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Differential Diagnosis of AH109A Tumor and Inflammation by Radioscintigraphy with L-[Methyl-^{11}C]methionine

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For the evaluation of tumor imaging with L-[methyl-^{11}C]methionine (^{11}C-Met), a basic study on the differentiation of tumor from inflammation with ^{11}C-Met and a comparison of the diagnostic value of the image with that obtained using ^{67}Ga citrate, a conventional scintigraphic agent, are important. ^{11}C-Met accumulations into inflammatory lesions, AH109A tumor and normal tissues of rats were examined by means of a tissue distribution study. Aseptic inflammatory lesions on the back of Donryu rats induced by croton oil and 1.5% carrageenan showed significantly lower accumulations of ^{11}C-Met than the AH109A tumor. Histologically, croton oil induced granulomatous inflammation and carrageenan, acute exudative inflammation. Whole-body autoradiography with ^{14}C-Met, a substitute for ^{11}C-Met, was negative in the carrageenan lesion and showed a slightly increased activity at the periphery of the croton oil lesion, in contrast with the high tumor activity. Whole-body autoradiography with ^{67}Ga citrate was performed to compare the imaging ability with that of ^{14}C-Met; it showed high activities in the tumor, bone, and intestine, and a broad increased activity at the periphery of the croton oil lesion, but was negative in the carrageenan lesion. ^{11}C-Met accumulations in the inflammations were very low and clinical application with positron emission tomography, should be useful for the differential diagnosis of tumor from inflammation.

Key words: L-[^{11}C]Methionine — ^{67}Ga citrate — AH109A tumor — Croton oil — Carrageenan

We have reported that L-[methyl-^{11}C]methionine (^{11}C-Met)[5] is a useful radiopharmaceutical among various labeled amino acids for the detection of cancer.[1] Tumor imaging with ^{11}C-Met using positron emission tomography has been reported in brain and lung tumors.[2,3] The extent to which ^{11}C-Met accumulated in a lung cancer was closely correlated to the tumor character, such as benign or malignant, viable or necrotic.[4] Also ^{11}C-Met accumulation is known to be correlated to the histopathology of lung cancer[5] and the grading of glioma.[6] For tumor detection with ^{11}C-Met, the differential diagnosis of tumor from inflammation is necessary, and a comparison of the diagnostic value with that of the conventional ^{67}Ga citrate image is required for the assessment of ^{11}C-Met imaging.

In this report, ^{11}C-Met accumulations in aseptic inflammatory lesions and rat AH109A tumor were examined by means of a tissue distribution study. Then, double-tracer whole-body autoradiography with ^{67}Ga citrate and L-[methyl-^{14}C]methionine (^{14}C-Met), a substitute for ^{11}C-Met, was performed in order to evaluate the imaging ability of these tracers for the differentiation of tumor from inflammation.

MATERIALS AND METHODS

Animals Thirty-eight male and 7 female Donryu rats weighing from 140 to 180 g were used for this study. A 0.1 ml suspension of 7×10^6 cells of ascitic hepatoma AH109A was sc inoculated on the back of the rats. The animals were used when the tumor had grown to about 1.5 cm in diameter. Aseptic inflammations were produced by sc injections of 0.1 ml of croton oil (Nakarai Kagaku, Kyoto) and 0.2 ml of 1.5% carrageenan (Tokyo Kasei Kogyo, Tokyo). They were used 4 days and 24 h after injections, respectively.

^{11}C-Met tissue distribution study ^{11}C-Met was synthesized as described previously.[4] The radiochemical purity was over 99%. The rats were fasted for 24 h and 100 μCi of ^{11}C-Met was injected via the tail vein. They were killed by cervical dislocation at 5, 10, 20 and 40 min after injection. Organs and tissue samples were excised, blotted to remove adhering blood, and weighed, and the radioactivity was counted with a well-type NaI(Tl) auto-

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[5] Abbreviations: ^{11}C-Met, L-[methyl-^{11}C]methionine; ^{14}C-Met, L-[methyl-^{14}C]methionine; DUR, differential uptake ratio.

