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AUTHOR(S) S. Packer, M.D., R. Lambrecht, H. L. Atkins and A. P. Wolf.

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SHORT-LIVED RADIOPHARMACEUTICALS FOR THE DIAGNOSIS OF OCULAR MELANOMA*

S. Packer, M.D., R. M. Lambrecht, Ph.D., H. L. Atkins, M.D.,
and A. P. Wolf, Ph.D.

The Medical Research Center (Dr. Packer, Dr. Atkins)
and the Chemistry Department (Dr. Lambrecht, Dr. Wolf)
Brookhaven National Laboratory Upton, New York 11973

and the

Department of Surgery (Ophthalmology),
The North Shore University Hospital (Dr. Packer)
Manhasset, N. Y. 11030

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INTRODUCTION

A major advantage in the use of radionuclides with short physical half-lives in nuclear ophthalmology is the low radiation dose to the lens. This is particularly important with a compound that selectively localizes in an ocular melanoma and has a long biological half-life. With the use of short-lived radiopharmaceuticals a small amount of radiation from each procedure would permit tests with these short-lived radiopharmaceuticals to be repeated and therefore suspicious lesions could be followed. By selecting the appropriate radionuclide, an adequate photon energy level can be obtained (50-300 keV) that would have adequate tissue penetration to leave the eye, and non-invasive techniques could be employed[1]. Those patients with lesions that were not obviously malignant melanomas would be more readily evaluated, as would patients with opaque media. The half-life of the radionuclide cannot be too short because time must elapse before the differential uptake between tumor and background tissues is large enough to allow detection.

The ratio between uptake of the radiopharmaceutical by a tumor vs. background will reach a maximum after a certain time has elapsed. The time course will determine how long a half-life is appropriate as well as determine when and if detecting a tumor in a given organ is possible. Wagner and Emmons[2] state that the ideal radiopharmaceutical should have an optimum physical half-life equal to ln2 x the time of the observation. Thus the biological behavior must be ascertained. The bond between the tumor seeking agent and the radioisotope must be stable in vivo. The ideal compound would be one that is tumor specific. Radiolabeled immune substances which are tumor associated have been used clinically, but have quantitative limitations. The number of available

receptor sites for these compounds is crucial if it is to be detectable. However, the high degree of specificity would be a favorable factor[3]. At present we are dealing with agents of only relative value in tumor localization. Therefore factors such as biological behavior and performance of detection devices become very important.

Pre-clinical evaluation of radiopharmaceuticals with an animal model and a phantom permits screening of compounds and comparison of the relative value of various agents. In addition the problem of background can be delineated, that is, does the agent also go to choroid, retina, bone, blood, muscle, etc. By looking at the time course of radioactivity in the tumor relative to these other tissues one can find the best time for detection.

There are several categories of compounds which can be used as tumor localizing agents. Blanquet and Safi[4] have reviewed the development of radiopharmaceuticals for use in nuclear ophthalmology up to 1974. Of these we have concentrated on the development of new nuclides and labeled compounds with suspected pigment affinity, e.g. iodinated chloroquine analogs (^{123}I-4,3 DMQ), heavy metals (lead-203 and thallium-201), antibiotics (^{57}Co-bleomycin) and metabolites (^{32}P). Our results obtained with ^{123}I-4,3-DMQ (i.e. 4-(3-dimethylaminopropylamino) - 7 - iodoquinoline) are the topic of another paper[5]. This paper will focus attention on our recent work with an inorganic coordination compound of ^{203}Pb.

Lead-203 seems to offer several advantages as a potential radionuclide in radiopharmaceutical applications. Certain heavy metals localize in tumors[6,7,8]. There is additional evidence that heavy metals have an affinity for melanin[9]. However localization of metals in tumors is not a constant finding[10,11]. We are searching for a chemical form of lead-203 which is appropriate for localization in malignant melanoma.

