INVESTIGATIVE NUCLEAR MEDICINE

Different Actions of Deferoxamine and Iron on Ga-67 Abscess Detection in Rats

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The contrast-enhancing properties of iron (Fe) and deferoxamine (DFO) in abscess imaging with Ga-67 citrate were compared in rats bearing turpentine-induced abscesses. Iron administration shifted Ga-67 from plasma into tissues such as muscle and fat. As a result, the abscess-to-plasma ratio increased whereas the abscess-to-muscle ratio decreased. DFO enhanced the abscess-to-muscle and abscess-to-plasma ratios by increasing urinary Ga-67 excretion. In contrast to Fe, DFO removed abscess-bound Ga-67, thus representing a disadvantage of DFO compared with Fe. As a result, the abscess-to-plasma ratio was more effectively enhanced by Fe than by DFO. We conclude that abscess imaging with Ga-67 citrate may be improved by administration of (a) Fe for detection of abscesses masked by blood activity, or (b) DFO for detection of abscesses surrounded by muscle tissue.

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The use of gallium-67 for abscess detection is hampered by poor-quality images due to low abscess-to-background ratios and by the long delay between injection and optimal imaging time. Because of the similar biophysical properties of Ga-67 and iron (*1*), iron (*2,3*) and iron-chelating agents like deferoxamine (DFO) (*4,5*) have been used for contrast enhancement in abscess imaging with Ga-67. Iron (*2,3*) and DFO (*4,5*) have shown promise as contrast-enhancing agents in Ga-67 abscess imaging and in an attempt to define the specific benefits of Fe and DFO administration for abscess detection with Ga-67, we determined the effects of Fe and DFO on Ga-67 biodistribution in abscess-bearing rats.

MATERIALS AND METHODS

Animal model. Female Wistar rats (230–250 g) were kept in metabolic cages with free access to food and water. Abscesses were induced by subcutaneous injection of 0.5 ml turpentine into the thigh 4 days before each experiment.

Ga-67 distribution. Blood samples were obtained in anesthetized animals from the retro-orbital vein plexus. Animals were killed by exsanguination, multiple tissue samples were removed, weighed wet, and counted against a standard in a well scintillation counter. In preliminary studies, the completeness of blood removal by exsanguination was confirmed with erythrocytes labeled with chromium-51.

Plasma analyses. Plasma Fe and total plasma Fe-binding capacity were measured with colorimetric kits. Protein binding of Ga-67 was determined using an ultrafiltration method (*6*). The nonfilterable Ga-67 was considered to be protein bound. The Ga-67 fraction passing the filter is considered bound to DFO, or to be "free" Ga-67 in the case of Fe administration.

Preliminary studies and experimental design. Carrier-free Ga-67 citrate (100 μCi/kg or 3.7 MBq/kg), DFO, and Fe as FE(III) saccharate were administered by tail vein. In preliminary studies, the maximum effect of DFO on renal Ga-67 excretion was achieved with a dosage of 150 mg/kg DFO, this effect lasting for approximately 3.5 hr. To saturate plasma Fe-binding ca-

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TABLE 1. EFFECTS OF IRON (Fe) AND DEFEROXAMINE (DFO), ADMINISTERED 3.5 hr AFTER Ga-67, ON Ga-67 BIODISTRIBUTION

	Ga-67 distribution at 7 hr after its injection (% injected dose/g tissue, mean ± s.e.m.)			
	Controls (n = 6)	10 mg/kg Fe (n = 5)	12.5 mg/kg DFO (n = 6)	150 mg/kg DFO (n = 6)
Liver	1.8 ± 0.1	1.5 ± 0.1*	1.0 ± 0.0§	0.63 ± 0.04§
Femur	1.9 ± 0.1	1.9 ± 0.1	1.2 ± 0.1§	0.48 ± 0.08§
Muscle	0.22 ± 0.01	0.32 ± 0.03§	0.087 ± 0.007§	0.024 ± 0.004§
Abscess	2.5 ± 0.2	2.1 ± 0.2	1.6 ± 0.1‡	0.90 ± 0.01§
Plasma	3.0 ± 0.1	0.20 ± 0.04§	1.0 ± 0.1§	0.11 ± 0.01§
Urine†	8.8 ± 0.4	18 ± 2§	47 ± 4.2§	60 ± 5§
Ratio of abscess to:				
Plasma	0.82 ± 0.04	12 ± 2§	1.5 ± 0.3§	9.3 ± 2.2§
Muscle	12 ± 1	7.6 ± 0.9*	19 ± 2‡	38 ± 7‡

* $p < 0.05$.
† % of total injected dose.
‡ $p < 0.01$.
§ $p < 0.001$ compared with controls.

pacity for a period of comparable duration, administration of 10 mg/kg Fe was required.