The research described in this report, involving animals maintained in the animal care facility of our institute, was fully accredited by the laboratory animal care committee of Tohoku University.

gamma counter. Data were expressed as the differential uptake ratio (DUR).[7]

$$\text{DUR} = \frac{\text{tissue counts (cpm)/tissue weight (g)}}{\text{injected RI counts (cpm)/animal body weight (g)}}$$

The tumor/tissue ratios were calculated. Six male rats were used for 5 min after injection, 10 males for 10 min after, 10 males and 7 females for 20 min after, and 8 males for 40 min after. Four rats could not be used because of technical problems. Tissue samples of AH109A tumor, and croton oil and carrageenan inflammations were fixed, sectioned and stained with hematoxylin and eosin for histological examination.

^{67}Ga citrate tissue distribution study Eight rats with AH109A and inflammations were prepared as described for the ^{11}C-Met study. Ten μCi of ^{67}Ga citrate (Daiichi Radioisotope Lab., Tokyo) was iv injected and the rats were killed 24 h later and processed for tissue counting. Data were expressed as DUR.

Double-tracer whole-body autoradiography Whole-body autoradiography was performed using ^{14}C-Met (Amersham International plc, Buckinghamshire, UK) in place of ^{11}C-Met because the half life of ^{11}C, 20 min, is too short for the process of autoradiography. The labeling position of ^{14}C or ^{11}C is the same in the L-methionine. AH109A tumor cells, croton oil and carrageenan were separately injected into the back of a rat. One day after the iv injection of 100 μCi of ^{67}Ga citrate, 10 μCi of ^{14}C-Met was iv injected and 30 min later, the rat was killed by an overdose of chloroform anesthesia. The rat

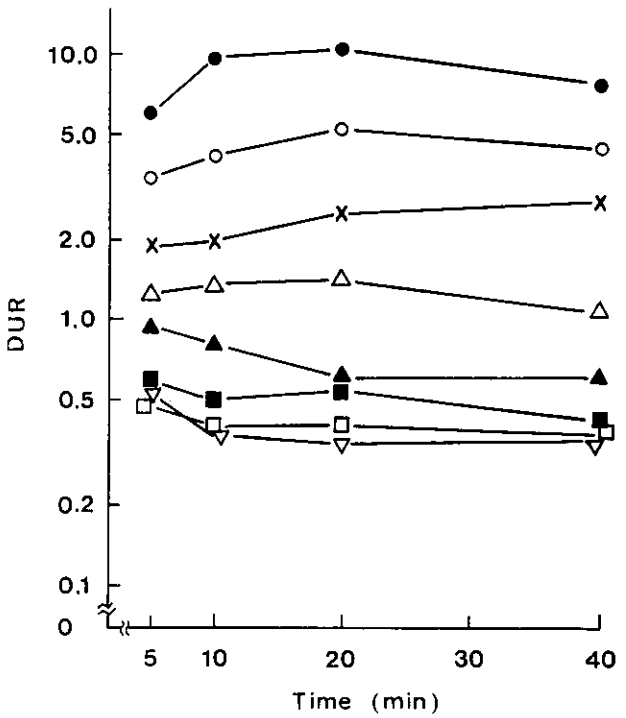

Fig. 1. Time activity curves of ^{11}C-Met tissue distribution in rats with AH109A tumor, and aseptic inflammations induced by croton oil and carrageenan. Mean DUR of 6 to 10 rats was plotted for each point. Pancreas (●), liver (○), tumor (×), lung (△), muscle (▲), carrageenan inflammation (■), croton oil inflammation (□) and blood (▽).

Table I. Tissue Distribution of ^{11}C-Met and ^{67}Ga Citrate in Donryu Rats

Tissue	^{11}C-Met (20 min after iv)		^{67}Ga citrate (24 h after iv)	
	DUR[a] (n=10)	Tumor/tissue ratio	DUR (n=8)	Tumor/tissue ratio
AH109A tumor	2.58±0.48	1.0	7.70±2.46	1.0
Croton oil inflammation	0.40±0.13	6.5	2.13±0.62	3.6
Carrageenan inflammation	0.51±0.06	5.1	1.34±0.23	5.7
Blood	0.34±0.05	7.6	0.35±0.16	22.0
Muscle	0.60±0.05	4.3	0.17±0.09	45.0
Lung	1.40±0.27	1.8	0.42±0.11	18.0
Myocardium	0.72±0.11	3.6	0.32±0.06	27.0
Liver	5.22±0.88	0.49	2.26±0.63	3.4
Pancreas	10.56±2.20	0.24	0.54±0.08	14.0
Kidney	2.11±0.21	1.2	1.54±0.63	5.0
Adrenal gland	2.13±0.25	1.2	—	—
Uterus	0.79±0.06	3.3	—	—
Overy	1.81±0.48	1.4	—	—

a) Mean±SD.