is optimal for testing since there is minimal necrosis and tumor size is adequate for biopsy. For ocular tumors a cell suspension was made from 1.0 gram of skin melanoma which was cut into small pieces, mixed with 5.0 ml of a tissue culture medium and forced through a microsieve (27 micron pores). This resulted in approximately 50 cells per µl with a moderate amount of cell clumping. Cell viability as determined with the trypan blue stain was approximately 75-80%. Animals were anesthetized with ether. With the aid of an operating microscope (25X) the globe was rotated and held firm with a small muscle hook, then a straight pin was used to penetrate conjunctive and aclera. Swirling the pen against a firm globe permitted a track to be made through the sclera while avoiding uncontrolled puncture of the globe and entry in the vitreous cavity. A 30 gauge needle was inserted into the track and passed posteriorly beneath the sclera for a short distance. A definite attempt was made to have the injection intrachoroidal. After 5.0 µl of the cell suspension was injected Eastman 910 glue was placed over the injection site while the needle was still in the eye. The glue was given time to harden slightly and the needle withdrawn. Antibiotic ointment was applied at the end of the procedure.

Dissections were done after 2 to 3 weeks of tumor growth. Various organs were taken from hamsters with either skin or eye melanoma. Detailed dissection of the various parts of the eye was done with the aid of the operating microscope. Large specimens such as liver or kidney were placed in larger vials. Small specimens, such as, cornea, retina, etc. were placed in gelatin capsules and then into small vials. Due to the light weight of the individual detailed parts of the eye each gelatin capsule contained four specimens. All vials, with or without gelatin capsules, were preweighed. After all tissues were in the vials they were reweighed to give tissue weights. The radioactivity was

The physical characteristics of ^{203}Pb are suitable for scintigraphy with available equipment[12]. It decays with a 52.1 hr half-life by electron capture with the emission of 279 keV photons in 95% abundance. The half-life of ^{203}Pb permits shipping to medical centers that do not have a cyclotron available. The 52.1 hr half-life also allows for biological time to elapse such that background concentration can decrease, while tumor concentration stabilizes thus favoring tumor detection.

METHODS

Carrier-free lead-203 was produced on the BNL 60' cyclotron using the $^{203}Tl(d,2n)$ ^{203}Pb nuclear reaction with the dueterons degraded from 22.7 → 0 MeV in a target of 99.99% purity thallium metal. The chemical separation of the ^{203}Pb from the target resulted in a radiochemical purity in excess of 99.99%[12]. Lead-203-tris was prepared by addition of an aqueous solution of 2-amino-2-hydroxymethyl, 1, 3-propanediol to the carrier-free ^{203}Pb[8]. The ligand is also known as tris, or tromethamine, and is commonly used in tris and Tham biological buffer. The solution was adjusted to pH 6.8-7.2. Passage through a millipore filter assured sterility of the labeled compound after its preparation. Details of the design of the radiopharmaceutical are found elsewhere[8,15]. Additional radiopharmaceuticals† tested were ^{57}Co-bleomycin and thallium-201 chloride.

The Greene melanoma in the male Syrian golden hamster was used as the experimental model[13]. Skin tumors are transplanted every 2 weeks. This time

†The ^{57}Co-bleomycin was provided by Dr. W. C. Eckelman, Department of Nuclear Medicine, Washington Hospital Center, Washington, D.C. Thallium-201 is available from New England Nuclear Corp.

assayed on an automatic gamma counter (1185 Series, Nuclear-Chicago, Des Plaines, Ill.). The data was corrected for radioactive decay, etc., and calculated as the percent uptake per gram of tissue relative to the total injected dose.

RESULTS

Table 1 has the % uptake for lead-203-tris by the skin and eye melanoma, parts of the eye and the various organs of the hamster. Figure 1 shows that the kidney, liver and bone have a substantial uptake. The uptake in eye melanoma peaks at 24 hours while the skin melanoma reaches maximum uptake at 6 hours.

