DISTRIBUTION OF ^{67}Ga FOLLOWING INTRAVENOUS ADMINISTRATION: EFFECTS OF DISODIUM EDETATE THERAPY

Sheldon R. Hurwitz*, Phillip L. Hagan, and Naomi P. Alazraki

University of California, San Diego, School of Medicine, La Jolla, California

Following intravenous administration of ^{67}Ga-citrate to normal and abscessed rats, the colon content of ^{67}Ga decreased with increasing oral doses of Na_2EDTA. The effects observed, however, were not thought to be of potential clinical value.

Since the introduction of ^{67}Ga-citrate as a radiopharmaceutical, observers have noted bowel activity obscuring accurate interpretation of abdominal views (*1–3*). For this reason patients routinely must undergo laxative and enema preparation for scanning. Even then, focal and diffuse areas of ^{67}Ga concentration frequently persist in the abdomen, usually in the distribution of the large bowel.

Persistent bowel activity of ^{67}Ga is often thought to result from an inadequate purge. The degree of localization of isotope in the bowel wall relative to stool has not been thoroughly investigated, however. This communication describes ^{67}Ga distribution following intravenous injection in rats with and without a model abscess and the effects of oral administration of a chelating agent, sodium ethylenediaminetetraacetic acid (Na_2EDTA).

MATERIALS AND METHODS

High-dose Na_2EDTA. Thirty-two Sprague-Dawley rats (Simonson Laboratory, Gilroy, Calif.) weighing 250–325 gm were divided into eight groups of four animals: "controls" (Groups 1–4) and "infected animals" (Groups 5–8). A model abscess was created in Groups 5–8 by injecting the left shoulder with 4×10^8 Escherichia coli (Type 0-111). Five days later, all animals received 100 μCi (400 μCi/kg) of ^{67}Ga-citrate (supplied as carrier-free ^{67}Ga-citrate by New England Nuclear) intravenously. Groups 1–4 and Groups 5–8 then received 0, 10, 50, and 100 mg/day, respectively, of Na_2EDTA (supplied by Abbott Laboratories) in divided doses (bid) administered orally for 4 days.

Urine and stool were collected separately using metabolic cages. Four days following ^{67}Ga injection, all animals were sacrificed with ether anesthesia and exsanguination by cardiac puncture. Samples of blood, abscessed shoulder, and liver were taken. In the normal animals, colon and ileum were excised and gently flushed with normal saline. All specimens were counted in a well scintillation counter using the 184-keV photopeak of ^{67}Ga with a 100-keV window. Duplicates of the injected dose (diluted to appropriate volume and range of activity) were used as standards to correct for decay.

Low-dose Na_2EDTA. Rats were studied next with a lower dose of Na_2EDTA (a "pharmacologic" dose used clinically for oral heavy-metal poisoning) (*4*). A more reliable and more easily quantitated infection model using Staphylococcus organisms was substituted and the dose of gallium was reduced.

A knee abscess was created in 24 Sprague-Dawley rats by injection of the right knee with 4.0×10^9 Staphylococcus aureus (Type 502A) and 4.0 mg talc, USP, in a volume of 0.4 cc. The injection was made with a 25-gage needle through the patellar ligament. Within 5 days, all rats had an acute pyarthrosis of the knee with swelling of the joint to twice normal in diameter.

Twenty-five microcuries (100 μCi/kg) of ^{67}Ga-citrate was injected into the tail vein of each animal on the fifth day after creation of the abscess. The rats were divided into four groups of six animals. Beginning on the day of the ^{67}Ga injection, Groups 1–4 received 0, 1, 5, and 10 mg/day, respectively, of Na_2EDTA in divided doses (bid) administered orally for 4 days.

Urine and stool were collected as with the high-dose

Received Sept. 4, 1974; original accepted Nov. 10, 1974.

For reprints contact: Naomi P. Alazraki, V.A. Hospital, 3350 La Jolla Village Dr., San Diego, Calif. 92161.

* Present address: Dept. of Nuclear Medicine, Naval Regional Medical Center, Oakland, Calif. 94627.

