A Comparative Study on Lysosomal Accumulation of Gallium-67 and Indium-111 In Morris Hepatoma 7316A

Shumpei Takeda, Tsuneo Uchida, and Taiju Matsuzawa

Research Institute for Tuberculosis, Leprosy and Cancer, Sendai, and Kobe University School of Medicine, Kobe, Japan

Intracellular localization of Ga-67 and In-111 was investigated in Morris hepatoma 7316A and in normal Buffalo rat liver cells by a cell fractionation method at 48 hr after an intraperitoneal injection of the nuclides. Lysosomal fractions of the tumor and normal liver cells had the highest relative specific radioactivities of the nuclides ($p < 0.001$). In a gradual solubilization experiment, the release of the nuclides at the same time as the acid phosphatase from the lysosomal fractions ($p < 0.001$) was thought to indicate that lysosomes are the site of accumulation for both nuclides whether in tumor or normal liver cells. Fragility of the tumor lysosomes might be inferred from the significantly greater regression coefficient in relation to the lysosomal fraction of tumor cells than that of normal liver cells when labeled with Ga-67 ($p < 0.001$). The poorer confidence limit for the regression coefficient in relation to the lysosomal fraction of tumor cells labeled with Ga-67 seemed to indicate that Ga-67 determines lysosomal functions of tumor cells more precisely than In-111.

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Tumor scintiscanning with several radionuclides plays an important role in clinical practice. This use of Ga-67 citrate was first reported by Edwards and Hayes (*1,2*) and of In-111 chloride by Hunter et al. (*3,4*). Although the mechanism and sites of localization of Ga-67 in malignant tumors have been extensively investigated, there have been few such studies of In-111. To compare the intracellular localization of these tracers in tumor cells, we fractionated Morris hepatoma 7316A cells by the method of De Duve et al. (*5,6*), believing it to be an appropriate method of preserving various subcellular organelles.

MATERIALS AND METHODS

Carrier-free Ga-67 citrate in water, pH 6.0–8.0, and carrier-free In-111 chloride in water, pH 1.0–3.0, were obtained commercially.

Preparation of tumor and normal liver. Sixteen Buffalo strain rats, weighing about 150 g, were divided into two groups. Morris hepatoma 7316A was transplanted intramuscularly into eight of them by introducing minced viable tumor tissue through a trocar. By about the 14th day of transplantation, the tumor usually reached about 2 cm in diameter. Rats bearing the tumor each received an intraperitoneal injection 0.2 mCi of Ga-67 or In-111, and the tumor was excised about 48 hr later. Tumors consisted chiefly of viable cells with a minimum of necrotic masses, and were used whole.

Normal livers were obtained from the eight disease-free Buffalo rats. The administration of the radionuclides was carried out in the same way in all the animals studied.

Subfractionation procedure (*5,6*). Tumor and normal liver were homogenized in 3 vol of 0.25 *M* sucrose in a Teflon homogenizer. The tumor homoge-

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For reprints contact: Shumpei Takeda, Dept. of Radiology and Nuclear Medicine, Research Inst. of Tuberculosis, Leprosy and Cancer, Tohoku University, 4-12 Hirosemachi, Sendai 980, Japan.

TABLE 1. SUBCELLULAR DISTRIBUTION OF Ga-67 AND ACID PHOSPHATASE IN MORRIS HEPATOMA 7316A CELLS

	N	HM	LM	M	C
Protein (%)*	10.2 (5.0 –13.8)	10.2 (7.2 –13.8)	8.2 (7.8 – 8.8)	16.5 (13.0 –19.7)	55.0 (47.7 –66.0)
Ga-67 (%)*	11.0 (8.1 –13.8)	19.8 (19.6 –20.1)	27.5 (26.4 –29.5)	9.3 (8.8 – 9.7)	32.5 (31.2 –33.5)
Relative specific radioactivity of Ga-67 (%/%)†	1.22 (0.90– 2.04)	2.06 (1.43– 2.79)	3.35 (3.31– 3.40)	0.57 (0.49– 0.68)	0.60 (0.51– 0.67)
Acid phosphatase (%)*	9.0 (6.7 –10.7)	15.8 (14.6 –17.6)	25.3 (24.6 –25.8)	16.6 (13.1 –18.0)	33.3 (31.7 –34.9)
Relative specific enzyme activity of acid phosphatase (%/%)†	1.02 (0.70– 1.78)	1.64 (1.28– 2.26)	3.09 (2.93– 3.24)	1.03 (0.80– 1.35)	0.62 (0.48– 0.70)

