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PREPARATION AND DISTRIBUTION OF $^{195}Pt^{m}$-LABELED BLEOMYCIN

FRANCIS K.V. LEH and WALTER WOLF

Radiopharmacy Program, Cancer Hospital and Research Institute, and School of Pharmacy, Los Angeles County, University of Southern California Medical Center, Los Angeles, Calif. 90033 (U.S.A.)

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SUMMARY

Radiolabeled bleomycin is known to localize in soft tumors, and is believed to serve as a specific radiopharmaceutical for this diagnosis. In the present study ^{195}Pt-bleomycin complexes were synthesized with $PtCl_4K_2$ or $Pt(NH_3)_2$ as metal ligands, and the distribution of these complexes studied in mice bearing Ehrlich's ascites carcinoma. The results obtained suggest that these ^{195m}Pt complexes of bleomycin are very stable in the body as they remain unmetabolized for up to 96 hr in vitro and up to 48 hr in vivo, and that they complex much better to bleomycin than any of the $^{99}Tc^{m}$ complexes studied to date. As much as the highest concentration of ^{195m}Pt bleomycin radioactivity 1 hr post-injection is in the blood, it may also be traced in the lung, tumor, liver, and skin. Its blood clearance seems to be very slow. The possible use of ^{195}Pt-bleomycin for tumor diagnosis and management is discussed.

INTRODUCTION

Ever since the discovery that radiolabeled bleomycin localizes in a number of soft tumors (Grove et al., 1973a; Nouel et al., 1973; Uimo et al., 1973) attempts have been made to synthesize radiolabeled metal chelates of bleomycin in a search for specific radiopharmaceuticals for the diagnosis and management of malignant tumors. Bleomycin has been labeled with ^{57}Co (Grove et al., 1973a; Nouel et al., 1972; Watanabe et al., 1974), ^{64}Cu (Renault et al., 1971), ^{67}Ga (Grove et al., 1973a and b), $^{99}Tc^{m}$ (Lin et al., 1973 and 1974; Mori et al., 1973; Odori et al., 1974), and ^{111}In (Grove et al., 1973b; Emmerson et al., 1973; Goodwin et al., 1973; Thakur, 1973; Robbins et al., 1974; Yeh et al., 1974). Of these $^{99}Tc^{m}$-bleomycin is found to be too unstable to be a tumor scanning agent (Orii et al., 1974). The most popular agent of this series being used clinically is ^{111}In-bleomycin, although it is weakly complexed and dissociates readily, with ^{111}In binding instead to blood transferring, which in turn localizes in the bone marrow and other iron binding sites (Thakur et al., 1974). ^{57}Co-bleomycin is far superior to ^{111}In-, ^{67}Ga- and $^{99}Tc^{m}$-bleomycin due to its rapid clearance from the blood and liver (Grove et al., 1973a)

and its greater stability (Orii et al., 1974), but its very long physical half-life (270 days) imposes a necessary limitation on its use, whereas the half-life of the other radionuclides are so much shorter ($^{195}Pt^m$: 4.0 days; ^{67}Ga: 3.25 days; ^{111}In: 2.81 days; and $^{99}Tc^m$: 6 hr).

In a continued search for more strongly bound radionuclides to enhance bleomycin imaging, we have synthesized $^{195}Pt^m$-bleomycin complexes.

MATERIALS AND METHODS

Radionuclides. $^{195}Pt^m$ (Oak Ridge National Laboratory, Tenn.) * is obtained as $K_2{}^{195}Pt^mCl_6$ in 0.1 M HCl, usually at a concentration of 1 mCi/ml. The K_2PtCl_6 is reduced with hydrazine to give K_2PtCl_4. This intermediate is allowed to react with KI and ammonia to yield *cis*-$[Pt(NH_3)_2I_2]$ which is then converted to *cis*-$[Pt(NH_3)_2Cl_2]$ (*cis*-DDP) upon aquation with $AgNO_3$ and reaction with NaCl (Wolf et al., 1973; Leh and Wolf, unpublished).

Bleomycin. Lyophilized bleomycin (Bristol Laboratories, Syracuse, N.Y.) was supplied in individual vials of 15 mg. It is a mixture containing A_2 as the main component and B_2 as the next major component (Umezawa et al., 1974).

