A Transferrin-Mediated Uptake of Gallium-67 by EMT-6 Sarcoma. II. Studies in Vivo (BALB/c Mice): Concise Communication

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The EMT-6 sarcoma-like tumor of BALB/c mice can be grown as a solid subcutaneous transplantable tumor in vivo or as a monolayer culture in vitro. We have studied the uptake of gallium-67 by this tumor growing subcutaneously on the backs of 6-week-old BALB/c mice. After i.v. administration of Ga-67 citrate, tumor uptakes were as high as any others reported for mouse tumors. Also, for unknown reasons, there was appreciable reduction in tumor uptake with increasing amounts of Ga-67 citrate, even in the microcurie range. Furthermore, when mouse serum is prelabeled with Ga-67 and then injected, the EMT-6 uptake is greater than with Ga-67 administered as citrate ($p < 0.02$). We believe that the finding of avid Ga-67 uptake in vivo helps to establish this unique in vivo/in vitro tumor system as a valid experimental model for studies regarding the mechanism of Ga-67 accumulation by neoplastic tissue.

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In the preceding paper (*1*), we described a transferrin-mediated uptake of Ga-67 by EMT-6 tumor cells growing in vitro. Since the EMT-6 tumor also grows as a solid, transplantable, subcutaneous tumor of BALB/c mice, we wondered whether the cellular uptake of Ga-67 in vitro was matched by a correspondingly active uptake in vivo. Accordingly, we studied uptake of Ga-67 after i.v. injection of Ga-67 citrate. Also, since transferrin is important to Ga-67 uptake in vitro, we compared the in vivo tumor uptake after i.v. injection of equal microcurie amounts of Ga-67 as citrate and Ga-67-labeled mouse serum.

MATERIALS AND METHODS

Inbred strain BALB/c mice were used*. Because a variety of host factors, including age and sex, affect tissue distribution of Ga-67 citrate, we used mice from a single supplier*, all being cohorts for age and sex. We selected tumors of about 100 mg (range 50-200 mg). We used the same microcurie dose of Ga-67 citrate from the same lot. We randomly allocated mice to experimental groups, which were large enough so that meaningful statistics could be obtained. Animals were obtained at 5 weeks of age, and were held for 2 wk before use. Each weighed about 20 g. The tumor was transplanted subcutaneously on the back by injecting 2 x 10^5 EMT-6 tumor cells in 0.1 ml of Hank's balanced saline solution. "Takes" were highly reproducible and occurred in virtually all the animals, but some spontaneous regressions also occurred. By 9-12 days posttransplant, the tumor weighed approximately 100 mg. An i.v. injection of Ga-67 citrate was made through the tail vein. Animals were killed at various times after injection—2,4, 6,24, and 48 hr. Just before death, 100 μl of blood

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were drawn from the retro-orbital plexus. At necropsy the tumor, liver, and muscle samples were removed and counted to < 1% relative error. By reference to the appropriate standard the results were expressed as percentage injected dose of Ga-67 per gram of tissue. Ratios of tumor concentration to the concentration in other tissues were also computed.

The Ga-67-labeled serum protein used for these experiments was prepared by incubating 10 μl of a Ga-67-citrate solution with 1.5 ml of nonhemolyzed serum from tumor-free BALB/c mice. The mixture was allowed to incubate for 3 hr at 37°. The mouse serum was then diluted one part serum to two parts 0.9% NaCl (pH 7.4). An aliquot was used to determine percentages of Ga-67 bound to serum protein. The remainder of the Ga-67-labeled serum was used for biodistribution studies.

The percentage of protein labeling was measured using an ultrafiltration cell† with a membrane designed to exclude molecules of greater than 30,000 mol wt (PM-30). The measured labeling percentage was 88%.

Gallium-67 as citrate was obtained within 72 hr of production and measured 3.01 mCi/ml on the day of use. Depending on the dose to be administered, a microcurie amount of the Ga-67 solution was taken with a Hamilton syringe and added to a volumetric flask. Dilution was made with saline so that the administered volume would always be 200 μl. Initial experiments used 0.5-4μCi per mouse. When it became evident that the larger amounts of radioactivity were associated with a reduction in the uptake, 0.25 μCi per mouse was used in all subsequent experiments. The biodistributions of Ga-67 as citrate and as labeled mouse serum (Ga-67 presumably bound to transferrin) were compared in an age cohort group of 38 BALB/c mice bearing the EMT-6 tumor. The experiment was performed as follows. Mice bearing 100-mg EMT-6 sarcoma tumors located subcutaneously on the back were randomly allocated to two groups. One of the groups received 0.25 μCi Ga-67-labeled mouse serum per mouse. Six animals from each of the two experimental groups were killed at 6 hr after injection. An additional 24 animals were killed at 24 hr, 11 from the Ga-67 citrate group and 13 from the Ga-67 serum group. Biodistribution was determined and the results expressed as above.

