IV. 4. Dynamic Study of Methionine Uptake in Glioma Using Positron Emission Tomography


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Introduction

It is widely held that positron emission tomography (PET) is useful for making quantitative diagnosis of brain tumors\(^2,3,5,12\), but no metabolic model suitable for quantitative analysis has yet been established. Although accurate determination of the serum concentration of free methionine is an important factor in the models which have been proposed thus far, it is commonly assumed that individual variability in metabolism is small and therefore that the free methionine activity can be calculated as a fixed percentage of the total serum activity\(^2,3,4\). Individual differences have, however, been found in the percentage of various metabolites several minutes following \(^1^1\)C-methionine administration\(^6,7,11\) suggesting\(^6\) that significant errors due to individual differences in methionine metabolism may have been included in previous studies. In the present study, we have made sequential measurements of free serum methionine in brain tumor patients. Those values were used as an input function for graphical analysis and comparisons were made with the result when a constant methionine metabolism was assumed.

Clinical Materials

Clinical materials were 19 cases of glioma, 17 male and 2 females. Ages ranged from 16 to 63 years, with a mean of 43. Diagnosis of the glioma were as follows: eight astrocytoma grade II, six grade II, five grade IV. Three grade II cases, one grade III and one grade IV case were studied 1-36 months after treatment. Serum metabolites were measured in 12 of these cases and histological diagnosis was made on the basis of a surgical sample or stereotaxic biopsy in all cases.
Methods

1. Measurement of $^{11}$C-methionine

Arterial blood was sampled 20 and 40 seconds, and 1, 1.5, 2, 2.5, 3, 4, 5, 7.5, 10, 15, 20, 30, 40, 50 and 60 minutes after the injection of 14-27 mCi of $^{11}$C-methionine. Blood samples was obtained after three minutes centrifugation and the specific weight then measured. The radioactivity was measured in a well counter. In 12 cases, the percentage of free methionine was also measured using a method which we have previously reported. Briefly, after sedimentation of the protein-bound fraction using HCLO$_4$, the non-protein-bound (supernatant) fraction was analyzed using HPLC and the proportion of methionine was determined.

2. PET study

The equipment used for the PET study was an ECAT II (EG&G, Ortec) or PT-931 (CTI, Knoxville, Tennessee). The spatial resolution was 15 and 18 mm and the slice thickness was 18 and 9 mm (FWHM), respectively. A continuous series of 5-minute scans over the course of 45 minutes were taken immediately following intravenous injection of 14-27 mCi of $^{11}$C-methionine. The PET images was reconstructed using the measured attenuation correction and each pixel corresponded to units of mCi/ml of radioactivity. In each scan, measurements were made at the site of the tumor and at a corresponding site of contralateral white matter of the same size. In the follow-up for the same patients, region of interest of the same size as that studied prior to therapy were used.

3. Graphical analysis

For the 12 cases in which the free serum methionine was also measured, graphical analysis was done following the method of Patlak et al.\textsuperscript{13} using the PET values for the radioactivity localized to the brain tumor and the serum radioactivity. The analysis was made both for the values obtained from whole plasma and for the free methionine values. Comparison was also made of the corrected value using mean of the cases for which analysis of the proportion of free methionine in the plasma was done.

The uptake rate (UR), distribution volumes (DV) and the correlation coefficients were calculated from the equation:

$$\frac{\text{Ci}(t)}{\text{Cp}(t)} = K_i$$

$$\int \frac{\text{Cp}(t)\,dt}{\text{Cp}+V_p}$$

Here, Ci is the tissue radioactivity as measured in the PET, Cp is the serum radioactivity, Ki corresponds to the UR, and Vp corresponds to the DV.\textsuperscript{14} As a consequence, graphs were plotted where the abscissa value, x, is equal to $\int \frac{\text{Cp}(t)\,dt}{\text{Cp}}$ and the ordinate value, y, is equal to $\frac{\text{Ci}(t)}{\text{Cp}(t)}$. The slope, Ki, of the linear regression of x on y gives the UR value and the y-intercept, Vp, gives the DV.
Result

1. Serum Analysis

The proportion of free methionine in the blood for each case is shown in Figure 1. The mean value (and standard deviations, SD) at 5, 10, 15, 20, 30, 60 minutes after injection of $^{11}$C-methionine were 97.2±1.4, 92.3±1.9, 89.7±4.1, 82.6±2.3, 72.0±10.1 and 42.5±16.3 percent respectively. An increase in the SD with time was observed.