The amount of uptake by background tissues is important if one is considering scintigraphy. Therefore an inspection of the uptake by other ocular tissues reveals that the primary competitor is the choroid. Figure 2 shows that the greatest ratio between ocular tumor and choroid (i.e. $\sim$ 3.6) occurs at 24 hours. The ratio of % uptake per gram of tumor eye to the normal eye was $\sim$ 25:1. The obvious implication is that ocular tumor scanning or counting with a collimated probe should be done at this time. However the <u>in vivo</u> problem is much more complex and background consists also of orbital bone, blood, brain, muscle, etc. (Table 1). For certain organs with high uptake, such as the liver and kidney, it is doubtful from results with this tumor model, that melanoma imaging with ^{203}Pb-tris is feasible. Figure 3 is a scan taken with a Nuclear Data gamma camera with a pinhole collimator, at 24 hours after an intraperitoneal injection of 50 μ Ci of ^{203}Pb-tris. The arrow is pointing to the area of an ocular melanoma.

A more limited analysis of ^{57}Co-bleomycin and thallium-201 chloride is given on Table 2 and Table 3. The ratio of ^{57}Co-bleomycin in eye melanoma to choroid of $\sim$ 3.8 is favorable. The ratio of tumor to choroid was similar to

the ^{203}Pb-tris. However, the ratio of % uptake/g in the tumor to normal eye was significant, i.e. ~ 57 to 1. However the % uptake of the labeled bleomycin (0.52 ± 0.14 %/g) is not so great as for the ^{203}Pb-tris (1.61 ± 0.31 %/g) (Figure 4). The % uptake of thallium-201 in skin melanoma is lower, and the ratio of uptake in melanoma to skin is unfavorable for detection. There was greater uptake of thallium-201 in the normal eye than with the other labeled compounds tested.

DISCUSSION

The Greene Melanoma was selected because of its degree of pigmentation and initially radiopharmaceuticals with pigment affinity were investigated for melanoma localization, that is, quinoline analogs. Therefore comment cannot be made as to amelanotic melanoma and the compounds that we have used. In addition human confirmation is necessary.

Heavy metals have been previously found in higher concentration in neoplastic tissue[6,7], although these findings have not been consistent[10,12]. These were with different tumor models; i.e. mammary carcinoma[10] and S-91 melanoma[7]. Another factor that may lead to lead-203 localizing in a melanotic tumor is the findings of Browness[9] that metals associate with melanin and a melanin-protein complex. Lead-203 tris has also been found to localize to varying degrees in other tumor models[15] in mice including ependymoma and adenocarcinoma of the breast.

Potts found that thallous ion concentrated in melanin-containing tissues[14]. Table 3 shows that the concentration of thallium-201 in melanoma is not so great as either ^{203}Pb-tris or ^{57}Co-bleomycin. Potts[14] also found a high lens concentration of thallium, whereas our data show a low lens concentration of ^{203}Pb-tris (Table 1). Thus it would appear that ^{203}Pb-tris is more appropriate for ocular tumor localization than thallium-201-chloride.

Bleomycin, as a prototype as an antibiotic with tumor affinity, was investigated with the ^{57}Co label because of its availability. Cobalt-57 has a long half-life (271 days) and is not the most suitable for a widespread clinical use. Table 2 and Figure 4 show that ^{57}Co bleomycin and ^{203}Pb-tris both exhibit ocular tumor specificity.

It then appears, that there are several compounds which have adequate melanoma specificty to allow detection when combined with the appropriate radionuclide. Each appears to have its own *in vivo* time course probably due to differeneces in selective partitioning and/or due to different metabolic breakdown. Imaging is best performed when the difference between tumor concentration vs. background concentration is adequate (Figure 3). All the data obtained serve as a basis for appropriate clinical application.

The problem of detecting ocular melanoma involves not only the tumor apecifity of compound but its detectability, i.e., is the energy emitted appropriate for counting or imaging. Very low energy gamma emitters do not have sufficient tissue penetration. Lead-203 (T 1/2 = 52.1 h. γ = 279 keV (95%) or iodine-123 (T 1/2 = 13.3 hr, γ = 159 keV) should allow for scintigraphy if tumor uptake is adequate. High purity iodine-123 and lead-203 have nuclear decay characteristics appropriate for nuclear ophthalmology. The matrix into which either nuclide is incorporated, and its biological behavior will determine which nuclide and compounds are preferable. Since several days may be needed to elapse before the ratio of tumor concentration to background is great enough to allow detection, the 13.1 hour half-life of iodine-123 may be less desirable than the 52.1h half-life of lead-203. Lead-203 and ^{123}I have physical half-lives which favors an observation time at 75.2 hours and 18.9 hours, respectively, after administration of the labeled compounds to the patient.

