Diagnosis of Malignant Tumor with ^{67}Ga -Citrate (2nd Report)

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This paper is intended to report the results of whole-body autoradiography and microautoradiography of mice having Ehrlich's tumor, and also to report the summerized clinical data so far studied. The accumulation of ^{67}Ga -citrate in the tumor cells was elucidated both by chemical analysis in preliminary report and by autoradiogram in this report. The mechanism of accumulation is still unknown but there seems to be some correlation to the cell function. Clinical studies on about 50 cases of malignant tumors with ^{67}Ga -citrate have proved to be satisfactory. Especially, ^{67}Ga -citrate is valuable to establish the diagnosis of cancer of lung, breast and maxilla, however, it is rather difficult, at present, to obtain positive scintigram in the case of cancer of stomach, pancreas, liver and esophagus. There is a possibility that ^{67}Ga -citrate may be uptaken into the benign tumor or inflammatory lesion. According to our experience, however, all of the benign lesion consisting of 2 cases of fibroadenoma and 5 cases of cystic disease of the breast, except one case, showed negative scintigram.

We believe that clinical usage of ^{67}Ga -citrate is valuable to make a differential diagnosis of benign and malignant diseases.

Uptake into the inflammatory lesion seems not to be significant comparing with that into the malignancy. However, further investigations might be necessary, because uptake into the inflammatory lesion seems to be different depending upon the site and type of the lesion.

1. Introduction

In 1969, Edwards and Hayes unexpectedly noticed accumulation of ^{67}Ga -citrate in the neck lymph nodes of a patient with Hodgkin's disease while doing a bone scintiscan survey¹⁾.

For several years we have been applying the activation analysis technique to find trace elements of metals within cancer tissues²⁾, and subsequently ^{67}Ga -citrate was administered to animals as well as humans having malignant tumors. We reported the results of our preliminary studies last year on ^{67}Ga -citrate in connection with its accumulation within tumor tissues³⁾.

Recently, Hisada⁴⁾ reported that ^{67}Ga -citrate

also accumulated not only in malignant tumors but also in inflammatory lesions. We experienced a similar case and detail description of this case will be published later. This paper is intended to report the results of whole-body autoradiography and microautoradiography of mice having Ehrlich's tumor, and also to report the summerized clinical data so far studied.

2. Animal Experiment

Method:

2.1 Whole-body autoradiography

Whole-body autoradiogram was taken to investigate the distribution of ^{67}Ga -citrate in mice having Ehrlich's tumors. The mice were

sacrificed by immersing in acetone which had been cooled to about -70°C with solid carbon dioxide 24 hours after intraperitoneal administration of $100\ \mu\text{Ci}$ of ^{67}Ga -citrate.

The frozen mice were then transferred to a cryostat kept at -20°C . Sagittal sections ($40\ \mu$ thickness) of mice were prepared by microtome. The section were dried in the cryostat and pressed on Sakura X-ray film Type N for 2 days.

2.2 Microautoradiography

The mice were sacrificed 48 hours after intramuscular administration of $100\ \mu\text{Ci}$ of ^{67}Ga -citrate using anesthesia. A microautoradiography was taken on the tumor specimen by the dipping method.

Results:

Whole-body autoradiogram:

Figure 1 (a),(b) shows a whole-body autoradiogram of accumulation of ^{67}Ga -citrate in bone, tooth, salivary gland, thymus, liver, kidney, stomach mucosa, intestinal mucosa, and transplanted tumor in the thigh.

However, the radioactivity in the brain,

Fig. 1(a),(b) Whole-body autoradiograms showing the distribution of ^{67}Ga -citrate in mice having Ehrlich's tumor. Figure shows accumulation of ^{67}Ga -citrate in the bone, salivary gland, liver, spleen, kidney, stomach mucosa, intestine mucosa and tumor.

spinal cord, heart, lung, adrenal, skin and muscle was minimal.

Microautoradiogram:

Accumulations of ^{67}Ga -citrate were clearly identified in the cytoplasm as well as in the nucleus of the tumor cells as seen in Figure 2.

