Decreased Tumor Uptake of Gallium-67 in Animals after Whole-Body Irradiation

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The mechanism of decreased Ga-67 citrate retention and serum binding after whole-body irradiation is unknown. To investigate this mechanism and to determine the effects of prior irradiation on tumor uptake of Ga-67, Sprague-Dawley rats bearing a subcutaneous Walker-256 carcinosarcoma were exposed to whole-body Co-60 irradiations of 250–1000 rads. Each animal received 10 μCi of Ga-67 citrate intravenously 24 hr after exposure. Control animals received Ga-67 but were not irradiated. Animals were killed at 48 hr and the uptakes (percentage ID/g) in the tumor and other tissues were determined. A blood sample was also obtained to determine the serum iron, unsaturated iron-binding capacity (UIBC) and transferrin level. Tumor uptake and serum UIBC were decreased in irradiated animals, whereas serum iron levels and Ga-67 urinary excretion were increased. There was a significant correlation between the UIBC and the Ga-67 tumor uptake ($r = 0.78$, $p < 0.001$, $n = 49$). Transferrin levels in the irradiated group were not different from control values. The results indicate that the decreased Ga-67 retention and tumor uptake seen after whole-body irradiation are related—at least in part—to the saturation of transferrin by increased levels of circulating iron.

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In 1973, Swartzendruber and Hübner (*1*) reported that mice had decreased soft-tissue retention and increased urinary excretion of Ga-67 citrate following whole-body x-irradiation. Fletcher et al. reported similar findings in rats and related this to a decreased serum binding of Ga-67 (*2*). However, the mechanism responsible for the decreased serum binding and body retention was not established, and the relationship of this phenomenon to tumor uptake was not investigated. Other investigators have shown that injected Ga-67 citrate binds to the serum protein transferrin (*3–6*), the carrier for iron. There are many reports in the literature of increased serum iron levels and decreased unsaturated iron-binding capacity (UIBC) in animals following irradiation (*7–14*). Injected iron also has been shown to have a dramatic effect on the distribution of Ga-67 (*15*). Thus it seemed possible that the reported effects of irradiation on tissue localization of Ga-67 could be due to the effects of radiation-induced hyperferremia. The current studies were therefore undertaken, using irradiated, tumor-bearing rats, to determine the relationship of Ga-67 tumor uptake and body retention to serum iron.

MATERIALS AND METHODS

Initially, a series of in vitro experiments on Ga-67 binding were performed. Nontumor-bearing Sprague-

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Dawley rats (400–450 g) were exposed to 250, 500, or 750 rads of irradiation of a Co-60 whole-body exposure facility. The irradiation was delivered at the rate of 40 rads/min. Serum was obtained from the irradiated rats (n = 30) 24 hr after exposure. Serum iron and UIBC were determined by the ferrozine reaction using commercially available reagents. Transferrin levels were determined by the Mancini technique (*16*) using commercially available rat transferrin and anti-rat transferrin. The serum was labeled with 0.1 μCi/ml of Ga-67 citrate and was incubated for 30 min at 37°C. Serum (1.8 ml) was placed in cellulose dialysis bags, 12,000 mw exclusion and dialyzed for 24 hr against 5 ml of saline. Dialyses were carried out at 4°C on a platform shaker. All in vitro experiments were performed using plastic equipment that was carefully counted to exclude retention of Ga-67. Additional dialyses were performed using pooled normal rat serum to which had been added increasing levels of iron, similar to the levels observed in the irradiated animals.

For in vivo studies, the Walker-256 carcinosarcoma, maintained in the ascitic form, was transplanted subcutaneously to the flanks of adult male Sprague-Dawley rats (350–400 g). In preliminary experiments, this tumor was shown to be Ga-67-avid (Fig. 1). One week after transplantation, when the tumor weighed 7–12 g, animals were exposed to 250, 500, 750, or 1000 rads of Co-60 irradiation in the whole-body exposure facility. The irradiation was delivered at the rate of 40 rads/min. Twenty-four hr later, irradiated and control animals received a 10-μCi i.v. injection of carrier-free Ga-67 citrate. Before and after irradiation, rats had access to standard laboratory chow and tap water *ad libitum*. The rats were placed in metabolic cages, and two consecutive 24-hr urine collections were obtained to determine percentage of the injected Ga-67 dose (%ID) excreted. When the urine collection was completed, the animals were killed by exsanguination under halothane anesthesia, and various normal tissues and the tumor were removed. Tissue samples were counted in a well counter using standard technique. To minimize tissue-sampling errors, the entire tumor was removed, serially sectioned, and all sections were weighed and counted to determine the average tumor uptake (%ID/g). Tumor-to-blood ratios were also determined. In addition, serum iron and unsaturated iron-binding capacity were determined in control and irradiated rats.

