Gallium: Mechanisms

Paul Hoffer

Yale University Medical School, New Haven, Connecticut

J Nucl Med 21: 282–285, 1980

In 1969 Edwards and Hayes (*1*) first described localization of Ga-67 in human tumors; subsequently other clinical investigators (*2,3*) demonstrated its localization in inflammatory lesions. However, after a decade of clinical use, there is still no general agreement on the exact mechanisms of localization in either tumor or inflammation. Experiments by Hartman and Hayes (*4*), Gunasekera et al. (*5*), and Hara (*6*) indicate that intravenously injected Ga-67 associates with transferrin. Tissue-bound Ga-67 is also associated with iron-binding proteins including ferritin and transferrin (*7–9*), and is found primarily in microvesicles of tumor cells and lysosomes of normal liver cells (*10*). It is the mechanisms facilitating the transfer from plasma to cell that represent the chief mystery in understanding Ga-67 localization.

Gallium-67 is a Group III_b transition metal that resembles the ferric ion in atomic radius, charge, and in the types of inorganic complexes these two atoms form (*11,12*). Elemental gallium is a solid with low melting point (29.8°C). Gallium-67 is usually adminstered as the citrate to facilitate solubilization.

A major difference between gallium and iron is the inability of gallium to be reduced in vivo. Therefore, whereas ferric ion is easily reduced and interacts with protoporphyrin IX to form heme (*13*), gallium remains bound to iron-transport proteins and carrier molecules. This difference explains in large part why, in spite of their other physical similarities, the biologic distributions of gallium and iron differ.

Gallium binds to at least four iron-binding molecules: transferrin (TF), lactoferrin (LF), ferritin, and siderophores. Siderophores are compounds of low molecular weight (~600 daltons) that facilitate iron uptake by microorganisms. The dissociation constants for the gallium-macromolecular complexes vary with pH and relative concentration. Little is known about the relative affinity of gallium for ferritin. Its relative affinity for the other iron-binding macromolecules, however, ranks as follows: siderophore >LF >TF (*14–16*). This order of affinity is similar to that of ferric iron for these molecules, although of considerably different magnitude in most cases.

Ferric ion easily displaces gallium from TF and, to a lesser extent, from LF. The gallium-siderophore bond is considerably stronger, and gallium is only incompletely dissociated from siderophore when a small excess of ferric ion is added, which suggests a dissociation constant within two orders of magnitude of that for iron-siderophore (T Emery, P B Hoffer unpublished data).

General biological considerations. Under laboratory conditions, excess citrate ion inhibits cellular gallium uptake (RE Weiner, MS Cohen, PB Hoffer, et al., unpublished data) and the association of gallium with transferrin (*17*), and affects its chromatographic characteristics (*18*). Following i.v. injection, however, extensive dilution of excess citrate in the radiopharmaceutical occurs, and therefore the amount of carrier citrate in the preparation does not affect biologic localization (*19*).

When a large excess of ferric ion is administered before or coincident with Ga-67, tumor and tissue localization is inhibited (*20*) and urinary excretion enhanced. Similarly, presence of excess carrier gallium increases radionuclide excretion and inhibits localization except in bone. If, however, ferric ion or scandium is administered *after* Ga-67 administration, there is less inhibition of tumor localization, and tumor-to-blood ratios are actually increased (*21,22*). Unfortunately scandium is toxic to humans, and therefore is not useful as a contrast-enhancing agent. Excess apotransferrin also appears to inhibit tumor localization of Ga-67 (*23*). Paradoxically, increase in serum iron due to irradiation has been shown to inhibit Ga-67 localization in some tumors (*24*).

Following i.v. injection in humans, 10–25% of the dose

Received Oct. 8, 1979; accepted Oct. 10, 1979.

For reprints contact: Paul Hoffer, MD, Dept. of Nuclear Medicine, Yale Univ. School of Medicine, 333 Cedar St., New Haven, CT 06510.

is excreted in the urine within the first 24 hr. Subsequent excretion is slower and is primarily via the bowel. Clinical observations indicate that most of the gallium retained in the body is normally localized in the liver and the skeleton. Localization of gallium in tumor or inflammatory lesions alters this distribution, frequently causing decreased hepatic uptake.

