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Intrasubject correlation between static scan and distribution volume images for [^{11}C]flumazenil PET

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Accumulation of [^{11}C]flumazenil (FMZ) reflects central nervous system benzodiazepine receptor (BZR). We searched for the optimal time for a static PET scan with FMZ as semi-quantitative imaging of BZR distribution. In 10 normal subjects, a dynamic series of decay-corrected PET scans was performed for 60 minutes, and the arterial blood was sampled during the scan to measure radioactivity and labeled metabolites. We generated 13 kinds of "static scan" images from the dynamic scan in each subject, and analyzed the pixel correlation for these images versus distribution volume (DV) images. We also analyzed the time for the [^{11}C]FMZ in plasma and tissue to reach the equilibrium. The intra-subject pixel correlation demonstrated that the "static scan" images for the period centering around 30 minutes post-injection had the strongest linear correlation with the DV image. The ratio of radioactivity in the cortex to that in the plasma reached a peak at 40 minutes after injection. Considering the physical decay and patient burden, we conclude that the decay corrected static scan for [^{11}C]FMZ PET as semi-quantitative imaging of BZR distribution is to be optimally acquired from 20 to 40 minutes after injection.

Key words: [^{11}C]flumazenil, benzodiazepine receptor, static scan, positron emission tomography, pixel correlation

INTRODUCTION

CENTRAL BENZODIAZEPINE RECEPTOR (BZR) has been studied with [^{11}C]flumazenil (FMZ) PET and with [^{123}I]iomazenil SPECT in various diseases.[1–7] Quantitative [^{11}C]FMZ PET is usually performed with a kinetic model.[8–11] We have performed the [^{11}C]FMZ PET with a dynamic scan for one hour and computed the distribution volume (DV) image as the BZR binding capacity by kinetic analysis. But we are obliged to force the subjects to remain still for 1.5 hour with their head fixed in a PET machine and to cannulate their radial artery to sample the arterial blood. Some patients are not suitable for this protocol. It is desirable to develop an easier method of measuring the BZR distribution with [^{11}C]FMZ PET.

A previous study reported a comparison of FMZ DV images with the static images acquired for 15–20 minutes post injection as "pseudoequilibrium scans."[8] The authors reported, however, that the scans indicated greater apparent receptor binding in the thalamus, basal ganglia and cerebellar cortex than estimated from the DV images. Another study reported that early activity images measured during the first three minutes after injection correlated with the K_1 images, and that delayed static images obtained in 24 to 39 minutes after injection correlated with the DV images,[11] but no other scanning time periods were examined in previous studies. The purpose of the present study is to search for on optimal scan time for

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Table 1 Frame time for generating "static scan" images

"static scan" image	Data acquisition period of the frames that were summed up
12-minute data	
2–14:	2.5–3.0, 3.5–4.0, 4.5–5.0, 5.5–6.0, 7.0–8.0, 9.0–10.0, 12.0–14.0
10–24:	12.0–14.0, 16.0–18.0, 22.0–24.0
24–36:	26.0–28.0, 30.0–32.0, 34.0–36.0
36–48:	38.0–40.0, 42.0–44.0, 46.0–48.0
48–60:	50.0–52.0, 54.0–56.0, 58.0–60.0
20-minute data	
2–24:	2.5–3.0, 3.5–4.0, 4.5–5.0, 5.5–6.0, 7.0–8.0, 9.0–10.0, 12.0–14.0, 16.0–18.0, 22.0–24.0
6–28:	7.0–8.0, 9.0–10.0, 12.0–14.0, 16.0–18.0, 22.0–24.0, 26.0–28.0
20–40:	22.0–24.0, 26.0–28.0, 30.0–32.0, 34.0–36.0, 38.0–40.0
28–48:	30.0–32.0, 34.0–36.0, 38.0–40.0, 42.0–44.0, 46.0–48.0
40–60:	42.0–44.0, 46.0–48.0, 50.0–52.0, 54.0–56.0, 58.0–60.0
32-minute data	
2–36:	2.5–3.0, 3.5–4.0, 4.5–5.0, 5.5–6.0, 7.0–8.0, 9.0–10.0, 12.0–14.0, 16.0–18.0, 22.0–24.0, 26.0–28.0, 30.0–32.0, 34.0–36.0
14–48:	16.0–18.0, 22.0–24.0, 26.0–28.0, 30.0–32.0, 34.0–36.0, 38.0–40.0, 42.0–44.0, 46.0–48.0
28–60:	30.0–32.0, 34.0–36.0, 38.0–40.0, 42.0–44.0, 46.0–48.0, 50.0–52.0, 54.0–56.0, 58.0–60.0

Values are start–end time (minutes post injection).