Figures in parentheses indicate range (n = 4).
* Results are expressed as percent of total contents of protein, Ga-67, and acid phosphatase in the filtered homogenate.
† Results are expressed as percent dose activity per percent protein content.

nate was filtered through gauze when labeled with Ga-67. The homogenates were centrifuged at 1,000 g for 10 min to sediment nuclei and cellular debris (N); at 3,300 g for 10 min to sediment a mitochondrial fraction (HM); at 10,000 g for 25 min to sediment a lysosomal fraction (LM); and at 105,000 g for 60 min to sediment a microsomal fraction (M) and to yield a soluble fraction (C) in the supernatant.

Gradual solubilization experiment. In order to disrupt the contained lysosomes gradually, the LM fraction was subjected to repeated freeze-thawing, or to incubation at 37°C, or to Triton X-100, and was then centrifuged at 105,000 g for 60 min. The release of activities of the nuclides and acid phosphatase into the supernatant was determined simultaneously.

Acid phosphatase. Activity was determined by the method of Igarashi et al. (*7,8*).

Protein content. This was determined by Lowry's procedure (*9*).

RESULTS

Uptake values. Of the Ga-67, 0.18 ± 0.00% dose/g wet weight (n = 4) was found in the tumor and 0.26 ± 0.06% dose/g (n = 4) in the normal liver. Of the In-111, 0.20 ± 0.01% dose/g (n = 4) was found in the tumor and 0.31 ± 0.02% dose/g (n = 4) in the normal liver.

Subcellular distribution of Ga-67, In-111, and acid phosphatase in tumor cells (Tables 1 and 2). Most of the total Ga-67 radioactivity was distributed in the C and LM fractions. Most of the In-111 was in the N, C, and LM fractions. All fractions other than the LM had a correspondingly high protein content. Thus the relative specific radioactivities of both nuclides, when defined as percent dose radioactivity per percent protein content, was highest in the LM fraction ($p < 0.001$). The distribution of both Ga-67 and In-111 was strikingly similar to that of acid phosphatase, which is a marker enzyme for lysosomes; this suggests that the tracers could have

TABLE 2. SUBCELLULAR-DISTRIBUTION OF In-111 AND ACID PHOSPHATASE IN MORRIS HEPATOMA 7316A CELLS

	N	HM	LM	M	C
Protein (%)*	44.9 (40.0 –56.1)	3.1 (1.9 – 5.7)	4.8 (3.7 – 5.5)	12.7 (8.5 –14.6)	34.5 (27.6 –38.2)
In-111 (%)*	30.5 (26.9 –33.0)	4.7 (3.6 – 5.3)	25.6 (24.1 –27.9)	5.6 (4.7 – 6.5)	33.7 (32.0 –37.2)
Relative specific radioactivity of In-111 (%/%)†	0.69 (0.54– 0.83)	1.74 (0.93– 2.47)	5.45 (4.77– 6.95)	0.46 (0.32– 0.65)	0.99 (0.87– 1.18)
Acid phosphatase (%)*	43.7 (40.3 –47.7)	5.5 (1.5 – 8.1)	16.8 (14.4 –19.0)	13.0 (6.0 –18.6)	21.1 (16.6 –26.8)
Relative specific enzyme activity of acid phosphatase (%/%)†	0.99 (0.82– 1.19)	1.85 (0.68– 2.67)	3.53 (3.13– 3.96)	1.14 (0.41– 2.19)	0.61 (0.53– 0.70)

Figures in parentheses indicate range (n = 4).
* Percent of total content in the homogenate.
† Percent dose activity per percent protein content.

