Relationship Between Histologic Type of Primary Lung Cancer and Carbon-11-L-Methionine Uptake with Positron Emission Tomography

Takehiko Fujiwara, Taiju Matsuzawa, Kazuo Kubota, Yoshinao Abe, Masatoshi Itoh, Hiroshi Fukuda, Jun Hatazawa, Seiro Yoshioka, Keiichiro Yamaguchi, Kengo Ito, Syoichi Watanuki, Toshihiro Takahasi, Kiichi Ishiwata, Ren Iwata, and Tatsuo Ido

Department of Radiology and Nuclear Medicine, The Research Institute for Tuberculosis and Cancer, Tohoku University; Division of Clinical Research, National Institute of Radiological Science; Department of Radiology, School of Medicine, Nagoya University; and Cyclotron and Radioisotope Center, Tohoku University, Japan

L-[Methyl-^{11}C]-methionine ([^{11}C]methionine) has proved to be one of the useful amino acids for the diagnosis of human cancer. We examined whether there was any correlation between [^{11}C]methionine uptake and histologic type of primary lung cancer. Sixteen patients with nine squamous cell carcinoma, five large cell carcinoma, one small cell carcinoma, and one adenocarcinoma were studied using positron emission tomography (PET). All patients had high accumulation of [^{11}C]methionine in lung tumors. We evaluated [^{11}C]methionine uptake into the tumor by a semiquantitative value, DUR (Differential Uptake Ratio). There was significant difference ($p < 0.01$, Mann-Whitney test) of [^{11}C]methionine uptake between large cell carcinoma (3.98 ± 0.27) and squamous cell carcinoma (2.92 ± 0.30). The result suggested that there was correlation between [^{11}C]methionine accumulation and the histologic type of lung cancer. This indicates a new potential applicability of [^{11}C]methionine PET for the study of lung cancer.

J Nucl Med 30:33–37, 1989

There are many modalities applied for cancer treatment such as radiotherapy, chemotherapy, hyperthermia, and immunotherapy. Most of the data for these therapies have been derived from in vitro and in vivo studies of animal models and from the careful observations of the patients. To increase our understanding of cancer and improve cancer treatment, we need more information on the nature of in vivo human cancer cells and their response to therapy. Positron emission tomography (PET) has become established as a technique to measure in vivo tissue function and to study both qualitative and quantitative differences between normal and cancer tissue. L-[Methyl-carbon-11]-methionine ([^{11}C]methionine) has proved to be one of the useful physiological amino acids for the diagnosis of human cancer. The increased methionine incorporation into tumor tissue was studied in human brain glioma (*1*) and lung cancer (*2,3*) using [^{11}C]methionine. Kubota (*3*) suggested the accumulation of [^{11}C]methionine into the lung tumor was closely correlated to the viability of tumor such as benign or malignant, viable or necrotic.

In this report, we focused on the correlation between [^{11}C]methionine uptake and histologic type of primary lung cancer.

MATERIALS AND METHODS

Radiopharmaceutical

Synthesis of [^{11}C]methionine was described previously (*3*). Quality assurance tests of [^{11}C]methionine for clinical use were performed according to the safety guidelines of the clinical research committee of our institution (*4*). The specific

Received Apr. 13, 1987; revision accepted Aug. 31, 1988.

For reprints contact: Takehiko Fujiwara, MD, Dept. of Radiology and Nuclear Medicine, The Research Institute for Tuberculosis and Cancer, 4-1 Seiryocho, Sendai 980, Japan.

activity of [^{11}C]methionine varied from 5 to 10 mCi/μmol at the time of injection and its radiochemical purity was over 99%.

Patients

Sixteen patients with lung cancer were studied using PET (EG&G Ortec ECAT II). The diagnosis of lung cancer was based on histopathological examinations with biopsy and/or confirmed by autopsy. Patients were classified according to the histologic type, nine squamous cell carcinoma, five large cell carcinoma, one small cell carcinoma, and one adenocarcinoma (Table 1). Nine of these patients had been given radiotherapy previously. Among the treated patients, Case 6, Case 7, and Case 10 were recurrent cases and radiotherapy had ended more than 3 mo before the PET study. In Case 13, the tumor showed a poor response to 60 Gy of irradiation and remained in the lung. In this case, PET study had performed 16 days later the last irradiation. The schedule of radiotherapy was not changed or interrupted for the PET study. The other seven patients had received no treatment before the PET study. Written informed consent was obtained from all patients.

