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TUMOR UPTAKE OF RADIORUTHENIUM COMPOUNDS

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A. Introduction

The role of radiopharmaceuticals in clinical oncology has been expanding rapidly. As "tumor-scanning" agents, they are useful in the initial staging of tumors and in providing a means of evaluating response to therapy and detecting metastasis or recurrence at early stages before it becomes clinically overt. With the development of improved radiopharmaceuticals, tumor scanning can provide the clinician with a highly sensitive, easy, and safe noninvasive supplement to, or substitute for, other diagnostic procedures. Radionuclides, by themselves or incorporated into tumor seeking compounds, also have additional potential as therapeutic agents.

The use of ruthenium-97 as a scintigraphic agent, in particular for tumor localization, offers several advantages over other isotopes in present use (1-3). The half life of 2.9 days is sufficiently long to allow limited chemical synthesis and purification and not too long to cause excessive radiation dose to the patient. Ruthenium-97 is a pure gamma emitter with an essentially monoenergetic emission of 216 keV (86%). This allows for its use in presently available imaging equipment. Ruthenium-97 has been produced at the Brookhaven Linac Isotope Producer (BLIP) by the ^{103}Rh (p, 2p5n) ^{97}Ru reaction (4). Sufficiently large quantities ($\geq$ 100 mCi/day) can be produced routinely and economically. The chemistry of ruthenium in its various oxidation states is uniquely suited for the incorporation of this element into a variety of diagnostically useful ligands including biological molecules. A number of ruthenium coordination compounds have recently shown promise in cancer chemotherapy(5).

Also, reports have appeared on the potential of ruthenium compounds as tumor-localizing agents (2, 6-8). All these facts taken together provide an excellent combination for the application of ruthenium-97 in nuclear medicine, particularly for the development of successful tumor-imaging agents.

Gallium-67 citrate is presently the most widely used agent for tumor localization. This agent has serious disadvantages, however. It does not concentrate selectively in tumors, the background remains high, and its imaging properties are far from ideal. A great deal of work has been reported on the mechanisms of uptake and the clinical applications of gallium citrate (9). Evidence has been presented that specific macromolecules, including transferrin, may be involved and that some gallium is incorporated intracellularly, perhaps in lysosomes. The role of transferrin in the gallium uptake has been studied in detail by Larson et al. in the EMT-6 sarcoma both in vivo and in vitro (10-13), and by others in a variety of tumor models (14-18). Increased uptake was observed with gallium-labeled serum, supporting the hypothesis of transferrin-mediated transport of gallium into tumor tissue in vivo. In various mouse tumors that have been studied, the gallium uptake expressed as per cent dose per g has varied from 3.8 for Ehrlich's carcinoma growing subcutaneously on the thigh to 10.5 for KHJJ carcinoma on the flank. In the EMT-6 sarcoma used by Larson and coworkers, the uptake has been in the highest range (7-9% at 24 hr), clearly demonstrating the suitability of this convenient mouse tumor model for investigative purposes (10).

A collaborative program at Brookhaven and the VA Hospital, Seattle has begun on the development of ruthenium-97 based tumor-localizing agents. Initial studies have demonstrated high concentration of a number of ruthenium compounds into the mouse EMT-6 sarcoma and in several other animal tumor models. The uptake on a per gram basis of carrier-free ruthenium-103 chloride and a variety of other ruthenium compounds in the EMT-6 sarcoma was comparable to that of gallium-67 citrate and the uptake of ruthenium labeled transferrin was almost twice as high as that of gallium at comparable time periods. It is likely that the simple ruthenium compounds are transported to various tissues (including tumor) after being predominantly bound to transferrin. If, according to prevailing hypothesis, the EMT-6 sarcoma concentrates gallium via a transferrin-mediated mechanism, it could follow that ruthenium, because of its strong binding to transferrin, will localize preferentially in tumor tissue and, in addition, that transferrin labled in vitro with ruthenium would produce a higher degree of tumor uptake. A knowledge of the mechanisms of ruthenium uptake by the tumors and the nature of the binding of ruthenium to various proteins and other tissue components will greatly facilitate further development of highly specific ruthenium agents for tumor localization. Availability of successful ruthenium-97 agents will no doubt result in improved diagnostic performance with high information content in the collected images and reduced patient radiation dose when compared to radiopharmaceuticals presently in use. Also,

high and selective uptake of ruthenium compounds may have implications for the use of β-emitting radionuclides, ruthenium-103 or ruthenium-106, in the radiation therapy of cancer.