The localization of Ga-67 in tumors and abscesses, as well as in most normal tissues, appears to occur in at least two phases. During the early phase (up to about 6 hr) the Ga-67 that has localized in the tissue can be extracted by iron-binding chelates such as Desferal (*26*). However, when Desferal is administered 24 hr after Ga-67, it is no longer capable of leaching the radionuclide from most tissues (*27,28*). This suggests that Ga-67 localizes by an early weak binding or diffusion mechanism followed by a firmer intracellular binding phase. Parenthetically, Desferal has been used in an effort to improve lesion contrast, since it reduces the blood level of Ga-67 even at 24 hr following Ga-67 administration (*27,28*). In animals, lesion contrast is improved by using relatively large doses of Desferal. However, clinical studies in humans have produced disappointing results, with no significant improvement in contrast (PB Hoffer, unpublished data). This is due primarily to the relatively small doses of Desferal that can be administered over short time intervals at a level of safety consistant with a noninvasive study. Also, while Desferal is still effective in lowering the blood level of Ga-67 at 24 hr following injection of the radionuclide, it is much less effective in lowering Ga-67 levels in other normal tissues.

Specific mechanisms of uptake. *Infection.* At least three mechanisms by which Ga-67 may localize in infections have been postulated. These include leukocyte labeling (*29*), lactoferrin binding at the site of infection (*30*), and direct bacterial uptake (*31*). For the latter two mechanisms it is assumed that the Ga-67 is available at the site of infection in either usual or increased amounts. Increased availability of Ga-67 at sites of inflammation can easily be explained by increased vessel permeability at such sites.

Leukocyte localization. Gallium-67 has been shown to be incorporated into leukocytes, which in turn localize at sites of inflammation (*32*). Absence of leukocytes is associated with decreased uptake of Ga-67 at inflammatory sites in monkeys (*29*). Leukocytes are rich in lactoferrin, and Ga-67 taken up by leukocytes is primarily bound to lactoferrin (*33*). It is doubtful, however, whether this one mechanism fully explains Ga-67 localization in inflammatory lesions. Gallium localization has been demonstrated in patients with absence of circulating leukocytes (*34*), and the quantitative uptake of Ga-67 in leukocytes is highly variable (*35*) (RE Weiner, PB Hoffer, ML Thakur, unpublished data). While some of this variability may represent the inhibitory effect of excess citrate ion under the experimental conditions used (RE Weiner, MS Cohen, PB Hoffer, et al., unpublished data), even under ideal circumstances direct cellular migration of labeled leukocytes is, in and of itself, an inadequate explanation for the extent of Ga-67 localization in most inflammatory lesions.

Lactoferrin binding at the site of infection. The lactoferrin (LF) contained in leukocytes is located within the secondary granule (*36,37*). When leukocytes localize at sites of inflammation, they not only ingest bacteria but also excrete some of the content of the secondary granules, including LF (*38*). The discharged LF tends to remain localized, sticking to receptor sites in tissue macrophages (*39–41*). Gallium-67, either in ionic form or bound to TF, may be delivered to the site of inflammation by leakage through permeable vessel endothelium and subsequently be retained by binding to apolactoferrin (LF). Recent studies demonstrate increased Ga-67 binding to fluid surrounding leukocytes that have been stimulated to excrete LF, lending additional support to this concept (*30*). In addition some tissues, e.g., salivary glands and breast, may also be capable of producing increased quantities of lactoferrin when stimulated by local inflammation.

Direct bacterial uptake. Finally, infective organisms may take up Ga-67 directly, which has been demonstrated in vitro (*31*). Microorganisms grown in low-iron environments produce siderophore (*42–44*). Since there is very little free iron present in most tissues, it is assumed that pathogenic microorganisms produce siderophore. The siderophore molecules have extraordinary binding affinity for Ga-67 as well as for iron. The Ga-67-siderophore complex is rapidly transported into the cell (T Emery, P B Hoffer unpublished data). Once captured within the cell the Ga-67-siderophore complex cannot be released without metabolic destruction of the entire molecule.

It is possible that other proteins such as ferritin may also be involved in inflammatory uptake of Ga-67. Potential mechanisms involving ferritin have not been adequately explored.

The exact quantitative role these three mechanisms play in localization of Ga-67 is unclear. Tissue injury without bacterial infection will result in Ga-67 localization; infection in the absence of leukocyte response will also result in localization. It is probable that in most cases all three mechanisms function to bring Ga-67 to the site of inflammation.

Localization in tumor. Proposed mechanisms of Ga-67 localization in tumor are highly speculative and controversial, with much contradictory evidence. There is some agreement that the highly permeable walls of tumor vessels, combined with the large extracellular fluid space of most tumors, play some role in the initial localization of Ga-67 at the site of the tumor (*45–47*). Hayes and associates postulate that it is the free ionic Ga-67 fraction in the plasma that becomes incorporated

into the tumor rather than the transferrin-bound fraction (*48*). These findings are supported by studies demonstrating that as available TF binding sites are saturated with scandium or iron, relative tumor uptake of Ga-67 increases (*20,21*).