TABLE 1. DISTRIBUTION OF ^{67}Ga-CITRATE* IN NORMAL RATS GIVEN ORAL ("HIGH DOSE") DISODIUM EDETATE (% INJECTED DOSE/ORGAN ± STANDARD DEVIATION AT 96 HR AFTER INJECTION)

	Group 1†	Group 2	Group 3	Group 4
	0 mg Na_2EDTA	10 mg Na_2EDTA	50 mg Na_2EDTA	100 mg Na_2EDTA
Blood (total)	0.496 (0.139)	0.442 (0.030)	0.433 (0.055)	0.461 (0.104)
Liver	5.347 (1.691)	3.736 (1.824)	1.587 (0.475)	1.697 (0.184)
Small intestine	1.138 (0.401)	0.567 (0.006)	0.573 (0.229)	0.424 (0.078)
Colon	0.197 (0.137)	0.081 (0.026)	0.059 (0.024)	0.078 (0.027)
Urine‡	25.88 (2.01)	30.04 (6.28)	36.97 (7.57)	36.08 (4.80)
Stool‡	10.35 (1.08)	11.59 (2.37)	12.60 (3.16)	12.89 (2.47)
Stool:colon (at 96 hr)	12	22	32	23

* One hundred microcuries per rat ^{67}Ga-citrate intravenously.
† Four rats per group.
‡ Pooled 96-hr collection.
Stool-to-colon ratio calculated as percent dose ^{67}Ga excreted in the 72–96 hr interval divided by percent dose remaining in the (washed) colon at 96 hr after injection.

TABLE 2. DISTRIBUTION OF ^{67}Ga-CITRATE* IN RATS BEARING E. COLI ABSCESS AND GIVEN ORAL ("HIGH DOSE") DISODIUM EDETATE (% INJECTED DOSE/ORGAN ± STANDARD DEVIATION AT 96 HR AFTER INJECTION)

	Group 5†	Group 6	Group 7	Group 8
	0 mg Na_2EDTA	10 mg Na_2EDTA	50 mg Na_2EDTA	100 mg Na_2EDTA
Shoulder abscess	0.844 (0.454)	0.608 (0.152)	0.493 (0.026)	0.421 (0.127)
Blood (total)	0.343 (0.047)	0.477 (0.058)	0.603 (0.026)	0.464 (0.145)
Liver	1.108 (0.257)	0.587 (0.114)	0.486 (0.133)	0.357 (0.114)
Urine‡	6.02 (1.81)	8.15 (2.46)	8.20 (1.52)	11.65 (1.67)
Stool‡	6.71 (1.26)	7.68 (1.69)	8.43 (1.80)	8.71 (2.21)
Abscess:blood	6.3	3.4	2.0	2.0

* One hundred microcuries per rat ^{67}Ga-citrate intravenously.
† Four rats per group.
‡ Pooled 96-hr collections.
Abscess-to-blood ratio calculated as counts per gram abscess divided by counts per cubic centimeter blood, both specimens obtained at 96 hr after injection.

Na_2EDTA animals. All rats were sacrificed 4 days following ^{67}Ga injection, and specimens of abscessed and normal knees, liver, blood, colon, and ileum were taken and counted. Bowel was prepared with a gentle saline flush prior to counting.

RESULTS

High-dose Na_2EDTA. The distribution of ^{67}Ga as percent of administered dose per organ is presented in Tables 1 and 2. High doses of the chelator in normal rats (Table 1) decreased counts in colon and small intestine and increased urine and stool excretion of gallium but liver activity also fell significantly. Blood activity remained unchanged. Table 2 presents the effect of the chelator on gallium distribution in rats with abscesses. The abscess-to-blood ratio fell as much as threefold ($p < 0.01$). Up to two times more ^{67}Ga was excreted in the urine ($p < 0.05$) although blood activity remained unchanged. Liver activity and total abscess activity fell.

Low-dose Na_2EDTA. The effects of low doses of chelator on the distribution of ^{67}Ga in rats with abscesses are presented in Table 3. Again, chelation with significant reduction of colonic mucosal ^{67}Ga ($p < 0.05$) was observed.

Gallium-67 uptake by the abscessed knee also dropped significantly ($p < 0.05$) at these lower doses of oral Na_2EDTA (1–10 mg/day). Liver activity and urinary excretion were not changed. Stool activity was always greater than eight times that of the entire (washed) colon.