SUMMARY

An experimental procedure has been established to evaluate radiopharmaceuticals for the specific purpose of melanoma detection. By using the Greene melanoma in the hamster several labeled compounds were compared. Specifically the tumor uptake along with detailed analyses of uptake by various parts of the eye and body were determined in a hamster model. Of those short-lived radionuclides investigated in this laboratory ^{203}Pb-tris is the most promising as a non-invasive localizing agent for ocular melanoma and it should allow for ocular scintigraphy.

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TABLE I. Distribution of Carrier-Free Lead-203 Tris in Percent Uptake per Gram of Tissue at Various Times*

Tissue	Time 1	3	6	24	72	144
Melanoma-Eye	1.048 ±0.214	0.976 ±0.190	1.609 ±0.306	1.314 ±0.188	0.724* ±0.260	0.821 ±0.150
Normal-Eye	0.200 ±0.037	0.086 ±0.010	0.111 ±0.011	0.054 ±0.006	0.084 ±0.005	0.047 ±0.007
Melanoma-Skin	1.095 ±0.132	1.051 ±0.137	1.209 ±0.154	0.845 ±0.075	1.010 ±0.154	0.749 ±0.068
Normal-Skin	0.401 ±0.055	0.256 ±0.055	0.286 ±0.058	0.090 ±0.009	0.122 ±0.018	0.071 ±0.008
Cornea	0.834 ±0.269	0.173 ±0.102	0.891 ±0.717	0.200 ±0.059	0.084*	0.062
Lens	0.017 ±0.013	0.023 ±0.012	0.021 ±0.017	0.005 ±0.006	0.006*	0.005
Vitreous	0.031 ±0.016	0.028 ±0.007	0.036 ±0.015	0.031 ±0.012	0.014*	0.018
Retina	0.169 ±0.106	0.074 ±0.007	0.251 ±0.132	0.206 ±0.002	0.091*	0.127
Choroid	1.109 ±0.631	0.950 ±0.855	0.337 ±0.163	0.361 ±0.103	0.127*	0.131 ±0.124
Kidney	25.32 ±2.55	18.156 ±1.205	17.703 ±2.069	10.508 ±0.481	17.443 ±1.766	3.904 ±0.217
Liver	5.463 ±0.721	4.273 ±0.350	7.188 ±0.701	3.729 ±0.346	4.841 ±0.398	2.331 ±0.457
Gonads	2.076 ±1.017	0.238 ±0.041	0.663 ±0.142	0.164 ±0.029	0.135 ±0.020	0.079 ±0.004
Brain	0.107 ±0.025	0.067 ±0.006	0.135 ±0.123	0.139 ±0.019	0.067* ±0.008	0.109 ±0.010
Muscle	0.159 ±0.024	0.100 ±0.020	0.090 ±0.015	0.026 ±0.003	0.041 ±0.005	0.027 ±0.011
Blood	3.870 ±0.749	3.635 ±0.404	5.847 ±0.828	3.007 ±0.267	3.792 ±0.224	0.852 ±0.175
Bone	3.977 ±0.648	3.991 ±0.641	5.320 ±0.717	5.342 ±0.758	9.761 ±2.016	5.757 ±0.602
Intestines	0.841 ±0.238	0.653 ±0.111	1.593 ±0.265	1.664 ±0.457	2.064* ±0.597	0.217 ±0.095

*Averages and standard deviations for an average of 8-17 hamsters per time group, except * data which represents results from 4 animals.