FIG. 1. Gallium-67 image of rat bearing 1-wk-old, subcutaneously implanted, Walker 256 carcinosarcoma on the right flank (arrow). Image was obtained 48 hr after i.v. injection of 50 μCi of Ga-67 citrate. Diverging collimator was used and 75,000 counts recorded.

To determine whether changes in tumor uptake were secondary to direct effects of irradiation, six rats were exposed to 500 rads (40 rad/min) confined only to the tumor site. This radiation was delivered using a standard Maxitron unit. In addition, five rats were exposed to 750 rads in the whole-body room with the tumor-bearing extremity shielded by an array of lead bricks. Finally, the ability of hyperferremia to alter Ga-67 tumor uptake was tested by injecting tumor-bearing rats with 20 mg/kg of N-acetylphenylhydrazine, a chemical that causes hyperferremia by hemolyzing red cells. One group of rats was used to measure the serum iron, UIBC, and in vitro gallium binding 24 hr after injection of the drug. Another group of rats received Ga-67 citrate 24 hr after injection of N-acetylphenylhydrazine, and were killed 48 hr later for determination of the %ID/g of tumor and the tumor-to-blood ratio. This procedure was followed for two reasons: (a) after injection of N-acetylphenylhydrazine, serum iron levels rise and return to normal in 72 hr; and (b) it was felt that drawing blood at the time of Ga-67 injection could in itself alter iron metabolism.

Statistical analyses in this study were performed using Dunnett's analysis of variance test.

RESULTS

Table 1 shows the mean values for serum iron, UIBC, transferrin, and in vitro Ga-67 binding for control and irradiated nontumor-bearing rats. Except for the transferrin levels, all values were significantly different from controls. Within the irradiated population there were no significant dose-related differences in serum iron, UIBC, or percentage Ga-67 binding. The relationship between percentage Ga-67 binding and UIBC is shown in Fig. 2.

TABLE 1. SERUM IRON, UIBC, TRANSFERRIN, AND IN VITRO PERCENTAGE Ga-67 BINDING, 24 HR AFTER IRRADIATION*

Radiation dose (rad)	n	Serum iron (μg/dl)	Unsaturated iron-binding capacity (μg/dl)	Transferrin (mg/dl)	In vitro % gallium binding
0	10	160.5 ± 25.7	249.1 ± 36.0	373 ± 65	96.0 ± 1.2
250	10	310.8 ± 47.8†	86.9 ± 46.4†	350 ± 47	78.4 ± 12.1‡
500	10	305.7 ± 46.2†	92.8 ± 51.5†	369 ± 56	77.0 ± 21.0¶
750	10	309.4 ± 34.1†	76.1 ± 31.0†	339 ± 43	67.1 ± 23.7‡

* Results given as mean ± s.d.
† Significantly different from control ($p < 0.001$).
‡ Significantly different from control ($p < 0.01$).
¶ Significantly different from control ($p < 0.05$).

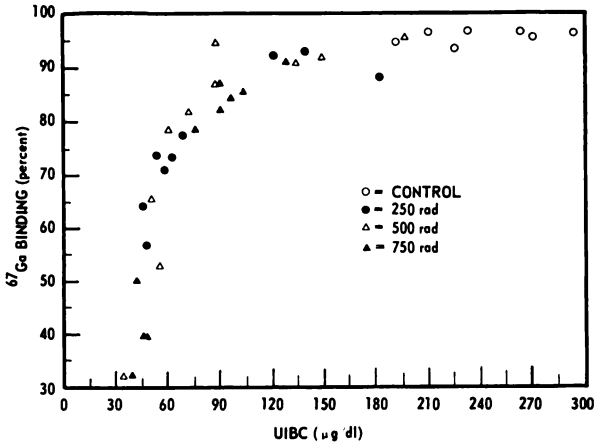

FIG. 2. Relationship between in vitro Ga-67 serum binding as measured by a 24-hr dialysis and serum unsaturated iron-binding capacity. Serum was obtained 24 hr after exposure in a whole-body Co-60 irradiation facility. Each point represents one rat.

This curve was duplicated in the experiment using normal rat serum with added iron—i.e., Ga-67 serum binding decreased as the iron-binding capacity was saturated by addition of iron.