Table 2 Estimates of K_1, k_2 and DV (= K_1/k_2) for [^{11}C]FMZ

	K_1 (ml/mg/min)	k_2 (/min)	DV (ml/mg)
pons	0.23 ± 0.07	0.23 ± 0.09	0.96 ± 0.23
cerebellum	0.32 ± 0.03	0.11 ± 0.09	3.65 ± 0.52
frontal lobe	0.30 ± 0.03	0.08 ± 0.08	5.15 ± 0.89
temporal lobe	0.33 ± 0.04	0.09 ± 0.10	5.60 ± 0.84
occipital lobe	0.32 ± 0.04	0.09 ± 0.09	5.52 ± 0.81
parietal lobe	0.32 ± 0.04	0.08 ± 0.09	5.41 ± 0.94
thalamus	0.32 ± 0.04	0.13 ± 0.08	3.01 ± 0.43

Values are mean ± standard deviation of 10 normal subjects.

[^{11}C]FMZ PET as a measure of relative DV by pixel correlation analysis, with a view to finding the semi-quantitative benzodiazepine receptor distribution. Although the correlation may depend on the types of pathological changes, we first investigated the intrasubject correlation in normal subjects.

MATERIALS AND METHODS

Subjects

We recruited 10 normal subjects, four men and six women, without a history of neurological disease or any abnormalities on physical or neurological examinations (mean age ± SD, 42.0 ± 20.9). They were all right-handed. They had neither medication known to affect brain function nor a history of alcoholism. All the subjects gave written informed consent to the study.

No abnormalities were found in the MRI, which was obtained at the First Hospital of Nippon Medical School with a MAGNEX 1.5 Tesla machine (Shimadzu Co., Kyoto, Japan) or in the Tokyo Metropolitan Geriatric Hospital with a SIGNA 1.5 Tesla machine (General Electric Inc., WI, US).

Positron emission tomography imaging

A dynamic series of PET data acquisition with decay correction was performed for 60 minutes starting at the time of [^{11}C]FMZ injection with a HEADTOME IV scanner (Shimadzu Co., Kyoto, Japan), which collected 6.5 mm-interval 14 contiguous planes with a spatial resolution of 7.5 mm full width at half maximum (FWHM) and a Z-axis resolution of 9.5 mm FWHM.[12] As this machine provided only seven 13 mm-interval slices in one frame, the data were acquired in the $Z = 2$ mode to get 14 slices, in which the detector assembly was alternately moved to and fro for 6.5 mm in the Z axis. Thus each odd frame had an odd number of slices, and each even frame had an even number of slices. For data storage capacity, the data acquisition was divided into two parts: 18 minutes (30-second × 12 frames; 60-second × 4 frames; 120-second × 4 frames) and 40 minutes (120-second × 20 frames) with approximately a 2-minute interruption in between. [^{11}C]FMZ was prepared as described before.[13] Specific activity at the time of injection ranged from 5.7 to 45.7 GBq/μmol (22.9 ± 11.6 GBq/μmol).

Measurement of unmetabolized [^{11}C]flumazenil in plasma

After the [^{11}C]FMZ injection, the arterial blood was sampled at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 135 and 150 seconds, and 3, 5, 7, 10, 15, 20, 30, 40, 50 and 60 minutes. Each volume sample was 0.5 m*l*. To analyze the labeled metabolites, 1 m*l* of additional blood was taken at 3, 5, 10, 15, 20, 30, 40 and 60 minutes. The plasma was separated, weighed and measured for radioactivity with an NaI (Tl) well scintillation counter. The percentage of unmetabolized FMZ fraction was measured by high-performance liquid chromatography.[13]