Fig. 2 Microautoradiogram showing ^{67}Ga localization in cells of an Ehrlich's tumor.

Note the localization of silver grains over the cytoplasm and the nucleus of the cancerous cells.

3. Clinical cases

Clinical survey using ^{67}Ga -citrate was performed to the benign and malignant tumors, and the inflammatory lesions of maxillary sinus, salivary gland, thyroid gland, lung, mammary gland, esophagus, stomach, colon, pancreas and liver. Results were summarized in Table 1,2 and 3.

At present, ^{67}Ga -citrate is useful to establish a diagnosis of cancers in maxillar sinus, lung and breast, however, there is a difficulty to obtain positive scintigram of the cancers of stomach, liver and intestine. As far as the benign tumors were concerned, although the number of analyzed cases were minimal, fibroadenoma and cystic disease of the breast, and mixed tumor of the palate demonstrated negative scintigram. There was a exceptional case of cystic disease of the breast which showed diffusely positive scintigram, however, this was considered to be due to the presence of inflammation. Therefore, we would like to consider that accumulation of ^{67}Ga -citrate in the benign tumor is less in comparison to that of in malignant tumor. As for inflamma-

Table 1 Case and result (lung tumor)

Case No.	Name	Sex	Site	Diagnosis	Dose	Scintigram
1	T. T.	♂	R-lung	Anaplastic carcinoma	2.0	(+)
2	T. T.	♂	L-lung	Squamous cell carcinoma	2.0	(+)
3	M. O.	♂	L-lung	Squamous cell carcinoma	2.0	(+)
4	M. Z.	♀	R-lung	Adenocarcinoma	2.0	(+)
5	Z. U.	♂	"	Oat cell carcinoma	2.0	(+)
6	H. M.	♂	"	Squamous cell carcinoma	2.0	(+)
7	M. I.	♀	"	Anaplastic carcinoma	2.0	(+)
8	T. I.	♂	"	Carcinoma cell(+)	2.0	(+)
9	Y. I.	♂	"	Unknown	2.0	(+)
10	G. O.	♂	"	Unknown	2.0	(+)
11	S. K.	♂	L-lung	Unknown	2.0	(+)
12	T. N.	♀	Lung Metastasis	Adenopapillary carcinoma	1.8	(±)
13	Y. S.	♂	L-lung	Unknown	1.8	(±) Radiation 7000 R
14	S. T.	♂	L-lung	"	1.8	(-) Radiation 7000 R

(inflammation)

Case No.	Name	Sex	Site	Diagnosis	Dose	Scintigram
1	K. Y.	♂	L-lung	Tuberculoma	1.5	(-)
2	T. Y.	♂	"	"	1.8	(-)
3	S. K.	♂	R-lung	Active tuberculosis	2.0	(±)
4	I. F.	♀	L-lung	"	1.5	(±)
5	K. U.	♀	R-lung	"	1.5	(±)
6	K. Y.	♂	"	"	1.8	(±)
7	K. A.	♂	Lung	"	1.8	(±)
8	H. S.	♂	R-lung	"	1.8	(+)
9	M. I.	♂	R-lung	Nonactive tuberculosis	1.8	(-)
10	S. N.	♂	L-lung	"	1.5	(-)
11	K. T.	♂	L-lung	Pneumonia	1.8	(-)
12	R. O.	♂	"	"	1.8	(±)
13	Y. T.	♂	L-lung	Pleuritis	1.5	(-)

tion, Hisada and his colleagues reported a case of pneumonia with strong positive scintigram⁴⁾. In our series of cases, the focus of active tuberculosis (being treated with chemotherapy), showed slightly positive scintigram, but old tuberculous lesion and tuberculoma showed negative scintigram. Pneumonia, in the course of treatment, showed slightly positive or negative scintigram. It can be considered that there is a difference of uptake of ^{67}Ga -citrate into the inflammatory lesion depending upon whether or not chemotherapy is applied.