However, the studies of Larson and associates (*23,49*) and of Sephton and Harris (*50*) suggest that TF may play a key role in Ga-67 localization in tumors. Larson has shown that Ga-67 uptake by tumors in tissue culture is dependent on TF concentration, increasing initially in direct proportion to added TF, and subsequently decreasing as the TF begins to saturate the number of available TF-binding sites on the tumor-cell membrane (*23*).

While it is possible that Ga-67 uptake in tumors is due to reactive cellular infiltration of leukocytes, this explanation is unlikely, since autoradiographic studies of tumor tissue indicate localization within the tumor cells themselves (*51*); moreover, direct tumor uptake of Ga-67 occurs in pure tumor-cell cultures.

The work of Anghileri (*52,53*) suggests a link between Ga-67 localization and calcium metabolism in tumors. However, this theory conflicts with the clinical observation that Ga-67 uptake and Ca^{2+} content in tumors are not associated—e.g., in neuroblastomas, which are frequently calcified but exhibit a low incidence of Ga-67 localization. Hayes and associates have demonstrated tumor Ga-67 bound to a specific 40,000-dalton metalloprotein (*54*). Other investigators have shown tumor-bound Ga-67 to be associated with fractions of higher molecular weight (*7*). Fernandez-Pol has recently described a siderophore-like growth factor (SGF) present in virally transformed tumors (*53*). This substance has strong binding affinity for ferric ion and a number of other cations including gallium. Finally, lactoferrin (LF) has also been demonstrated in tumors known to localize Ga-67, including Hodgkin's disease, Burkitt's lymphoma (*56*), and melanoma.

In view of the strong evidence available to support many of the competing theories for gallium localization in tumor, it is possible that more than one mechanism is operating. It seems doubtful, however, that all of the proposed mechanisms play some role. One of the most intriguing aspects of many of the mechanisms is the similarity between the kinetics of Ga-67 and ferric iron within the tumor. The concept that tumors have extraordinary need for ferric ion is not new; it is well known that tumor cells contain high levels of ribonucleotide reductase, an iron-dependent enzyme. It is possible that gallium localization in tumors is exposing a pathway by which the needed iron is derived.

ACKNOWLEDGMENTS

Ronald Weiner, PhD, provided numerous helpful suggestions in the preparation of this manuscript. The many drafts and final manuscript were prepared by Rose Ann Cherlin. This work is supported by D.O.E. Contract No. EP-78-S-02-4625.