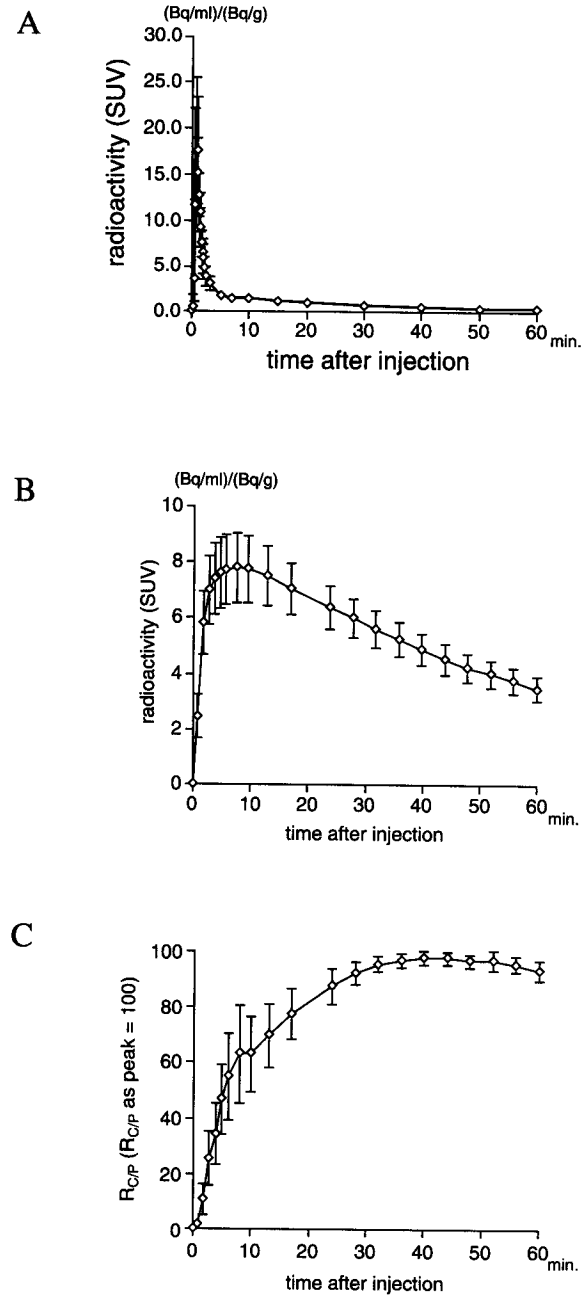

Fig. 1 Decay-corrected time–radioactivity curve for unmetabolized [^{11}C]FMZ in plasma (A) and that for the cerebral cortex (B) and their ratio $R_{C/P}$ (C) for 10 normal subjects. The unit of $R_{C/P}$ is percentage of peak value for each subject. Mean and SD of 10 subjects.

Data processing

Image manipulations were carried out on an Indy workstation (Silicon Graphics Inc., CA, US) with medical image processing software Dr. View (Asahi Kasei Joho System Co. Ltd., Tokyo, Japan) and a locally developed pixel correlation module that worked on Dr. View.

As the input function for the kinetic analysis, the time course of the plasma concentration of unmetabolized [^{11}C]FMZ was computed from the plasma radioactivity and the fraction of unmetabolized [^{11}C]FMZ interpolated through the scanning period. The kinetic analysis was carried out with a single-compartment two-parameter model to estimate the rate constant of tracer incorporation (K_1) and washout (k_2) with the Marquardt algorithm. The ratio of K_1 to k_2 was taken as the distribution volume (DV), which is considered to be linearly related to BZR density.[9] Parametric images of K_1, k_2 and DV were generated. Circular regions of interest (ROIs) 10-mm in diameter were placed in the parametric images over the frontal lobe, temporal lobe, occipital lobe and parietal lobe for each subject. Values for K_1, k_2 and DV in these regions were taken as the mean pixel value in the circular ROIs.

With the above-mentioned circular ROIs above, we also calculated the average time-radioactivity curve for the cerebral cortex. We computed the ratio of radioactivity in the cerebral cortex to radioactivity for unmetabolized [^{11}C]FMZ in the plasma ($R_{C/P}$) for each time frame.

To search for the optimal time for static scan, we generated 13 kinds of "static scan" images by adding up appropriate frames of the dynamic scan for each subject (Table 1). We used only even numbered frames corresponding to even numbered slices. The pixel value in the "static scan" images was expressed as the standardized uptake value (SUV), which is the radioactivity averaged across the period divided by dose per body weight ([Bq/ml tissue]/[Bq dose/g weight]). The SUV value for each pixel in each "static scan" image was plotted against the DV obtained in the kinetic analysis within the same subject over the entire brain, excluding the pixels with a DV less than 1.0 ml/mg. Then we analyzed the pixel correlation in the scattergrams.

RESULTS

Table 2 summarizes K_1, k_2 and DV values for each region obtained from ROIs over the parametric images. These values were almost the same as the parameter values previously reported.

Figure 1 shows the curve for the unmetabolized [^{11}C]FMZ in plasma (A), the time–radioactivity curve in the cerebral cortex (B) and their ratio $R_{C/P}$ (C). The $R_{C/P}$ reached 87.5 ± 6.3% of the peak at 24 minutes, reached the peak at 40 minutes, and then gradually decreased.

Figure 2 shows representative parametric images and "static scan" images. The "static scan" images were similar to the K_1 image in the early phase, but it had almost the same appearance as the DV image after 20 minutes. Noise increased in the late phase due to physical decay. Figure 3 shows the pixel-by-pixel correlation scattergram of the "static scan" image against the DV image. The one for 20–40 minutes post injection showed the smallest dispersion. Table 3 summarizes the results of pixel correlation analyses in 10 subjects. Of the various periods