RESULTS

In our initial experiments, various doses of Ga-67 citrate were used—from 0.5 μCi to 4.0 μCi per mouse. A least-squares linear correlation was sought between the percentage dose per gram of tumor and the μCi dose of Ga-67 citrate. There was a highly significant, negative correlation between uptake and dose ($r = 0.58$, $p < 0.0001$, $df = 67$).

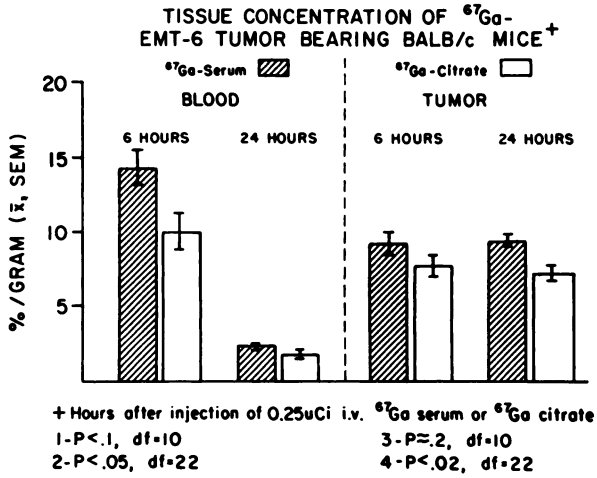

FIG. 1. Time course of concentration of Ga-67 (percentage dose per gram) for blood (dashed line) and EMT-6 sarcoma (solid line), after i.v. administration of 0.25 μCi of Ga-67 citrate in BALB/c mice. T/B = tumor-to-blood ratio.

Figure 1 shows the time course of Ga-67 uptake after i.v. injection of 0.25 μCi Ga-67 citrate. The solid line is the uptake into tumor; the dashed line shows blood activity. The uptake into tumor was relatively rapid, peaking (12% per gram) at 4-6 hr after injection. There was a modest decline in tumor content thereafter, but at 24 and 48 hr it was still high (7-10% dose per gram). The blood activity fell progressively, so that by 24 hr tumor-to-blood activity was about 4:1, and by 48 hr more than 10:1. Subsequent comparisons were made at 24 hr as a convenient interval for killing.

In a separate series of experiments the biodistributions of Ga-67 citrate and Ga-67-labeled mouse serum were compared (Table 1). The injected radioactivity (0.25 μCi per mouse) was the same throughout. Some statistically significant differences were observed in biodistribution. In the serum group at 24 hr, there was significantly more activity retained in the carcass ($p < 0.01$)*. This suggests that there was less of the pre-labeled Ga-67 excreted over the interval of study. By 24 hours there was significantly more radioactivity (about one-third) retained in the tumor ($p < 0.02$)‡ and the blood ($p < 0.05$)‡. Tumor-to-blood ratios are not greatly altered, however, because both tumor and blood change in the same direction. A summary of the comparative findings for blood and tumor is shown in Fig. 2. Data are presented for biodistribution at 6 hr (six mice per group) and 24 hr (11 mice in the citrate group and 13 given prelabeled serum). The average blood and tumor uptakes were higher with the labeled serum. Blood clearance was relatively rapid for both preparations, but tumor

TABLE 1. COMPARISON OF BIODISTRIBUTION OF GA-67 CITRATE AND GA-67 SERUM

		Blood*	Tumor*	Liver*	Whole body†	Muscle*	Ratios
Ga-67 citrate‡	$\bar{X}$	1.76	7.13	13.45	42.3	0.78	T/B[1], T/L[2], T/M[3]
	SD	0.44	2.22	3.27	4.47	0.37	4.05, 0.53, 9.14
	SEM	0.13	0.67	0.99	1.35	0.11	
Ga-67 serum‖	$\bar{X}$	2.20	9.48	11.11	49.40	0.72	T/B[1], T/L[2], T/M[3]
	SD	0.55	2.04	3.07	6.87	0.22	4.31, 0.85, 13.17
	SEM	0.15	0.56	0.85	1.90	0.06	
	p§	< 0.05	< 0.02	< 0.10	< 0.01	NS	

* Percent injected dose 0.25 μCi per mouse per gram tissue, at 24 hrs.
† Dose percentage.
‡ Data from 11 mice.
‖ Data from 13 mice.
§ Student's t-test for differences between means.
1- tumor-to-blood; 2- tumor-to-liver; 3- tumor-to-muscle.

uptake remained relatively stable. At 6 hr the differences were of borderline significance, but by 24 hr there was a clear-cut difference for blood and tumor levels, and the retention of Ga-67 was clearly greater in the mice that received prelabeled mouse serum. Significant concentration in tumor relative to blood and muscle is seen for both dosage forms at 24 hr. The liver seems to concentrate Ga-67 more effectively than tumor.

DISCUSSION

In vivo, Ga-67 citrate uptake has been studied for several mouse tumors. In terms of percentage dose per gram of tumor, uptake has varied from 3.8% per gram for Ehrlich's carcinoma growing subcutaneously on the thigh of the mouse (*3*) to 10.5% per gram for KHJJ carcinoma growing subcutaneously in the flank (*4*). Thus the uptake we observed in the EMT-6 sarcoma was in the highest range reported for mouse-tumor uptakes.