Evaluation of the tumor uptake of ruthenium chloride and ruthenium-labled transferrin is the subject of this work; data obtained from studies of several other ruthenium compounds are also summarized.

B. Experimental

Most initial investigations were carried out using ruthenium-103 due to its convenient 39.6 day half life. Carrier-free ruthenium-103 chloride was obtained from the Oak Ridge National Laboratory as a solution in 3.5 N hydrochloric acid. Ruthenium-97 was prepared as needed at the BLIP from proton spallation of high purity (>99.9%) rhodium foil (4). The target was bombarded with 200 MeV protons from the Linac. After the bombardment, the target was transferred to a processing hot cell and dissolved by a.c. electrolysis in 6 N hydrochloric acid ($0.3A/cm^2$, ~15 hr). After evaporating the solution to near dryness, radiochemical separation of ruthenium-97 was achieved by distillation of RuO_4 from a sulfuric acid medium in the presence of permanganate. The distillate was collected in ice cold ethanol-hydrochloric acid (1:1). Recovery of the ruthenium-97 from the target was almost quantitative.

All chemicals used were high purity reagent grade. Radiosynthesis of potassium pentachloroaquoruthenate(III), potassium trisoxalatoruthenium(III), chloropentaammineruthenium(III) chloride, cis-dichlorotetraammineruthenium(III) chloride, cis-dichloro bis (ethylenediamine)-ruthenium(III) chloride, and several other ammine complexes was carried out according to suitably modified literature procedures (19), using added stable ruthenium carrier. In vitro characterization of these compounds was carried out by elemental analysis as well as by measurement of the UV, visible, and IR spectra. Oxine (8-hydroxyquinoline) 7-carboxylic acid was purified by

recrystallization from acetic acid. Ruthenium complex of this ligand was prepared as follows. To a suitable quantity of the tracer, ^{103}Ru or ^{97}Ru, both available in 3.5 N HCl neutralized to pH 2, an appropriate amount of the ligand was added, and following an incubation period of 10 min, the pH was raised to ~7. The resulting solution was heated on a boiling water bath for 30 min. The complex was characterized by cellulose acetate electrophoresis (borate/KCl buffer, pH 8), thin-layer chromatography (polyamide sheets, chloroform), and by solvent extraction into chloroform.

Transferrin was labeled with either ruthenium-103 or ruthenium-97 as follows. Twenty mg purified iron-free human transferrin (Sigma Chemical Company) was dissolved in 3 ml of a 0.1 M sodium acetate solution. The desired amount of carrier-free ruthenium activity (in 3N HCl) was added. The pH was adjusted to about 7 with sodium hydroxide and the mixture (final volume 5 ml) heated at 40°C for 2 hr. This preparation was purified on a 0.9 x 100 cm G-150 Sephadex column to provide pure monomeric labeled transferrin (~95% by polyacrylamide gel electrophoresis) in approximately 65-70% yield based on radioactivity.

Tissue distribution data were obtained in normal and tumor-bearing mice. The tumor model used most extensively in these studies was the subcutaneous EMT-6 sarcoma in Balb/c mice (10).

C. Results

Analysis of ruthenium-103 chloride by paper chromatography in methyl ethyl ketone/conc HCl (70:30 v/v) (20) indicated the presence of ruthenium (III) and ruthenium (IV) species in the hydrochloric acid solution. Conversion of the ruthenium (IV) to ruthenium (III) was effected by refluxing the mixture in ethanol overnight. Tissue distribution of the two species in normal as well as tumor-bearing animals was not found to be significantly different. The mixture as received was therefore used in all subsequent experiments involving ruthenium chloride. Tissue uptake of the ruthenium chloride (pH 2) is shown in Table 1. The results are expressed as per cent injected dose per g of the various tissue samples. The tumor uptake remains essentially level at 6-72 hours after injection. Significant background activity persists, however, mainly in blood, muscle, kidneys, and the liver.

