycin (△), and Ga citrate (◆).

^{111}In and ^{67}Ga, however, were nearly equivalent at 24 hr, probably as a consequence of the breakdown of ^{111}In-bleomycin with subsequent formation of ^{111}In-transferrin (*15*).

These differences in tumor and blood kinetics are illustrated in Fig. 3. The Tc- and In-bleomycin and Ga citrate have indistinguishable tumor-to-blood ratios for the first 6 hr, after which the ratios for In-bleomycin and Ga citrate rise. The ratio for Tc-bleomycin never exceeds unity. The tumor-to-blood ratios for I- and Co-bleomycin closely parallel each other, although the ratio for I-bleomycin is slightly higher, especially at 2 and 48 hr. A major problem associated with the clinical use of Ga citrate is the large accumulation in abdominal organs. The tumor-to-liver ratios of Tc-, In-, and Co-bleomycin were equivalent to, or lower than the ratio for Ga citrate, but I-bleomycin had a markedly higher ratio.

The similar in vivo kinetics and exceptional stability of I- and Co-bleomycin indicate that, of the nuclides evaluated, only iodine and cobalt provide legitimate radiolabels for tracer studies of bleomycin. Furthermore, the results of our mouse experiments suggest that ^{123}I-bleomycin is the best tumor-localizing radiodiagnostic agent when imaging is performed within the first day after injection. A clinical comparison of ^{123}I-bleomycin with ^{111}In-bleomycin and ^{67}Ga citrate has been initiated.

A number of pharmacokinetic investigations of oncophilic radiodiagnostic agents in animals have been reported. Lin and Goodwin (*16*) studied ^{99m}Tc-bleomycin in the KHJJ tumor model and found slightly higher tumor and blood concentrations than we found. However, their method of labeling did not involve the use of ascorbate as a stabilizer, and their specific activity was one-tenth of ours. We followed Odori's procedure for preparing Tc-bleomycin because the report of his clinical experience was enthusiastic.

Our tumor, organ, and blood concentrations for ^{111}In-bleomycin were intermediate between those of other investigators (*13,15,17,18*). Our KHJJ tumor concentrations averaged 1.8 times those in Ehrlich tumors (*13*) but were lower than in others. Our tumor-to-blood ratios in rodents were comparable with those reported by other investigators, except for one result in rats at 24 hr after injection (*15*). This result was significantly higher than our comparable measurement.

The reports of Grove et al. (*13*) and Higashi (*19*) agree quantitatively on the pharmacokinetics of ^{57}Co-bleomycin and ^{67}Ga citrate in mice with an Ehrlich carcinoma model. The KHJJ blood clearance of ^{67}Ga citrate in our studies was not significantly different from theirs, but tumor concentrations in the KHJJ model averaged 2.4 times those for the Ehrlich carcinoma at comparable times.

While the KHJJ tumor accumulated ^{67}Ga citrate and ^{111}In-bleomycin more avidly than did the Ehrlich tumor, we did not find an equivalent result for ^{57}Co-bleomycin. The tumor concentrations at 1 and 6 hr reported by Grove (*13*) and Higashi (*19*), respectively, were higher than ours, but our tumor concentration at 48 hr was greater than theirs. The 3-, 4-, and 24-hr measurements of all three groups are in agreement. The blood levels reported by Grove and Higashi were consistent with each other, but at times later than 1 hr after injection they averaged only one-twentieth of ours. Consequently, they obtained much higher tumor-to-blood ratios with the Ehrlich model. By way of reference, their blood clearances are faster than the clearance of ^{99m}Tc-Sn-DTPA reported by Konikowski et al. (*20*).

Furthermore, as part of another investigation designed to elucidate differences in tumor biology, we maintain a mouse mammary carcinoma in which we found tumor and blood concentrations for ^{57}Co-bleomycin at 2 and 24 hr equivalent to those in the KHJJ model.

Another difference between the Co-bleomycin investigations is the higher muscle-to-blood ratios in the Ehrlich model compared with KHJJ. Table 2 gives comparable ratios for divalent chelate-labeled bleomycin, tritiated bleomycin, and $CoCl_2$ in several tumor models. The gross difference in ratios correlates with the Ehrlich model rather than with the

TABLE 2. TUMOR-TO-MUSCLE RATIOS FOR RADIOLABELED BLEOMYCINS IN VARIOUS TUMOR MODELS

Radiopharmaceuticals	Tumor models	Muscle-to-blood ratio	Time range studied (hr)
^{57}Co-bleomycin (13)	Ehrlich/mice	3.2 ± 1.1	1–24
^{57}Co-bleomycin (19)	Ehrlich/mice	2.1 ± 1.2	3–48
^{57}Co-bleomycin†	KHJJ/mice	0.39 ± 0.14	1–48
^{57}Co-bleomycin (21)	Renal ca/rats	0.2*	17
$^{57}CoCl_2$ (21)	Renal ca/rats	0.3*	17
^{3}H-bleomycin (22)	Breast ca/rats	0.37 ± 0.21	2–72
^{64}Cu-bleomycin (22)	Breast ca/rats	0.24 ± 0.08	2–72

* Estimated from published graphs.
† This investigation (n = 18).

radiopharmaceutical or the host animal, which leads us to wonder whether there is something unique about the pharmacokinetics of ^{57}Co-bleomycin in the Ehrlich model. Unfortunately, one cannot readily ascertain which tumor model most closely approximates the human situation.

CONCLUSION

Iodinated bleomycin prepared by the iodine monochloride method is chemically and biologically the best radiolabeled bleomycin that we tested. It appears to be at least as useful as ^{67}Ga citrate for tumor localization. The nearly ideal nuclear properties of ^{123}I complement the biologic properties of bleomycin and suggest that ^{123}I-bleomycin may be a useful tumor radiodiagnostic agent.

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