Influence of Dimercaprol on the Early Hepatic Uptake of ^{111}In-Bleomycin in the BALB/c Mouse

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Dimercaprol (BAL) administered 1 hr before ^{111}In-bleomycin in the normal BALB/c mouse produced an early and preferential hepatic loading of ^{111}In-bleomycin without a loading of the spleen, skin, bone, or muscle. Liver-to-muscle ratios were increased about threefold under the influence of BAL. Liver (c BAL)/liver (s BAL) ratios also increased threefold at 3 hr whereas relative muscle uptake remained at about unity. Indium-111 chloride (colloid, pH 6.5) used as a control did not show a similar increase. The findings suggest that the kinetics and distribution of ^{111}In-bleomycin in the normal BALB/c mouse can be influenced by pretreatment with BAL.

J Nucl Med 17: 1083–1087, 1976

The use of the antitumor antibiotic, bleomycin (*1–4*), labeled with ^{111}In, ^{57}Co, or ^{99m}Tc for tumor localization, has been well documented (*5–15*). The advantages associated with the ^{111}In label include a relatively short half-life of 2.8 days, and gamma emissions at 173 keV (87%) and 247 keV (93%) with high photon-per-rad characteristics. These features are offset, however, by the disadvantage of several days' wait following administration of the radiopharmaceutical to allow for maximum tissue localization and elimination of unwanted background.

Evidence from studies of the mechanisms of action of bleomycin in growing cultures of bacteria and tumor cells suggested that certain thiols might influence the pharmacokinetics of bleomycin.

For example, it has been shown that one of the mechanisms of action of bleomycin is the production of single-strand DNA breaks (*16*). Frequency of these breaks is markedly increased on neutral and alkaline sucrose gradients in the presence of mercaptoethanol (*17*). Bleomycin also induces a severalfold increase in thymidine (H-3) incorporation into the DNA of isolated rat liver nuclei, presumably due to DNA-polymerase activity in response to DNA damage caused by bleomycin (*18,19*). This incorporation can be further increased severalfold by the addition of the monothiol, mercaptoethanol, and also by dimercaprol or "BAL" (2,3-dimercapto-1-propanol, a dithiol). These agents apparently enhance the DNA-damaging effects of bleomycin at the nuclear level, possibly by facilitating binding of bleomycin to DNA at the site at which damage is to occur.

The experiments reported below were conducted to determine whether an organic thiol would influence the kinetics and distribution of bleomycin in the nontumor-bearing (normal) BALB/c mouse. Indium-111 chloride was used as a control and as an index of the specificity of the interaction of the thiol and bleomycin. Since BAL is a heavy-metal chelating agent used in the treatment of heavy-metal poisoning in man (*20,21*), it was selected for study because of the possibility that its chelating effect might influence the distribution of the metal-containing bleomycin.

MATERIALS AND METHODS

The ^{111}In-bleomycin and ^{111}In chloride used in these experiments were obtained from Medi-Physics, Inc., Emeryville, Calif. The BAL* was diluted in peanut oil (10 mg/cm^3) just before use. Normal female BALB/c mice, weighing 20–25 gm and 12–16 weeks of age, were divided into four groups:

Received Oct. 31, 1975; revision accepted July 15, 1976.

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TABLE 1. INFLUENCE OF DIMERCAPROL (BAL) ON LIVER AND MUSCLE UPTAKE OF ^{111}In-BLEOMYCIN

Time* (hr)	BAL	Liver	t	Liver (c BAL)/ liver (s BAL)	Muscle	t	Muscle (c BAL)/ muscle (s BAL)	Liver/muscle	L/M (c BAL)/ L/M (s BAL)
1	+	4.65 ± 1.17	2.19	2.35 ± 0.73	0.66 ± 0.05	0.45	1.04 ± 0.09	7.07 ± 1.87	2.25
	−	1.99 ± 0.34			0.63 ± 0.03			5.63 ± 0.63	
2	+	3.48 ± 0.61	2.77	1.96 ± 0.36	0.41 ± 0.04	0.00	1.00 ± 0.18	8.42 ± 1.70	1.96
	−	1.77 ± 0.03			0.41 ± 0.06			5.96 ± 1.56	
2½	+	4.06 ± 2.21	1.04	2.32 ± 1.28	0.32 ± 0.07	0.60	0.88 ± 0.21	12.83 ± 7.78	2.65
	−	1.74 ± 0.11			0.36 ± 0.04			3.48 ± 0.90	
3†	+	6.20 ± 1.14	3.65	3.27 ± 0.81	0.71 ± 0.11	1.12	1.31 ± 0.33	8.72 ± 2.00	2.50
	−	1.90 ± 0.30			0.54 ± 0.11			4.85 ± 0.57	
3½	+	1.76 ± 0.43	0.79	1.24 ± 0.31	0.33 ± 0.05	1.41	1.41 ± 0.35	5.26 ± 1.56	0.88
	−	1.41 ± 0.06			0.24 ± 0.04			4.30 ± 0.66	
4	+	1.49 ± 0.16	0.04	1.00 ± 0.14	0.24 ± 0.04	0.67	0.90 ± 0.15	6.30 ± 1.17	1.12
	−	1.48 ± 0.13			0.26 ± 0.02			3.14 ± 0.56	

