RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Effect of Iron Deficiency on the Biodistribution and Tumor Uptake of Ga-67 Citrate in Animals: Concise Communication

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To investigate the effect of iron deficiency on the biodistribution and tumor uptake of Ga-67 citrate, 20 weanling Sprague-Dawley rats were maintained for 6-8 wk on a low-iron diet. Eighteen littermates were maintained on a normal iron diet and served as controls. Animals received 10 μCi Ga-67 citrate, and urine and feces were collected for 48 hr. The animals were then killed, tissue samples were obtained, and serum iron and unsaturated iron-binding capacity (UIBC) were measured. The accumulation of Ga-67 in the liver and spleen (% ID/g) was markedly increased in iron-deficient animals and urinary excretion was reduced. Tumor uptake was not significantly different in iron-deficient and control animals, but tumor-to-blood ratios were elevated ($p < .001$) in the iron-deficient animals because of low blood levels of Ga-67. The liver and spleen accumulation of Ga-67 correlated significantly ($p < .001$) with the UIBC. The results show that iron deficiency alters the distribution of Ga-67 citrate, and suggest that the variable liver-spleen uptake seen in clinical Ga-67 images may be explained, in part, by changes in serum iron and UIBC.

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The biodistribution of Ga-67 citrate seems to be strongly influenced by the presence of iron-binding proteins such as transferrin and lactoferrin (*1–3*). Whole-body irradiation transiently increases serum iron and decreases the unsaturated iron-binding capacity (UIBC). This results in increased urinary excretion and decreased tumor uptake of Ga-67 (*1*). Since transient elevations in serum iron levels and decreases in the UIBC can alter Ga-67 distribution, it seemed possible that iron deficiency might also have an effect. Accordingly, we investigated the effect of iron-deficiency anemia on the distribution of Ga-67 citrate.

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MATERIALS AND METHODS

Three-week-old male Sprague-Dawley rats (n=38) were weaned and maintained for 6-8 wk on distilled water and either a normal (n=18) or a low-iron diet (n=20). The normal diet* consisted of a formulation of all essential amino acids, minerals, and vitamins in a corn, wheat, and soybean base. No antibiotics, estrogens, or artifical preservatives were present. The diet contained 340 ppm iron. The average iron intake of animals on the control diet was approximately 3 mg/day. The low-iron diet† also contained all essential nutrients, except iron, in a low-bulk milk base. In order to minimize dietary bulk differ-

TABLE 1. COMPARISON OF ANEMIC AND CONTROL RATS ON LOW-BULK DIET

				Ga-67 %ID/g				Ga-67 % excreted	
	Hct	Fe (μg/dl)	UIBC (μg/dl)	Blood	Liver	Spleen	Femur	24-hr Urine	48-hr Feces
Control (n=5)	45.9 ±2.5	141.5 ±33.2	286.9 ±35.1	.052 ±.011	.562 ±.047	1.085 ±.182	.572 ±.054	10.5 ±1.3	3.4 ±2.4
Anemic (n=6)	31.8 ±3.1	68.5 ±20.8	545.6 ±37.6	.025 ±.008	1.889 ±.199	2.552 ±.078	.503 ±.070	4.7 ±1.0	8.0 ±3.5
P	<.001	<.01	<.001	<.001	<.001	<.001	NS	<.001	<.05

ences that might alter excretion data, the animals were prepared for the biodistribution study in two ways. In one experiment, animals that were fed the normal diet were switched to the low-bulk, low-iron diet 72 hr before the Ga-67 injection. The tissue distribution of Ga-67 in these animals was then compared with that of iron-deficient animals who had been on the low-bulk diet for 6-8 wk (Table 1). In the other experiment, animals that had been fed the low-bulk, low-iron diet were switched to the normal diet 24 hr before the Ga-67 injection. Their Ga-67 distribution was then compared with that of control animals receiving the normal diet throughout the study (Table 2).

All animals received an i.v. injection of 10 μCi of Ga-67 citrate and were placed in metabolic cages for 48 hr to allow separate collection of urine and feces. At 48 hr animals were killed under methoxyflurane‡ anesthesia and tissue samples (liver, spleen, whole femur) were removed. Urine, feces, and tissue samples were assayed in a well counter using standard techniques. Percentages injected dose (% ID) excreted and %ID/g of tissue were calculated. Serum iron and unsaturated iron-binding capacity (UIBC) were determined from the serum samples.

