Quantitative Evaluation of L-[methyl-C-11]Methionine Uptake in Tumor Using Positron Emission Tomography

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Introduction

Positron emission tomography and L-[methyl-^{11}C]methionine (^{11}C-Met) as a tracer has been used to measure in vivo amino acid metabolism in human brain[1-3], brain tumor [11] and lung cancer.[8-10]

Although an accurate measurement of plasma radioactivities of introduced tracer is essential for the quantitative evaluation using any compartment analysis, there were no reports including such measurements in individual cases. We previously measured the metabolic products of ^{11}C-Met in human plasma during PET-methionine study and found remarkable individual difference in clearance pattern of plasma ^{11}C-Met.[11] Here, we evaluated the uptake rate and distribution volume of ^{11}C-Met in brain tumor and lung cancer using the graphical analysis[12] after the correction for plasma metabolites of ^{11}C-Met in individual cases. Importance of the plasma metabolites analysis for the quantitative assessment is demonstrated. The effect of ^{11}C labeled metabolites in plasma on the evaluation of tumor uptake of ^{11}C-Met is discussed.

Materials and Methods

Patients

Six patients with brain tumor and three patients with lung cancer were studied with positron emission tomography and ^{11}C-Met. Clinical informations are summarized in Table 1. All the patients with brain tumor had partial resection of tumor or stereotaxic biopsy. One patient with lung cancer (ID 590) was studied repeatedly during radio-and

chemotherapy. Each patient was fasted prior to the study. Informed consent was obtained from the patients and relatives. The present profect was approved by the Committee for clinical PET study of Tohoku University.

Scanner and Procedure

The ECAT II (EG&G, Ortec)[13] and PT-931 (CTI, Knoxville, Tennessee)[14] were employed. The special resolution of the image was 15 mm and 8 mm for the ECAT II and PT-931, respectively, and slice thickness was 18 and 9 mm in FWHM, respectively. Three to four images were obtained with the ECAT II at 1 cm center to center spacing and 14 images were obtained with PT-931 at 8 mm spacing. The sequential scan was performed in one of these slices where the tumor was most visible in X-ray CT or MRI.

Before scanning, a short 21-gauge cannule was inserted to a brachial or radial artery for arterial blood sampling. Fourteen to 24 mCi of ^{11}C-Met were administrated within 30 seconds through contralateral hand vein. Repeated scanning started just after administration. Eight to ten sequential images with 5 minutes date acquisition were obtained. During the scanning, 1 ml of arterial blood samples was taken at 0.33, 0.67, 1, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 7.5, 10, 15, 20, 40, 50, 60 min after the i.v. administration and at the end of scanning. These blood samples were centrifuged for 3 min and plasma samples were weighed and counted for radio- activities using cross-calibrated well counter with ECAT II and PT-931. At 5, 15, 30 and 60 min, additional 3 ml of arterial blood were obtained for the analysis of protein-bound ^{11}C and ^{11}C-Met in plasma using HPLC.

Analysis of metabolites of ^{11}C-Met in plasma

Protein-bound and protein-free metabolites of ^{11}C-Met in plasma were analyzed with the same conditions as described previously.[11] Briefly, after counting total radioactivity, plasma samples taken at 3, 4, 5, 7.5, 10, 15, 20, 30, 40, 50 and 60 min after i.v. injection were treated with 5 ml of ice-cold 0.2 M $HClO_4$ to precipitate plasma proteins. The samples were centrifuged for 3 min. The precipitate was resuspended in 5 ml of 0.2 M $HClO_4$, and centrifuged again. This procedure for washing was repeated twice. The final precipitate was counted for radioactivity and corrected for decay as protein-bound for radioactivity and corrected for decay as protein-bound fraction.

For the samples taken at 5, 15, 30 and 60 min, supernatants obtained with the procedure described above were combined as protein-free fraction. Radioactivity of this fraction was then measured. The supernatant was applied on an Aminex A-6 column. The column was eluted with 0.2 N sodium citrate. The column was reequilibrated and elution profile was measured with radioactivity monitor (Romona-D equipped with an IM-

2020X flow cell, Raytest). The collected effluent in 1.0 mL fraction was then counted for radioactivity with well counter.

Uptake rate and distribution volume of ^{11}C-Met

We obtained three different input functions of ^{11}C-radioactivity for one study, total ^{11}C radioactivity, protein-free ^{11}C (total minus protein-bound fraction) and radioactivity for ^{11}C Met. For total ^{11}C in plasma, integrated radioactivity from injection time to each mid time of sequential scanning was calculated using a BLD computer program.[15] For protein-free ^{11}C and ^{11}C-Met, plasma radioactivities against sampling time were fitted best to double exponential curves using a Dampnig Gaus Newton method.