* Three mice per group.
† Nine mice in this group (three trials of three mice each).
Tissue data $\bar{X}$ % dose/gm ± 1 s.e.m.
t value for organ with (+) and without (−) BAL. Values < 2.77 are not significant for $n = 3$.
Ratio ± 1 s.e.

TABLE 2. INFLUENCE OF DIMERCAPROL (BAL) ON THE DISTRIBUTION OF ^{111}In-BLEOMYCIN AT 3 HR

	^{111}In-bleomycin (% dose/gm)					
	Skin	Muscle	Spleen	Bone (femur)	Liver	L/M
Drug alone*	1.43 ± 0.15	0.76 ± 0.06	1.07 ± 0.11	0.06 ± 0.01	1.94 ± 0.27	2.55
BAL followed by drug*	1.43 ± 0.18	0.77 ± 0.04	0.96 ± 0.21	0.08 ± 0.01	9.63 ± 1.57	12.41
t	0.03	0.13	0.44	1.76	4.81	

Results are expressed as $\bar{X}$ % dose/gm tissue ± 1 s.e., except for "bone" where they are expressed as $\bar{X}$ % dose per whole femur. Underlining indicates statistically significant differences between groups given BAL and those given no BAL at $p = 0.05$ level.
t value for organ with (+) and without (−) BAL.
* Three mice per group.

^{111}In-bleomycin, with and without BAL pretreatment, and ^{111}In chloride, with and without BAL pretreatment.

BAL (1.0 mg) was administered intraperitoneally 1 hr prior to the i.v. administration of ^{111}In-bleomycin and ^{111}In chloride (about 1 μCi/gm of mouse weight; pH 6.5 ± 0.25, 0.2 ml). At each preselected time interval after injection, the mice were killed by cervical dislocation, and the organs to be sampled were excised, lightly blotted on paper toweling to remove external blood or fluid, weighed, and counted by gamma spectrometry in a well counter. The whole femur was removed, cleaned of muscle and fat, and counted. Since mouse femurs contain a fairly constant proportion of marrow, and since the solid bone should not be involved in significant uptake (*22*), weights were not taken. Results are expressed as percent of dose per femur. For the other organs, the results were expressed as percent of dose per gram of tissue.

Data for groups consisting of three mice each, given the same treatment, were pooled and expressed as means ± 1 s.e. The 3-hr data for ^{111}In-bleomycin consist of three trials of three mice each (Table 1), and include the data from Table 2. When $n = 3$, t values of 2.77 and 4.60 or higher imply statistical significance at $p \leq 0.05$ and $p \leq 0.01$, respectively. When ratios between measurements were calculated, the s.e. was estimated as one-half the difference between the approximate upper and lower 68% confidence limits as previously described (*23*).

RESULTS

Results of the effects of prior administration of BAL on the uptake of ^{111}In-bleomycin in selected organs at 3 hr are illustrated in Table 2. When ^{111}In-bleomycin alone was used, hepatic uptake exceeded that in skin, muscle, and spleen on a percent dose/gm basis but did not exceed that in bone. (Comparison to bone could not be made on a percent dose/gm basis since this tissue was not weighed.) The liver-to-muscle ratio was 2.55, showing a greater