Three study protocols were adopted. The first studied the changes of Ga-67 distribution at 7 hr after injection caused by Fe (10 mg/kg) compared with DFO (12.5 and 150 mg/kg), each injected at 3.5 hr after the gallium injection. The second protocol assessed the effect of short-term Fe loading (10 mg/kg Fe injected simultaneously with Ga-67) on plasma Fe concentration and Ga-67 biodistribution during 48 hr. The third protocol determined the effect of prolonged Fe loading (repeated injections of 10 mg/kg Fe at 3.5, 9, and 16 hr after injection of Ga-67) on Ga-67 biodistribution at 24 hr after Ga-67 administration.

Statistical analysis was performed using the unpaired Student's t-test.

RESULTS

Comparison of the effects of Fe and DFO (Table 1). Either dose of DFO removed tissue- and abscess-bound Ga-67. Fe seemed to increase Ga-67 muscle activity.

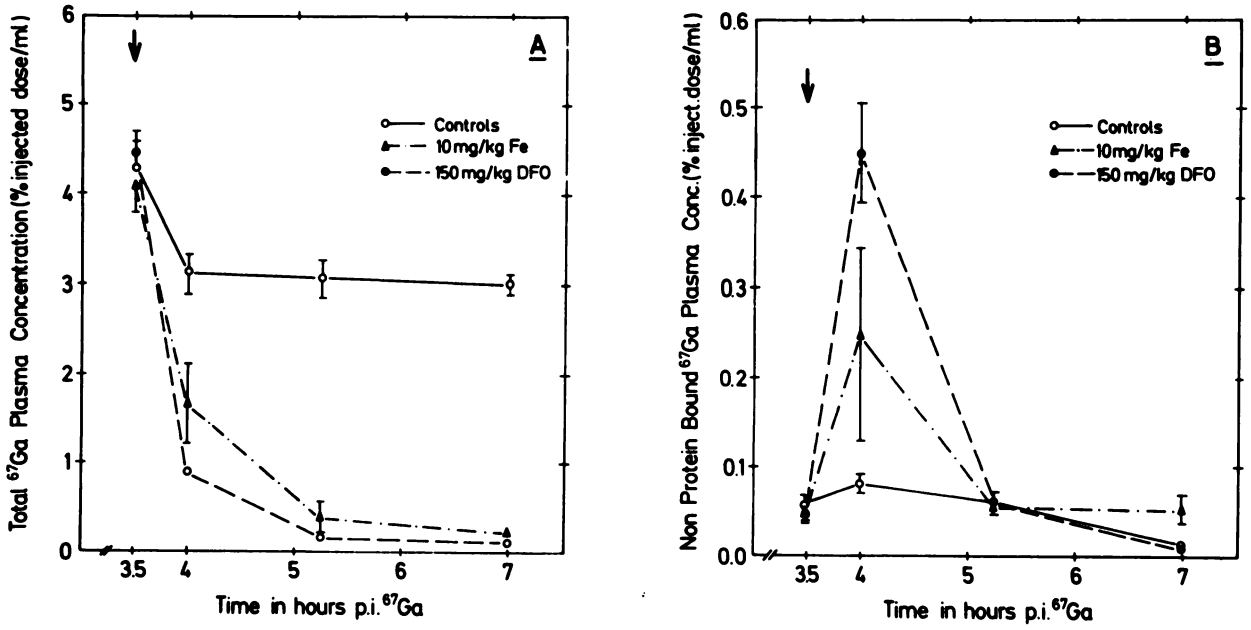

FIG. 1. Effects of iron (Fe) and deferoxamine (DFO) on total Ga-67 plasma concentration (A) and on nonprotein-bound Ga-67 plasma concentration (B). 10 mg/kg Fe, or 150 mg/kg DFO, were administered at 3.5 hr after Ga-67 dose (arrow). Each point represents mean ± s.e.m. of determinations in at least 5 animals.

