The Effect of Scandium on the Tissue Distribution of Ga-67 in Normal and Tumor-Bearing Rodents

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In rats and mice the intravenous administration of scandium before or with Ga-67 produces an increase in Ga-67 excretion and bone deposition, coupled with pronounced decreases in the uptake of Ga-67 in soft tissues. These effects result from the blocking by scandium of Ga-67 plasma-protein binding sites, which forces Ga-67 into an unbound or loosely bound state. This increases Ga-67 excretion and bone deposition, which in turn acts to produce greatly reduced Ga-67 uptake in soft tissues. When tumor-bearing rats and mice are administered scandium, similar effects occur, but the uptake of Ga-67 by tumor tissue remains unchanged. This suggests that Ga-67 enters tumor and normal soft tissues by different routes. With tumor, an unbound or loosely bound form of gallium is primarily involved, whereas with normal soft tissues this route is apparently of minor importance.

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We have previously reported that the simultaneous administration of scandium with Ga-67 resulted in pronounced decreases in the concentration of Ga-67 in normal soft tissues whereas the concentration in a transplanted rat tumor remained essentially unchanged (*1*). Based on our subsequent experience with this scandium effect, and on other supporting observations, we recently proposed that different routes are involved in the initial uptake of Ga-67 by tumor and by normal tissues (*2*). We report here the results of our detailed studies of the effect of scandium on the tissue distribution of Ga-67 in rats and mice, and discuss the significance of these findings.

MATERIALS AND METHODS

Gallium-67 was obtained as the chloride,* and converted to the citrate form as previously reported (*3*). The citrate was also obtained commercially.† Gallium-68, eluted from a commercial generator,‡ was converted to the chloride form by an ion-exchange technique (*4*). Scandium-46 was procured* with a specific activity of 0.3 mCi/μg Sc.

Scandium oxide was purchased commercially,‖ with a purity stated by the supplier to exceed 99.9%. It was dissolved in concentrated HCl, and most of the excess HCl removed by multiple evaporations following additions of distilled water. After assay for scandium content by the xylenol orange technique (*5*), the chloride solution was diluted to give a stock preparation containing 50 mg Sc/ml in approximately 0.1 *N* HCl.

The experimental animals were Fischer-344 and Buffalo rats§ and $CD2F_1$ and DBA/2 mice.¶ Only male animals were used, in order to avoid any sex effect on Ga-67 tissue distribution. In each study, animals were of the same age ±2 wk. The RFT transplantable tumor (host, Fischer-344 rats) was developed in our own laboratory (*3*). The 5123C and 7777 Morris hepatomas (host, Buffalo rats) were obtained from Dr. Fred Snyder of our Division staff. The P-1798 mouse lymphosarcoma** (host $CD2F_1$ mice) was donated, and the SaD_2 mouse fibrosarcoma†† (host, DBA/2 mice) purchased. Transplantation of tumors was made by intramuscular trocar implantation in the upper hind leg. In each study the animals used had been implanted with tumor at the same time and from the same tumor. The tumor weights

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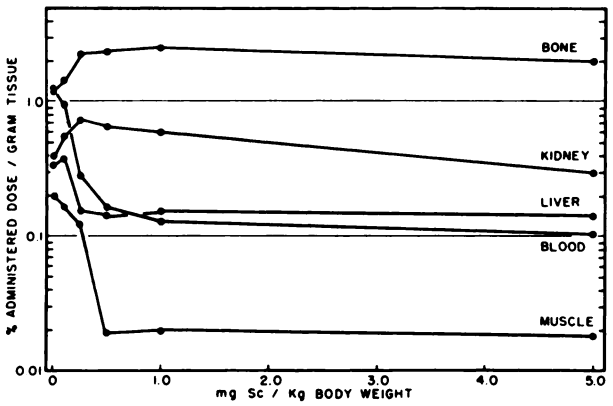

FIG. 1. Effect of scandium administration on 2-hr tissue distribution of Ga-68 in Fischer-344 rats. Points are means of measurements on four animals.

did not exceed 5% of the body weight at the time of sacrifice.