As only a few percent of protein-free metabolites and undetectable amount of protein-bound metabolites were found in the samples taken at 5 min after administration, total radioactivity before 5 min were incorporated as protein-free fraction and ^{11}C-Met fraction for curve fitting. The integrated radioactivity from injection time to mid time of scanning and the radioactivity at the mid time were obtained using this fitted curves.

The PET images were reconstructed using a measured attenuation correction, and radioactivity concentration in each pixel was converted to nCi/ml unit. Ovale regions of interest were located on brain tumor, brain matter in the contralateral hemisphere in the sequential images. For lung tumor, region of interest was put on the tumor mass which was visible in the transmission image.

Plasma and tissue time activity curves were treated with graphical analysis which provides evaluation of unidirectional transfer process.[12] The operational equation is

$$Ci(t) / Cp(t) = Ki \int Cp(t)\,dt / Dp(t) + Vp$$

where Ci (t) is the total amount of ^{11}C radioactivity at time t in the tissue, Ki is the rate constant for tracer transfer from blood to tissue of interest, Cp is the tracer concentration in blood at time t, and Vp is the distribution volume of tracer. When a linear portion of the curve was identified, the slope (Ki) and the intercept (Vp) were calculated.

Result

Mean radioactivity ratio of plasma ^{11}C-Met to total 11C was 0.67 (SD=0.19) at 30 min and 0.36 (SD=0.17) at 60 min after administration. There was marked individual difference in the clearance rate of plasma ^{11}C-Met.

Tissue distribution of the tracer injected in patient with brain tumor (ID 619) and lung cancer (ID 552) was demonstrated in Figure 1. Besides the initial distribution of ^{11}C-Met in large vessels, accumulations to tumor tissue were visualized in both cases.

Figure 2 illustrates graphical analysis of brain tumor and lung cancer for total ^{11}C and ^{11}C-Met in plasma as an input function. The slopes for total ^{11}C and ^{11}C-met were visually identical in four patients (ID 552, 606, 333 and 758). In two studies (ID 590-1, 590-2), it was difficult to find a linear part in the plots without the metabolites correction. In four cases (ID 600, 619, 629 and 714), there was a discrepancy of the slopes obtained with and without the correction. Table 2 shows uptake rates and distribution volumes for ^{11}C-Met in plasma as an input. In one case with lung cancer (ID 590), the values were calculated only after the metabolites correction.

Mean uptake rate and distribution volume of ^{11}C-Met in the cerebral cortex was 0.022 (SD=0.08) and 0.36 (SD=0.09), respectively.

Discussion

As realized by Lundqvist et al.[16] and Ishiwata et al.[11], a fraction of ^{11}C radio activity was detected in protein fraction and in protein-free fraction (methionine, serine and unknown origin). Therefore, when total amount of ^{11}C radioactivity in plasma is employed as an input function, serious error to measure physiological parameters might be anticipated.

Bergstrom et al.[6] estimated methionine accumulation in glioma and normal brain tissue using graphical analysis. In their study, plasma radioactivity was corrected mathematically for labeled plasma proteins by subtracting a fraction according to Lundqvist et al.. However, as reported here, there was large variation in clearance pattern of ^{11}C-Met among individuals. Another group[7] evaluated the slope and intercept of the initial straight line of the plot after administrating ^{11}C-Met to avoid the presence of plasma metabolites in the late phase of the study. However, irreversible fraction of total radioactivities in the tumor or ^{11}C-Met incorporated into protein might increase during the scanning. Therefore, more reliable values for incorporated ^{11}C-Met could be obtained by measuring tissue radioactivities as long as possible.

In the present study, a fraction of ^{11}C-Met in plasma was directly determined using liquid chromatography in individual cases. Since the time for metabolites analysis is limited because of short half life of ^{11}C, only four plasma samples taken at 5, 15, 30 and 60 min were analyzed. This would induce some inaccuracy of curve fitting. We assumed that until 5 min all the radioactivity in plasma came from ^{11}C-Met because protein-bound ^{11}C was not detectable at 5 min and 97.3 % of radioactivity was detected as ^{11}C-Met. These data were incorporated for curve fitting to increase the accuracy.

Small fraction of serin was detected in the HPLC analysis. Because Brain Uptake Index of serin is almost one forth of that of methionine[18], this fraction was not considered as an input.