TABLE 3. INFLUENCE OF DIMERCAPROL (BAL) ON LIVER AND MUSCLE UPTAKE OF ^{111}In CHLORIDE

Time* (hr)	BAL	Liver	t	Liver (c BAL)/ liver (s BAL)	Muscle	t	Muscle (c BAL)/ muscle (s BAL)	Liver/muscle	L/M (c BAL)/ L/M (s BAL)
1	+	12.29 ± 0.84	4.43	0.75 ± 0.05	5.81 ± 0.97	2.28	1.82 ± 0.48	2.11 ± 0.39	0.41
	−	16.34 ± 0.35			3.19 ± 0.62			5.12 ± 1.05	
2	+	16.30 ± 0.90	3.13	1.49 ± 0.22	2.10 ± 0.26	9.80	0.41 ± 0.05	7.77 ± 1.08	0.62
	−	10.93 ± 1.46			5.11 ± 0.16			2.14 ± 0.29	
2½	+	13.96 ± 0.86	1.27	1.14 ± 0.08	2.73 ± 0.17	2.59	0.56 ± 0.04	5.11 ± 0.45	2.07
	−	11.66 ± 1.60			5.20 ± 0.94			2.50 ± 0.15	
3	+	16.07 ± 1.76	1.86	1.38 ± 0.25	3.33 ± 0.30	1.90	0.64 ± 0.13	4.83 ± 0.68	2.15
	−	11.66 ± 1.60			5.20 ± 0.94			2.24 ± 0.52	
3½	+	27.82 ± 3.96	3.52	2.02 ± 0.30	2.24 ± 0.25	10.30	0.46 ± 0.05	12.42 ± 2.28	4.44
	−	13.75 ± 0.56			4.92 ± 0.05			2.80 ± 0.12	
4	+	22.86 ± 4.57	1.45	1.41 ± 0.28	3.39 ± 0.32	2.64	0.70 ± 0.09	6.74 ± 1.50	1.60
	−	16.22 ± 0.43			4.83 ± 0.44			3.36 ± 0.32	

* Three mice per group.
Tissue data $\bar{X}$ % dose/gm ± 1 s.e.m.
t value for organ with (+) and without (−) BAL. Values < 2.77 are not significant for n = 3.
Ratio ± 1 s.e.

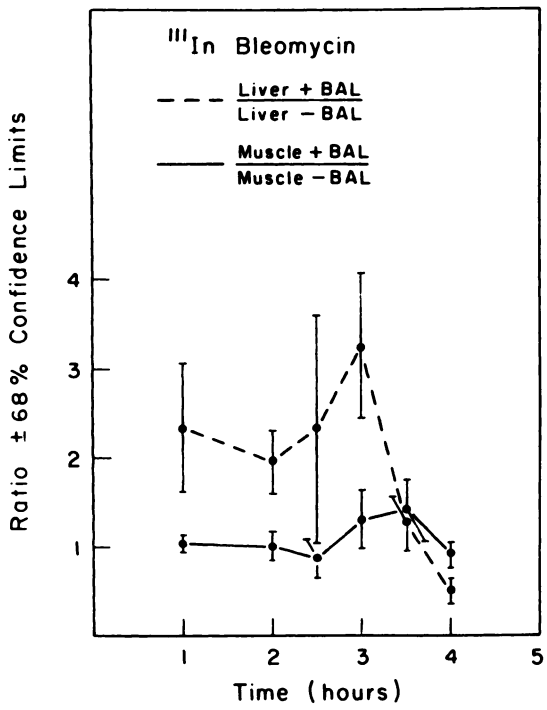

FIG. 1. Influence of BAL on uptake of ^{111}In-bleomycin as function of time. Organ ratio ± 1 s.e. with (+) and without (−) BAL administration 1 hr before ^{111}In-bleomycin.

concentration in the liver. Prior administration of BAL did not significantly influence ^{111}In-bleomycin uptake in skin, muscle, spleen, or bone ($p = 0.05$). However, BAL pretreatment did produce an approximately fivefold increase in hepatic uptake of ^{111}In-bleomycin, significant at the $p = 0.01$ level. Since BAL did not significantly influence muscle uptake, this advantage was observed in the liver-to-muscle ratio (12.4).

When ^{111}In chloride was used instead of ^{111}In-bleomycin (Table 3), the preferential localization of liver over muscle, in the absence of BAL, was again noted at 3 hr (L/M = 2.24), but BAL did not produce a large increase in hepatic localization at this time.

The influence of BAL on the hepatic and muscle uptake of ^{111}In-bleomycin as a function of time is illustrated in Table 1. In these experiments there is a consistent trend at all time periods for hepatic uptake of ^{111}In-bleomycin to be greater in the BAL-pretreated than in the nonpretreated group. These data are statistically significant ($p = 0.05$) at the 2- and 3-hr points. The muscle (c BAL)/muscle (s BAL) ratio (lower curve, Fig. 1) approximates unity at all time intervals and shows no statistically significant change.

Even in the absence of BAL, all liver-to-muscle ratios for ^{111}In-bleomycin were considerably greater than unity (3.1–6.0), reflecting the expected greater localization in liver as compared to muscle (Fig. 2). In the presence of BAL, the preferential hepatic loading effect was pronounced, with liver-to-muscle ratios of 5.3–12.8. At the 1-, 2-, 2.5-, and 3-hr intervals, this represented a 2–2.6-fold increase of uptake in comparison to that of mice not pretreated with BAL. The large s.e. seen at the 2.5-hr interval is not surprising, since this is kinetically a transition point with larger potential for variation due to sampling errors.