Animals (four per group) were injected intravenously by tail vein. Rats received 2–10 μCi of Ga-67, Ga-68, or Sc-46, and mice 0.5–1 μCi of Ga-67. Those given scandium received citrate at a citrate-to-scandium molar ratio of 3:1. Gallium-67, Ga-68, and Sc-46 were administered with citrate at a level of 1 mg/kg. All injectates were adjusted to pH ~7. Normal saline was used as a diluent and the volumes injected did not exceed 1 ml with rats or 0.3 ml with mice. Animals were killed by exsanguination following brief ether anesthesia. Blood was obtained by puncture of the abdominal aorta, and tissue samples were blotted as free as possible of blood before being weighed and counted in a well scintillation counter. Only obviously viable tumor tissue was chosen for counting. The concentrations of Ga-67, Ga-68, and Sc-46 were calculated as percentage of administered dose per gram of tissue, normalized to a body weight of 250 g with rats and 25 g with mice. Body retention of Ga-67 was measured by whole-body counting, using the geometry-independent method of Gibbs et al. (*6*); approximately 50 μCi of Ga-67 were administered for this purpose.

RESULTS

Our initial studies of scandium were concerned with the development of methods for altering the tissue distribution of 68-min Ga-68 to promote the rapid and preferential uptake of this radionuclide by bone without having to administer stable gallium (*7*). Figure 1 shows the effect of scandium citrate administration on the 2-hr tissue distribution of Ga-68 in the Fischer-344 rat.

Following the observation that Ga-67 showed an unusual affinity for malignancies in humans (*8*) and transplanted tumors in animals (*3*), we tested scandium for its effect on the tissue distribution of Ga-67 in rats bearing RFT tumors. The results of our initial study are shown in Table 1. It is apparent that scandium reduced Ga-67 concentrations in normal soft tissues, as expected, but did not affect the concentration in the tumor tissue. A subsequent study indicated that the most effective scandium dose for producing this effect was approximately 0.5 mg Sc/kg. A test of the effectiveness of scandium administration in increasing tumor-to-soft-tissue ratios was then carried out using a variety of transplanted tumors in both rats and mice. Both early (Table 2, 4 hr) and late (Table 3, 24 hr) effects were studied. It was apparent that scandium was effective in both tumor-bearing rats and mice at early time periods, but at longer time periods in rats only. Figure 2 shows a Ga-67 body retention study carried out in Fischer-344 rats and $CD2F_1$ mice. Administration of scandium caused an elevated excretion of Ga-67 that was particularly pronounced in the rat. A further study (Table 4) showed that scandium could be administered as much

TABLE 1. EFFECT OF SCANDIUM ON TISSUE DISTRIBUTION OF Ga-67 IN RATS BEARING RFT TUMORS

	Time, scandium dose, and significance					
	4 hr			24 hr		
	Sc (mg/kg)		Sig.	Sc (mg/kg)		Sig.
Tissue	0.0	0.5	P*	0.0	0.5	P
Tumor	2.30 (1.80–2.50)†	2.80 (2.30–3.50)	—	2.30 (1.80–3.00)	3.30 (3.10–3.80)	MS
Liver	0.83 (0.79–0.88)	0.17 (0.16–0.18)	HS	0.86 (0.76–0.94)	0.21 (0.19–0.22)	HS
Spleen	0.92 (0.86–0.95)	0.12 (0.11–0.13)	HS	1.30 (1.10–1.40)	0.20 (0.19–0.21)	HS
Marrow	1.20 (1.20–1.30)	0.20 (0.14–0.30)	HS	0.77 (0.69–0.88)	0.17 (0.16–0.18)	HS
Muscle	0.23 (0.21–0.26)	0.03 (0.02–0.03)	HS	0.14 (0.09–0.18)	0.01 (0.01–0.01)	HS
Femur	0.91 (0.84–0.96)	1.50 (1.40–1.70)	HS	0.95 (0.76–1.20)	1.50 (1.20–1.60)	HS
Blood	1.70 (1.60–1.80)	0.25 (0.14–0.31)	HS	0.12 (0.09–0.16)	0.08 (0.07–0.09)	MS

* MS = marginally significant, P = 0.01–0.05; HS = highly significant, P < 0.001.

† Percent administered Ga-67/g; mean (range).