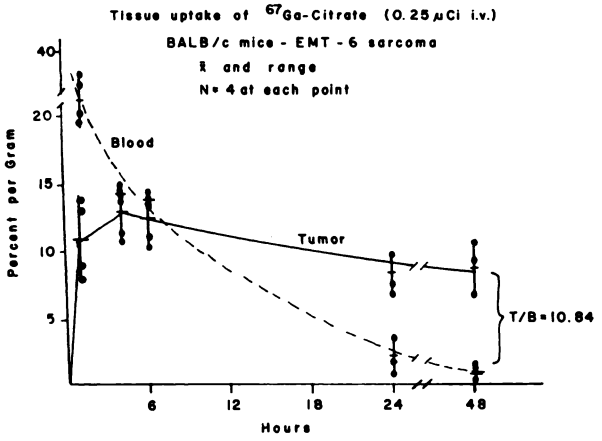

FIG. 2. Comparison of Ga-67 activity in blood and tumor, at 6 and 24 hr after equal i.v. injections of Ga-67 as citrate (clear columns) and as prelabeled mouse serum (hatched columns). Tumor and blood concentrations are both greater with labeled serum.

A possible role of transferrin in in vivo uptake was suggested by our in vitro experiments with the EMT-6 tumor (*1*). We did observe some increase in Ga-67 tumor uptake with labeled serum (presumably Ga-67 transferrin) (*6,7*), and these results are therefore consistent with the hypothesis that it is the Ga-67 transferrin that is the active radiopharmaceutical form in vivo. The differences observed were not great in absolute terms, however, and the clinical utility of Ga-67 transferrin as a dosage form for Ga-67 tumor imaging must await further studies.

A possible role for transferrin has also been suggested by studies in which tumor uptake of Ga-67 was inversely correlated with the degree of saturation of iron-binding capacity (*8*). When Sprague-Dawley rats bearing Walker-256 sarcoma were subjected to whole-body irradiation, this treatment reduced uptake, whereas local radiation to the tumor did not. The whole-body irradiation increased iron levels in plasma. In these studies, the lower the iron-binding capacity of serum, the lower the uptake of Ga-67 by tumor.

On the basis of our in vitro study of EMT-6 (*1*), it appears that at the usual concentration of transferrin in serum (2.5 mg per milliliter) the transferrin receptors on the tumor cells would be completely occupied in vivo. Assuming a relatively rapid turnover of transferrin on and off these receptors, the fractional tumor uptake of Ga-67 in vivo should depend on a) the proportion of the total transferrin pool that is labeled with Ga-67, and b) the proportion of total transferrin receptor sites that are contained in the tumor. In addition, the receptors that turn over more rapidly would sample more of the total transferrin pool. Thus, tumor cells must compete with other tissues that have transferrin recep-

tors. The transferrin receptors will limit the rate of uptake, provided that the cell has sufficient capacity to bind the Ga-67 that is taken up. In the preceding paper (*1*), we observed that Ga-67 equilibrated across the EMT-6 cell membrane in the absence of added human transferrin. This equilibration, presumably by diffusion, implies a two-way movement of Ga-67 across the cell membrane. Thus, the Ga-67 that got into the cell freely could also diffuse out, not being bound to the specific macromolecules known to bind Ga-67. Similarly, we might expect that, if the capacity of the cell for Ga-67 binding to intracellular macromolecules should be saturated once the Ga-67 transferrin that entered the cell was dissociated, the ionic Ga-67 would freely diffuse out of the cell. Thus tissues that either do not contain specific subcellular gallium acceptor molecules or that have already been saturated with gallium would not concentrate Ga-67, even if the tissues had abundant transferrin receptors. Of course, this hypothesis for the role of transferrin receptors in determining distribution of Ga-67 is still highly speculative. Nonetheless, it is consistent with the experimental observations so far reported. Moreover, it is amenable to further testing.

Animal tumor models such as EMT-6, having both in vitro and in vivo growth forms, provide powerful tools for the study of the uptake mechanisms of tumor-seeking tracers. A number of such systems are available (*9*) and as these are more widely applied to investigative nuclear medicine, we may anticipate a better understanding of those aspects of tumor physiology that permit concentration of oncologic radioagents.

In conclusion, Ga-67 citrate, upon i.v. administration, was avidly concentrated by EMT-6, a subcutaneous, transplantable, sarcoma-like tumor of BALB/c mice. Also, tumor uptake of Ga-67 was even greater with i.v. injection of Ga-67 prelabeled to mouse serum. This finding suggests that binding of Ga-67 to serum proteins (presumably transferrin) plays an important role in the in vivo tumor uptake of Ga-67.

FOOTNOTES

* Simonson Laboratory, Gilroy, CA.
† Amicon, Scientific Systems Division, Lexington, MA.
† Student's t-test.

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