Fate of Gallium *in Vivo* and Its Significance in Tumour Diagnosis and Therapy Respectively with Gallium-67 and Cold Gallium

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Early tumour diagnosis with high quality images is obtained by injecting gallium-67 made free before intravenous administration. No healthy organ uptake of the radionuclide administered in this form takes place. Since free cationic gallium is readily hydrolyzed in aqueous solution, the solution of gallium-67 should be made just before injection from commercial solutions. Both strongly-bound and hydrolyzed gallium-67 concentrate in healthy organs, like liver.

Free cold gallium solutions have been successfully used in the treatment of experimental mammary tumour (TGS) in mice.

Introduction

Of 106 elements known today, gallium is the only one which has lent itself to both noninvasive diagnosis and therapy of tumours. Although the first report on the clinical application of gallium dates back as far as 1931 [1], it was the observation by Edwards and Hayes [2] in 1969 that gallium-67 concentrates in several soft-tissue tumours, which aroused great interest in this radionuclide and today it has become the most widely used radionuclide for tumour imaging [3]. Two years later Hart and colleagues [4, 5] reported the potential usefulness of cold gallium nitrate for treatment of solid tumours both in rodents and humans. Although the literature reports less work on the antitumour activity of cold gallium compared with that on the diagnostic use of gallium-67, it has been felt that if the aqueous solution chemistry of both gallium-67 and cold gallium is well studied, better results can be obtained both in the early diagnosis and treatment of cancer with gallium [6].

Gallium, having a high charge density (4.84), is highly amphoteric and is readily hydrolyzed in aqueous solution. Citrated gallium-67 and cold gallium solutions have been used for clinical purposes [7]. Hayes and Edwards [8] and Hammersley and Zivanovic [9] have found that the uptake of gallium-67 in healthy tissues and tumours is independent of citrate ion concentration in the gallium-67 formulation. The results from our laboratories, on the other hand, have shown [10, 11] that the citrate ion both *in vitro* and *in vivo* plays an important role in the biological distribution of both gallium-67 and cold gallium.

Many studies have been reported to show that the ion transport protein *in vivo* plays the important role of transporting gallium from the site of its administration to the tumour site [6]. There is little agreement, however, on the role of the ion storage protein, ferritin, on the biological behaviour of gallium and on its tumour affinity [12]. Our results have shown that the stability of gallium complexes with the three ligands with which the cation comes in contact in vivo follows the order [13]: gallium–ferritin complex $\gg$ citratogallate $>$ gallium–transferrin complex. These results have explained the biological behaviour of gallium known so far [14], and have led us to improve the quality of tumour images with lower dose of the radiopharmaceutical. The present paper reports some results in rodents and humans.

Experimental

A detailed description of the tracer, tumour-bearing animals and the procedure for scintigraphic imaging is already available [10]. The quality control of the radiopharmaceutical and its solution chemistry was studied chromatographically in the system: Whatman-3MM-physiological saline. Scintigrams were obtained with an Italelettronica scanner. Whole-body distribution of the radionuclide was also examined with a Siemens gamma camera.

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Results and Discussion

Quality Control of Commercial Gallium-67 Citrate

Quality control of the commercial gallium-67 citrate solutions received weekly for tumour imaging showed that two samples of the radiopharmaceutical received from different suppliers or from the same supplier are not the same and always give different chromatographic and electrophoretic patterns. The gallium-67 formulation for the same concentration of gallium-67 from the Amersham Radiochemical Centre, England, and Sorin Biomedica, Saluggia, Italy, has respectively 26 mg/ml and 1.75 mg/ml of sodium citrate. Chromatographic analysis of the two solutions has shown that the Amersham gallium-67 solution contains 92% gallium-67 as citratogallate-67 while that from Sorin has only 28%. The distribution of the two gallium-67 in Morris hepatoma-3924A-bearing rats injected with Amersham gallium-67 and Sorin gallium-67 is shown respectively in Figs. 1 and 2. It is evident from the scintigrams that the higher