The effect of BAL on the distribution of ^{111}In chloride is illustrated in Table 3. A small increase in hepatic uptake is seen at 2, 2.5, and 3 hr as the result of administration of BAL. A twofold maximum increase was seen at 3.5 hr. Once again, reduction of muscle localization by 30–60% was seen at

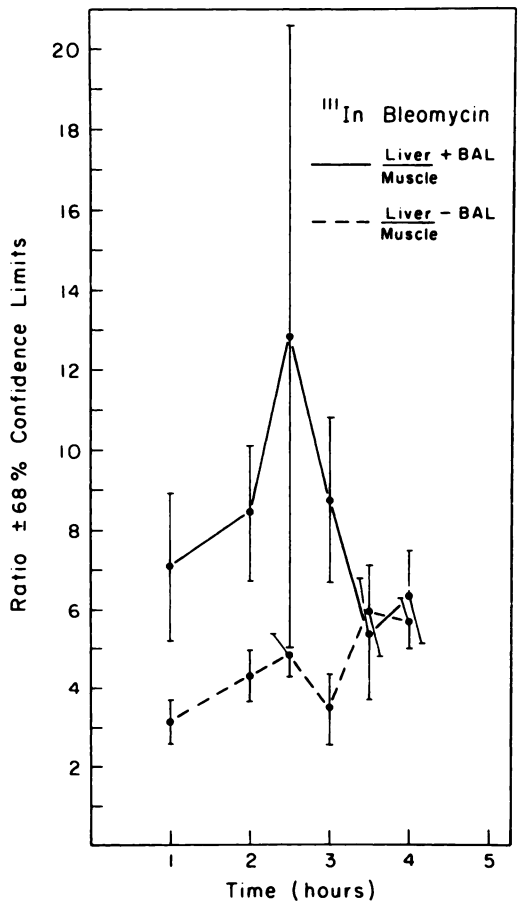

FIG. 2. Influence of BAL on liver-to-muscle ratios (± 1 s.e.) of ^{111}In-bleomycin as function of time.

all points except 1 hr [muscle (c BAL)/muscle (s BAL)]. The interaction of the slight hepatic localization, combined with the reduced muscle uptake, led to an increased liver-to-muscle ratio, resulting from BAL, at all points except 1 hr, producing an increase in the ratio [liver/muscle (c BAL)] : [liver/muscle (s BAL)].

DISCUSSION

These experiments show that pretreatment with 1 mg BAL, 1 hr before administration of ^{111}In-bleomycin, significantly increases the hepatic uptake of radiolabeled bleomycin in the mouse. This effect was time-dependent, peaking at 3 hr, and was largely due to specific hepatic loading, since it did not occur in skin, spleen, muscle, or bone. Since uptake in muscle was not significantly altered, the hepatic effect is one of loading.

Although a similar but smaller amount of liver localization occurs after the administration of ^{111}In chloride, this effect is exaggerated when the liver-to-muscle ratio in the BAL-pretreated group is considered, since muscle uptake is about half that of non-BAL-treated controls. We have seen no deaths in the mouse following the administration of BAL at this dose level.

The data suggest that the hepatic loading of ^{111}In-bleomycin at 2–3 hr following pretreatment with BAL is due largely to an influence on ^{111}In-bleomycin itself, since this loading effect is not elicited during this time interval following ^{111}In chloride administration. It is known that ^{111}In, administered as indium chloride, binds to transferrin in vivo (*5*). Since the loading effect induced by BAL is seen during the first 3 hr, it seems unlikely that this is due to the effect of BAL on indium that has dissociated from ^{111}In-bleomycin. However, we cannot rule out the possibility that BAL removes indium from bleomycin. This seems unlikely, however, since the distribution of ^{111}In chloride is not similarly influenced by BAL.

It is not known whether the loading effect exerted by BAL on the distribution of ^{111}In-bleomycin is related to alterations in circulation, membrane permeability, intracellular binding, drug–drug interaction, or nonspecific hepatic injury. The dose of BAL used in these experiments produces transient hyperactivity and diuresis in BALB/c mice, but it is tolerated, even on daily administration up to 14 days, without visible permanent side effects or fatality.

ACKNOWLEDGMENTS

We would like to thank Lee Scott, Cynthia Phillips, Marilyn Walter, and Jane Segal for their technical assistance, and Linda Tolstoi and Ginny Saville for typing the manuscript. We also acknowledge the cooperative support of Willie W. Smith, NCI Investigator. This work was supported in part by the American Cancer Society Institutional Research Grants IN58N and IN58M. Dr. Boggs is a Scholar of the Leukemia Society of America.

FOOTNOTE

* Supplied through the courtesy of Hynson, Westcott, and Dunning Corporation.

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