PET Study

After transmission scanning, 4.6 to 15.6 mCi (mean 9.3 mCi) of [^{11}C]methionine in ~10 ml of physiological saline was injected intravenously as a bolus. Following the injection, serial tomographic scans were performed at the slice level through the tumor as determined by x-ray CT and transmission PET images.

Quantitation of [^{11}C]methionine uptake was performed using the differential uptake ratio (DUR) (*3*) which was obtained from mean counts per pixel data calibrated by the injection dose (mCi), body weight (kg), and PET-well calibration factor. The correction of [^{11}C]methionine uptake by blood volume was not performed.

$$\text{DUR} = \frac{\text{mean PET counts per pixel volume}}{\text{injected dose / body weight} \times \text{scan time} \times \text{calibration factor}}.$$

RESULTS

All 16 patients had high accumulation of [^{11}C]methionine in lung tumors from early scans and there were no other hot area in the same cross-sectional images. The region of interest (ROI) of the tumor on the PET image was easily determined. X-ray computed tomography (CT) and transmission scans were used as references.

Since some tumors showed inhomogenous [^{11}C]methionine accumulation, ROI was selected so that the mean count of ROI (3 × 3 pixels, 1.31 cm^2) data showed maximum value in the tumor. ROI data of back muscle was evaluated in the same manner. Thus, the data showed relatively high values than those of the previous report (*3*). Figure 1 shows the time activity curves for each tumor. In Case 7 and Case 8, the sequential emission scans were performed at other levels and the ROI data of tumor were obtained from the scans 40 min after injection. DUR curves of large cell carcinoma are higher than those of squamous cell carcinoma. Since DURs of tumor and muscle were almost constant during the scan period, the data of tumor and muscle of patients at 30 min after injection are shown in Table 1. In Case 7 and Case 8, the DUR value at 40 min after injection were used.

Although injected dose of [^{11}C]methionine per body weight varied from 0.08 to 0.30 mCi/kg, the DURs of the muscle of each patient remained constant with a 10% variation (0.81 ± 0.08, mean ± s.d.). Thus DUR was considered to be a reliable marker for [^{11}C]methionine accumulation in this study. The highest value of DUR was seen in a case of large cell carcinoma (Case 1), and the Case 16, with adenocarcinoma, showed the lowest uptake of [^{11}C]methionine. The average DUR values (Table 2) in squamous cell carcinoma and large

TABLE 1
Clinical Study of Primary Lung Cancer with [^{11}C]Methionine

Patient no.	Cell type	Radiation dose (time from the last therapy)	DUR at 30 min		Dose (mCi/kg)
			Tumor	Muscle	
1	Large cell	20Gy (5 days)	4.52	0.77	0.11
2	Large cell	6Gy (2 days)	3.90	0.86	0.20
3	Large cell	—	3.89	0.80	0.10
4	Large cell	—	3.81	0.71	0.08
5	Large cell	20Gy (7 days)	3.79	0.79	0.09
6	Squamous cell	Recurrence (48Gy, 6 mo)	3.35	0.75	0.19
7	Squamous cell	Recurrence (67Gy, 3 mo)	3.32	0.84	0.13
8	Squamous cell	30Gy (22 days)	3.18	0.80	0.30
9	Squamous cell	—	3.03	0.84	0.16
10	Squamous cell	Recurrence (30Gy, 3 mo)	2.85	0.96	0.28
11	Squamous cell	—	2.77	0.85	0.13
12	Squamous cell	4Gy (3 days)	2.73	0.72	0.15
13	Squamous cell	60Gy (16 days) Noneffective	2.64	0.96	0.17
14	Squamous cell	—	2.40	0.78	0.25
15	Small cell	—	2.00	0.75	0.23
16	Adeno	—	1.63	0.70	0.17