TABLE 3. SUBCELLULAR DISTRIBUTION OF Ga-67 AND ACID PHOSPHATASE IN NORMAL LIVER CELLS

	N	HM	LM	M	C
Protein (%)*	20.7 (18.6 –22.9)	15.9 (13.2 –19.0)	7.0 (5.4 – 8.2)	13.4 (11.7 –14.2)	43.1 (38.1 –46.6)
Ga-67 (%)*	11.0 (10.5 –11.5)	31.9 (30.8 –34.1)	36.3 (34.6 –39.5)	3.6 (3.6 – 3.7)	9.8 (9.0 –10.7)
Relative specific radioactivity of Ga-67 (%/%)†	0.54 (0.48– 0.62)	2.10 (1.62– 2.46)	5.31 (4.26– 6.41)	0.27 (0.25– 0.31)	0.23 (0.21– 0.24)
Acid phosphatase (%)*	12.1 (11.7 –12.7)	21.8 (21.0 –23.2)	26.3 (25.0 –27.3)	10.5 (10.1 –10.7)	29.3 (29.0 –29.8)
Relative specific enzyme activity of acid phosphatase (%/%)†	0.59 (0.56– 0.64)	1.40 (1.11– 1.59)	3.84 (3.33–4.63)	0.79 (0.75– 0.86)	0.69 (0.64– 0.77)

Figures in parentheses indicate range (n = 4).
* Percent of total content in the homogenate.
† Percent activity per percent protein content.

had this same intracellular location.

Subcellular distribution of Ga-67, In-111, and acid phosphatase in normal liver cells (Tables 3 and 4). The LM fractions had the highest radioactivities but the lowest protein content. Thus the highest relative specific radioactivities of both nuclides were observed in this fraction ($p < 0.001$). The distributions of both Ga-67 and In-111 were similar to that of acid phosphatase.

Comparison of activities of Ga-67 and acid phosphatase released from the LM fractions. In the gradual solubilization experiment, the release of Ga-67 into the supernatant from the LM fractions of both tumor and normal liver cells paralleled that of acid phosphatase. In the case of the tumor cells, however, the slope of the line of regression (1.23, Fig. 1A) was significantly greater ($p < 0.001$) than the slope with the normal liver cells (0.82, Fig. 1B). This suggests a relatively greater fragility in the tumor lysosomes (*10*).

Comparison of activities of In-111 and acid phosphatase released from the LM fractions. In much the same way, In-111 and acid phosphatase were released in parallel fashion from the LM fractions of tumor and normal liver cells, except that in this case the slopes of the two regression lines (0.88 for tumor, Fig. 2A, and 0.75 for normal, Fig. 2B) were not significantly different ($0.4 < p < 0.5$).

DISCUSSION

There are various opinions about the mechanism of accumulation of Ga-67 in tumor tissues, implicating increased regional blood flow (*11*), increased local protein metabolism (*12*), abnormal tumor vasculature (*13*), altered alkaline-earth metabolism (*14,15*), and increased lysosomal function (*16*). On the other hand, few reports exist concerning the mechanism of the localization of In-111 in tumors (*12*).

We observed the highest activity of In-111 in the

TABLE 4. SUBCELLULAR DISTRIBUTION OF In-111 AND ACID PHOSPHATASE IN NORMAL LIVER CELLS

	N	HM	LM	M	C
Protein (%)*	32.2 (28.4 –40.6)	7.5 (5.5 – 9.3)	6.7 (2.9 – 8.9)	15.0 (11.4 –17.0)	38.6 (33.7 –42.5)
In-111 (%)*	13.8 (11.7 –17.2)	13.4 (11.2 –14.2)	41.5 (37.5 –47.2)	6.8 (5.5 – 7.3)	24.6 (22.8 –27.0)
Relative specific radioactivity of In-111 (%/%)†	0.43 (0.41– 0.47)	1.93 (1.23– 2.58)	7.85 (4.58–16.28)	0.45 (0.44– 0.48)	0.64 (0.54– 0.75)
Acid phosphatase (%)*	18.5 (13.5 –22.8)	10.3 (8.1 –12.9)	30.3 (27.7–32.5)	14.1 (11.2 –16.3)	27.2 (23.8 –30.2)
Relative specific enzyme activity of acid phosphatase (%/%)†	0.57 (0.48– 0.69)	1.51 (0.89– 2.35)	5.60 (3.11–11.21)	0.95 (0.84– 1.05)	0.71 (0.59– 0.82)

Figures in parentheses indicate range (n = 4).
* Percent of total content in the homogenate.
† Percent activity per percent protein content.