Standard labeling procedure. Fifteen milligrams of bleomycin powder were dissolved in 2 ml of sterile water and this solution was divided equally into two parts, each mixed with 0.5 ml (1.5 mCi) of $^{195}Pt^m$-potassium tetrachloroplatinate or $^{195}Pt^m$-*cis*-dichlorodiammine-platinum (II) at pH 6.8. The mixtures were incubated at room temperature for 2 hr and then passed through a 0.22 μm millipore filter to sterilize the products.

The radiochemical purity and tagging yield were determined using thin-layer chromatography on Baker silica plates in 1 : 1 mixture of 10% ammonium chloride and methanol. The potassium tetrachloroplatinate, $K_2{}^{195}Pt^mCl_4$, and *cis*-dichlorodiammine-platinum(II), *cis*-$[^{195}Pt^m(NH_3)_2Cl_2]$, had R_f values of 1.0 and their bleomycin derivatives R_f values of 0.6 to 0.9, as determined by radiochromatogram scanning.

Stability. The stability of $^{195}Pt^m$-bleomycin complexes were determined by incubating the preparation for 2, 26, 48, 72 and 96 hr at room temperature. Thin-layer chromatography of these preparations before and after incubation was done to determine the amount of platinum complexed to bleomycin. Chromatograms were also made of the urines of animals to whom $^{195}Pt^m$-labeled bleomycin had been administered.

Distribution studies in mice. Swiss mice bearing Ehrlich's ascites carcinoma were used in distribution studies. The tumors were subcutaneous transplants to the right lower quadrant of the abdomen and were allowed to develop at least 10 days before injection of the $^{195}Pt^m$-labeled bleomycin. 50–100 μCi of either of the $^{195}Pt^m$-bleomycin complexes were injected into the tail vein at a dose of 15 mg bleomycin per kg weight. The mice were sacrificed by cervical dislocation 1, 24 and 48 hr post-injection. Samples of blood were withdrawn immediately by cardiac puncture. Samples of the lung, tumor, muscle, liver, skin, heart, spleen and kidney were removed from the mice and weighed in tared counting tubes. The $^{195}Pt^m$ activity in each sample was counted in a Beckman

* This radionuclide has been supplied by Dr. K. Poggenburg, Medical Cooperative Programs, AEC.

gamma counting system with window setting to count the activity from 50 to 140 KeV photons. The counting rates were corrected for background and for decay of $^{195}Pt^{m}$ to the time of injection. Standards were prepared in triplicate by transferring 0.1 ml aliquots of $^{195}Pt^{m}$-bleomycin to volumetric flasks and dilution to an activity level approximately equal to the sample. The standards were counted at the same time and the counting rates were corrected in the same manner as the samples. The data are expressed as percent dose per gram tissue.

RESULTS

The complexation of $^{195}Pt^{m}$-labeled tetrachloroplatinate and *cis*-dichlorodiammine-platinum(II) to bleomycin is quantitative, as determined by thin-layer chromatography. A representative radiochromatogram of the $^{195}Pt^{m}$-bleomycin preparation is shown in Fig. 1. Using 10% NH_4Cl and methanol as the developing solvent, most of the applied platinum migrated in a manner similar to bleomycin A (the dominant peak centered at about $R_f = 0.8$) and to bleomycin B (part of the shoulder at $R_f = 0.60$–0.75). No free $^{195}Pt^{m}Cl_4^{2-}$ or *cis*-$[^{195}Pt^{m}(NH_3)_2Cl_2]$ could be detected.

The stability of $^{195}Pt^{m}$-bleomycin complexes was excellent. Samples incubated at room temperature as long as 96 hr were chromatographed and showed no change during this time. The 'in vivo' stability is equally suggested by the absence of any observable chromatographic peak in the excreta, other than that of the injected $^{195}Pt^{m}$-labeled bleomycin. Specifically, no free $^{195}Pt^{m}$-K_2PtCl_4 or $^{195}Pt^{m}$-$[Pt(NH_3)_2Cl_2]$ could be observed in urines of animals up to 48 hr post-injection.