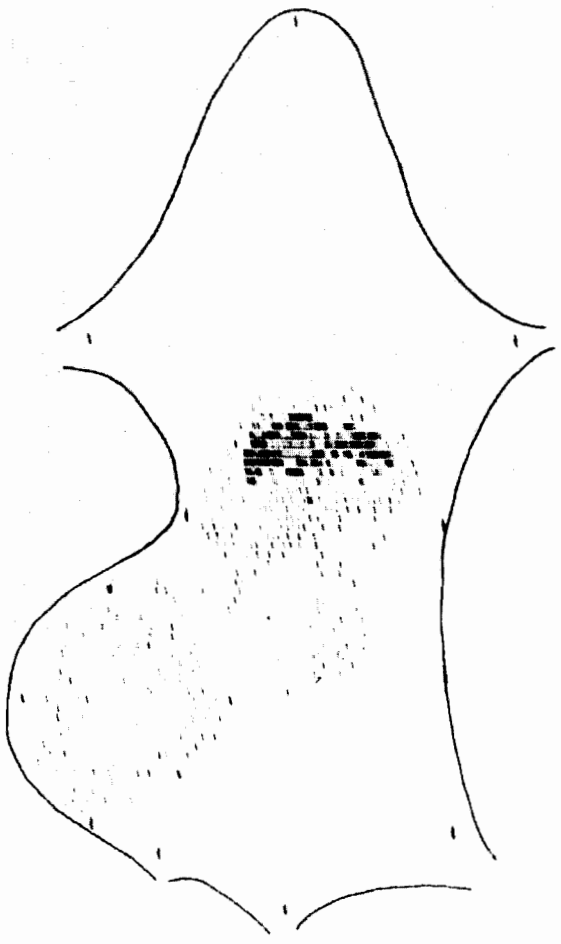

Fig. 1. Scintigram of a Morris hepatoma-3924A-bearing rat injected with Amersham gallium-67 containing 26 mg/ml sodium citrate.

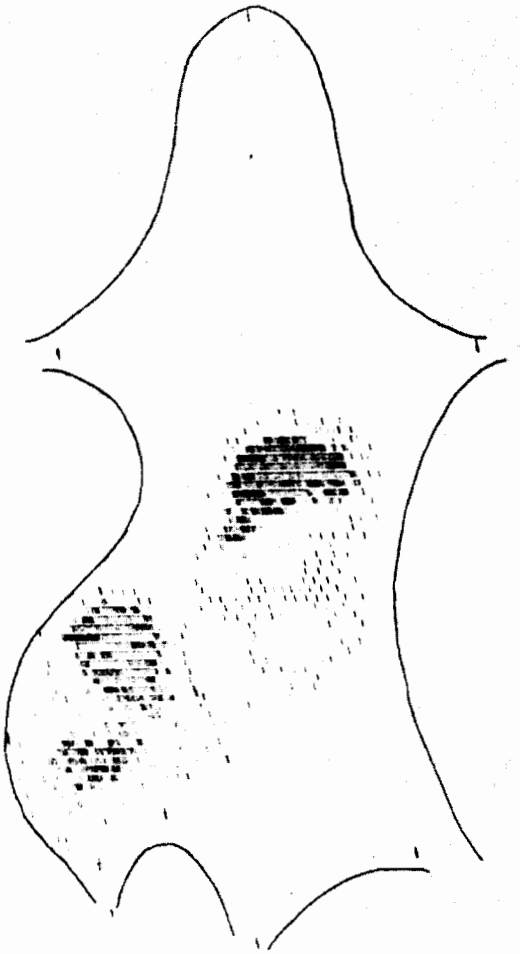

Fig. 2. Scintigram of a Morris hepatoma-3924A-bearing rat injected with Sorin gallium-67 containing 1.75 mg/ml sodium citrate.

is the amount of stable citratogallate-67 in the solution injected, the lower is the concentration of the radionuclide in the tumour and the more intense the uptake in healthy liver. Similar results were obtained when the radioactivity in different organs and in the tumour was counted in a gamma counter after the death of the animal.