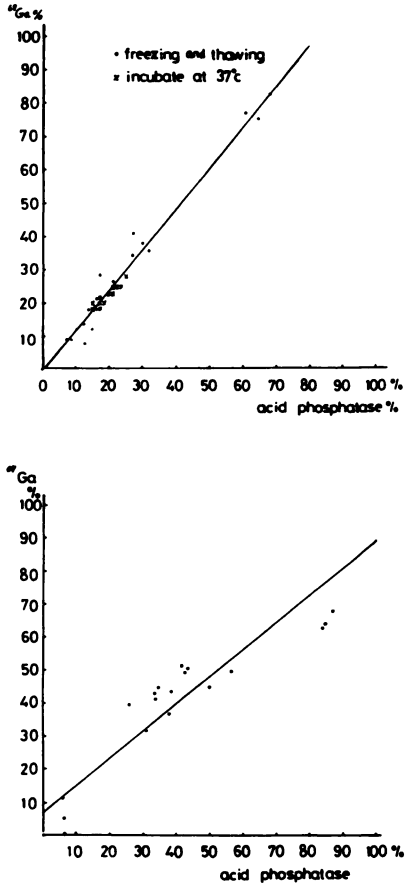

FIG. 1. Comparison of activities of Ga-67 and acid phosphatase released from LM fractions of tumor (top) and normal liver cells (bottom) into supernatant in gradual solubilization experiment. Correlation coefficient is 0.99, $p < 0.001$ in both cases.

N fraction of tumor cells, which contains nuclei as well as cellular debris and connective-tissue remnants that may trap some of the radioactivity. Few counts remained after removal of such remnants by filtration, as was seen in the N fraction of filtered homogenate of the tumor when labeled with Ga-67 (*17*).

Not only high activities of both Ga-67 and In-111, but also greater concentration of acid phosphatase were found in the C fraction of tumor cells (correlation coefficient: 0.81, $p < 0.01$ for Ga-67; 0.95, $p < 0.01$ for In-111. Therefore most of the counts in this fraction may have come from the lysosomes ruptured during the subfractionation procedure.

The LM fraction showed comparatively high activities of the tracers but almost the lowest protein content, whether in tumor or normal liver cells. Thus both Ga-67 and In-111 were specifically concentrated most highly in this fraction (*18,19*). The anatomic localization of the nuclides in lysosomes was ascertained by the release of their activities at the same time as acid phosphatase in the gradual solubilization experiment with the LM fraction. As we mentioned earlier, our evidence suggests greater than normal fragility of tumor lysosomes when labeled with Ga-67 ($p < 0.01$), but such a difference was not found when they were labeled with In-111. The size of confidence limit of the regression coefficient in relation to the LM fraction of tumor cells when labeled with Ga-67 ($1.15 < \beta < 1.31$, $p < 0.05$) was smaller than that when labeled with In-111 ($0.75 < \beta < 1.01$, $p < 0.05$). The close relationship between Ga-67 and acid phosphatase suggests that this nuclide is more completely engulfed into lysosomal vesicles than is In-111. Accordingly one would expect Ga-67 to be better as an indicator of lysosomal function of tumor cells than In-111.

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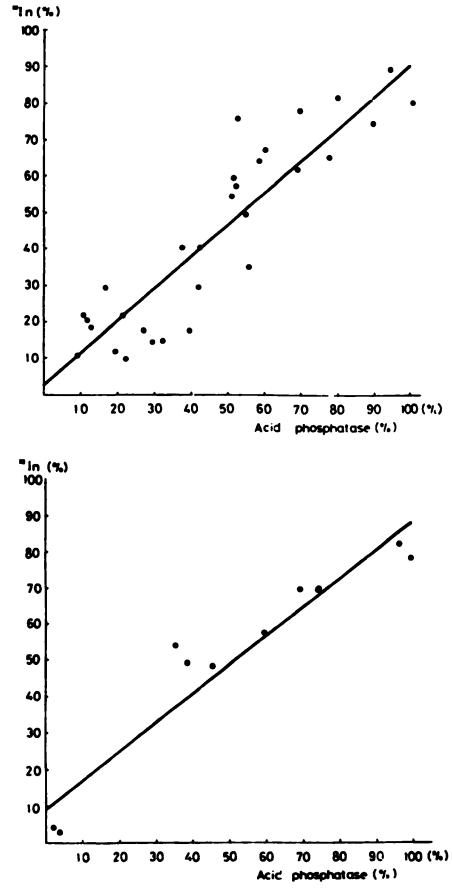

FIG. 2. Comparison of activities of In-111 and acid phosphatase released from LM fractions of tumor (top) and normal liver cells (bottom) into supernatant in the gradual solubilization experiment. Correlation coefficients are 0.93, $p < 0.001$ (top) and 0.95, $p < 0.001$ (bottom).

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