Results on the uptake of ruthenium-103-transferrin are described in Table 2. Even though the non target activity (blood, liver, spleen, kidneys) remains substantial, the tumor uptake of this agent is quite high at 24-72 hours after injection resulting in good tumor-to-tissue ratios (Table 4). The distribution of gallium-67 citrate is shown in Table 3 for comparison.

Table 5 provides a summary of the organ distribution data obtained with a number of the ruthenium compounds that appear promising as tumor-localizing agents. Table 6 describes the tumor concentration index (TCI) of various compounds. This index which

provides a useful correlation between the tumor uptake and the mean body concentration is defined as the ratio of percent injected dose per g of the tumor to percent injected dose per g remaining in the whole body at any given time period.

D. Discussion

Ruthenium, a member of the second triad of the group VIII transition metals, is a highly reactive element and displays as many as ten oxidation states in its compounds. The common oxidation states are 2, 3, 4, and 8. The chemical reactiveness of ruthenium and the excellent radiophysical properties of ruthenium-97 provide a valuable combination for application to nuclear medicine (1). In the area of tumor-localizing agents, ruthenium-97 offers exceptional promise, especially since the chemical reactivity allows for its incorporation into a variety of oncostatic ligands and biological molecules. The potential of a number of ruthenium coordination compounds in cancer chemotherapy has recently been recognized (5). Many of these compounds act by preferential localization in the tumor tissue. An additional possibility thus exists for developing successful tumor-localizing agents, by labeling such compounds with ruthenium-97.

Initial evaluation of a series of simple coordination complexes of ruthenium in the EMT-6 sarcoma mice demonstrated varying degrees of promise (2). These results are summarized in Tables 5 and 6. Uptake by the tumor, expressed as percent dose per gram, ranges between 1.5 and 6.8, and compares favorably with gallium (3.5-7.1; Table 3). The general body background of cis-dichlorotetraammine-ruthenium(III) chloride and Ru-oxine 7-carboxylic acid is much lower than that of gallium citrate; the other compounds are comparable to gallium in this respect. The tumor-to-blood (T/B)

and tumor-to-muscle (T/M) ratios with several compounds are high enough for delineating tumors by imaging. The uptake of gallium by the tumor reaches a plateau at about 24 hr; thereafter, significant loss of the activity is seen without comparable excretion from the body (Table 3). This results in a decrease in the value of the tumor concentration index (Table 6). This index reaches a maximum value at different time periods with different compounds. The TCI of various ruthenium compounds at 24-96 hr ranges between 1.37 and 2.68 and is comparable to that of gallium citrate (1.45-1.81). Ruthenium-oxine 7-carboxylic acid (TCI 96 hr 2.50; T/B 3.2; T/M 7.7) and cis-dichlorotetraammineruthenium(III) chloride (TCI 96 hr 2.0; T/B 4.4; T/M 4.2) look particularly promising when compared to gallium citrate (TCI 24 hr 1.81; T/B 4.2; T/M 13.4).

The most promising compound in terms of the total tumor uptake, target to non-target ratios, and the tumor concentration index, was ruthenium labeled transferrin (Tables 2, 4, and 6). When ruthenium chloride is administered intravenously, a major portion of it becomes bound to transferrin and is initially transported to various tissues intact as the ruthenium-transferrin complex. Apparently, the in-vivo kinetics of this binding is not very favorable; the result is non-specific localization of a significant part of the ruthenium activity in other tissues. When in-vitro labeled ruthenium transferrin is injected, a more specific tumor uptake results with diminished background activity. This result is expected, if one assumes the hypothesis of the

presence of transferrin receptor sites on the tumor cell surface. Considerable evidence has been accumulated recently in support of a transferrin mediated uptake of gallium citrate by tumor tissue (9-18) and it is likely that a similar mechanism is operative in the case of ruthenium-labeled transferrin. The difference between the relative uptakes of gallium and ruthenium could be due to the relative affinities that the transferrin complexes of these elements may have for the tumor cell membrane binding site. Recent studies on the ruthenium-labeled transferrin point to the predominance of monoruthenium transferrin in our preparations. It has been shown (21, 22) that transferrin which has both available metal binding sites occupied binds to the cell surface receptors more avidly than monometallic transferrin. The active transport of ruthenium could then be enhanced by metallating both the transferrin metal binding sites with ruthenium; in addition, the total uptake of the activity by the tumor thus could be doubled. We are looking into these various possibilities at the present time. Detailed studies on the nature of the ruthenium-transferrin binding and the mechanisms of uptake by <u>in vitro</u> tumor cell cultures are in progress. Efforts to further reduce the general body background are also continuing. It appears that (i) chemical modification, (ii) administration of suitable chelating agents, and (iii) computer background subtraction, will result in lowering the non-target tissue activity and thus yield good images.