TABLE II

DISTRIBUTION OF ^{57}CO-BLEOMYCIN IN PERCENT UPTAKE PER GRAM OF TISSUE AT VARIOUS TIMES[1,2]

Tissue \ Time	1	3	6	24	144
Melanoma-Eye		0.220 ± .217	0.518 ± .141	0.422 ± .049	0.003 ± .003
Normal-Eye	0.076 ± .006	0.008 ± .003	0.009 ± .003	0.0024 ± .0004	0.001 ± .000
Melanoma-Skin	1.344 ± .218	0.363 ± .185	0.358 ± .030	0.211 ± .067	0.003 ± .002
Normal-Skin	1.089 ± .486	0.161 ± .328	0.085 ± .037	0.016 ± .007	0.003 ± .000
Cornea		0.160	0.053	0.007	-*
Lens		0.003	0.003	0.002	-*
Vitreous		0.011	0.006	0.001	0.001
Retina		0.002	0.004	0.009	0.003
Choroid		0.077	0.135	0.064	0.003
Kidney	1.567 ± .376	0.038 ± .020	0.259 ± .031	0.140 ± .020	0.021 ± .005
Liver	0.431 ± .021	0.088 ± .065	0.188 ± .056	0.101 ± .024	0.009 ± .003
Gonads	0.381 ± .077	0.013 ± .012	0.020 ± .005	0.156 ± .122	0.003 ± .001
Brain		0.005 ± .004	0.004 ± .002	0.003 ± .001	
Muscle	0.101 ± .004	0.189 ± .012	0.013 ± .004	0.002 ± .000	0.001 ± .000
Blood	0.446 ± .088	0.017 ± .008	0.006 ± .001	0.002 ± .001	
Bone	0.044 ± .027	0.006 ± .002	0.017 ± .005	0.007 ± .002	0.006 ± .001
Intestines	0.389 ± .013	0.055 ± .016	0.241 ± .036	0.069 ± .027	0.002 ± .001

1. Average and standard deviation for 3-5 animals per group, data grouped.
2. Loading dose = 0.1 unit per Kg body weight.
*Less than 0.000 %/g for parts of the eye. See text.

TABLE III

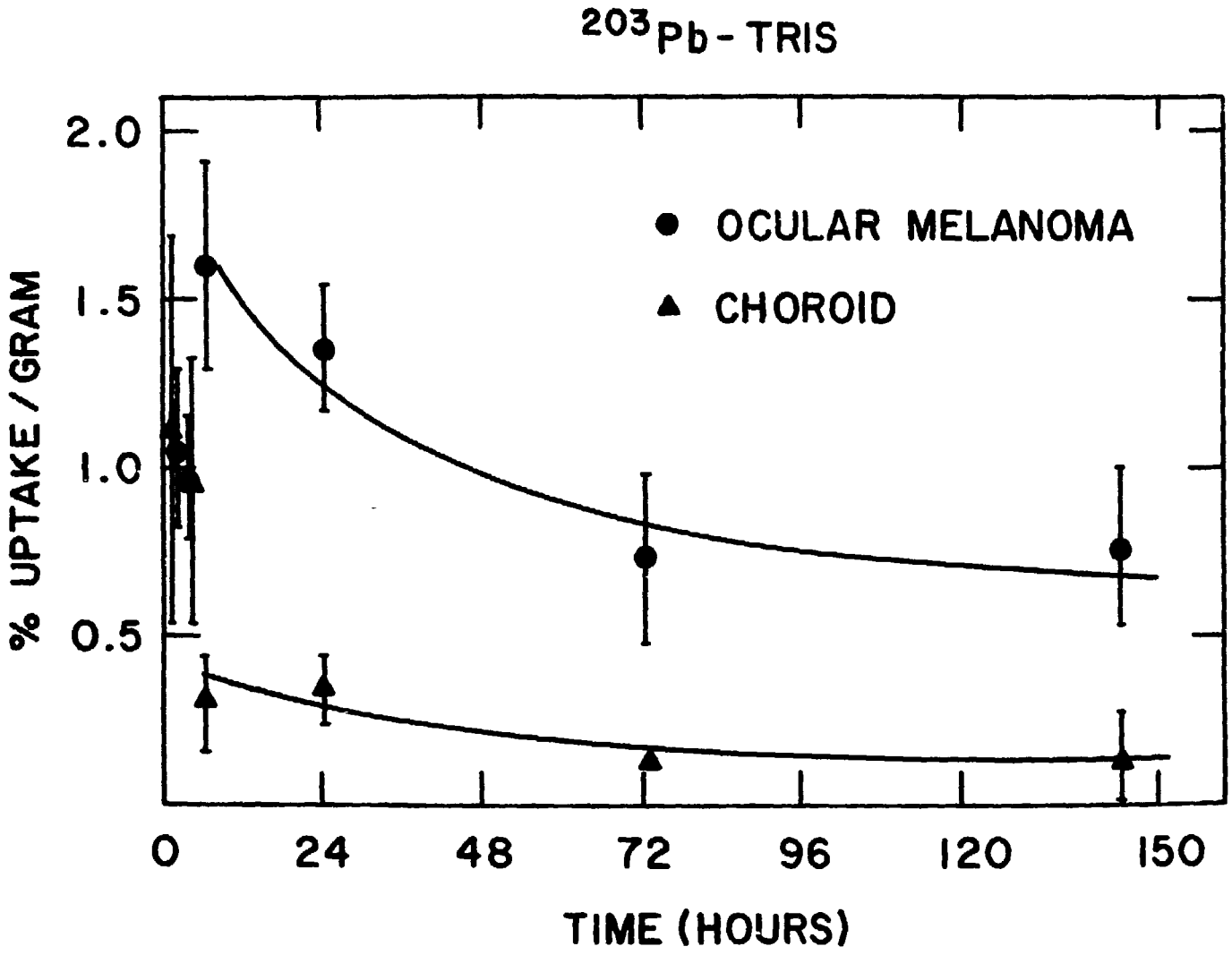
DISTRIBUTION OF CARRIER-FREE THALLIUM-201 CHLORIDE IN PERCENT UPTAKE PER GRAM OF TISSUE AS A FUNCTION OF TIME[1]