The effect of corrections for ^{11}C metabolites in plasma on the graphical analysis was remarkable. In one patient with lung cancer (ID 590), uptake rate in tumor decreased from 0.025 to 0.016 after the chemo- and radio therapy indicating the therapy was effective. In the two studies of this patient, no straight portions of the plotting were identified without the correction indicating no active incorporation of methionine.

As expected, in patients with rapid clearance of ^{11}C-met in plasma, differences in uptake rate with and without the correction were large. Probably, nutritional conditions and liver function might have some influence to individual differences in clearance pattern of ^{11}C-Met in plasma. It is difficult to predict the clearance rates without the measurement of ^{11}C metabolites.

When protein-free ^{11}C radioactivity was employed as an input function, the magnitude of error was much smaller than that obtained for total ^{11}C. However, it still included 5 % difference as a mean ranged from 0.3 to 12%. This might suggest that protein-free ^{11}C, which was much easier to measure than ^{11}C-Met with HPLC, could not substitute ^{11}C-Met as an input when the accurate measurements are requested.

We previously reported that accumulation of ^{11}C-Met methionine was found in lung cancer.[8] ^{11}C radioactivity in the tumor tissue corrected for administrated dose and body weight was considered to be an indicator of tumor viability. Total radioactivities in the tumor were obtained by only PET measurements at some appropriate time after administration. No information was included regarding the fraction of incorporated ^{11}C-Met. Tumor viability should be evaluated by irreversible fraction of ^{11}C-Met incorporated into amino acid. Especially, responses of tumors to chemo- and radiotherapy should be assessed quantitatively by comparing changes before and after the therapy.

In conclusion, because of considerable variation in clearance patterns of 11C-Met administrated, the corrections for plasma metabolites should be performed to obtain 11C-Met plasma radioactivity as an input when quantitative informations of methionine metabolism is extracted in the PET study.

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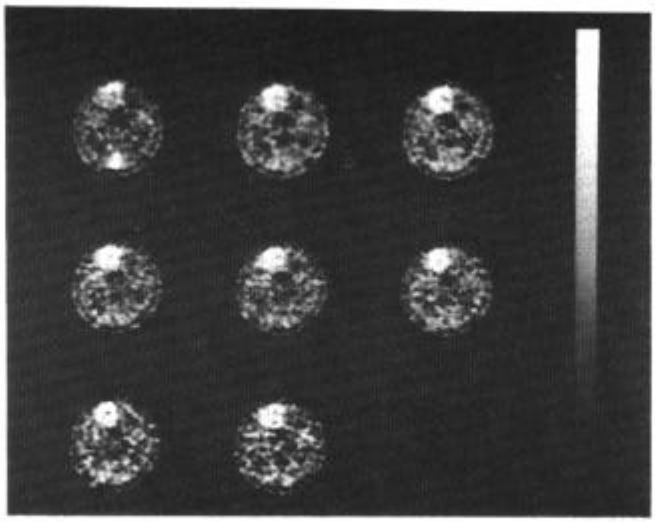
Table 1 Patients data

ID	age & sex		disease	diagnosis	therapy
552	72	M	lung cancer	squamous cell carcinoma	radio-chemotherapy
590	39	M	lung cancer	adenocarcinoma	radiotherapy (42 Gy)
590	39	M	lung cancer	adenocarcinoma	radiotherapy (69 Gy) chemotherapy
600	49	M	brain tumor	astrocytoma grade II	radiochemotherapy
606	61	F	lung cancer	oat cell carcinoma	none
619	28	M	brain tumor	neuroblastoma	none
629	46	F	brain tumor	astrocytoma	radiotherapy grade II
714	16	F	brain tumor	gliocytoma	radiotherapy (50 Gy)
333	40	M	brain tumor	astrocytoma grade II	radio and chemotherapy
758	63	M	brain tumor	glioblastoma	none

Table 2. Uptake rate and distribution volume of ^{11}C-Met in tumors calculated with and without correction for metabolites in plasma.

