**DESFERAL (DFO) INDUCED Ga-67 WASHOUT FROM NORMAL TISSUE,
TUMOR AND ABSCESS IN EXPERIMENTAL ANIMALS**

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ABSTRACT

In the experimental animal desferal (DFO) given intravenously washes out Ga-67 from all tissues. This effect is not uniform: blood activity is reduced very markedly, while liver activity is less affected. Maximal effect of DFO occurs if given close to the Ga-67 injection. When the time interval between the two is increased, the absolute amount of Ga-67 excreted in the urine in excess of the spontaneous excretion is reduced. Administration of DFO does not effect Ga-67 gastrointestinal excretion.

In three animal tumor models (EMT-6 sarcoma in Balb/c mice, spontaneous adenocarcinoma in mice and spontaneous adenocarcinoma in the rabbit) and in sterile abscess-bearing rats, the administration of DFO twenty-four hours after Ga-67-citrate improves significantly the target-to-nontarget ratio.

Animals given 50mg/kg DFO I.V. after Ga-67 citrate showed a significant reduction in the whole-body activity as seen in a one-week follow up.

INTRODUCTION

Desferal (DFO) isolated from microorganisms of the Actinomycetes group is a highly potent and specific iron-chelating agent (1) which has been used for iron overload treatment (2,3). It was also shown that DFO can bind many other cations (1). We have shown earlier that the administration of DFO to animals given Ga-67 citrate previously, induces a rapid decrease in Ga-67 blood levels with subsequent excretion of the activity in the urine (4). This observation was extended to abscess-bearing animals (5) as well as to tumor-bearing animals (6,7).

Because to the present time Ga-67 seems to be the best available agent for tumor and abscess localization it seems worthwhile to direct efforts for improving this technique. The studies on the effect of DFO on Ga-67 biodistribution had the following goals: to improve the target to nontarget ratio by promoting blood clearance through urinary excretion, to investigate the possibility of reducing intestinal excretion by increasing urinary excretion and finally to determine the decrease in radiation burden when DFO is given after Ga-67 citrate. The purpose of this paper is to describe the results of our efforts in these directions.

MATERIALS AND METHODS

The effect of DFO on the biodistribution of Ga-67 citrate was studied in 12 random-bred female rabbits, weighing 1.2-1.5 kg. Ga-67 citrate, 0.3mCi/kg body weight was injected intravenously. Six animals received 50 mg/kg body weight DFO in 1 ml normal saline intravenously, while six controls were given the same volume of saline. The animals were sacrificed 4 hours after the injection of Ga-67 by an overdose of pentothal. Samples of blood, liver, spleen, kidney, muscle and bone were obtained and assessed for Ga-67 activity in a well counter. Results were expressed as percentage of injected dose/gram of tissue.

In vivo studies were performed in 1.0-1.2 kg female random-bred rabbits using ketamine HCL/promazine HCL anesthesia. Butterfly needles were inserted into ear-veins of two rabbits positioned side by side under a gamma-camera, equipped with a medium energy collimator. The 93- and 184-KeV peaks were used, with 20% and 30% windows respectively. A simultaneous bolus injection of Ga-67 citrate (0.3mCi/kg body weight) was given to the two animals and data acquisition was started on the magnetic disc of a dedicated minicomputer system. A 128 x 128 matrix at a rate of two frames/minute was used. After a 30 minute

baseline acquisition, 50mg DFO in 1 ml saline was given to the experimental animals, the controls receiving 1 ml of saline intravenously. In the first experiments acquisition was continued for 180 minutes, but later a 30 minute period was found adequate to observe the action of DFO. Small sized animals were used so that most of the trunk was included in the field of view. Time-activity curves were generated by delineating regions of interest over the heart, left kidney, bladder and background. The right kidney was omitted because of liver superimposition. A body background area was selected in the right flank between the liver and the pelvic bones. In addition to the above described experimental set-up, three paired experiments each were performed by varying the time interval between the injection of radiogallium and DFO as follows: 2, 4, 5 and 24 hours.