REFERENCES

1. EDWARDS CL, HAYES RL: Tumor scanning with ^{67}Ga citrate. *J Nucl Med* 10: 103-105, 1969
2. LAVENDER JP, LOWE J, BARKER JR, et al: Gallium 67 citrate scanning in neoplastic and inflammatory lesions. *Br J Radiol* 44: 361-366, 1971
3. LITTENBERG RL, TAKETA RM, ALAZRAKI NP, et al: Gallium-67 for the localization of septic lesions. *Ann Intern Med* 79: 403-406, 1973
4. HARTMAN RE, HAYES RL: Gallium binding by blood serum. *Fed Proc* 26: 780, 1967 (abst)
5. GUNASEKERA SW, KING LJ, LAVENDER PJ: The behavior of tracer gallium-67 towards serum proteins. *Clin Chim Acta* 39: 401-406, 1972
6. HARA T: On the binding of gallium to transferrin. *Int J Nucl Med Biol* 1: 152-154, 1974
7. CLAUSEN J, EDELING C-J, FOGH J: ^{67}Ga binding to human serum proteins and tumor components. *Cancer Res* 34: 1931-1937, 1974
8. AULBERT E, GEBHARDT A, SCHULZ E, et al: Mechanism of ^{67}Ga accumulation in normal rat liver lysosomes. *Nucl Med* 15: 185-194, 1976
9. 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
10. BROWN DH, BYRD BL, CARLTON JE, et al: A quantitative study of the subcellular localization of ^{67}Ga. *Cancer Res* 36: 956-963, 1976
11. COTTON FA, WILKINSON G: *Advanced Inorganic Chemistry*. New York, Interscience, 3rd ed, 1972, pp 260-282
12. LATIMER WM: *The Oxidation States of the Elements and Their Potentials in Aqueous Solutions*. New York, Prentice-Hall Inc. 1952, pp 158-161
13. MAINES MD, KAPPAS A: Metals as regulators of heme metabolism. *Science* 198: 1215-1221, 1977
14. WEINER RE, THAKUR ML, GOODMAN MM, et al: Relative stabilities of In-111 and Ga-67 desferrioxamine and transferrin complexes. *Am J Roentgenol* 132: 489, 1979 (abst)
15. WEINER RE, THAKUR ML, GOODMAN M, et al: Relative stability of In-111 and Ga-67 desferrioxamine and human transferrin complexes. Proceedings 2nd International Symposium on Radiopharmaceuticals, Seattle, WA, March 1979, (in press)
16. WEINER RE, HOFFER PB, THAKUR ML: Effect of ferric ion in Ga-67 binding to lactoferrin. Second International Congress of the World Federation of Nuclear Medicine and Biology, Washington, DC 1978, p 73 (abst)
17. HARTMAN RE, HAYES RL: The binding of gallium by blood serum. *J Pharmacol Exp Ther* 168: 193-198, 1969
18. WAXMAN AD, KAWADA T, SIEMSEN JK, et al: Are all gallium citrate preparations the same? *J Nucl Med* 16: 580, 1976 (abst)
19. DAVIS MA, TAUBE RA, CARMEL AD: Comparison of the biologic distribution of three commercially available Ga-67 citrate preparations in normal and diseased rats. *J Nucl Med* 18: 617, 1977 (abst)
20. OSTER ZH, LARSON SM, WAGNER HN JR: Possible enhancement of ^{67}Ga-citrate imaging by iron dextran. *J Nucl Med* 17: 356-358, 1976
21. HAYES RL, BYRD BL, CARLTON JE, et al: Effect of scandium on the distribution of ^{67}Ga in tumor-bearing animals. *J Nucl Med* 12: 437-438, 1971 (abst)
22. LARSON SM, RASEY JS, ALLEN DR, et al: A transferrin-mediated uptake of gallium-67 by EMT-6 sarcoma. 1. Studies in tissue culture. *J Nucl Med* 20: 837-842, 1979