Even if there is a uptake into the inflammatory lesion, it is considered that the turnover rate is quite different from that of malignancy. The typical cases were demonstrated in the following.

(1) Cancer of maxillary sinus.

Case 1 (Fig. 3) T.T. 62 y.o.

Squamous cell carcinoma

(2) Breast cancer

Case 2 (Fig. 4) S.K. 58 y.o.

adenocarcinoma

(3) Lung cancer

Case 3 (Fig. 5) M.Z. 32 y.o.

Table 2 Case and result (breast tumor)

Case No.	Name	Sex	Site	Diagnosis	Dose	Scintigram
1	S. S.	♀	L-breast	Adenocarcinoma	2.0	(+)
2	U. W.	♀	"	"	1.8	(+)
3	S. K.	♀	R-breast	"	2.0	(+)
4	K. S.	♀	"	"	1.8	(+)
5	H. M.	♀	"	"	2.0	(+)
6	Y. S.	♀	"	"	1.5	(+)
7	T. T.	♀	"	"	1.5	(+)
8	H. Y.	♀	L-breast	"	1.5	(-)
9	T. I.	♀	"	"	1.5	(-)
10	S. N.	♀	"	"	1.5	(-)
11	A. I.	♀	L-breast	Mastopathy	1.5	(-)
12	S. T.	♀	"	"	1.5	(-)
13	S. T.	♀	"	"	1.8	(-)
14	M. T.	♀	R-breast	"	1.8	(+)
15	H. I.	♀	"	"	1.8	(-)
16	E. Y.	♀	R-breast	Fibroadenoma	1.5	(-)
17	K. Y.	♀	L-breast	"	1.8	(-)

Table 3 Case and result (digestive organs and thyroid gland)

Case No.	Name	Sex	Site	Diagnosis	Dose	Scintigram
1	H. S.	♀	Stomach	Adenocarcinoma	1.5	?
2	M. K.	♀	"	"	1.5	(-)
3	S. K.	♀	Pancreas	"	2.0	(+)
4	K. K.	♀	"	"	2.0	?
5	K. S.	♀	Liver	Hepatoma	2.0	?
6	M. K.	♂	"	"	2.0	?
7	S. T.	♂	Oesophagus	Squamous cell carcinoma	2.0	(+) ?
8	T. Y.	♂	"	"	2.0	?
9	T. T.	♂	Thyroid gland	Anaplastic carcinoma	1.5	(+)
10	T. I.	♀	"	Simple goiter	1.5	(-)

(maxillary sinus, salivary gland)

Case No.	Name	Sex	Site	Diagnosis	Dose	Scintigram
1	T. T.	♀	R-sinus	Squamous cell carcinoma	1.5	(+)
2	S. T.	♂	"	"	1.5	(+)
3	K. G.	♂	L-sinus	"	1.5	(+)
4	M. I.	♂	R-sinus	Inflammation	1.5	(-)
5	S. K.	♂	L-sinus	"	1.5	(-)
6	K. K.	♂	Parotid gland	Micklicz's disease	1.5	(-)
7	K. T.	♂	L-submaxillary gland	Inflammation	1.5	(+)
8	M. H.	♀	"	"	1.5	(+)
9	A. N.	♂	"	"	1.5	(+)
10	F. A.	♀	Neck	Hodgkin's disease	1.5	(+)

Fig. 3 Case 1(T.T.) Carcinoma of right maxillary cavity (squamous cell carcinoma). A scintigram taken 48 hours after the administration of 1.5 mCi of ^{67}Ga -citrate revealed definite positive finding. The accumulation of the ^{67}Ga -citrate corresponded to the location of the metastatic cervical lymph nodes(indicated by arrow).

adenocarcinoma

Case 4 (Fig. 6 & 7) M.I. 58 y.o.

anaplastic carcinoma

(4) Pulmonary tuberculosis and pneumonia

Case 5 (Fig. 8) H.K. 63 y.o.

active tuberculosis

Case 6 (Fig. 9) R.O. 70 y.o.

bacterial pneumonia

4. Summary

On the basis of the results of biochemical experiments of preliminary report using tumor bearing animals, and of the morphological experiments by this time using autoradiography, ^{67}Ga -citrate definitely accumulates in the tumor cells. This was also observed by Hayes and his colleagues who reported their result recently⁵⁾. The mechanism of uptake is still unknown. At present, we would like to consider that instead of active uptake, ^{67}Ga -citrate was taken into intracellularly because of the abnormal permeability of cell membrane of the tumor cells, and combined with cytoplasmic protein and accumulated in the cells.