TABLE 2. EFFECT OF SCANDIUM ON 4-HR TISSUE DISTRIBUTION OF Ga-67 IN VARIOUS TUMOR-BEARING RATS AND MICE

	Tumor type, scandium dose, and significance					
	Rat-7777			Rat-5123C*		
	Sc (mg/kg)		Sig.	Sc (mg/kg)		Sig.
Tissue	0.0	0.5	P†	0.0	0.5	P
Tumor	2.80 (1.70–4.00)‡	5.90 (3.80–9.20)	—	4.20 (3.10–5.10)	4.00 (3.40–5.10)	—
Liver	0.57 (0.38–0.75)	0.24 (0.14–0.35)	MS	0.58 (0.51–0.68)	0.14 (0.12–0.15)	HS
Spleen	0.76 (0.49–1.00)	0.19 (0.12–0.25)	S	1.20 (1.10–1.40)	0.14 (0.13–0.15)	HS
Marrow	1.10 (0.97–1.40)	0.18 (0.13–0.23)	HS	1.10 (0.94–1.20)	0.17 (0.14–0.20)	HS
Muscle	0.18 (0.12–0.26)	0.04 (0.02–0.08)	S	0.17 (0.14–0.19)	0.02 (0.02–0.02)	HS
Femur	0.81 (0.58–0.98)	1.40 (1.20–1.80)	S	0.65 (0.61–0.68)	1.25 (1.10–1.40)	HS
Blood	1.10 (0.89–1.30)	0.35 (0.24–0.42)	HS	1.00 (0.92–1.10)	0.31 (0.29–0.32)	HS
	Mouse P-1798			Mouse-SaD2		
Tumor	6.70 (5.30–8.00)	10.00 (9.60–11.00)	S	8.60 (7.00–12.00)	10.00 (7.80–12.00)	—
Liver	4.00 (3.20–4.40)	1.20 (0.91–1.50)	HS	4.90 (4.40–5.90)	2.70 (2.30–3.60)	S
Spleen	3.50 (3.10–4.20)	0.97 (0.76–1.20)	HS	5.40 (4.50–6.90)	2.50 (2.00–3.40)	S
Muscle	0.80 (0.50–0.99)	0.09 (0.06–0.13)	HS	2.00 (0.96–4.80)	0.32 (0.21–0.63)	—
Femur	4.40 (2.70–5.90)	6.80 (5.60–8.50)	—	5.90 (3.70–9.20)	13.00 (11.00–15.00)	S
Blood	5.00 (3.70–6.30)	1.90 (1.30–2.60)	S	9.50 (8.50–11.00)	5.00 (1.90–7.90)	MS

* Previously reported in different format in Ref. *1*.

† MS = marginally significant, P = 0.01–0.05; S = significant, P = 0.001–0.01; HS = highly significant, P < 0.001.

‡ Percent administered Ga-67/g; mean (range).

as 4 hr before Ga-67 and still exert an enhancing effect on relative Ga-67 tumor uptake.

These experiments raised the question of whether scandium was being taken up by tumor tissue itself. The data in Table 5 clearly show that scandium has no preferential affinity for the RFT tumor up to a level of 0.5 mg Sc/kg.

DISCUSSION

The studies reported here clearly indicate that in the rat and mouse, administration of scandium makes the Ga-67 uptake higher in bone and lower in soft tissues (Fig. 1 and Tables 1–4). This alteration in tissue distribution is in turn accompanied by a greatly reduced body retention of Ga-67 (Fig. 2).

Ford-Hutchinson and Perkins (*9*) have reported results from both in vitro and in vivo studies that show that scandium binds preferentially to transferrin, a major binder of Ga-67. Gallium has also been reported to bind to lactoferrin (*10*). We have also observed a strong binding of Sc-46 by plasma proteins in in vitro experiments (unpublished results). We feel that the increased excretion, increased bone deposition, and decreased soft-tissue uptake of Ga-67 that is produced by scandium administration result from a blocking by scandium of Ga-67 binding sites on plasma proteins. Similar alterations in tissue distribution are produced by the administration of stable gallium (*7*), and such alterations have been shown to result from the saturation of Ga-67 binding sites on plasma proteins by stable gallium (*11*). Thus with both scandium and stable gallium administration, Ga-67 appears to be forced into an unbound or loosely bound state, which enhances its renal excretion and bone deposition, resulting in turn in decreased Ga-67 uptake in normal soft tissues.