The data obtained so far support the conclusion that ruthenium-97-labeled transferrin is a potential new agent for scintigraphic delineation of tumors. Uptake of this material in several mouse tumor models is almost twice as high as that of gallium citrate. Additionally, the imaging characteristics of ruthenium-97 are far superior to those of gallium-67. Continued evaluation of ruthenium-97-transferrin in other tumor models, particularly in larger animals, is in progress.

E. References

1. Srivastava, SC, Richards, P, Som, P, et al: Ruthenium-97 labeled compounds - a new class of radiopharmaceuticals, in Frontiers in Nuclear Medicine; Heidelberg, Springer-Verlag, 1980, p. 123.

2. Srivastava, SC, Richards, P, Meinken, G, et al: Evaluation of radiolabeled ruthenium compounds as tumor-localizing agents, in Radiopharmaceuticals II: Proceedings of the Second International Symposium on Radiopharmaceuticals, New York, Society of Nuclear Medicine, 1979.

3. Srivastava, SC, Richards, P, Larson, SM, et al: Radiolabeled ruthenium compounds as potential tumor localizing agents. J Nucl Med 20: 684, 1979.

4. Ku, TH, Richards, P, Srivastava, SC, et al: Production of ruthenium-97 for medical applications. Second International Congress, World Federation of Nuclear Medicine and Biology, Washington, DC, September 18-21, 1978.

5. Clarke, MJ: Oncological implications of the chemistry of ruthenium, in Sigel H. (ed): Metal Ions in Biological Systems, vol. 11. New York, Marcel Dekker, 1980.

6. Anghileri, LJ: Radioactive ruthenium red accumulation by tumors: a potential scanning agent. Strahlentherapie 149: 173, 1975.

7. Tanabe, M: Clinical trials on tumor scanning with ^{103}Ru. Radioisotopes 25: 44-49, 1976.

8. Tanabe, M, Yamamoto, G: Tissue distribution of ^{97}Ru and ^{103}Ku in subcutaneous tumor of rodents. Acta medica Okayama 29: 431-436, 1975.

9. Hoffer, P: Gallium: mechanisms. J Nucl Med 21: 282-285, 1980, and references cited therein.

10. Larson, SM: Mechanisms of localization of gallium-67 in tumors. Sem Nucl Med 8: 193, 1978.

11. Larson, SM, Rasey, JS, Allen, DR, et al: A transferrin-mediated uptake of gallium-67 by EMT-6 sarcoma II. Studies in-vivo (Balb/c mice): Concise communiation. J Nucl Med 20: 843-846, 1979.

12. Larson, SM, Rasey, JS, Allen, DR, et al: A transferrin-mediated uptake of gallium-67 by EMT-6 sarcoma I. Studies in tissue culture. J Nucl Med 20: 837-842 (1979).

13. Larson, SM, Rasey, JS, Grunbaum, Z, et al: Pharmacologic enhancement of gallium-67 tumor to blood ratios for EMT-6 sarcoma (Balb/c mice). Radiology 130: 241-244, 1979.

14. Noujaim, AA, Lentle, BC, Hill, JR, et al: On the role of transferrin in the uptake of gallium by tumor cells. Int J Nucl Med Biol 6: 193-199, 1979.

15. Terner, UK, Wong, H, Noujaim, AA, et al: ^{67}Ga and ^{59}Fe uptake in human melanoma cells. Int J Nucl Med Biol 6: 23-28, 1979.

16. Wong, H, Terne, UK, English, D, et al: The role of transferrin in the in vivo uptake of gallium-67 in a canine tumor. Int J Nucl Med Biol 7: 9-16, 1980.