This may also explain the uptake of ^{67}Ga -citrate into the inflammatory lesion. All the cases of inflammatory lesion in our series of

Fig. 4 Case 2 (S.K.) Breast carcinoma (adenocarcinoma). A scintigram taken 48 hours after the administration of 1.5 mCi of ^{67}Ga -citrate revealed definite positive finding (indicated by arrow).

Operation at 3 days after administration of 2.0 mCi of ^{67}Ga -citrate

(1) normal tissue	cpm/g	ratio
a)	116	1.0
b)	120	
(2) cancer tissue		
a)	2124	
b)	2409	13.0
c)	1414	

Case 2 (S.K.): Three days later operation was performed.

Table demonstrates 13.0 times higher radioactivity in the tumor than in normal tissue.

case were treated with chemotherapy and uptake of ^{67}Ga -citrate was not significant, however, there is a possibility that ^{67}Ga -citrate may accumulate in the severe inflammatory lesion as equal as in malignancy. Further studies are necessary.

Distribution of ^{67}Ga -citrate in the body of mice bearing Ehrlich's tumor was studied by whole-body autoradiography. Result was coincidental to the preliminary report. It was revealed that ^{67}Ga -citrate accumulated in thymus, mucosa of stomach and intestine, but not in brain, muscle, skin and adrenal gland.

Experiments using mice bearing Ehrlich's tumor, revealed that ^{67}Ga -citrate accumulated more in the peripheral area than in the center of the tumor. This can be explained by the vascular architecture and blood flow. Similar observations were made in clinical cases. So that the uptake of the center of the tumor is

Fig. 5 Case 3 (M.Z.) Pulmonary carcinoma (adenocarcinoma). A scintigram taken 48 hours after the administration of 2.0 mCi of ^{67}Ga -citrate revealed a slightly positive finding (indicated by arrow).

Operation at 7 days after administration of 2.0 mCi of ^{67}Ga -citrate

(1) normal tissue	cpm/g	ratio
a)	520	1.0
b)	360	
(2) cancer tissue		
a)	911	2.0
b)	1604	3.6
c)	1471	3.3

Case 3 (M.Z.): Seven days later operation was performed. Resected specimen was about $2.0 \times 1.7 \text{ cm}^2$ in size. Table demonstrates 2.0~3.6 times higher radioactivity in the tumor than in the normal tissue.

Fig. 6 Case 4 (M.I.) Pulmonary carcinoma (anaplastic carcinoma). A scintigram taken 48 hours after the administration of 2.0 mCi of ^{67}Ga -citrate revealed a definite positive finding (indicated by arrow).

not as great as that of peripheral tumor shadow.

In clinical study, most of the lung cancer

Fig. 7 Case 4 (M.I.) Cross section of lung specimen removed at autopsy. As seen in Figure 6, there is a definite accumulation of ^{67}Ga -citrate in the area corresponding to the tumor (indicated by arrow).

Fig. 8 Case 5 (S.K.) Active tuberculosis. A scintigram taken 48 hours after the administration of 2.0 mCi of ^{67}Ga -citrate revealed a slightly positive finding.

Fig. 9 Case 6 (R.O.) Bacterial pneumonia. A scintigram taken 48 hours after the administration of 1.8 mCi of ^{67}Ga -citrate revealed a slightly positive finding.

showed a positive scintigram. More strong positive scans were obtained in a case of oat cell cancer and in two cases of undifferentiated carcinoma in comparison to the adenocarcinoma. Smallest cancerous lesion which was

detected by scintigram was $2.0\text{ cm} \times 1.7\text{ cm}$ nodular adenocarcinoma measured in resected specimen. ^{67}Ga -citrate accumulated in cancerous tissue approximately 3.6 times as great as in the normal surrounding tissue.