When we tested the effect of scandium on the tissue distribution of Ga-67 in a tumor-bearing rat (Table 1), we obtained the expected decreased concentration of

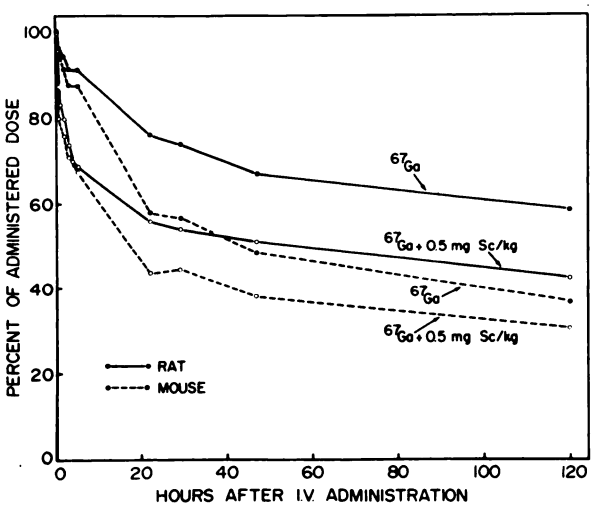

Fig. 2. Effect of scandium administration on body retention of Ga-67 in Fischer-344 rat and $CD2F_1$ mouse. Points are means of measurements on four animals.

TABLE 3. EFFECT OF SCANDIUM ON 24-HR TISSUE DISTRIBUTION OF Ga-67 IN VARIOUS TUMOR-BEARING RATS AND MICE

	Tumor type, scandium dose, and significance					
	Rat-7777			Rat-5123C*		
	Sc (mg/kg)		Sig.	Sc (mg/kg)		Sig.
Tissue	0.0	0.5	P†	0.0	0.5	P
Tumor	5.80 (5.40–6.10)‡	7.70 (6.80–8.80)	S	7.10 (5.50–9.30)	5.90 (3.90–7.60)	—
Liver	0.80 (0.78–0.82)	0.25 (0.15–0.36)	HS	0.55 (0.52–0.59)	0.25 (0.16–0.33)	HS
Spleen	1.20 (0.98–1.30)	0.23 (0.15–0.35)	HS	1.50 (1.30–1.70)	0.29 (0.23–0.33)	HS
Marrow	1.20 (1.00–1.30)	0.29 (0.13–0.57)	HS	1.00 (1.00–1.10)	0.26 (0.19–0.29)	HS
Muscle	0.17 (0.15–0.19)	0.02 (0.01–0.02)	HS	0.08 (0.06–0.11)	0.01 (0.01–0.01)	HS
Femur	1.30 (1.20–1.30)	1.60 (1.40–1.70)	HS	0.51 (0.43–0.59)	0.96 (0.69–1.10)	S
Blood	0.18 (0.15–0.19)	0.09 (0.06–0.10)	HS	0.06 (0.04–0.08)	0.07 (0.04–0.09)	—
	Mouse P-1798			Mouse SaD2		
Tumor	6.00 (4.10–8.10)	4.70 (3.80–6.10)	—	13.00 (11.00–15.00)	12.00 (11.00–13.00)	—
Liver	5.00 (3.80–5.80)	2.50 (1.70–3.10)	S	6.70 (5.80–7.80)	5.80 (4.70–6.70)	—
Spleen	4.40 (3.30–5.40)	2.70 (2.10–3.50)	MS	4.40 (3.80–4.90)	5.50 (4.10–7.40)	—
Muscle	0.64 (0.40–1.20)	0.16 (0.11–0.21)	MS	0.31 (0.27–0.38)	0.17 (0.12–0.25)	MS
Femur	9.60 (5.60–13.0)	12.00 (9.80–13.0)	—	8.60 (7.80–10.00)	8.90 (8.20–11.00)	—
Blood	0.92 (0.71–1.10)	0.65 (0.39–0.87)	—	2.30 (1.70–3.10)	2.10 (1.20–2.80)	—

* Previously reported in different format in Ref. *1*.