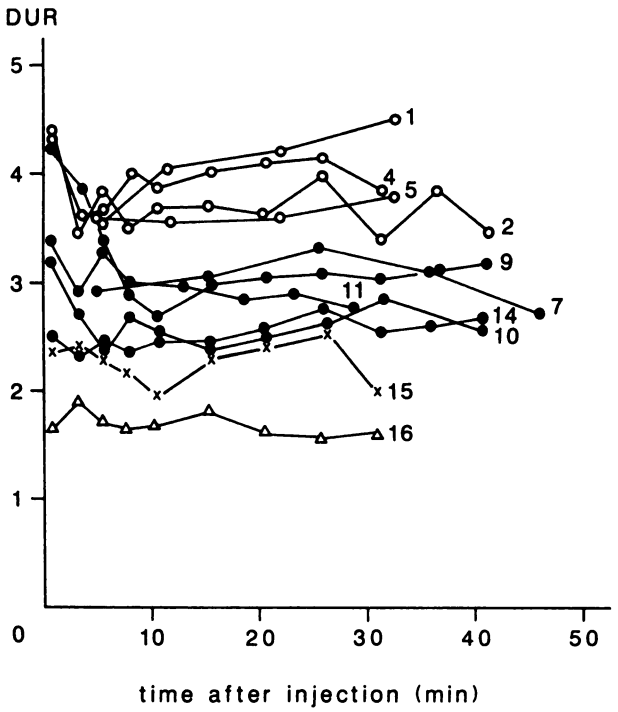

FIGURE 1
Time-activity curves of primary lung cancers using [^{11}C] methionine. (○) Large cell carcinoma; (●) Squamous cell carcinoma; (×) Small cell carcinoma; (△) Adenocarcinoma. In Case 6 and Case 7, the sequential emission scans of tumor were not performed. Case 3, Case 6, Case 8, Case 12, and Case 13 were omitted because they were too crowded. The definition of DUR was shown in the text.

cell carcinoma were 2.92 ± 0.30 and 3.98 ± 0.27, respectively. There was a significant difference of DURs between large cell carcinoma and squamous cell carcinoma ($p < 0.01$, Mann-Whitney test). The DUR of small cell carcinoma was 2.00 and that of adenocarcinoma was 1.63.

DISCUSSION

Our results confirmed the previous observation (*3*) of increased uptake of [^{11}C]methionine in lung cancer. The method of the quantitation of [^{11}C]methionine uptake into the tissue was reported in the study of local brain protein metabolism using compartment model analysis (*5*). In our study, ROI data of tumor had not enough accuracy for curve fitting of compartment analysis. The reason may be an artifact from respiratory movement of the tumor in the lung during the scan period. So we evaluated [^{11}C]methionine uptake into the tumor by a semiquantitative value, DUR. It was reported that this technique was valid for tumor study (*3,6*).

The time-activity curve of each patient with DUR showed that [^{11}C]methionine distribution in the tumor

TABLE 2
Relationship Between [^{11}C]Methionine Uptake (DUR) and Histologic Type of Primary Lung Cancer

Histologic type	DUR (mean ± s.d.)
Large cell carcinoma	3.98 ± 0.27 (five cases)*
Squamous cell carcinoma	2.92 ± 0.30 (nine cases)*
Small cell carcinoma	2.00 (1 case)
Adenocarcinoma	1.63 (1 case)

*$p < 0.01$ (Mann-Whitney test).

had no essential changes from 10 to 40 min. Therefore, the DUR at 30 min seemed to be reasonable assessment for the uptake of [^{11}C]methionine in the tumor, when the blood activity was sufficiently low (*3*) that scan data of tumor were not likely to be obscured.

The result suggested that there was correlation between [^{11}C]methionine accumulation and the histologic type of lung cancer and that [^{11}C]methionine uptake of large cell carcinoma was higher than that of squamous cell carcinoma. Methionine is one of the essential amino acids. The high accumulation of [^{11}C]methionine in the tumor seemed to be derived from the increased demand of amino acids for protein synthesis.