As the ability of scintiscanner is limited even with the latest model, however, in order to detect smaller lesions on the scans, it is necessary to make a device of direct measuring of radioactivity in the lesion or to use a superior radioisotopes in future.

To be interesting, two cases of pulmonary carcinoma treated with 7000 R of ^{60}Co radiation, showed a negative or slightly positive scintigram.

This was also observed in the cases of pulmonary and esophageal cancers treated with anticancer drugs.

These are noteworthy in connection with the studies of the mode of action of radiation and anticancer agents.

About 70% of the cases of breast cancer showed positive scintigram. Some of the tumors less than 2 cm in diameter showed negative scan. In the cases of breast cancer, it is important to establish a diagnosis, whether the tumor is benign or malignant, because the tumor can be palpated and measured. Small amount of ^{67}Ga -citrate seems to accumulate functionally in the normal breast, therefore, careful reading would be required for the scanning of the breast of the young female.

Cancer of maxillary sinus was demonstrated as definitely positive scan by the use of ^{67}Ga -citrate. The uptake of ^{67}Ga -citrate in the metastatic lymphnodes was 3.0-5.0 times greater than that of normal lymph nodes. It is also valuable for the detection of the metastatic lesion.

At present, however, it was rather difficult to obtain positive scintigram in the cases of cancer of the gastrointestinal tract, which we often encountered (e.g. cancer of stomach, intestine, colon, liver, esophagus and pancreas, of which diagnosis was usually difficult).

Positive scan was obtained only in one case of cancer of the pancreas.

In the resected specimen of the stomach cancer, the radioactivity of gallium was 2.0-

3.2 times greater comparing to the normal tissue. In other words, ^{67}Ga -citrate also accumulates in the cancerous lesion of the gastrointestinal tract. It is necessary to develop a device to measure the radioactivity of the cancerous lesion directly. We made a catheter type semiconductor detector and now used clinically. (clinical application will be reported in the subsequent paper).

Accumulation of ^{67}Ga -citrate in malignant tumor is definite, however, its mechanism is still unknown. It seems to correlate the cell function. Further studies and many speculations would be necessary until the days when the entity of cancer would be clarified.

5. Conclusion

Studies were made on the accumulation of ^{67}Ga -citrate in the tumors using experimental tumor bearing mice. The accumulation of ^{67}Ga -citrate in the tumor cells was elucidated both by chemical analysis in preliminary report and by autoradiogram in this report. The mechanism of accumulation is still unknown, but there seems to be some correlation to the cell function.

In clinical studies, ^{67}Ga -citrate was valuable for the diagnosis of cancers of lung, breast and maxillary sinus.

Uptake of ^{67}Ga -citrate is less in benign tumor and it may be possible to make a differential diagnosis from malignancy.

^{67}Ga -citrate also accumulates in inflammatory lesion in various degree depending upon the severity and stage of inflammation. Further studies are necessary.

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要 旨

^{67}Ga -Citrate による悪性腫瘍の診断 (第2報)

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さきに, わたくしたちは ^{67}Ga -citrate による悪性腫瘍の診断をこころみて, 第1報を報告した。今回は, 動物腫瘍を用いて, ^{67}Ga -citrate の腫瘍細胞内分布をマイクロオートラジオグラムにより調べた。また, 体内分布をマウスの全身オートラジオグラムにより調べた。

その結果, ^{67}Ga -citrate は, 腫瘍細胞内に取り込まれていた。体内分布として ^{67}Ga -citrate は正常な骨, 肝臓, 唾液腺, 胃, 腸粘膜にも多く摂取されていた。

臨床例として, 肺がん, 乳がん, 上顎がんの診断には有効であった。消化器がんには不適當であった。炎症にも症状により摂取されるが, 今後の検討が必要である。