The results of the tissue distribution studies in mice are given in Tables 1 and 2. Table 1 presents results from the study of $K_2{}^{195}Pt^{m}Cl_2$-bleomycin complexes in Swiss mice bearing Ehrlich's ascites carcinoma, while Table 2 shows the results from $^{195}Pt^{m}$-*cis*-DDP-bleomycin injected into mice bearing the same tumor model. The data on ^{111}In-bleomycin are included in Table 1 for comparison. Tables 3 and 4 give a comparison of tumor to organ ratios in Swiss mice bearing Ehrlich's ascites carcinoma for bleomycin labeled with both $K_2{}^{195}Pt^{m}Cl_4$ and *cis*-$[^{195}Pt^{m}(NH_3)_2Cl_2]$.

It is apparent that the $^{195}Pt^{m}$-bleomycin concentration in Ehrlich's ascites tumor is comparable with that of ^{111}In-bleomycin, but that it is cleared more slowly from the

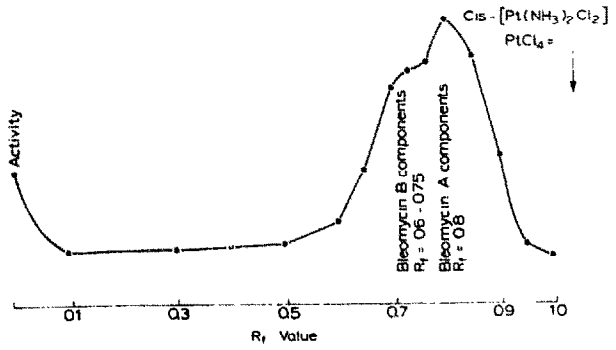

Fig. 1. Radiogram of Pt-bleomycin complex in 10% NH_4Cl.

TABLE 1

DISTRIBUTION OF $^{111}InCl$- AND $^{195}Pt^{m}Cl_4$-LABELED BLEOMYCIN IN MICE BEARING EHRLICH'S ASCITES CARCINOMA

Six animals were used for each group; the data are given with their ± S.D.

Isotope	Bleomycin (mg/kg)	Time (hr)	Dose % g^{-1}							
			Lung	Tumor	Muscle	Blood	Liver	Skin	Heart	Spleen
^{111}In [a]	15.0	1	1.11	1.26	0.40	1.89	1.30	1.13	–	–
($InCl_3$)	15.0	24	0.97	1.95	0.68	0.43	2.41	2.91	–	–
$^{195}Pt^{m}$	15.0	1	1.65 ± 0.21	1.36 ± 0.13	0.65 ± 0.12	1.53 ± 0.12	1.30 ± 0.04	1.18 ± 0.22	0.73 ± 0.07	0.48 ± 0.13
($PtCl_4^{2-}$)	15.0	24	1.38 ± 0.32	1.56 ± 0.17	0.56 ± 0.02	0.88 ± 0.05	1.63 ± 0.12	1.75 ± 0.11	0.68 ± 0.15	0.80 ± 0.23
	15.0	48	1.30 ± 0.18	1.85 ± 0.30	0.70 ± 0.03	0.50 ± 0.01	1.73 ± 0.02	1.38 ± 0.03	0.63 ± 0.11	0.78 ± 0.06

[a] Data from Emmerson et al. 1973.

TABLE 2

DISTRIBUTION OF $^{195}Pt^{m}$-LABELED BLEOMYCIN IN MICE BEARING EHRLICH'S ASCITES CARCINOMA

Three mice per group, average value ± one-half the range of the highest and lowest individual values.

Isotope	Bleomycin (mg/kg)	Time (hr)	Dose % g^{-1}								
			Lung	Tumor	Muscle	Blood	Liver	Skin	Heart	Spleen	Kidney
$^{195}Pt^{m}$	15.0	1	1.50	1.32	0.47	1.57	1.32	0.96	0.74	0.94	3.32
-[$Pt(NH_3)_2Cl_2$]			± 0.42	± 0.30	± 0.06	± 0.15	± 0.05	± 0.15	± 0.15	± 0.21	± 0.51
	15.0	24	1.30	1.77	0.49	1.09	1.52	1.35	0.52	0.59	5.35
			± 0.33	± 0.41	± 0.01	± 0.12	± 0.06	± 0.26	± 0.02	± 0.01	± 0.05
	15.0	48	0.94	1.94	0.58	0.56	1.65	1.10	0.62	0.90	4.40
			± 0.21	± 0.35	± 0.01	± 0.12	± 0.09	± 0.01	± 0.03	± 0.15	± 0.10