Ga-67 whole-body retention was determined in six rabbits using the same doses of Ga-67 citrate and DFO as in the imaging experiments. The DFO was injected in the experimental animals 20 minutes after Ga-67 citrate, and an equivalent volume of saline was given to the controls. Whole-body counting was performed with a gamma camera using a medium energy collimator and same spectrometer setting as described above. The animals were placed 160 cm away from the crystal face of the camera and counts were obtained from the experimental animals, from the controls and from room background at 24, 48, 72, 96, 192 and 216 hours after Ga-67 injection. Sufficient time was allowed to collect 100,000 - 300,000 counts from the animals after background subtraction. Results were expressed as the ratio of counts in the DFO-treated animals over counts in control animals.

Abscesses were produced by injecting 0.02 - 0.05 ml turpentine into the hind thigh of albino female rats weighing 250-300 g. Seventy-two hours after injection a small abscess could be palpated. Ga-67 citrate (0.05 mCi) was administered intravenously to all rats and half were given 25 mg DFO I.V. at varying time intervals after Ga-67 citrate injection, as follows: 2, 4, 24 hours. One hour after the DFO, animals were sacrificed by ether overdose. Samples were obtained from blood, spleen, liver, muscle, gut, kidneys as well as from the excised abscess. The "abscess sample" included the central necrotic material as well as a rim of tissue surrounding it which clearly differed in color from the normal muscle. Samples and organs were weighed and Ga-67 activity measured in a well-type counter. Results were expressed as percentage of injected dose per gram and per whole organ.

A transplantable spontaneous adenocarcinoma in Hale-Stoner strain mice, EMT-6 sarcoma in Balb/c mice and spontaneous transplantable adenocarcinoma in New Zealand rabbits were studied in an identical fashion. The tumor was implanted subcutaneously on the back or abdomen and excision from the surrounding tissues was easily performed. In addition, in the abscessed rats experiments were also carried out by injecting Tc-99m DPTA (10 μ Ci), Tc-99m RBC (10 μ Ci) Tc-99m Plasmin (10 μ Ci), I-125 Fibrinogen (5 μ Ci) and I-131 Albumin (5 μ Ci).

Unsaturated iron binding capacity (UIBC) was determined in normal mice, rats, rabbits and dogs using a commercially available kit.

RESULTS

Tissue distribution studies at 4 hours (figure 1) showed that when DFO is given 30 minutes after Ga-67 citrate there is a reduction in activity in all tissues examined. The most prominent effect observed is on blood (50% of control values, $0.001 < p < 0.01$) and muscle ($p < 0.001$). Renal activity was slightly higher ($p > 0.05$).

Figure 1: Normal distribution of Ga-67 in rabbits (open bars) at 4 hrs and 3.5 hrs after DFO (notched bars).

In vivo experiments showed that by this time (3.5 hrs after DFO) most of the activity has already been excreted through the kidneys and therefore the activity is not high. The differences in spleen, liver and bone concentrations were not significant statistically ($p > 0.05$). Results of three paired in vivo experiments are shown in figure 2. DFO was injected 20 minutes after Ga-67 citrate. The decrease in blood activity started to be visible 2-3 minutes after DFO injection and continued for 20-30 minutes. The differences between the experimental and control animals were highly significant ($p < 0.001$).

As seen in figure 3 there is spontaneous washout of Ga-67 from all organs shown. The decrease in blood levels (71% to 84% washout) and muscle (50% to 81% washout) is most marked and less from tumor, abscess (13% to 36% washout) and liver (13% to 27% washout) as shown in table 1. When DFO is given, the target/blood ratio increases both in tumor and abscess-bearing animals as shown in table 2. At 5 hours the ratio increase is between 1.8 (adenocarcinoma of rabbit) to 6.3 (abscess in rats). The target to muscle ratio is either unchanged (EMT-6 sarcoma and rabbit adenocarcinoma) or slightly improved (mouse adenocarcinoma) and significantly improved in the rat abscess (2.8). The target to liver ratio is decreased by DFO in EMT-6 sarcoma and rabbit adenocarcinoma and slightly increased in rat abscess and mouse adenocarcinoma. The latter is mainly due to the lesser washout of Ga-67 from the liver as compared to the abscess or tumor. At 24 hours, DFO increases the target to blood ratio ranging from 1.7 (mouse adenocarcinoma) to 5.4 (EMT-6 sarcoma) and 4.0 (rat abscess). The target to muscle and target to liver ratio are only slightly affected.