Table 2 shows the mean values for serum iron, UIBC, %ID/g tumor, tumor-to-blood ratio, and the %ID excreted in the urine in the tumor-bearing rats. Values obtained in rats receiving doses of 250–1000 rads are combined, since there were no significant dose-related differences. The irradiated animals had significantly decreased tumor uptake (%ID/g) and tumor-to-blood ratios, but the serum iron levels and urinary excretion of Ga-67 were increased. Figure 3 shows the significant correlation that was found between serum UIBC and %ID/g of tumor for 49 irradiated and control rats ($r = 0.78$, $p < 0.001$). The normal tissues from irradiated rats (500 rads) that had decreased Ga-67 uptake were the liver (0.463 ± 0.288 %ID/g; control = 0.888 ± 0.046) and lung (0.132 ± 0.053 %ID/g; control = 0.345 ± 0.065). Other tissues studied (blood, kidney, fat, and muscle) did not appear significantly altered.

Tumor uptake and urinary excretion of Ga-67 in six rats receiving 500 rads confined to the tumor were not significantly different from those of nonirradiated rats (Table 3). However, rats receiving "whole-body" exposure with the tumor shielded showed decreased tumor uptake, as did rats whose tumor was included in the radiation field.

The ability of hyperferremia to reduce Ga-67

TABLE 2. EFFECT OF WHOLE-BODY IRRADIATION ON TUMOR UPTAKE AND URINARY EXCRETION OF Ga-67*

	n	% ID/g tumor	Tumor-to-blood ratio	Serum iron (μg/dl)	UIBC (μg/dl)	% ID in 48-hr urine
Control	6	.806 ± .198	14.1 ± 4.7	160.7 ± 43.0	295.5 ± 41.6	10.18 ± 2.47
Irradiated	16	.284† ± .108	5.8† ± 1.9	251.8† ± 65.7	186.6† ± 49.7	20.90† ± 6.95

* Results given as mean ± s.d.
† Significantly different from control ($p < 0.01$).

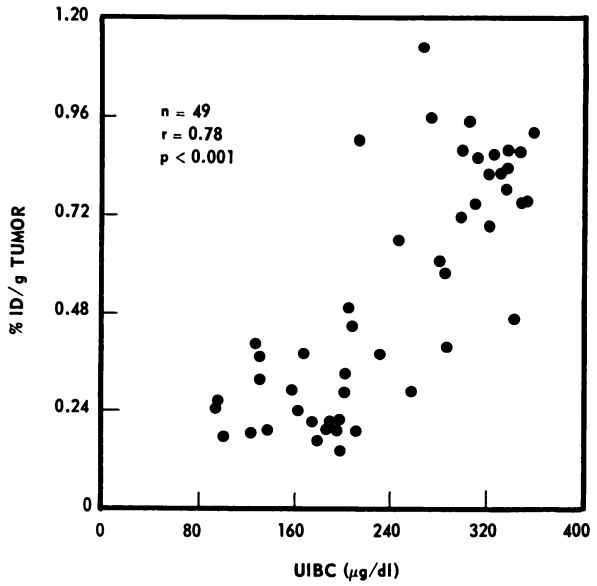

FIG. 3. Relationship between unsaturated iron-binding capacity and percentage injected dose per gram of tumor in control and irradiated rats. UIBC was determined at time of sacrifice, 72 hr after irradiation, 48 hr after injection of Ga-67.

tumor uptake was confirmed by the N-acetylphenylhydrazine experiments. Animals receiving 20 mg/kg of the drug 24 hr before Ga-67 showed a mean %ID/g tumor similar to that of irradiated animals (Table 4). These animals also showed elevated iron, decreased UIBC, and decreased Ga-67 serum binding 24 hr after injection.

DISCUSSION

In 1975, Fletcher et al. (*2*) postulated that the decreased Ga-67 retention seen after whole-body irradiation might be due to radiation-induced changes in the serum carrier for gallium, or to the release of a new carrier or a competing gallium-binding ligand. In a subsequent report (*17*), they offered two additional hypotheses: (a) that gallium may form polymeric complexes of low molecular weight due to radiation-caused alterations in plasma citrate or buffer-anion concentrations; and (b) that radiation-induced hyperferremia may prevent gallium from binding to normal binding sites. The results of the current study demonstrate that serum transferrin levels do not change 24 hr after irradiation and that the alteration of the gallium carrier is due to saturation with iron. The data support the hypothesis that hyperferremia contributes to a decreased whole-body retention and serum binding of Ga-67 following whole-body irradiation. Most studies have found that gallium binds in part to transferrin, the plasma carrier for iron (*3–5*). However, iron is bound tightly to transferrin whereas gallium is bound loosely. Therefore, iron may displace or prevent gallium binding, allowing the unbound Ga-67 to be removed through the kidneys. This may

TABLE 3. EFFECT OF LOCAL TUMOR IRRADIATION OR SHIELDING ON Ga-67 UPTAKE*

	n	% ID/g tumor	Tumor-to-blood ratio	Serum iron (μg/dl)	Serum UIBC (μg/dl)
Control	5	.844 ± .190	15.9 ± 3.2	143.2 ± 15.2	308.0 ± 29.4
500 rad, tumor only	6	.763 ± .170	11.5 ± 4.8	177.5 ± 57.9	280.5 ± 64.0
750 rad, whole body	5	.346 ± .133†	7.2 ± 2.8†	298.4 ± 29.5†	135.6 ± 46.2†
750 rad, whole body except tumor	5	.278 ± .137†	4.1 ± 2.0†	270.2 ± 40.7†	181.2 ± 43.9†

* Results are mean ± s.d.
† Significantly different from control ($p < 0.001$).