TABLE 3

TUMOR/ORGAN RATIOS OF $K_2{}^{195}Pt^mCl_4$-LABELED BLEOMYCIN IN MICE BEARING EHRLICH'S ASCITES CARCINOMA [a]

Ratio	Time after injection		
	1 hr	24 hr	48 hr
Tumor/Blood	0.89	1.77	3.70
Tumor/Muscle	2.09	2.79	2.64
Tumor/Lung	0.83	1.13	1.42
Tumor/Liver	1.05	0.96	1.07
Tumor/Skin	1.15	0.89	1.34
Tumor/Kidney	0.35	0.30	0.44

[a] Ratios of averaged data.

TABLE 4

TUMOR/ORGAN RATIOS OF *cis*-$[^{195}Pt^m(NH_3)_2Cl_2]$-LABELED BLEOMYCIN IN MICE BEARING EHRLICH'S ASCITES CARCINOMA [a]

Ratio	Time after injection		
	1 hr	24 hr	48 hr
Tumor/Blood	0.84	1.62	3.46
Tumor/Muscle	2.81	3.61	3.35
Tumor/Lung	0.88	1.36	2.04
Tumor/Liver	1.00	1.16	1.18
Tumor/Skin	1.38	1.31	1.76
Tumor/Kidney	0.41	0.33	0.44

[a] Ratios of averaged data.

blood. At 48 hr post-injection, the tumor-to-blood ratios are 3.5–3.7 with both Pt bleomycins. Otherwise, the distribution of $^{195}Pt^m$-bleomycin in organs is very similar to that of ^{111}In-bleomycin (Table 1).

DISCUSSION

One approach to the search of tumor-localizing radiopharmaceuticals of greater specificity and diagnostic accuracy is to investigate available chemotherapeutic drugs, such as bleomycin. The structures of the two platinum compounds used in this study are shown in Fig. 2. The chloride leaving ligand can can be replaced by bleomycin to form a bleomycin-to-metal bond. The central platinum cation is bivalent and has the d configuration. In this oxidation state, the Pt(II) compounds are square planar and are kinetically inert. In addition, *cis*-dichlorodiammine-platinum(II) is itself a chemotherapeutically active agent which is currently in Phase (II) clinical trial (Lokich et al., 1974; Talley et al., 1973; Higby et al., 1973).

Tetrachloroplatinate *cis*-dichlorodiammine platinum (II)

Fig. 2. Structures of platinum compounds.

The $^{195}Pt^{m}$-bleomycin complexes have considerably greater stability than ^{111}In-bleomycin. They remain unchanged for up to 96 hr 'in vitro' and to 48 hr 'in vivo'. However, ^{111}In-bleomycin was reported to be stable only up to 48 hr (Goodwin et al., 1973) and is readily dissociated 'in vivo' (Robbins et al., 1974). The $^{195}Pt^{m}$ is also a better complexing radionuclide for labeling bleomycin than $^{99}Tc^{m}$. The tagging yield for $^{195}Pt^{m}$-bleomycin is practically 100%, while the yield for $^{99}Tc^{m}$-bleomycin in such preparations is only as high as 50% (Lin et al., 1973) and the product is not very stable.

The distribution of $^{195}Pt^{m}$-bleomycin complexes in mice bearing Ehrlich's ascites carcinoma is similar to that of ^{111}In-bleomycin. The highest concentration of radioactivity 1 hr post-injection is in blood, followed by lung, tumor, liver, and skin. The concentration of $^{195}Pt^{m}$-bleomycin complexes in blood is not as high as that of ^{111}In-bleomycin, but decreases more slowly than the latter (Tables 1 and 2).

However, while these results show that the distribution of two $^{195}Pt^{m}$-bleomycins is similar to that of ^{111}In-bleomycin, we do not propose that the platinum-labeled agent replace the indium-labeled bleomycin as a tumor imaging agent. Rather, the results reported here suggests that a common mechanism for metal binding may exist, a concept that we have further developed into a theory of 'tumeroponic' agents (Wolf et al., 1977).

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