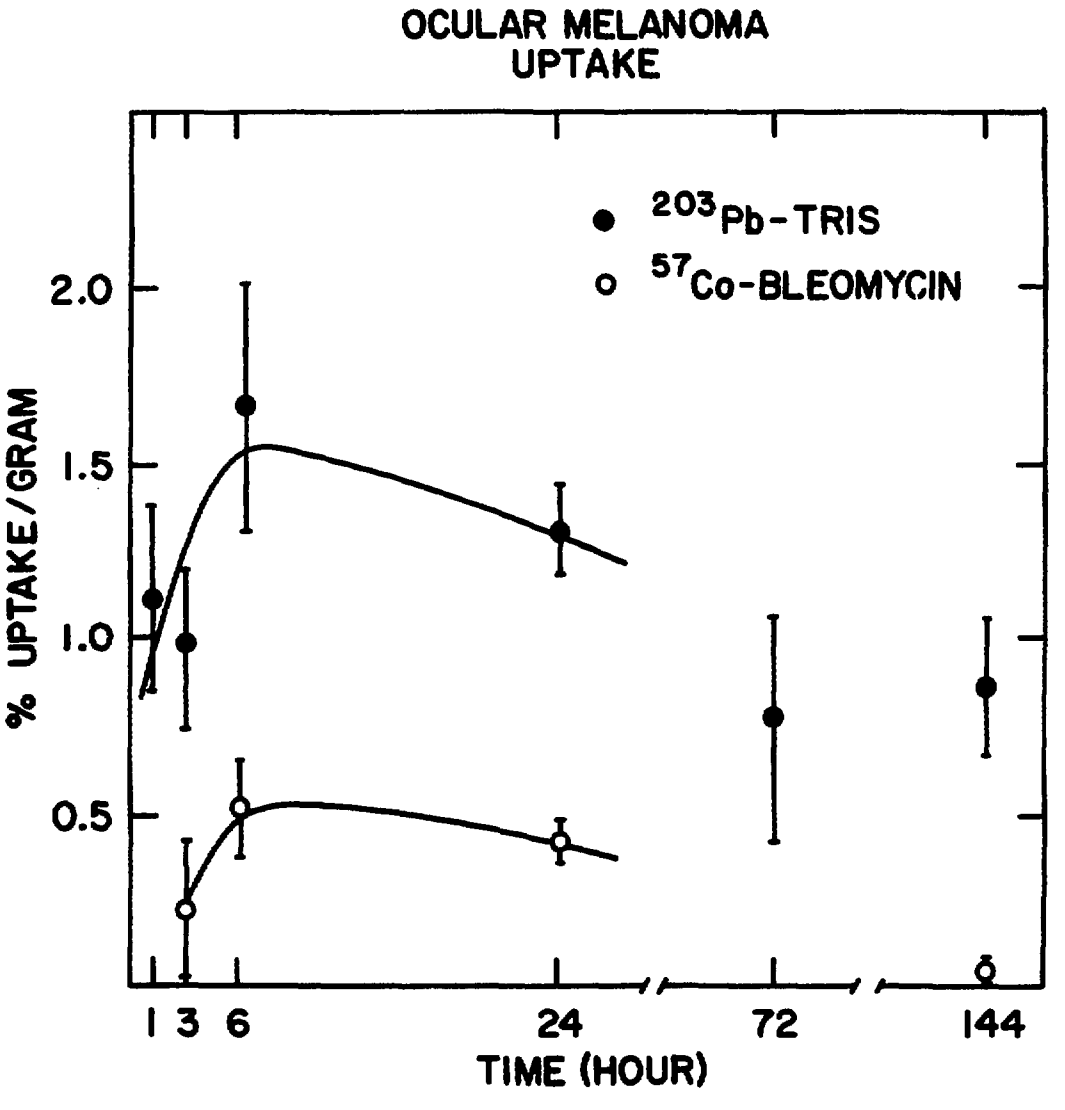
Tissue \ Time, hr	1	3	6	24	48	72	144
Normal-Eye	0.239 ± .037	0.321 ± .038	0.479 ± .067	0.456 ± .038	0.354 ± .100	0.295 ± .028	0.119 ± .034
Melanoma-Skin	0.327 ± .062	0.718 ± .269	0.982 ± .159	0.478 ± .057	0.389 ± .132	0.299 ± .036	0.128 ± .063
Normal-Skin	0.225 ± .121	0.295 ± .079	0.283 ± .048	0.190 ± .039	0.164 ± .055	0.143 ± .025	0.047 ± .011
Kidney	12.177 ± 2.136	17.904 ± 2.192	17.931 ± .378	10.732 ± 4.267	6.047 ±1.484	4.430 ± .481	2.301 ±0.947
Liver	2.736 ± .055	1.806 ± .090	1.567 ± .120	0.798 ± .151	0.649 ± .135	0.465 ± .091	0.196 ± .035
Muscle	2.287 ± .846	0.354 ± .038	0.805 ± .142	0.699 ± .110	0.409 ± .137	0.461 ± .111	0.216 ± .058
Blood	1.004 ± .486	0.144 ± .048	0.488 ± .071	0.069 ± .005	0.073 ± .036	0.074 ± .023	0.030 ± .013
Bone	0.283 ± .075	0.358 ± .182	0.507 ± .038	0.703 ± .083	0.598 ± .161	0.499 ± .230	0.226 ± .070
Intestines	4.309 ± .053	3.033 ± 1.566	2.011 ± .273	1.387 ± .506	0.661 ± .102	0.487 ± .117	0.219 ± .034

1. 4 hamsters per group.

FIGURE CAPTIONS

1. Uptake of ^{203}Pb-tris in various organs of the hamster at 1 to 144 hrs.
2. Uptake of ^{203}Pb-tris in the ocular melanoma and choroid of the hamster at 1 to 144 hrs.
3. Scintiphoto and photo of the ocular Greene melanoma in the hamster at 24 hours post i.p. administration. The arrows indicate the location of the tumor.
4. A comparison of the uptake of ^{203}Pb-tris and ^{57}Co-bleomycin in the ocular melanoma as a function of time.


^{203}Pb - TRIS
2.0
1.5
1.0
0.5
% UPTAKE / GRAM
OCULAR MELANOMA
CHOROID
0
24
48
72
96
120
150
TIME (HOURS)


OCULAR MELANOMA
UPTAKE
^{203}Pb-TRIS
^{57}Co-BLEOMYCIN
% UPTAKE/GRAM
2.0
1.5
1.0
0.5
1
3
6
24
72
144
TIME (HOUR)