was embedded in 3% solution of carboxymethylcellulose sodium salt (Wako Pure Chemical, Osaka), frozen in acetone-dry ice, and sectioned with a cryotome (NA200, Nakagawa, Tokyo) at −20°C. The following process was carried out at −20°C. Whole-body sections 30 μm thick were taken up on adhesive tape (NA75, Nakagawa) and exposed for 12 h to the autoradiography film (MARG Tritium type, Konica, Tokyo) to obtain the ^{67}Ga citrate image, then stored for one month to allow the decay of ^{67}Ga (78 h half life ×10). The second exposure for ^{14}C took 10 days. The cross talk of ^{14}C into the ^{67}Ga autoradiographic image measured by using the standard exposure was less than 12%.

RESULTS

Figure 1 shows the time-activity curves of ^{11}C-Met tissue distribution. Both inflammations induced by croton oil and carrageenan showed lower uptakes than pancreas, liver, kidney, tumor and muscle during the experiment. Their curves showed gradual excretion patterns. The activities of pancreas and liver increased until 20 min after injection, then decreased. Tumor activity was increasing even at 40 min. Table I shows the DUR values of each tissue and the tumor/tissue ratios at 20 min for ^{11}C-Met and 24 h for ^{67}Ga citrate. The tumor/blood ratio was 7.6 and the tumor/muscle ratio, 4.3 with ^{11}C-Met. DURs of both inflammations were significantly lower than in muscle but higher than in blood. Adrenal gland and ovary showed more than double the activity of muscle. ^{67}Ga citrate showed very high tumor to muscle, blood and myocardium ratios. But the tumor to croton oil inflammation ratio was lower than that with ^{11}C-Met.

Figure 2-a, b, c shows photomicrographs of AH109A tumor, carrageenan inflammation and croton oil inflammation, respectively. The AH109A tumor contained many mitotic cells and its growth rate was very fast. It had abundant blood capillaries in the tumor matrix and a little necrotic tissue. Carrageenan inflammation (24 h after injection) showed edematous acute exudative inflammation characterized by migrating cells such as macrophages and neutrophils surrounding the abscess at the injection site. Croton oil inflammation (4 days after injection) showed granulation tissue of the healing stage of inflammation characterized by fibroblasts and lymphocytes surrounding the injection site.

Whole-body autoradiography with ^{14}C-Met (Fig. 3-a) showed high isotope accumulation in the tumor, pancreas and liver. Inflammation by carrageenan showed no specific accumulation, whereas the croton oil lesion showed a slightly increased accumulation in a narrow region of the periphery. The tumor and the carrageenan inflammation in the ^{67}Ga citrate image (Fig. 3-b) were similar to those in the ^{14}C-Met image, but the croton oil lesion showed an increased accumulation over a wide region at the periphery. Very high accumulation of ^{67}Ga citrate was observed in bone, such as vertebra, sternum, and skull, but the accumulation in the liver was lower.

Fig. 2. (a) Photomicrograph of AH109A tumor (×400), (b) carrageenan inflammation (×200), and (c) croton oil inflammation (×200).