Optimal Gallium-67 Formulation for Tumour Specific Early Uptake of the Radionuclide

Since the chemical form of gallium-67 also varied in the samples received on different days from the same supplier, it was difficult to obtain reproducible results from the commercial sample. A chromatographic prerequisite of the gallium-67 solution was therefore developed in our laboratories, and corresponds to the tumour specific uptake of the radionuclide. The solution containing 58% uncomplexed gallium-67 and only 42% as citratogallate-67 concentrated uniquely in the Morris hepatoma-3924A (Fig. 3).

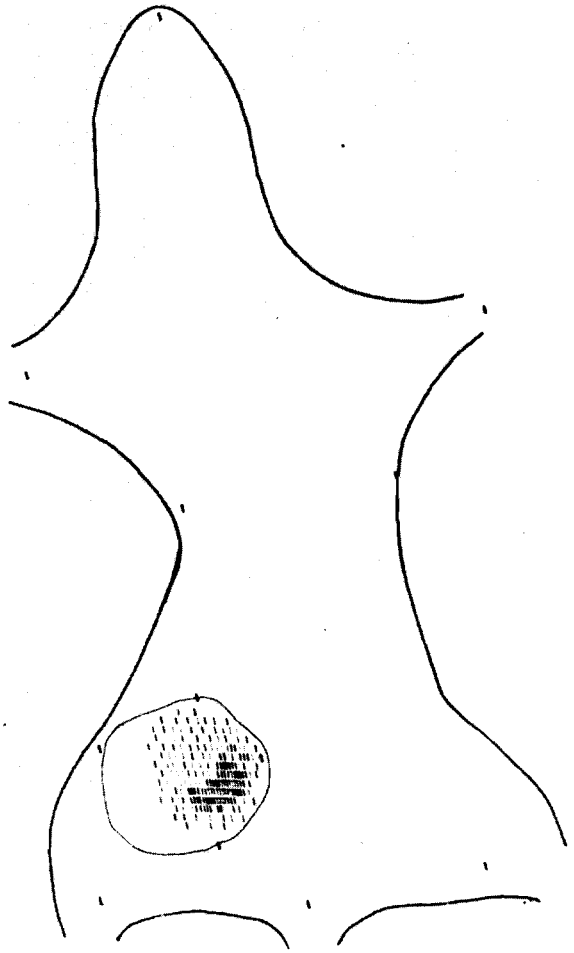

Fig. 3. Scintigram of a Morris hepatoma-3924A-bearing rat injected with our gallium-67 solution containing 58% unbound gallium-67. No hepatic uptake of the radionuclide.

This formulation of gallium-67 was found to be suitable for a wide variety of human tumours. Figure 4 shows the concentration of the radionuclide in lung tumour. No uptake of the radionuclide in the liver of the patient took place. Similar results are observed for Hodgkin's disease, neuroblastoma, bone tumour and liver tumours.

Since the unbound gallium-67 in physiological saline is easily hydrolyzed, the gallium-67 of our solution cannot be stored and should always be administered fresh. It is prepared at the time of its use from commercial gallium-67 solutions which are analysed chromatographically daily.

Antitumour Activity of Cold Gallium

The antitumour activity of cold gallium solutions containing different amounts of sodium citrate was examined in experimental mammary tumour (TGS) bearing mice. The gallium solutions were administered fresh and examined chromatographically for the gallium species present. Here again the solution containing 58% unbound gallium and 42% citratogallate proved to be most effective against tumour development. The average life of all animals given gallium intraperitoneally was prolonged. In 50% of cases the developed tumour was cured completely. Work is in progress to examine the antitumour activity of gallium in Morris hepatoma-3924A-bearing rats.