† MS = marginally significant, P = 0.01–0.05; S = significant, P = 0.001–0.01; HS = highly significant, P < 0.001.

‡ Percent administered Ga-67/g; mean (range).

Ga-67 in normal tissues, but, to our surprise, the concentration of Ga-67 in tumor tissue remained unchanged. This behavior of transplanted tumor tissue appears to be general for both rat and mouse tumors at 4 hr (Tables 1 and 2), but only for rat tumors at 24 hr (Tables 1 and 3). This difference between species is possibly accounted for by the more pronounced effect that scandium has on the body retention of Ga-67 in the rat (Fig. 2).

The pronounced difference in the behavior of tumor tisue and normal soft tissue produced by administration of scandium strongly suggests that in the initial biodistribution phase, Ga-67 enters tumor tissue and normal soft tissues by different routes. As indicated above, administration of scandium appears to force Ga-67 in the direction of an unbound or loosely bound state. Under such circumstances, tumor tissue can apparently compete effectively with the renal excretory process and the deposition of Ga-67 in bone. This suggests that uptake of Ga-67 by tumor tissue involves primarily an unbound or loosely bound form of gallium. Apparently this route is not of importance in the entry of Ga-67 into normal soft tissues.

Since we are attributing the effect of scandium to its blocking of Ga-67 binding sites on plasma proteins, our observation that the uptake of Ga-67 by different tumor tissues does not change when scandium is administered, appears to be in conflict with a study by Bradley et al. (*12*). They reported a decrease in Ga-67 uptake in both tumor and normal soft tissues in rats bearing Walker-256 carcinosarcomas when the serum iron-binding capacity was decreased by irradiation or administration of the hemolyzing agent, N-acetylphenylhydrazine. Their study differed basically from ours in that they assayed the entire tumor for Ga-67 content rather than just its viable portion, as we did. Walker-256 tumors tend to be rather necrotic, and necrotic portions of tumors show much less Ga-67 uptake than do viable portions (*1*). This probably accounts for the fact that they observed a considerably lower uptake of Ga-67 in the Walker-256 tumor than we did (*3*). Differences in amounts of necrosis in treated and control animal tumors might account for their observation of a lower overall tumor uptake of Ga-67 in their treated animals. In other experiments (to be reported in another communication) we have observed that serum iron loading by administration of iron agents produces effects similar to those produced by scandium administration in tumor-bearing animals, although to a less pronounced degree.

NOTE

Although the enhancing effect of scandium on tumor-to-nontumor ratios would appear to be of use in clinical studies, it would be hazardous, since, in spite of the low toxicity observed in animals (including a primate), scandium was found to produce hemolysis of human red blood cells (*13*).

FOOTNOTES

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TABLE 4. EFFECT OF SCANDIUM ADMINISTRATION TIME ON 4-HR TISSUE DISTRIBUTION OF Ga-67 IN RATS BEARING MORRIS 7777 HEPATOMAS

	Time of scandium administration (0.5 mg/kg)			
Tissue	No Sc	Sc with Ga-67	Sc 2 hr before Ga-67	Sc 4 hr before Ga-67
Tumor	3.00 (2.70–3.20)*	5.80 (5.00–6.50)	6.00 (5.20–6.70)	6.70 (6.20–7.70)
Liver	0.70 (0.66–0.74)	0.15 (0.13–0.17)	0.18 (0.12–0.21)	0.39 (0.35–0.44)
Spleen	0.89 (0.76–1.00)	0.12 (0.10–0.15)	0.18 (0.12–0.24)	0.37 (0.32–0.41)
Marrow	1.10 (1.10–1.20)	0.20 (0.10–0.41)	0.25 (0.14–0.33)	0.50 (0.42–0.62)
Muscle	0.23 (0.19–0.30)	0.03 (0.02–0.04)	0.03 (0.03–0.04)	0.08 (0.06–0.12)
Femur	0.94 (0.92–0.98)	1.50 (1.30–1.70)	1.40 (1.20–1.50)	1.50 (1.20–1.70)
Blood	1.10 (1.00–1.20)	0.27 (0.23–0.34)	0.43 (0.29–0.55)	0.77 (0.37–1.10)

* Percent administered Ga-67/g; mean (range).