ID	Uptake rate		Distribution volume	
	not corrected	corrected	not corrected	corrected
552	0.0524	0.0560	0.6678	0.6903
590	-	0.0248	-	0.8063
590	-	0.0169	-	0.3448
600	0.0150	0.0188	0.5593	0.6619
606	0.0576	0.0583	0.4796	0.4759
619	0.0260	0.0283	0.3543	0.3412
629	0.0213	0.0282	0.5490	0.4755
714	0.0238	0.0294	0.6910	0.4964
333	0.0157	0.0174	0.2943	0.2207
758	0.0400	0.0424	0.6035	0.4737

(a)



(b)

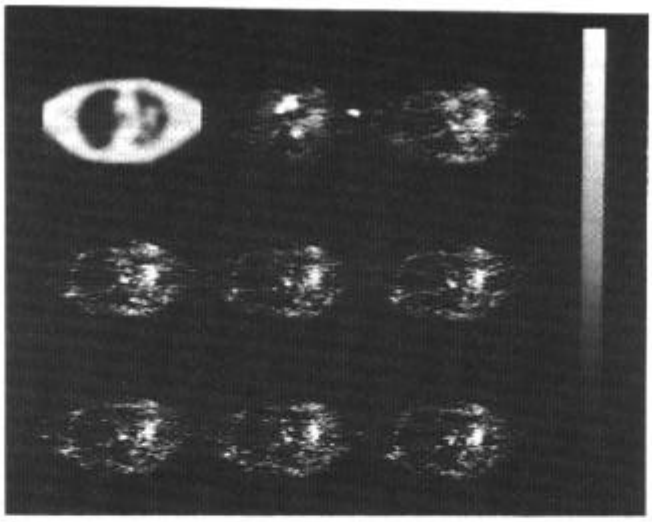


Fig.1. Sequential images of brain tumor (ID 619, 1a) and lung cancer (ID 552, 1b) scanned with 5 min data aquisition. In both studies, increased radioactivities in the tumor tissue were observed. However, it is difficult to know ^{11}C-met incorporated to protein synthesis. From the top left to the bottom right, decay- corrected PET images obtained sequentially were shown. In 1b, transmission image of the chest was also shown.

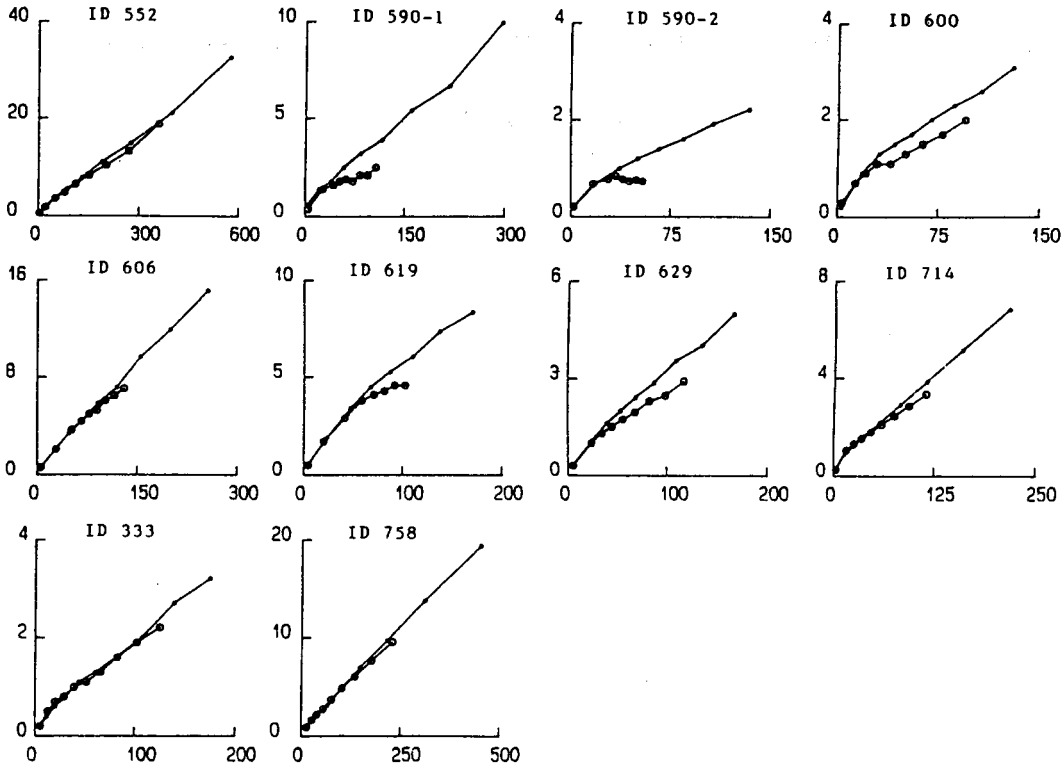


Fig.2. Graphical analysis of all the patients obtained for ^{11}C-met (•) and (○) total ^{11}C as an input. abscissa: normalized time (min) ordinate: Ci (t)/Cp(t)