Figure 2: Semilog time-activity curves of Ga-67 in heart (blood), kidney and bladder of control rabbits and DFO treated rabbits. DFO was injected 30 minutes after radiogallium as indicated by arrow. Note marked and immediate decrease in blood and increase in kidney and bladder activity in DFO-treated animals.

In table 3 are listed some parameters of the experimental abscesses. It can be seen that the albumin and DTPA spaces are increased more than 8 times as compared to normal muscle, indicating extravasation and edema; the red cell space is twice that of normal muscle most probably because of the hyperemia surrounding the abscess. Deposition of clotting factors is also prominent, as seen from the abscess to muscle ratios of I-125 fibrinogen and Tc-99m plasmin.

We observed a wide variation in the Ga-67 blood levels of the 4 animal models as shown in table 4. Furthermore, it can be seen that there is a linear correlation between the initial blood level of Ga-67 and the early (5 hrs) and late (24 hrs) concentration in the tumor models and abscess ($r = 0.93$). This in turn correlated with the percent of unsaturated iron binding capacity (UIBC) of the animals: the higher the UIBC, the higher the Ga-67 blood level and the higher the concentration of Ga-67 in the target.

Figure 3: Ga-67 activity in blood, abscess or tumor, muscle and liver in controls (left-hand-side bars) and in DFO treated animals (right-hand-side bars) at different time intervals. Notice the nonuniform effect of DFO on Ga-67 activity in the different tissues.

TABLE 1: TIME RELATED Ga-67 CITRATE CONCENTRATION

Spontaneous Washout (5 hr. vs. 24 hr.)

	<u>BLOOD</u>	<u>MUSCLE</u>	<u>TUMOR OR ABSCESS</u>	<u>LIVER</u>
EMT-6 SA, MOUSE	83%	67%	36%	13%
ADENOCAR, MOUSE	71%	50%	15%	15%
ABSCCESS, RAT	84%	81%	13%	27%

After DFO administration this relationship of Ga-67 level in the blood and in the target was not as obvious as in the untreated animals but the effect can still be observed at the extreme values, as seen in table 5.

The effect DFO on Ga-67 excretion in the G.I. tract is shown in table 6. It can be seen that the differences are small and are not significant statistically.

Total-body Ga-67 activity in DFO treated and control animals is shown in figure 4. At 24 hours the DFO treated animals have 50% less activity than the controls and when followed up for one week this group has less than 10% of the activity in the controls.

TABLE 2: EFFECT OF DFO ON TARGET/NON-TARGET RATIOS OF Ga-67-CITRATE

RATIOS	TARGET/BLOOD		TARGET/MUSCLE		TARGET/LIVER	
	CONT.	DFO	CONT.	DFO	CONT.	DFO
EMT-6 SA, MOUSE						
5 HOURS	0.48	1.95	2.01	1.95	0.39	0.21
24 HOURS	1.80	10.73	3.90	5.79	0.24	0.32
ADENOCA, MOUSE						
5 HOURS	0.51	1.29	3.76	5.42	0.89	1.01
24 HOURS	1.95	3.49	6.41	5.35	0.88	0.73
ADENOCA, RABBIT						
5 HOURS	0.44	0.81	7.28	7.80	1.60	0.80
ABSCCESS, RAT						
5 HOURS	0.39	2.47	2.14	6.00	0.44	0.59
24 HOURS	3.11	12.50	14.75	12.50	0.83	0.77

TABLE 3: SOME PARAMETERS OF TURPENTINE ABSCESSSES IN RATS

RADIOPHARMACEUTICAL	TIME (HRS.)	CONCENTRATION (% DOSE/GRAM)	RATIOS	
			ABSC/MUSL	ABSC/BLD
Tc-99m-RBC	2.0	0.06±0.02	2.00	0.01
I-131-ALBUMIN	2.0	0.93±0.28	8.45	0.21
Tc-99m-DTPA	2.0	0.05±0.01	8.33	1.21
I-125-FIBRINOGEN	4.0	1.30±0.41	10.83	0.26
Tc-99m-PLASMIN	4.0	0.21±0.00	10.50	0.50

*Each data point represents the mean ± S.D. of 6 rats.