The difference of DURs among tumors was thought to be brought from the different growth rate of tumor. The concept of tumor growth measurement of lung cancer by studying serial x-ray films is well established (*7,8*). The first systematic radiologic measurements of the growth rate of human lung cancers by Collins et al. (*9*) was followed by numerous studies (*10–14*) confirming that volume doubling time is related to histologic type of the tumor. The four major histologic types of lung cancers were ranked in order by their increasing doubling times: small cell carcinoma, large cell carcinoma, squamous cell carcinoma, adenocarcinoma (*13, 14*). The significant difference of [^{11}C]methionine uptake between large cell carcinoma and squamous cell carcinoma in this study corresponded to the reported growth rate of each cell type of lung cancer. However, further studies are necessary. We have little information on the growth rate of lung cancer of the patients in this group. Their lung tumors were found in the advanced stage and few x-ray films were available to calculate the doubling times.

It has been reported that gallium-67 (^{67}Ga) citrate uptake by lung cancers differs somewhat according to the histologic type. Higashi et al. (*15*) reported that the [^{67}Ga]citrate uptake of small cell carcinoma was greater than that of adenocarcinoma. Thesingh et al. (*16*), using autoradiographic techniques, studied the relationship between the accumulation of [^{67}Ga]citrate in the tumor and the histologic type of lung cancer. They found that the highest gamma counts for tissue samples occurred in the groups of undifferentiated and squamous cell

carcinomas. Adenocarcinomas have lower grain counts in autoradiograms and lower gamma counts in tissue samples. Although many factors are proposed to influence the ^{67}Ga uptake, the exact mechanism of the uptake of ^{67}Ga by cancer cells is still obscure, while [^{11}C]methionine uptake reflects protein synthesis in the cancer cells (*17*).

In this study, PET of some cases were performed during or after anti-cancer treatment. Irradiation up to 30 Gy produced no change of uptake of [^{11}C]methionine in squamous cell carcinoma and large cell carcinoma. Since the radiation dose in this study were not enough to kill all of tumor cells, the tumor cells were thought to be still alive and able to take up [^{11}C] methionine in the treated cases. The recurrent tumors of squamous cell carcinoma showed no different uptake of [^{11}C]methionine from nontreated tumors. Therefore the difference in [^{11}C]methionine uptake among tumors seemed to be due to the different metabolism of tumor cells according to their histological differences. In Case 13, in spite of 60 Gy of irradiation the tumor had remained and showed high uptake [^{11}C]methionine. The patient became symptomatic and died 8 mo later. The recurrent growth of lung tumor was proved by autopsy.

Blood flow is one of the factors that modifies [^{11}C] methionine uptake of tumor cells. Knapp et al. (*18*) reported that nitrogen-13 (^{13}N) glutamate uptake by malignant tumors in the extremities was related to blood flow comparing [^{13}N]glutamate uptake with the initial distribution of thallium-201. In our preliminary study using experimental rat tumor (AH109A) with double labeled autoradiography of L-[methyl-^{14}C]methionine and 4-^{18}F-fluoroantipyrine, methionine distribution in tumor was related to blood flow distribution. It has been reported that radiotherapy will change tumor blood flow (*19*). However we did not find any changes of uptakes of [^{11}C]methionine between irradiated and nonirradiated group. It seems that tumor uptake of methionine is not only governed by the blood flow but may be affected by the amino acid transport and metabolism.

Di Chiro et al. (*20*) reported the correlation between rate of glycolysis and tumor grade of human cerebral glioma by [^{18}F]-2-fluoro-2-deoxyglucose and PET. Schober et al. (*21*) reported that comparing the malignancy of brain tumors with the tumor-to-nontumor ratio, the [^{11}C]methionine uptake increases as the gliomas increase in grade. In the present study, we reported the possible correlation between [^{11}C]methionine accumulation and the histologic type of lung cancer. Lung cancer is one of the major causes of death among malignant tumors. The results of this study suggest a new potential applicability of [^{11}C]methionine PET for the diagnosis of lung cancer and for the evaluation of metabolic activity of tumor cells.

ACKNOWLEDGMENT

This work was supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan.

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