17. Harris, AW, Sephton, RG: Transferrin promotion of ^{67}Ga and ^{59}Fe uptake by cultured mouse myeloma cells. Cancer Res 37: 3634-3638, 1977.

18. Tzen, K, Oster, ZH, Wagner, HN et al : Role of iron-binding proteins and enhanced capillary permeability on the accumulation of gallium-67. J Nucl Med 21: 31-35, 1980.

19. Mercer, EE, Buckley, RR: Inorg Chem 4: 1692, 1965; Raichart, DW, Taube, H: Inorg Chem 11: 999, 1972; Broomhead, JA, Kane-MacGuire, LAP: J Chem Soc (A): 546, 1967; Krentzien, H: Ph.D. Dissertation, Stanford Univ., Stanford, CA, 1976; Lever, FM, Powell, AR: J Chem Soc (A): 1477, 1969; Ford, PC, Rudd, DFP, Gaunder, R, et al.: J Amer Chem Soc 90: 1187, 1968.

20. Burstall, FH, Davies, GR, Linstead, RP, et al: Inorganic chromatography in cellulose. Part II. The separation and detection of metals and acid radicals on strips of absorbent paper. J Chem Soc: 516, 1950.

21. Harris, DC, Aisen, P: Biochem J 14: 262, 1975.

22. Verhoef, NJ, Noordeloos, P: J Clin Sci Molec Med 52: 87, 1977.

Table 1

Tissue Uptake of Ruthenium-103 Chloride[a] in EMT-6 Sarcoma Mice

	Percent dose per g (n=6)					
	Time after injection, hr					
Tissue	1	6	24	48	72	96
Blood	12.43 ± 0.90	7.98 ± 0.36	3.60 ± 0.29	1.82 ± 0.11	1.32 ± 0.09	0.91 ± 0.13
Tumor	4.17 ± 0.23	4.71 ± 0.21	5.25 ± 0.21	4.89 ± 0.43	4.67 ± 0.29	3.61 ± 0.25
Liver	10.08 ± 1.31	10.74 ± 0.89	5.56 ± 0.47	5.18 ± 0.26	5.62 ± 0.48	4.32 ± 0.49
Kidney	19.06 ± 1.19	17.21 ± 0.68	10.23 ± 0.53	8.59 ± 0.54	7.22 ± 0.30	6.16 ± 0.51
Muscle	2.26 ± 0.17	1.97 ± 0.25	1.24 ± 0.09	1.32 ± 0.16	1.17 ± 0.07	1.07 ± 0.07
Heart	4.37 ± 0.17	4.30 ± 0.21	3.62 ± 0.06	3.17 ± 0.16	2.63 ± 0.22	2.47 ± 0.26
Spleen	4.69 ± 0.53	5.56 ± 0.52	3.89 ± 0.40	3.25 ± 0.22	3.50 ± 0.35	3.30 ± 0.65
Bone	4.59 ± 0.43	4.24 ± 0.26	3.45 ± 0.29	2.69 ± 0.32	2.73 ± 0.19	2.32 ± 0.37
% Dose remaining in whole body	-	-	59.29 ± 0.88	45.36 ± 1.88	47.36 ± 0.84	40.74 ± 2.66

a. The injection solution was a mixture of Ru(III) and Ru(IV) chlorides. The pH was adjusted to 2.0 with a NaCl/HCl buffer (μ = 0.154).

Table 2

Tissue Uptake of Ruthenium-103 Labeled Transferrin[a,b] in EMT-6 Sarcoma Mice

	Percent dose per g (n=6)				
	Time after injection, hr				
Tissue	1	6	24[c]	48	72
Blood	43.27 ± 5.11	23.73 ± 2.20	12.89 ± 0.58	4.56 ± 0.34	2.49 ± 0.37
Tumor	4.90 ± 0.39	8.67 ± 0.52	12.75 ± 0.70	13.34 ± 0.60	12.39 ± 1.59
Liver	10.36 ± 0.76	12.26 ± 1.20	8.85 ± 0.77	7.46 ± 0.57	6.06 ± 0.44
Kidney	11.55 ± 1.47	8.92 ± 0.48	8.42 ± 0.45	7.16 ± 0.49	6.14 ± 0.41
Muscle	0.83 ± 0.12	1.38 ± 0.19	1.57 ± 0.21	1.32 ± 0.14	1.07 ± 0.13
Heart	6.97 ± 0.67	4.60 ± 0.32	3.81 ± 0.25	2.47 ± 0.20	1.92 ± 0.10
Spleen	9.81 ± 0.73	9.12 ± 0.56	8.74 ± 0.52	7.30 ± 0.77	5.77 ± 0.71
Bone	5.81 ± 0.74	4.26 ± 0.38	3.56 ± 0.22	2.61 ± 0.24	2.50 ± 0.27
% Dose remaining in whole body	81.63 ± 2.26	80.02 ± 1.95	66.61 ± 3.59	55.88 ± 2.62	44.21 ± 2.80