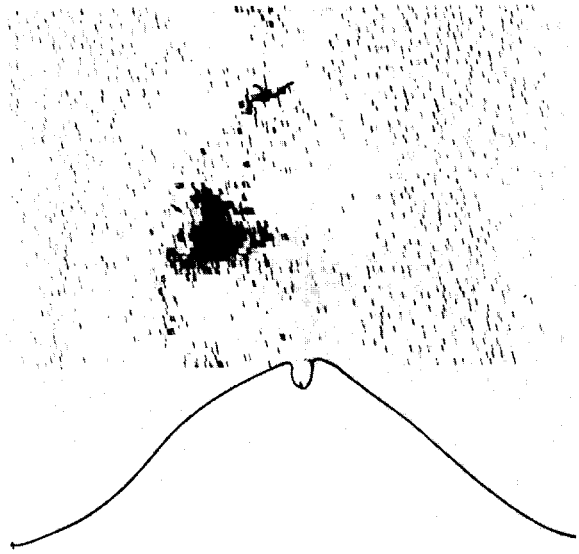

Fig. 4. Scintigram of a patient with lung tumour (arrow head) injected with our gallium-67 solution containing 58% unbound gallium-67. No hepatic uptake of the radionuclide.

After the animals die, the gallium in the tumour and different organs is quantified by flameless atomic absorption spectrometry. In animals given gallium in the optimal formulation the concentration of the element in the tumour is the highest, which explains its antitumour activity.

Conclusion

Commercial gallium-67 solutions for tumour imaging should always be examined chromatographically in order to bring the radionuclide to the same chemical form. Only unbound gallium has a high affinity for the tumour tissue. Since the unbound gallium is unstable in aqueous solution, the solutions for tumour imaging or tumour therapy should be administered fresh.

References

1 C. Levditi, J. Bardet, A. Tchakirian and A. Vaisman, *Compt. Rend. Acad. Sci. Paris, 192,* 1142 (1931).
2 C. L. Edwards and R. L. Hayes, *J. Nucl. Med., 10,* 103 (1969).

3 S. M. Moerlein, M. J. Welch and K. N. Raymond, *J. Nucl. Med., 23,* 501 (1982).
4 M. M. Hart and R. H. Adamson, *Proc. Nat. Acad. Sci., U.S.A., 68,* 1623 (1971).
5 M. M. Hart, C. F. Smith, S. T. Yancy and R. H. Adamson, *J. Nat. Cancer Inst., 47,* 1121 (1971).
6 S. K. Shukla, L. Castelli, I. Blotta and S. Caroli, 'Role of Anion in Tumour Diagnosis and Therapy with Gallium', Book of Abstracts, *Int. Conf. on Gallium and Related Elements: Basic Science and Clinical Applications,* Banff, Alberta, Canada, Sept. 12–14, 1980, pp. 22–23.
7 J. D. Perkinson, J. S. Eldridge and B. M. Cooper, *Radiology, 61,* 543 (1953).
8 R. L. Hayes and C. L. Edwards, In 'Medical Radioisotope Scintigraphy', IAEA, Vienna, 1973, pp. 531–552.
9 P. A. G. Hammersley and M. A. Zivanovic, *Nucl.-Med., 19,* 25 (1980).
10 S. K. Shukla, L. Castelli, C. Cipriani and G. B. Manni, *Radioakt. Isotope Klin. Forsch., 14* (I), 179 (1980).
11 S. K. Shukla, L. Castelli, I. Blotta, G. B. Manni and C. Cipriani, *Radioakt. Isotope Klin. Forsch., 15* (I), 303 (1982).
12 K. Samezima, K. Nakamura and H. Orii, *Int. J. Nucl. Med. Biol., 8,* 94 (1981).
13 S. K. Shukla, I. Blotta, R. Masella, C. Cipriani and G. B. Manni', Proc. Nat. Symp. on Ferritin', Ravenna, 1982, (in press).
14 L. Castelli, I. Blotta, S. K. Shukla, C. Cipriani and G. B. Manni, *Radiol. Med., 69,* 103 (1983).