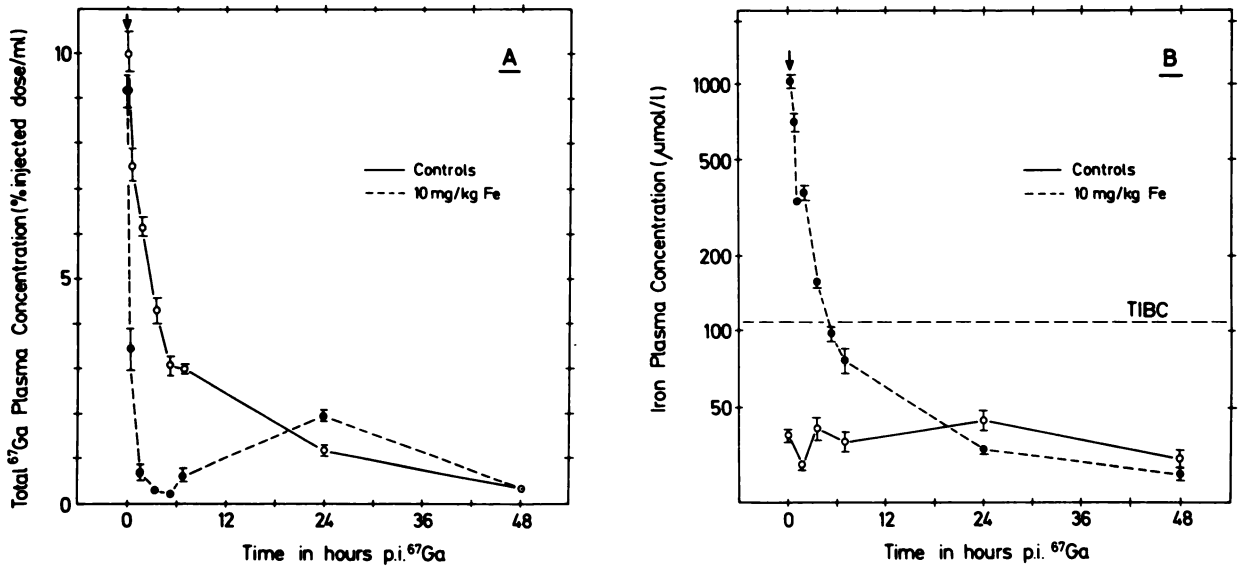

FIG. 2. Time course of total Ga-67 concentration (A) and iron concentration (B) in plasma after injection of 10 mg/kg iron, administered simultaneously with Ga-67 (arrow). Each point represents mean ± s.e.m. of determinations in at least 5 animals. Protein-bound Ga-67 accounted for 98 ± 0.2% of total plasma Ga-67 in controls; it decreased transiently to minimum (57 ± 4%) at 3.5 hr after Fe injection. Total Fe-binding capacity (TIBC) was found to be 108 ± 4 µmol/l.

Comparison of urinary Ga-67 excretion revealed that DFO reduced Ga-67 body retention more effectively than did Fe. Administration of Fe or DFO resulted in a rapid decrease in total Ga-67 plasma concentration (Fig. 1A) and caused release of Ga-67 bound to plasma proteins (Fig. 1B).

In contrast to Fe, Ga-67 muscle activity did not increase after DFO. Separate experiments showed that even 30 min after 150 mg/kg DFO (4 hr after Ga-67), when plasma levels of Ga-67 bound to DFO are high (Fig. 1B), Ga-67 muscle activity was lower in DFO-treated animals (mean 0.11% of injected dose (ID)/g, n = 5) than in controls (0.22% ID/g).

Short-term Fe loading decreased total Ga-67 plasma concentrations (Fig. 2A) rapidly and transiently. Plasma Ga-67 concentration started to rise again as soon as plasma Fe concentration had declined below the total plasma Fe-binding capacity (Fig. 2B). Muscle and fat showed an increase in Ga-67 activity relative to controls at 3.5 hr after Ga-67 injection, but not at 48 hr (Table 2). During prolonged Fe loading (Table 3) Ga-67 body retention was significantly reduced, as shown by the

TABLE 2. EFFECT OF SHORT-TERM IRON (Fe) LOADING ON Ga-67 BIODISTRIBUTION

	Ga-67 distribution (% injected dose/g tissue, mean ± s.e.m.)			
	3.5 hr after Ga-67 inj.		48 hr after Ga-67 inj.	
	Controls (n = 6)	10 mg/kg Fe* (n = 6)	Controls (n = 6)	10 mg/kg Fe* (n = 10)
Liver	1.4 ± 0.0	1.0 ± 0.1†	2.5 ± 0.1	2.2 ± 0.1
Femur	1.5 ± 0.1	1.1 ± 0.1†	2.2 ± 0.2	2.1 ± 0.2
Muscle	0.22 ± 0.01	0.32 ± 0.02¶	0.11 ± 0.02	0.12 ± 0.03
Fat	0.09 ± 0.01	0.13 ± 0.01†	0.032 ± 0.003	0.038 ± 0.004
Plasma	4.3 ± 0.3	0.30 ± 0.04¶	0.32 ± 0.02	0.33 ± 0.02
Abscess	2.3 ± 0.2	1.6 ± 0.1†	3.4 ± 0.1	2.3 ± 0.2¶
Urine§	nd**	nd	17 ± 0	22 ± 2†
Feces§	nd	nd	9.4 ± 0.4	6.5 ± 0.5†

* Injected simultaneously with Ga-67.
† $p < 0.01$.
¶ $p < 0.001$ compared with controls.
§ % of total injected dose.
** nd: not determined.