DISCUSSION

Gallium-67-citrate is a useful agent for detecting neoplastic and inflammatory lesions (*1–3*). Interpretation of abdominal views is difficult because of uncertainty in distinguishing normal bowel activity from pathologic lesions. A bowel prep consisting of enemas and laxatives is generally helpful but there is little prior evidence to document the degree of sequestered ^{67}Ga activity in the wall of the bowel itself. A study in 1972 by the group at Oak Ridge

TABLE 3. DISTRIBUTION OF ^{67}Ga-CITRATE* IN RATS BEARING S. AUREUS ABSCESS AND GIVEN ORAL ("LOW DOSE") DISODIUM EDETATE (% INJECTED DOSE/ORGAN ± STANDARD DEVIATION AT 96 HR AFTER INJECTION)

	Group 1†	Group 2	Group 3	Group 4
	0 mg Na_2EDTA	1 mg Na_2EDTA	5 mg Na_2EDTA	10 mg Na_2EDTA
Knee (abscessed)	2.005 (0.555)	1.861 (0.342)	1.688 (0.128)	1.428 (0.169)
Knee (normal)	0.855 (0.113)	0.814 (0.555)	1.166 (0.505)	0.763 (0.119)
Blood	0.309 (0.039)	0.319 (0.035)	0.324 (0.068)	0.309 (0.074)
Liver	1.660 (0.257)	1.689 (0.361)	1.318 (0.335)	1.451 (0.453)
Small intestine	0.237 (0.181)	0.250 (0.151)	0.189 (0.138)	0.338 (0.169)
Colon	0.105 (0.046)	0.060 (0.018)	0.053 (0.017)	0.033 (0.013)
Urine‡	14.38 (3.55)	15.99 (5.24)	14.01 (2.55)	13.73 (1.95)
Stool‡	7.42 (1.05)	7.11 (1.10)	6.70 (1.25)	6.55 (2.72)
Stool:colon (at 96 hr)	8	18	18	24
Abscess:blood	41	34	32	29

* Twenty-five microcuries per rat of ^{67}Ga-citrate intravenously.
† Six rats per group.
‡ Pooled at 96-hr collections.

TABLE 4. BINDING CONSTANTS OF Na_2EDTA WITH METAL IONS OF INTEREST IN NUCLEAR MEDICINE*

$Ca^{++} + EDTA^{-4} = CaEDTA^{--}$	$K_1 = 5.01 \times 10^{10}$
$Co^{++} + EDTA^{-4} = CoEDTA^{--}$	$K_1 = 2.04 \times 10^{16}$
$Ga^{+++} + EDTA^{-4} = GaEDTA^{-}$	$K_1 = 1.86 \times 10^{20}$
$In^{+++} + EDTA^{-4} = InEDTA^{-}$	$K_1 = 8.91 \times 10^{24}$
$Fe^{+++} + EDTA^{-4} = FeEDTA^{-}$	$K_1 = 1.3 \times 10^{25}$
$Pb^{++} + EDTA^{-4} = PbEDTA^{--}$	$K_1 = 1.10 \times 10^{18}$
$Zn^{++} + EDTA^{-4} = ZnEDTA^{--}$	$K_1 = 3.16 \times 10^{16}$

* After Fischer and Peters (7).

(*4*) was performed in humans to determine distribution of ^{67}Ga (including intestinal activity) but the population had a variety of pathologic lesions and only tissue concentrations are given, not absolute organ distribution.

Since chelation chemistry is well defined for many cations (*5–7*), we also explored the possibility of binding bowel wall activity of gallium to improve the quality of the scans. The ideal chelator for gallium would be administered during the interval between injection and imaging. It should have a high binding constant (Table 4), be nontoxic and nonabsorbable, and not affect the distribution of ^{67}Ga in any body compartment other than bowel. Less than 5% of ^{14}C-labeled Na_2EDTA was absorbed from the gastrointestinal tract in studies by Foreman in 1954 (*8,9*).

We therefore anticipated that orally administered Na_2EDTA would chelate colonic mucosal ^{67}Ga activity and enhance its removal. Although Na_2EDTA did indeed have the effect of clearing residual radioactivity (Fig. 1) from colon wall, other important effects were seen on body distribution of the radioisotope. Even the small amount of oral EDTA that was systemically absorbed significantly diverted gallium from distant areas of concentration such as liver and the model abscess.

Other chelators such as DTPA, dimercaprol, deferoxamine, and penicillamine were briefly evaluated. Although effective, preliminary testing showed that these agents either had undesirable side effects or were also absorbable with oral administration.