TABLE 5. EFFECT OF TIME AND STABLE SCANDIUM ON TISSUE DISTRIBUTION OF Sc-46 IN RATS BEARING RFT TUMORS

	Time and stable scandium dose			
	4 hr		24 hr	
	Sc (mg/kg)		Sc (mg/kg)	
Tissue	0.0	0.5	0.0	0.5
Tumor	0.21 (0.17–0.24)*	0.21 (0.17–0.24)	0.35 (0.29–0.45)	0.26 (0.20–0.29)
Liver	1.20 (1.10–1.30)	1.20 (1.10–1.30)	1.70 (1.50–2.00)	2.60 (2.40–2.80)
Spleen	1.50 (1.40–1.70)	1.10 (1.00–1.20)	2.20 (1.70–2.90)	2.20 (2.00–2.50)
Marrow	1.80 (1.70–2.00)	1.10 (0.98–1.30)	2.20 (2.00–2.60)	1.60 (1.10–2.00)
Muscle	0.19 (0.17–0.21)	0.22 (0.19–0.24)	0.17 (0.09–0.22)	0.20 (0.18–0.21)
Femur	0.57 (0.51–0.63)	0.57 (0.54–0.60)	0.84 (0.69–0.96)	0.96 (0.84–1.00)
Blood	2.00 (1.90–2.20)	2.00 (1.90–2.20)	0.31 (0.28–0.35)	0.28 (0.25–0.29)

* Percent administered Sc-46/g; mean (range).

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REFERENCES

1. HAYES RL, EDWARDS CL: New applications of tumour-localizing radiopharmaceuticals. In *Medical Radioisotope Scintigraphy 1972*, vol. II, Vienna, IAEA, 1973, pp 531-551
2. HAYES RL, RAFTER JJ, BYRD BL: Studies of the mechanism of gallium-67 uptake by tumor, abscess and normal tissues. *J Nucl Med* 20: 672-673, 1979 (abst)
3. HAYES RL, NELSON B, SWARTZENDRUBER DC, et al: Gallium-67 localization in rat and mouse tumors. *Science* 167: 289-290, 1970
4. CARLTON JE, HAYES RL: Rapid separation of generator-produced gallium-68 from EDTA eluate. *Int J Appl Radiat Isot* 22: 44-45, 1971
5. BERMAN SS, DUVAL GR, RUSSELL DS: The spectrophotometric determination of scandium in copper with xylenol orange. *Anal Chem* 35: 1392-1394, 1963
6. GIBBS WD, HODGES HD, LUSHBAUGH CC: Precise geometry-independent radioassay of large biological samples. *J Nucl Med* 9: 264-266, 1968
7. HAYES RL: Radioisotopes of gallium. In *Radioactive Pharmaceuticals*, Andrews GA, Kniseley RM, Wagner HN, eds., Symposium Series 6 CONF-651111. Springfield, VA, National Bureau of Standards, 1966, pp 603-618
8. EDWARDS CL, HAYES RL: Tumor scanning with ^{67}Ga citrate. *J Nucl Med* 10: 103-105, 1969
9. FORD-HUTCHINSON AW, PERKINS DJ: The binding of scandium ions to transferrin in vivo and in vitro. *Eur J Biochem* 21: 55-59, 1971
10. HOFFER PB, HUBERTY J, KHAYAM-BASHI H: The association of Ga-67 and lactoferrin. *J Nucl Med* 18: 713-717, 1977
11. HARTMAN RE, HAYES RL: The binding of gallium by blood serum. *J Pharmacol Exp Ther* 168: 193-198, 1969
12. BRADLEY WP, ALDERSON PO, ECKELMAN WC, et al: Decreased tumor uptake of gallium-67 in animals after whole-body irradiation. *J Nucl Med* 19: 204-209, 1978
13. HAYES RL, EDWARDS CL: The effect of stable scandium on red blood cells and on the retention and excretion of ^{67}Ga in humans. *South Med J* 66: 1339-1340, 1973 (abst)