To assess the effect of iron deficiency on tumor uptake of Ga-67, a Walker-256 carcinosarcoma known to be gallium-avid (*1*) was implanted subcutaneously on the flanks of anemic (n=7) and control (n=7) rats. One week later, when the tumors weighed 7-10 g, the control animals were placed on the low-bulk, low-iron diet (24 hr before injection), and 10 μCi of Ga-67 was given intravenously. Forty-eight hours later the animals were killed and the %ID/g tumor and other tissues were determined as described above.

Results are presented as mean ± 1 s.d. Statistical analyses were performed using Student's t-test.

RESULTS

The biodistribution of Ga-67 citrate was significantly different in controls and iron-deficient animals. The liver and spleen showed markedly increased Ga-67 uptake (%ID/g) in iron-deficient animals (Table 1, Fig. 1). The whole-spleen content of Ga-67 was also measured, and iron-deficient animals showed significantly higher ($p < .001$) Ga-67 activity: 1.83 ± 0.13 % in six iron-deficient animals compared with 0.96 ± 0.13% in five controls. The uptake of Ga-67 in the femur (bone and marrow) was not significantly different in the two groups (Table 1), but the mean bone-to-blood ratio was higher in the iron-deficient animals (20.1 against 11.0 in controls) due to low Ga-67 blood levels. Urinary Ga-67 excretion was significantly reduced in these animals, but colonic excretion was increased.

The serum iron and UIBC of iron-deficient animals exposed to a normal diet for 24 hr were markedly different from those of iron-deficient animals that remained on the low-iron diet. The anemic animals exposed to the normal iron diet had an elevated serum iron level and a low-normal UIBC (Table 2). These animals showed normal liver-spleen Ga-67 uptake (%ID/g). Whole-spleen Ga-67 activity was also normal: 0.80 ± 0.25% in seven

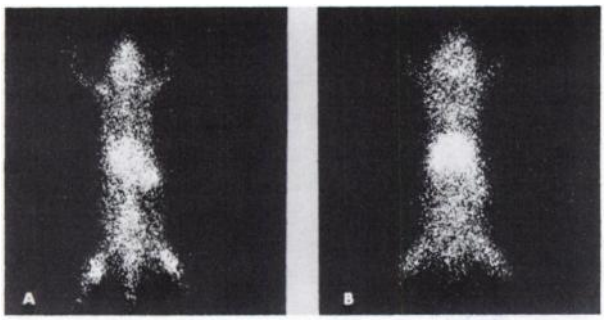

FIG. 1. Gallium-67 images of normal rat (A) and iron-deficient rat (B). Images were obtained 48 hr after i.v. injection of 100 μCi of Ga-67 citrate. A parallel-hole, medium-energy collimator was used and 30,000 counts recorded. Note increased liver and spleen activity in anemic animal.

TABLE 2. COMPARISON OF CONTROL AND ANEMIC RATS ON NORMAL DIET

				Ga-67 %ID/g				Ga-67 % excreted	
	Hct	Fe (μg/dl)	UIBC (μg/dl)	Blood	Liver	Spleen	Femur	24-hr Urine	48-hr Feces
Control (n=6)	47.0 ±1.3	161.9 ±16.3	235.0 ±19.9	.043 ±.006	.573 ±.083	.907 ±.124	.916 ±.125	12.9 ±1.7	8.3 ±1.3
Anemic (n=7)	35.6 ±1.6	312.4 ±93.5	179.6 ±79.4	.031 ±.017	.435 ±.085	.963 ±.229	.871 ±.055	18.8 ±1.6	3.6 ±0.8
P	<.001	<.01	NS	<.01	NS	NS	NS	<.001	<.001

anemic animals compared with 0.89 ± 0.08% in six controls. In addition, the anemic animals exposed to dietary iron showed increased Ga-67 urinary excretion compared with controls or iron-deficient animals not exposed to iron, and their fecal Ga-67 excretion was reduced. There was a significant correlation between UIBC and liver-spleen Ga-67 uptake in this group and in the iron-deficient animals kept on the low-iron diet before killing (Fig. 2).