TABLE 4. EFFECT OF N-ACETYLPHENYLHYDRAZINE ON SERUM IRON, UIBC, IN VITRO Ga-67 BINDING, AND IN VIVO TUMOR UPTAKE*

Dose (mg/kg)	n_1†	Serum iron (μg/dl)	UIBC (μg/dl)	In vitro % Ga binding	n_2†	% ID/g tumor	Tumor-to-blood ratio
0	8	169.6 ± 19.8	291.8 ± 39.9	98.6 ± .5	9	.813 ± .140	14.8 ± 2.7
20	6	419.2 ± 26.8‡	35.0 ± 9.5‡	33.0 ± 1.2‡	6	.309 ± .070‡	3.8 ± .9‡

* Results are mean ± s.d.
† In groups n_1, serum iron, UIBC, and gallium binding were determined. In groups n_2, % ID/g and tumor/blood ratios were determined.
‡ Significantly different from control ($p < 0.001$).

explain the decreased whole-body retention, and increased urinary excretion, seen in the current and past investigations. It is not known whether the unbound gallium forms polymeric complexes as suggested by Fletcher (*17*). The decreased binding of Ga-67 by serum from irradiated animals, however, can be duplicated by the in vitro addition of similar levels of iron to normal rat serum.

Previous investigations have shown decreased soft-tissue uptake and whole-body retention of Ga-67 injected after whole-body irradiation (*1,2,17*). Our data confirm these studies with regard to decreased whole-body retention and decreased uptake of gallium in the liver and lungs. Previously reported decreases in Ga-67 retention in blood and kidney after irradiation (*1,2*) apparently are seen soon after gallium injection and not at 48 hr. The current study extends previous findings by showing decreased Ga-67 tumor uptake after whole-body irradiation. The decrease in uptake correlates with a decrease in the serum UIBC and is not dependent upon direct radiation damage to the tumor. Other investigators have noted changes in Ga-67 uptake in lesions following administration of iron. Oster et al. (*15*), working with an abscess model in rabbits, showed a decreased Ga-67 abscess-to-muscle ratio when animals were preloaded with iron 24 hr before Ga-67 injection. However, Farrer and Saha (*18*) reported decreased tumor uptake, but an increased tumor-to-blood ratio, when mice bearing an Ehrlich ascites tumor were given an iron preload before Ga-67. The reasons for disagreement between the latter results and those of the current study are not clear.

The exact mechanism for radiation-induced hyperferremia has not been established. However, uptake of iron by the bone marrow is suppressed by irradiation (*19*). Therefore, the probable mechanism is that iron normally removed from the plasma by this route builds up, saturating the iron-binding capacity. Stanculescu et al. (*13*) have shown that whole-body irradiation in the range of 300–900 rads depresses the UIBC for about 6 days.

Our experiments with N-acetylphenylhydrazine indicate that hyperferremia from any source can also decrease tumor uptake of Ga-67. Hyperferremia could lead to decreased Ga-67 tumor uptake in several ways. The rapid excretion of unbound Ga-67 might lead to decreased tumor uptake simply because less Ga-67 is available to enter the tumor. It is also possible that Ga-67 must be attached to transferrin to enter tumor cells efficiently. Finally, the increased circulating iron could saturate gallium-binding proteins in the tumor, preventing Ga-67 uptake. Recently, lactoferrin has been identified as a gallium-binding protein (*20*). Saturation of this or a similar protein by iron would prevent normal Ga-67 accumulation.

The results of the current study suggest that conditions, including therapy, that affect iron metabolism in patients may interfere with the success of a Ga-67 scan. According to Davidsohn and Nelson (*21*), any agent that causes a hypoplastic or aplastic anemia is associated with an elevated serum iron level. This long list of agents includes antineoplastic drugs, antimetabolites, and some antimicrobials, as well as ionizing radiation. False-negative Ga-67 images have been reported frequently in patients with proven neoplastic disease, but the status of serum iron and UIBC in these patients has apparently not been noted. The results of this study suggest that (a) serum iron and UIBC should be determined before Ga-67 imaging in patients who may have altered iron metabolism, and (b) hyperferremia and low serum UIBC will diminish the chances for true-positive Ga-67 citrate tumor imaging in these patients.

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