a. Purified on Sephadex G-150 (0.9 × 100 cm) column. Fraction used here contained essentially monomeric transferrin with about 65% of the original ruthenium activity associated with it.
b. Dose of transferrin ~3.3 mg/Kg body wt.
c. n = 11.

Table 3

Tissue Uptake of Gallium-67 Citrate in EMT-6 Sarcoma Mice

	Percent dose per g (n=6)				
	Time after injection, hr				
Tissue	6	24	48	72	96
Blood	11.17 ± 1.03	1.81 ± 0.22	0.51 ± 0.07	0.44 ± 0.03	0.36 ± 0.07
Tumor	6.01 ± 0.74	7.09 ± 0.69	5.05 ± 0.46	4.53 ± 0.34	3.54 ± 0.62
Liver	8.52 ± 0.64	9.30 ± 0.95	9.49 ± 1.57	8.35 ± 1.09	8.19 ± 1.10
Kidney	7.44 ± 0.66	9.21 ± 0.82	8.03 ± 0.63	7.50 ± 0.73	7.14 ± 1.25
Muscle	0.87 ± 0.08	0.53 ± 0.002	0.47 ± 0.004	0.47 ± 0.08	0.37 ± 0.06
Heart	3.09 ± 0.29	2.21 ± 0.27	1.69 ± 0.10	1.82 ± 0.14	1.51 ± 0.52
Spleen	5.78 ± 0.81	8.37 ± 0.91	6.60 ± 0.78	6.52 ± 1.13	5.41 ± 1.06
Bone	16.16 ± 1.05	17.75 ± 2.50	17.55 ± 1.40	15.38 ± 2.30	12.66 ± 1.12
% Dose remaining in whole body	65.23 ± 0.79	59.58 ± 1.72	50.68 ± 0.21	43.91 ± 1.69	38.29 ± 2.69

Table 4
Tumor-to-Blood and Tumor-to-Tissue Ratios of Ruthenium-103 Chloride, Ruthenium-103-Transferrin, and Gallium-67 Citrate in EMT-6 Sarcoma Mice (n=6)[a]

Compound	Time post injection, hr	Ratio, tumor to Blood	Muscle	Liver	Kidney
Ruthenium-103 chloride	1	0.34 ± 0.01	1.86 ± 0.06	0.43 ± 0.04	0.22 ± 0.00
	6	0.59 ± 0.00	2.50 ± 0.24	0.45 ± 0.05	0.28 ± 0.02
	24	1.48 ± 0.07	4.28 ± 0.57	0.91 ± 0.09	0.51 ± 0.05
	48	2.67 ± 0.12	3.79 ± 0.27	0.96 ± 0.11	0.57 ± 0.05
	72	3.66 ± 0.44	4.11 ± 0.52	0.87 ± 0.13	0.66 ± 0.06
	96	4.36 ± 0.68	3.42 ± 0.29	0.89 ± 0.14	0.60 ± 0.06
Ruthenium-103 transferrin	1	0.12 ± 0.01	6.37 ± 0.89	0.48 ± 0.03	0.44 ± 0.04
	6	0.37 ± 0.02	6.61 ± 0.64	0.72 ± 0.04	0.97 ± 0.05
	24	1.00 ± 0.07	8.76 ± 1.10	1.48 ± 0.12	1.52 ± 0.07
	48	2.97 ± 0.14	10.63 ± 1.27	1.83 ± 0.15	1.89 ± 0.12
	72	5.04 ± 0.15	11.79 ± 1.16	2.04 ± 0.20	2.01 ± 0.18
Gallium-67 citrate	6	0.53 ± 0.003	7.30 ± 1.05	0.71 ± 0.06	0.82 ± 0.08
	24	4.17 ± 0.58	13.36 ± 1.52	0.81 ± 0.12	0.77 ± 0.06
	48	10.70 ± 1.86	11.04 ± 1.25	0.56 ± 0.06	0.63 ± 0.05
	72	10.66 ± 1.24	10.78 ± 2.07	0.57 ± 0.06	0.61 ± 0.04
	96	10.33 ± 1.36	10.55 ± 2.25	0.40 ± 0.07	0.51 ± 0.04