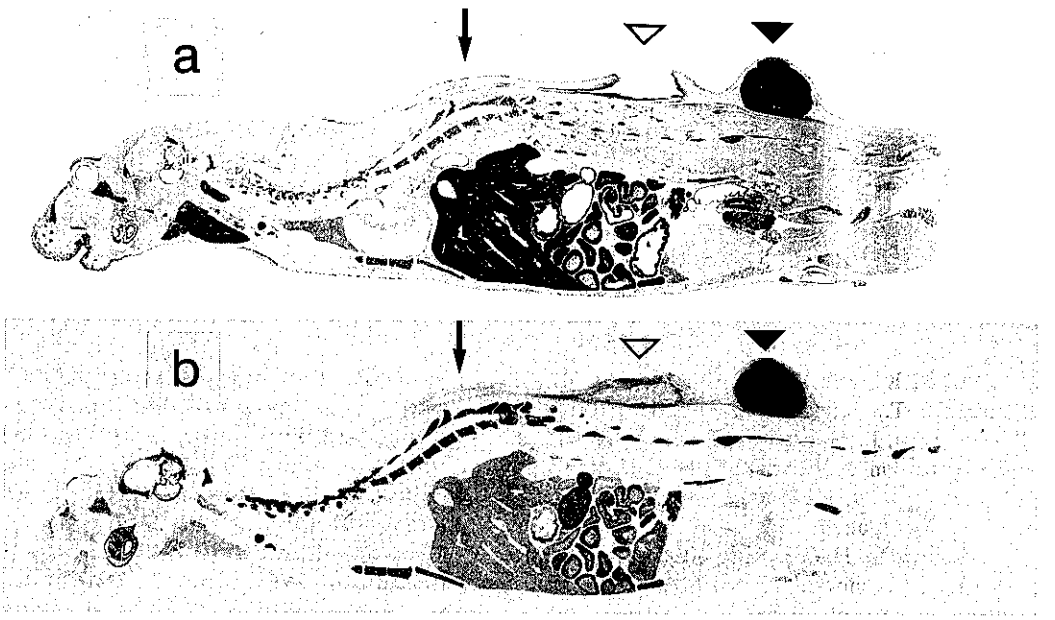

Fig. 3. Whole-body autoradiogram of a rat with AH109A tumor closed arrow, croton oil inflammation (open arrow) and carrageenan inflammation (arrowhead) on the back from caudal to cephalad. Double tracer images of (a) ^{14}C-Met exposure (as a substitute for ^{11}C-Met), and (b) ^{67}Ga-citrate exposure were compared.

DISCUSSION

In this tissue distribution study, the low levels of ^{11}C-Met accumulation in the two different types of inflammation, the acute exudative inflammation induced by carrageenan and the granulomatous one by croton oil, provided high contrast for the tumor. Malignant tumors are generally characterized by uncontrolled cell proliferation, which requires increased metabolic activity especially in relation to glucose and amino acids.[8] ^{11}C-Met uptake by the tumor is mediated by the increased amino acid transport and most of the ^{11}C-Met is incorporated into protein.[9, 10] The increasing tumor activity with time in our experiment is also explained by this mechanism. The pathophysiological correlation of tumor viability and ^{11}C-Met uptake has been demonstrated clinically.[4, 5]

There are two possible mechanisms for the increased activity at the periphery of the croton oil lesion observed in the whole-body autoradiography with ^{14}C-Met. The fibroblast proliferation which is a part of granulomatous inflammation may require amino acids. On the other hand, the increased blood flow with high vascular permeability may facilitate the increased concentration at the periphery. The latter mechanism is, however, would also be expected to operate in the carrageenan lesion, where no peripheral concentration was observed. The former mechanism may therefore operate. High physiological accumulation was also seen in the abdominal organs and submandibular gland. Tumor activity, however, can produce high contrast against lung, heart, brain, muscle and bone, consistent with the ^{11}C-Met tissue distribution study.

Whole-body autoradiography with ^{67}Ga citrate clearly showed some differences from that with ^{14}C-Met. ^{67}Ga citrate accumulated highly in the bone, tumor and intestine. The periphery of the croton oil lesion seen in the ^{67}Ga whole-body autoradiography was more intense than the liver, and wider than that in ^{14}C-Met whole-body autoradiography ^{67}Ga citrate has been used as a bone[11] and tumor scintigraphic agent.[12] However, ^{67}Ga has also been found to concentrate in aseptic and septic inflammatory areas, and repair tissue,[13] so that it does not have the desired specificity that had been anticipated.[14, 15] Also the mechanisms of its tumor accumulation are complex and the correlation to tumor pathophysiology is not yet clear.[16, 17]

From the viewpoint of the tumor vs. organ contrast, ^{67}Ga citrate may have some advantage over ^{14}C- or ^{11}C-Met especially for lesions in the abdominal area. But ^{11}C-Met imaging represents amino acid metabolism and it enables the diagnosis of tumor viability, in addition to the differential diagnosis from inflammation. A recent report showed successful differentiation of early lung cancer[18]

and a ^{11}C-Met tumor study with positron emission tomography seems to be a promising approach for pathophysiological imaging of tumors.

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