2. Graphical Analysis

Table 1 and Figures 2 and 3 show the UR, DV and correlation coefficients in the graphical analysis for each case. When the whole serum radioactivity was used as the input function for calculating UR, a mean error of 15.3 % (maximum 21.4 %) was produced, in comparison with when the actual measured free methionine radioactivity was used (Fig.2). When comparisons were made between the actual measured value of free methionine and the corrected value using mean of the cases for which analysis of the proportion of free methionine was done, it was found that mean error of 4.2 % and maximum error of 17.7 % was produced. In contrast, using the whole plasma value for calculating DV, a mean error of 25.9 % and a maximum of 92.5 % were produced (Fig. 3). Using the mean value, a mean error of 6.2 % and a maximum value of 25.9 % were found. The mean correlation coefficient was 0.992 when the actual free methionine value was used, whereas this fell to 0.939 when the whole plasma value was used. When, however, the mean value was used, the correlation coefficient was approximately the same as that found using the actual value (0.993).

3. Histological Grade and Graphical Analysis

In a study of the histological grade in relation to UR, as calculated from the results of serum analysis, the UR was found to be below 0.0244 in low grade and above 0.0251 in high grade case (Fig. 4). In one case of a grade II astrocytoma in which follow-up study was possible, a 28.8 % drop in UR was found due to combined radio- and chemotherapy (a drop from 0.0497 to 0.0354). Although DV values tended to be higher in grade III than grade II cases, a statistically significant difference was not found (Fig.5). In the one case for which follow-up was possible, however, a notable decrease was found.

Including those cases for which blood analysis was not done, and using the mean values of all cases in which the proportion of free methionine was determined, graphical analysis gave the following result (Fig.6). Prior to therapy, statistically significant differences were found between grade II and grade III cases (p<0.005), grade II and grade IV cases (p<0.05), and between low grade and high grade cases (p<0.001) for the UR values. Prior to the therapy, DV values showed significant differences between grade II and grade III cases (p<0.01)(Fig.7).
Discussion

It is known that, using L-[methyl-\(^{11}\text{C}\)]methionine, PET is an effective method diagnosing brain tumors.\(^2,3,5,8,9,11,12\) It has also been reported that qualitative diagnosis of glioma can be made by measuring the total activity over a fixed period following \(^{11}\text{C}\)-methionine injection or by measuring the radioactivity after a fixed interval following injection\(^5,9,12\), but such techniques measure only one aspect of the dynamic metabolic process and it would be desirable to analyze the kinetics of such metabolism in greater detail. Although a kinetic technique has not yet been established, several promising methods have been reported - notably, the 3-compartment model\(^3,4\), graphical analysis\(^2,4\), and study of the tissue activity curve immediately following \(^{11}\text{C}\)-methionine administration.\(^10\)