The overwhelming amount of ^{67}Ga activity seen on abdominal views is intraluminal and cleansing enemas remain essential. Conceivably, Na_2EDTA might prove useful as an adjunctive measure: administration of the chelator as a brief retention enema (substituting for the usual preparatory enemas) might be another approach to leaching any residual ^{67}Ga in the colonic mucosa.

Finally, it is interesting to note that the percent of injected ^{67}Ga excreted in urine by normal rats was 26% in the first 96 hr following injection but only

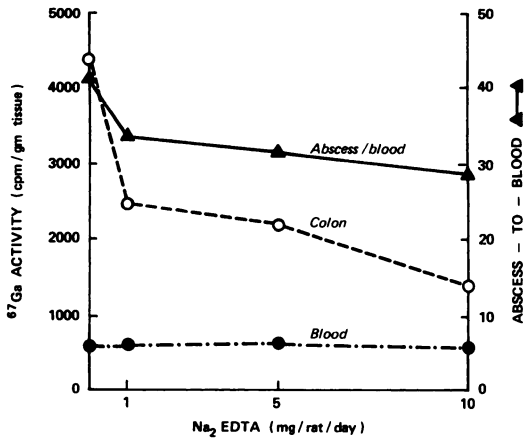

FIG. 1. Concentration of ^{67}Ga in colon, abscess, and blood related to Na_2EDTA dosage.

6% in rats with abscesses (receiving comparable doses of ^{67}Ga). The gallium in stool was also higher in the normal rats (10%) than in animals with abscesses (7%). For unknown reasons the presence of a small gallium-concentrating lesion alters organ distribution and body excretion of the isotope. Careful studies of tissue distribution of ^{67}Ga in normal mice and in tumor-bearing animals have been performed (*10,11*) but there has been no mention of such dramatic shifts of gallium distribution in the pathologic state.

The original intent of removing ^{67}Ga from the wall of the large bowel was achieved. The data presented indicate, however, that most "abdominal activity" seen on scans is localized in the stool. With increasing doses of oral Na_2EDTA, activity of ^{67}Ga in colonic mucosa was significantly decreased but activity in a model abscess and liver also fell. Enough oral EDTA is absorbed into the blood to leach gallium out of distant areas of concentration, and the chelated complex is then excreted in the urine. These effects of oral Na_2EDTA on ^{67}Ga distribution have no clinical utility.

REFERENCES

1. EDWARDS CL, HAYES RL: Scanning malignant neoplasms with gallium 67. *JAMA* 212: 1182–1190, 1970

2. LITTENBERG RL, ALAZRAKI NP, TAKETA RM, et al: A clinical evaluation of gallium-67 citrate scanning. *Surg Gynecol Obstet* 137: 424, 1973

3. LITTENBERG RL, TAKETA RM, ALAZRAKI NP, et al: Gallium-67 for localization of septic lesions. *Ann Intern Med* 79: 403–406, 1973

4. NELSON B, HAYES RL, EDWARDS CL, et al: Distribution of gallium in human tissues after intravenous administration. *J Nucl Med* 13: 92–100, 1972

5. LEVINE WG: Heavy-metal antagonists. In *The Pharmacological Basis of Therapeutics*, 4th ed, Goodman LS, Gilman A, eds, New York, Macmillan, pp 944–957, 1970

6. FOREMAN H: The pharmacology of some useful chelating agents. In *Metal-Binding in Medicine*, Seven MJ, ed, Philadelphia, Lippincott, 1960, pp 66–94

7. FISCHER RB, PETERS DG: *Chemical Equilibrium*, Philadelphia, Saunders, 1970, p 263

8. FOREMAN H, TRUJILLO TT: The metabolism of C^{14}-labeled ethylenediaminetetraacetic acid in human beings. *J Lab Clin Med* 43: 566–571, 1954

9. FOREMAN H, VIER M, MAGGEE M: The metabolism of C^{14}-labeled ethylenediaminetetraacetic acid in the rat. *J Biol Chem* 203: 1045–1053, 1953

10. SWARTZENDRUBER DC, BYRD BL, HAYES RL, et al: Preferential localization of gallium-67 citrate in tissues of leukemic mice. *J Natl Cancer Inst* 44: 695–700, 1970

11. HAYES RL, BYRD BL, CARLETON JE, et al: Factors affecting the localization of ^{67}Ga in animal tumors. *J Nucl Med* 11: 324, 1970