[a] For experimental conditions, refer to footnotes in Tables 1-3.

Table 5

Tissue Uptake of Various Other Promising Ruthenium-103-Labeled Compounds[a] in EMT-6 Sarcoma Mice (% Dose Per g) (n=6)

		Time (hr)	Tumor	Blood	Muscle	Liver	Kidney	Whole Body Retention (% Dose)	Ratio, Tumor to: Blood	Muscle
1.	$K_2[RuCl_5(H_2O)]$[b]	24	6.76	5.46	2.06	10.89	11.76	61.86	1.3	3.3
2.	$K_3[Ru(Ox)_3]1.xH_2O$[c]	24	5.97	3.81	2.03	5.22	6.32	57.77	1.6	3.0
3.	$[Ru(NH_3)_5Cl]Cl_2$[d]	24	3.29	1.97	0.79	2.37	5.89	25.70	1.7	4.2
		96	2.30	0.67	0.80	1.63	3.81	17.40	3.5	4.1
4.	cis-$[Ru(NH_3)_4Cl_2]Cl$[e]	24	5.38	5.18	1.54	5.12	9.23	49.61	1.1	3.5
		72	5.93	2.10	1.32	4.14	7.21	43.80	-	-
		96	4.92	1.15	1.20	3.23	5.35	37.06	4.4	4.2
5.	cis-$[Ru(en)_2Cl_2]Cl$[f]	24	5.97	5.67	1.69	5.76	11.15	52.80	1.1	3.6
6.	Ru-Oxine 7-CA[g]	24	2.38	2.34	0.39	3.03	2.88	19.01	1.0	6.3
		72	1.68	0.72	0.24	1.49	1.46	9.61	2.4	7.2
		96	1.48	0.47	0.21	1.54	1.38	8.42	3.2	7.7

a. All compounds were synthesized using stable ruthenium carrier, in collaboration with Dr. Michael Clarke of Boston College, Chestnut Hill, MA; ruthenium dose 2-6 mg/Kg body weight
b. Potassium pentachloroaquoruthenate(III)
c. Potassium tris-(oxalato)ruthenium(III)chloride
d. Chloropentaammineruthenium(III)chloride
e. cis-Dichlorotetraammineruthenium(III)chloride
f. cis-Dichloro bis(ethylenediamine)ruthenium(III)chloride
g. 8-Hydroxyquinoline 7-carboxylic acid

Table 6

Tumor Concentration Index[a] of Various Ruthenium-103-Labeled Compounds In EMT-6 Sarcoma (Balb/c mice)

Compound[b]	TCI			
	24 hr	48 hr	72 hr	96 hr
Ru-Transferrin	2.70	3.60	4.08	--
Ru-Oxine 7-Carboxylic Acid	1.90	2.39	2.68	2.50
Gallium-67 Citrate	1.81	1.48	1.57	1.45
cis-$[Ru(en)_2Cl_2]Cl$	1.70	--	--	--
$[Ru(NH_3)_5Cl]Cl_2$	1.70	1.46	1.37	1.49
$K_2[RuCl_5(OH_2)]$	1.65	--	--	--
$K_3[Ru(Ox)_3]$	1.64	--	--	--
cis$[Ru(NH_3)_4Cl_2]Cl$	1.56	1.74	2.03	2.00
Ruthenium Chloride	1.56	1.69	1.73	1.61

a. Tumor concentration index (TCI) is defined as the ratio of per cent injected dose per g in the tumor to per cent injected dose per g remaining in the whole body at any given time period. For inter-species comparisons, and for normalizing for different animal body weights, TCI could be expressed as per Kg body wt.

b. For explanations, refer to table 5.