TABLE 3. EFFECT OF PROLONGED IRON (Fe) LOADING ON Ga-67 BIODISTRIBUTION

	Ga-67 distribution 24 hr after Ga-67 injection % injected dose/g tissue, mean ± s.e.m.	
	Controls (n = 6)	3 X 10 mg/kg Fe* (n = 5)
Liver	2.0 ± 0.1	1.7 ± 0.2
Femur	2.4 ± 0.1	3.1 ± 0.2§
Muscle	0.14 ± 0.01	0.045 ± 0.009¶
Abscess	3.1 ± 0.2	2.9 ± 0.4
Plasma	1.2 ± 0.1	0.10 ± 0.04¶
Urine†	16 ± 1‡	37 ± 1¶
Feces†	3.6 ± 0.7‡	3.2 ± 0.8

* Administration of 10 mg/kg Fe at 3.5, 9, 16 hours after Ga-67 injection.
† % of total injected dose.
‡ n = 12.
§ $p < 0.01$.
¶ $p < 0.001$ compared with controls.

increase in urinary Ga-67 excretion and the lowered Ga-67 muscle activity.

DISCUSSION

The present results show that short-term Fe saturation of plasma Fe-binding capacity resulted in Ga-67 redistribution within the body. As a result, the abscess-to-plasma ratio was enhanced after Fe administration whereas the abscess-to-muscle and abscess-to-fat ratios decreased. This was probably due to diffusion of "free" Ga-67 out of the vascular compartment into tissues, such as muscle and fat, after Ga-67 had been displaced by Fe from its binding to plasma proteins (*7*). This redistributing effect of Fe on Ga-67 has not been recognized in other reports (*2,3*). It seemed predominant in the early phase after Fe administration. Prolonged Fe loading was required to reduce Ga-67 muscle activity through enhanced renal Ga-67 excretion, or by the previously shown increased Ga-67 fixation in bone (*2,3*), indicating that this was a delayed effect of Fe administration.

Short-term saturation of plasma Fe binding capacity with Fe in humans (*8,9*) produced changes in Ga-67 plasma activity similar to those observed in the present studies in rats (Fig. 2A), suggesting Fe-induced Ga-67 redistribution within the body. Hence it may be speculated that clinical application of Fe may decrease the abscess-to-muscle ratio, thereby potentially offsetting the benefit of the enhanced abscess-to-blood ratio.

Iron interfered with Ga-67 uptake by the abscess (Table 2), but (in contrast to DFO) it did not displace Ga-67 that had already accumulated in the abscess (Table 3). Indeed, removal of abscess-bound Ga-67 by DFO seemed to be the major drawback of DFO administration. As a result, DFO was inferior to Fe in enhancing the abscess-to-plasma ratio, although at high dosage it more efficiently reduced Ga-67 plasma activity than Fe. The lower potential of DFO, relative to iron, for enhancement of the abscess-to-plasma ratio is further demonstrated by the results obtained with moderate dosage of DFO (12.5 mg/kg). This dose possibly yielded a more reasonable estimate for the DFO effects achievable in humans. A dose of DFO equivalent to the maximum effective dose of DSO (150 mg/kg)that we used in rats does not seem to be a safe diagnostic procedure in humans (*10,11*).

DFO enhanced the abscess-to-muscle ratio by removing muscle-bound Ga-67 in preference to abscess-bound Ga-67, as was reported previously (*4,5*). In contrast to Fe, an increase in Ga-67 muscle activity did not occur after DFO, probably due to the efficient renal excretion of DFO-bound Ga-67. Delaying DFO administration until Ga-67 binding by the abscess had made it inaccessible to DFO (*4*) does not appear to improve the abscess-to-muscle ratio any further because, an increase of the time interval between Ga-67 and DFO administration has been shown to result in resistance of Ga-67 muscle activity to removal by DFO (unpublished results, *12*).

DFO and Fe have been introduced into abscess imaging with Ga-67 for contrast enhancement and to speed up the diagonstic procedure, thereby possibly providing a basis for the clinical application of Ga isotopes with shorter half-lives (Ga-66 and Ga-68). Thus, the present results suggest that Fe may be particularly suitable for detection of abscesses masked by blood activity (e.g., in mediastinal or pulmonary lesions), since Fe quickly depletes the Ga-67 plasma pool without removing Ga-67 from the abscess. DFO administration may improve detection of inflammatory lesions surrounded by muscle tissue. In this case, Fe would even impair abscess detection, since it was shown to increase Ga-67 muscle activity. Although this effect was overcome by prolonged Fe loading in the present studies, this procedure would be time-consuming and possibly not feasible in humans because of the toxicity of the large amounts of Fe required.

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