There was no significant difference in the %ID/g tumor between iron-deficient and control animals (Table 3). However, because of the lower blood levels of Ga-67 in the iron-deficient animals, the tumor-to-blood ratios were significantly higher ($p < .001$).

DISCUSSION

The current study shows that iron deficiency alters the distribution of Ga-67 and adds to the evidence that Ga-67 distribution and iron metabolism are closely related. Several studies (*4–7*) have shown that transferrin is one of the major carrier proteins for Ga-67 in plasma. Gallium-67 also binds to tissue lactoferrin (*3*) and is associated with ferritin in rabbit hepatocytes (*8*). The present study shows that iron-deficient rats with a large UIBC have abnormally high Ga-67 uptake in tissues that normally store iron (e.g., liver and spleen). In a previous study (*1*), whole-body irradiation led to increased serum iron and decreased UIBC and was associated with decreased liver uptake of Ga-67. This association of serum iron, UIBC, and liver-spleen uptake may help explain the variability of liver-spleen uptake seen in clinical Ga-67 studies (*9*). As expected, decreased liver uptake is also seen in patients with tissue iron-storage diseases like hemachromatosis, and within 24 hr of vincristine treatment, which transiently elevates serum iron and saturates the UIBC (*10*).

Gallium-67 excretion patterns were also altered in the iron-deficient animals. Urinary excretion was low, but fecal excretion was increased. This pattern may be a reflection of the elevated hepatic uptake in the iron-deficient animals, with secondarily increased biliary excretion. However, the data of Taylor et al. (*11*) suggest that biliary excretion of Ga-67 is insignificant under normal conditions. Perhaps biliary excretion does occur when hepatic Ga-67 concentrations are markedly elevated, or perhaps the Ga-67 gained access to the bowel by some other route. The excretion pattern in these animals

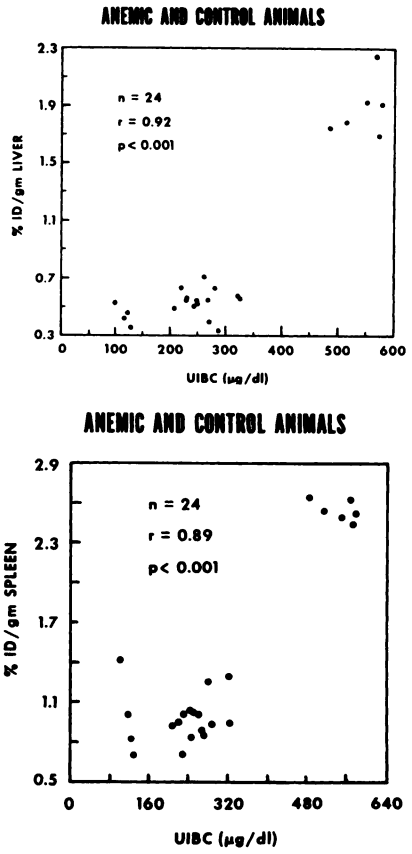

FIG. 2. Showing correlation of UIBC with uptake of Ga-67 (%ID/g) in liver (top) and spleen (bottom) in normal and iron-deficient rats.

TABLE 3. Ga-67 CITRATE TUMOR UPTAKE IN ANEMIC AND CONTROL RATS

	Hct	Fe (μg/dl)	UIBC (μg/dl)	% ID/g Tumor	Tumor/ blood ratio	Tumor weight (g)
Control (n=7)	39.7 ±1.8	114.7 ±33.0	267.6 ±46.3	.764 ±.113	13.5 ±1.8	10.1 ±1.9
Anemic (n=7)	22.3 ±4.3	45.0 ±10.9	563.1 ±100.3	.954 ±.238	27.7 ±8.0	7.6 ±3.6
P	<.001	<.001	<.001	NS	<.001	NS

was changed by short-term exposure to iron in the normal-bulk diet. The increased urinary excretion of Ga-67 probably occurred because serum iron levels were elevated and the UIBC was low-normal. The rapid changes in liver-spleen Ga-67 uptake in these animals also suggest that serum iron and UIBC have an important effect on organ uptake. Although it is possible that the brief exposure to dietary iron led to partial replenishment of tissue iron stores, it seems unlikely that this was a major factor in the observed changes in Ga-67 distribution.