23. LARSON SM, RASEY JS, ALLEN DR, et al: A transferrin-mediated uptake of gallium-67 by EMT-6 sarcoma. 1. Studies in tissue culture. *J Nucl Med* 20: 837–842, 1979
24. 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
25. LARSON SM, HOFFER PB: Chapt. 3, Normal patterns of localization. In *Gallium-67 Imaging,* Hoffer PB, Bekerman C, Henkin RE, eds, New York, John Wiley & Sons, 1978, pp 23–38
26. HOFFER PB, SAMUEL A, BUSHBERG JT, et al: Effect of desferoxamine on tissue and tumor retention of gallium-67: concise communication. *J Nucl Med* 20: 248–251, 1979
27. HOFFER PB, SAMUEL A, BUSHBERG JT, et al: Desferoxamine mesylate (Desferal): A contrast-enhancing agent for gallium-67 imaging. *Radiology* 131: 775–779, 1979
28. LARSON SM, RASEY JS, GRUNBAUM Z, et al: Pharmacologic enhancement of gallium-67 tumor-to-blood ratios for EMT-6 sarcoma (BALB/c mice). *Radiology* 130: 241–244, 1979
29. GELRUD LG, ARSENEAU JC, MILDER MS, et al: The kinetics of ^{67}gallium incorporation into inflammatory lesions: experimental and clinical studies. *J Lab Clin Med* 83: 489–495, 1974
30. WRIGHT DG, PIZZO PA, JONES AE, et al: Studies of ^{67}Ga uptake at sites of neutrophil exudation. *Clin Res* 27: 360A, 1979 (abst)
31. MENON S, WAGNER HN JR, TSAN M-F: Studies on gallium accumulation in inflammatory lesions: II. Uptake by *Staphylococcus aureus:* Concise communication *J Nucl Med* 19: 44–47, 1978
32. BURLESON RL, HOLMAN BL, TOW DE: Scintigraphic demonstration of abscesses with radioactive gallium labeled leukocytes. *Surg Gynecol Obstet* 141: 379–382, 1975
33. WEINER RE, HOFFER PB, THAKUR ML: Identification of Ga-67 binding component in human neutrophils. *J Nucl Med* 19: 732, 1978 (abst)
34. DHAWAN VM, SZIKLAS JJ, SPENCER RP: Localization of Ga-67 in inflammations in the absence of circulating polymorphonuclear leukocytes. *J Nucl Med* 19: 292–294, 1978
35. TSAN M-F, CHEN WY, SHEFFEL U, et al: Studies on gallium accumulation in inflammatory lesions: I. Gallium uptake by human polymorphonuclear leukocytes. *J Nucl Med* 19: 36–43, 1978
36. BAGGIOLINI M, DEDUVE C, MASSON PL, et al: Association of lactoferrin with specific granules in rabbit heterophil leukocytes. *J Exp Med* 131: 559–570, 1970
37. SPITZNAGEL JK, DALLDORF FG, LEFFELL MS, et al: Character of azurophil and specific granules purified from human polymorphonuclear leukocytes. *Lab Invest* 30: 774–785, 1974
38. WANG-IVERSON P, PRYZWANSKY KG, SPITZNAGEL JK, et al: Bactericidal capacity of phorbol myristate acetate-treated human polymorphonuclear leukocytes. *Infec Immun* 22: 945–955, 1978
39. VAN SNICK JL, MARKOWETZ B, MASSON PL: The ingestion and digestion of human lactoferrin by mouse peritoneal macrophages and the transfer of its iron into ferritin. *J Exp Med* 146: 817–827, 1977
40. BENNETT R, KOKOCINSKI T: Lactoferrin content of peripheral blood cells. *Br J Haematol* 39: 509–521, 1978
41. BENNETT RM, SPROUSE C: Lactoferrin cell interaction: Non-specific binding, cell aggregation and protein polymerization. *Clin Res* 27: 321A, 1979 (abst)
42. NIELANDS JB: A crystalline organo-iron pigment from a rust fungus (ustilago sphaerogena). *J Am Chem Soc* 74: 4846–4847, 1952
43. NIELANDS JB: Microbial iron transport compounds (siderochromes). In *Inorganic Biochemistry* Eichhorn G, ed. Amsterdam, Elsevier, 1973, pp 167–202
44. NIELANDS JB: Siderophores: biochemical ecology and mechanism of iron transport in enterobacteria. In *Bioinorganic Chemistry II; Advanced Chemistry Series,* Raymond KN, ed. Vol 162, Washington, DC, American Chemical Society, 1977, pp 3–32
45. ITO Y, OKUYAMA S, SATO K, et al: ^{67}Ga tumor scanning and its mechanisms studied in rabbits. *Radiology* 100: 357, 1971
46. WINCHELL HS: Mechanisms for localization of radiopharmaceuticals in neoplasms. *Semin Nucl Med* 6: 371–378, 1976
47. WINCHELL HS, SANCHEZ PD, WATANABE CK, et al: Visualization of tumors in humans using ^{67}Ga-citrate and the Anger whole-body scanner, scintillation camera and tomographic scanner. *J Nucl Med* 11: 459, 1970
48. HAYES RL, BROWN DH: Biokinetics of radiogallium. In *Nuklear Medizin,* Pabst HW, Hör G, Schmidt HAE, eds. New York, FK Schattauer Verlag, 1975, pp 837–848
49. LARSON SM, RASEY JS, ALLEN DR, et al: A transferrin-mediated uptake of gallium-67 by EMT-6 sarcoma. II. Studies in vivo (BALB/c mice): Concise communication. *J Nucl Med* 20: 843–846, 1979
50. SEPHTON RG, HARRIS AW: Gallium-67 citrate uptake by cultured tumor cells, stimulated by serum transferrin. *J Natl Cancer Inst* 54: 1263, 1974
51. THESINGH CW, DRIESSEN OMJ, DAEMS WTH, et al: Accumulation and localization of gallium-67 in various types of primary lung carcinoma. *J Nucl Med* 19: 28–30, 1978
52. ANGHILERI LB: The mechanism of accumulation of radiogallium and radioanthanides in tumors. *J Nucl Biol Med* 17: 177, 1973
53. ANGHILERI LB: On the similarity of alkaline earths metabolism and ^{67}Ga accumulation by liver as revealed by acute thioacetamide intoxication. *J Nucl Biol Med* 19: 145, 1975
54. HAYES RL, CARLTON JE: A study of the macromolecular binding of ^{67}Ga in normal and malignant animal tissues. *Cancer Res* 33: 3265–3272, 1973
55. FERNANDEZ-POL JA: Isolation and characterization of a siderophore-like growth factor from mutants of SV40-transformed cells adapted to picolinic acid. *Cell* 14: 489–499, 1978
56. HOFFER PB, MILLER-CATCHPOLE R, TURNER D: Demonstration of lactoferrin in tumor tissue from two patients with positive gallium scans. *J Nucl Med* 20: 424–427, 1979