TABLE 4: Ga-67 BLOOD LEVELS (%D/g) AND Ga-67 TARGET CONCENTRATION (%D/g)

TIME:	5 HRS.		24 HRS.	
	BLOOD	TARGET	BLOOD	TARGET
EMT-6 SA, MOUSE	15.80	7.65	2.73	4.91
ADENOCA, MOUSE	9.10	4.65	2.27	4.42
ABSCCESS, RAT	1.13	0.45	0.19	0.52
ADENOCA, RABBIT	0.23	0.06	----	----

TABLE 5: Ga-67 BLOOD LEVELS (XD/g) AND Ga-67 TARGET CONCENTRATION (XD/g)
IN DFO TREATED ANIMALS

TIME:	5 HRS.		24 HRS.	
	<u>BLOOD</u>	<u>TARGET</u>	<u>BLOOD</u>	<u>TARGET</u>
EMT-6 SA, MOUSE	1.75	3.42	0.48	5.15
ADENOCAR, MOUSE	2.31	2.66	1.09	3.84
ABSCISS, RAT	0.17	0.42	0.04	0.50
ADENOCAR, RABBIT	0.11	0.10	-----	-----

TABLE 6: GASTROINTESTINAL EXCRETION OF Ga-67 IN DFO-TREATED ANIMALS AND
CONTROLS

	<u>2 HRS.</u>	<u>5 HRS.</u>	<u>24 HRS.</u>
DFO*	6.20±0.84	9.23±1.36	4.64±0.63
CONTROLS*	7.93±0.82	8.54±0.57	4.47±0.98

*The data represents the mean ± S.D. of 6 animals in each group.

Figure 4: Ratios of total-body counts of controls vs. experimental rabbits.
DFO was injected 20 minutes after radiogallium. Values were
expressed as percentages of 24-hr. total-body count in controls.
Note marked decrease in activity in DFO-treated animals.

DISCUSSION

The galliuretic effect of DFO is another example of the biological similarities of gallium to iron. It has been known for a long time that in the serum, gallium is bound to the same proteins as iron (8,9). The effect of DFO on gallium demonstrates that similarly to its effect on iron, it also can remove protein bound gallium and increase urinary excretion. Removal of gallium by DFO is not limited to circulating gallium only; it was observed in all tissues that were examined, but the degree of gallium removal is not uniform: the highest removal of gallium was from the blood and the lowest washout was from the liver. The effect of DFO was greatest when given shortly after Ga-67 citrate and decreased as the time interval between the two injections increased.

With time, gallium is shifted from the circulation into the cells. DFO will probably affect the gallium both in the circulation and intracellularly. It is possible however, that once gallium becomes intracellular, the accessibility and strength of binding are different in various organs and therefore the DFO action is not uniform.

In two of the tumor models and the abscess, a comparison of the 5 and 24 hour gallium levels in the control animals shows that the spontaneous washout is highest in the EMT-6 sarcoma and lower in the mouse adenocarcinoma and abscess. It is of interest to point out that EMT-6 sarcoma was more vascular and had less necrosis than the two other pathological lesions.

Spontaneous and DFO induced washout from the liver was lowest as compared to other organs, possibly because gallium in the liver is bound to a high affinity binding site (?Ferritin).

DFO induces a marked washout of gallium from tumors and abscess. Because its effect on blood gallium is many times the effect on tumor or abscess, the net effect is an improved target-to-non-target ratio.

In the turpentine abscess, increased vascularity is not a prominent factor as seen from the RBC experiments. Edema with transudation is very marked as evidenced by the increased albumin and DTPA spaces. The importance of this factor in gallium localization in the viable portion of the abscess is probably very significant, at least initially. It may enable the cells a longer period for extracting the gallium. DFO, a polypeptide of low molecular weight is probably also delivered into these expanded extracellular spaces, but its effect at this level remains to be studied.

We conclude that DFO can improve the target to non-target ratio. When given after the conclusion of a gallium study it may reduce the radiation dose to the patient.

We did not find evidence that DFO decreases gastrointestinal excretion, despite the increase in urinary excretion.

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RATIO OF COUNTS IN DESFERAL
TREATED/CONTROL ANIMALS

TISSUE DISTRIBUTION OF ^{67}Ga IN RABBITS

^{67}Ga CONCENTRATION IN BLADDER ^{67}Ga CONCENTRATION IN KIDNEY ^{67}Ga CONCENTRATION IN "HEART"
(PERCENT OF INITIAL VALUE) (PERCENT OF INITIAL VALUE) (PERCENT OF INITIAL VALUE)