In tumor tissue, it has been found that \(^{11}\text{C}\)-methionine radioactivity in the protein fraction increase over time following \(^{11}\text{C}\)-methionine injection: 42 % after 5 minutes, 72.4 % after 30 minutes and 76.8 % after 60 minutes.\(^8\) In our result of the graphical analysis as well, the uptake into an irreversible compartment increases continuously for at least 45 minutes following \(^{11}\text{C}\)-methionine injection – indicating that sufficient understanding of brain metabolism cannot be obtained from measurements taken over only the first few minutes following injection of the radioactive substance. For this reason, use of the 3-compartment model and graphical analysis is thought to be appropriate, but question remain concerning dynamics of the relevant metabolites.\(^6,7,11\) That is, when \(^{11}\text{C}\)-methionine has been injected, since various metabolites, such as \(^{11}\text{C}\)-protein and \(^{11}\text{C}\)-serine arrive blood stream after several minutes, it is necessary to know precisely the free methionine concentration for use as an input function in the dynamic analysis.\(^6,11\) In PET studies using \(^{11}\text{C}\)-methionine which have been reported thus far, direct measurement of free methionine have not been made, and only a constant value as a percentage of the total plasma activity has been used.\(^2,3,4\) It is known, however, that the metabolism of \(^{11}\text{C}\)-methionine varies among individuals.\(^6,7\) Moreover, in the present study it was found that 30 minutes after \(^{11}\text{C}\)-methionine administration, free methionine accounted for at least 43.9 % and at most 89.3 % of the total plasma activity. After 60 minutes, these values changed to 16.4 % and 71.6 %. It is therefore concluded that when a calculated, rather than the actual measured, value is used as an input function, significant errors will be introduced.\(^6,7\) These individual differences in methionine metabolism do not reflect merely differences in rates of protein synthesis, but are also influenced by differences in the patient's nutritional state.\(^1\)
The difference between the uptake rate when the measured free methionine was used and the total activity, in 12 glioma cases, averaged 15.3 %, with a maximum difference of 20 %. As has previously been demonstrated2,3,4,6,7, this difference shows the difficulty in accurately evaluating the metabolic dynamics of methionine. However, a mean difference of 4.2 % (maximum of 17.7 %) was found between using the actual level of free methionine and that calculated when a constant methionine metabolism is assumed. Although this difference is smaller than that mentioned above, it is too large to be ignored. In previous PET studies of glioma using 11C-methionine, this difference between actual and assumed levels has not been considered when calculating the uptake rate. As a consequence, care must be taken when evaluating reports results concerning the grading and therapeutic effectiveness.2,3,4)

The error in the distribution volume averaged 23.4 % (a maximum of 92.5 %) when the whole plasma value was used as an input function, whereas a mean error of 9.2 % (maximum 25.9 %) was found when the mean value was used. Discussion of the significance of the distribution volume have not been previously reported, but clearly our results indicate that these difference in DV need to be considered in any such discussion. Moreover, we found much lower correlation coefficients when the whole plasma value was used for calculations—again meaning that the reliability of such results is low. In comparison, when the mean value was used, these was almost no difference from the correlation coefficient obtained with the measured methionine levels.

Graphical analysis of the preoperative state of 14 patients using the mathematically corrected value as an input function, assuming the methionine metabolism is constant, showed significant differences in the uptake rate of grade II and grade III, grade II and grade IV and low grade high grade cases. Moreover, for the two cases in which follow-up study was possible, both showed notable decrease in UR postoperatively. Similarly, a significant difference in DV was found for the grade II and grade III cases, and both follow-up cases showed postoperatively decreases. However, similar level were seen in the grade II and grade IV cases - indicating that even when graphical analysis is done under the assumption of a constant methionine metabolism, statistically significant differences in UR and DV according to grade will be found. Therefore, qualitative diagnosis of glioma and evaluation of therapeutic results is possible under this assumption, but mean errors of 4.2 % and 9.2 % in estimates metabolic dynamics in individual cases - particularly for follow-up of the changes in an individual case, it is essential to measure the actual concentration of free methionine.

References

Table 1. Results of graphical analysis

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post: post treatment
UR: uptake rate (/min)
DV: distribution volume (ml/ml tissue)
r: correlation coefficient
y: year
m: month
measured: result using measured free methionine as input function
mean: result using corrected value from mean proportion of free methionine in total activity
total: result using total activity as input function
*,#: the same case
Fig. 1. Percentages of free methionine in plasma

Fig. 2. Uptake rate comparing 3 input functions
- ◊ result using measured free methionine as input function
- ○ result using corrected value from mean proportion of free methionine in total activity
- △: result using total activity as input function
Fig. 3. Distribution volume comparing 3 input functions
○: result using measured free methionine as input function
●: result using corrected value from mean proportion of free methionine in total activity
△: result using total activity as input function

Fig. 4. Uptake rate using free methionine as input function
Fig. 5. Distribution volume using free methionine as input function

Fig. 6. Uptake rate using corrected value from mean proportion of free methionine as input function
Fig. 7. Distribution volume using corrected value from proportion of free methionine as input function