Tumor uptake of Ga-67 was not altered by the presence of iron deficiency. Although tumor-to-blood activity ratios for Ga-67 were higher in iron-deficient animals ($p < .001$), the actual tumor uptake (%ID/g) was not significantly elevated. Previous work with irradiated Sprague-Dawley rats showed a significant ($p < .001$) positive correlation between %ID/g Ga-67 in this tumor (Walker-256 carcinosarcoma) and UIBC. The UIBCs in that series ranged from approximately 80 to 370 μg/dl. The UIBC in the anemic rats in the current study was 563 ± 100 (mean ± 1 s.d.). Thus, a higher tumor uptake of Ga-67 would have been predicted. The work of Sephton and Harris (*12*) with tissue cultures of mouse tumor cells showed that transferrin stimulated Ga-67 tumor uptake. Transferrin was not specifically measured in the current study, but it is likely that unsaturated transferrin levels were increased in the animals with a high UIBC. The recent work of Larson et al. (*13*) also investigated transferrin-mediated uptake of Ga-67 in a tissue culture of EMT-6 sarcoma cells. They demonstrated that tumor-cell uptake of Ga-67 leveled off as the transferrin concentration increased, suggesting that tissue receptors for Ga-67 transferrin might become saturated at certain concentrations. It is possible that the same kind of saturation phenomenon existed in vivo in the tumor model used in the current study.

The close association between Ga-67 distribution, tumor uptake, and the availability of iron-binding proteins may be of clinical importance. Iron deficiency may occur in patients and its presence could alter Ga-67 image findings. Iron overload, with saturation of the UIBC, could result in rapid Ga-67 excretion and a decreased probability of lesion uptake. These findings suggest that knowledge of a patient's UIBC may be valuable in the interpretation of images that show an unusual distribution of Ga-67.

ACKNOWLEDGMENT

Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council.

FOOTNOTES

*Wayne Lab-Blox®, obtained from Allied Mills, Inc., Specialty Feeds Dept., Chicago, IL.
†ICN Life Sciences, 26201 Miles Road, Cleveland, OH.
‡Metafone, Pitman-Moore, Washington Crossing, NJ.

REFERENCES

1. 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
2. OSTER ZH, LARSON SM, WAGNER HN JR: Possible enhancement of ^{67}Ga-citrate imaging by iron dextran. *J Nucl Med* 17: 356–358, 1976
3. HOFFER PB, HUBERTY J, KHAYAM-BASHI H: The association of Ga-67 and lactoferrin. *J Nucl Med* 18: 713–717, 1977
4. GUNASEKERA SW, KING LJ, LAVENDER PJ: The behaviour of tracer gallium-67 towards serum proteins. *Clin Chim Acta* 39: 401–406, 1972
5. HARTMAN RE, HAYES RL: The binding of gallium by blood serum. *J Pharmacol Exp Ther* 168: 193–198, 1969
6. CLAUSEN J, EDELING C-J, FOCH J: ^{67}Ga binding to human serum proteins and tumor components. *Cancer Res* 34: 1931–1937, 1974
7. HARA T: On the binding of gallium to transferrin. *Int J Nucl Med Biol* 1: 152–154, 1974
8. HEGGE FN, MAHLER DJ, LARSON SM: The incorporation of Ga-67 into the ferritin fraction of rabbit hepatocytes in vivo. *J Nucl Med* 18: 937–939, 1977
9. MILDER MS: Scintigraphic interpretation. In *Atlas of Gallium-67 Scintigraphy*, Johnston GS and Jones AE, eds. New York, Plenum Press, 1973, pp 11–29
10. LARSON SM, HOFFER PB: Normal patterns of localization.

In *Gallium-67 Imaging*, Hoffer PB, Bekerman C, Henkin RE, eds. New York, John Wiley and Sons, 1978, pp 23–38

11. TAYLOR A, MULLEN S, CHAFETZ N, et al: The source of fecal gallium-67 in rats. *J Nucl Med* 18: 621, 1977 (abst)
12. SEPHTON RG, HARRIS AW: Gallium-67 citrate uptake of cultured tumor cells stimulated by serum transferrin. *J Natl Cancer Inst* 54: 1263–1266, 1975
13. LARSON SM, RASEY JS, ALLEN DR: A transferrin mediated uptake of gallium-67 by EMT-6 sarcoma. *J Nucl Med* 19: 715–716, 1978 (abst)