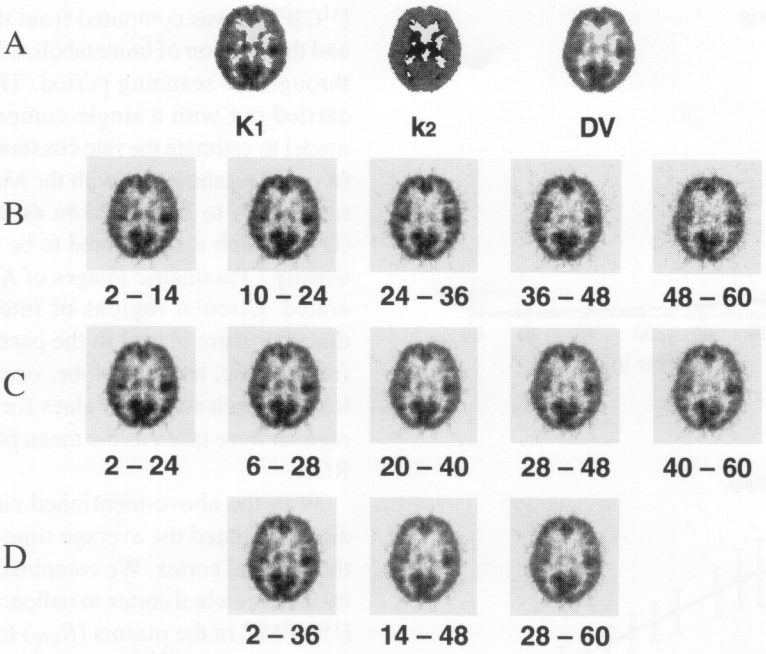

Fig. 2 Parametric images (A) and "static scan" images obtained by summing up frames for 12 minutes (B), 20 minutes (C) and 32 minutes (D) for a 58-years-old woman. The gray scales for the "static scan" images were adjusted so that the gray tone in the cerebral cortex would roughly equal that for the DV image. Note similarity of the static activity images to K_1 image in the early phase, and to DV image after 20 minutes post injection. Noise increased in the late phase due to physical decay.

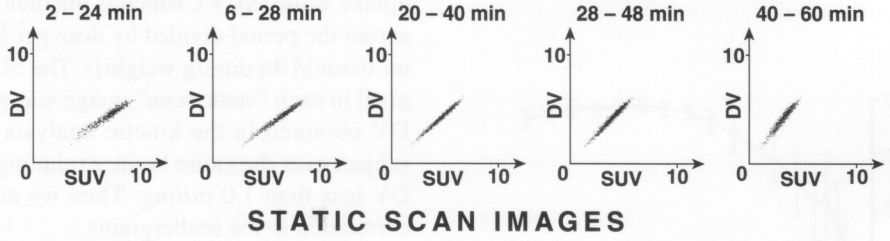

Fig. 3 Pixel-by-pixel correlation scattergrams of 20-minute "static scan" images at various time after injection against the DV image for a 58-years-old woman. Scattergram for 20–40 minutes showed the smallest dispersion.

studied, the pixel value for the image for 14–48 minutes had the strongest linear correlation with that of the DV images. Regardless of the summation period (12, 20 or 32 minutes), the "static scan" images for the phase centering around 30 minutes showed the largest correlation coefficient against the DV image. The Y intercept for the regression lines was close to zero in the images around 20 minutes, but gradually increased afterward.

DISCUSSION

In this study, we adopted a single-compartment two-parameter model to estimate K_1 and k_2.[9] The regional change in the FMZ concentration is described as:

$$\frac{dC_T}{dt} = K_1 C_P - k_2 C_T \quad (1)$$

where C_P is the FMZ concentration in plasma, and C_T is the FMZ concentration in tissue. When the input of FMZ is kept constant for a sufficiently long time, FMZ in the tissue and the plasma are in equilibrium, i.e. $\frac{dC_T}{dt}$ is equal to zero. Using equation (1);

$$\frac{K_1}{k_2} = \frac{C_T}{C_P} \quad (2)$$

Since we defined DV as K_1/k_2 and $R_{C/P}$ as C_T/C_P, DV is equal to $R_{C/P}$ in equilibrium, but the input function gradually decreased after a sharp peak (Fig. 1A). Therefore, $\frac{dC_T}{dt}$ is negative, and DV is smaller than $R_{C/P}$ even after a sufficiently long time in the late phase. Neither the input function nor the tissue activity reached an idealistic equilibrium during the observed period. The gradual decrease in the ratio $R_{C/P}$ after 40 minutes shows that $R_{C/P}$ is higher than DV (Fig. 1C).

Table 3 Regression line and correlation coefficient of pixel value of static activity images and DV images

time (min.)	slope (ml/g)	*Y* intercept (ml/mg)	correlation coefficient
2–14	0.728 ± 0.117	−0.176 ± 0.217	0.901 ± 0.034
10–24	0.749 ± 0.108	0.133 ± 0.165	0.960 ± 0.023
24–36	0.878 ± 0.124	0.427 ± 0.218	0.960 ± 0.024
36–48	1.004 ± 0.155	0.665 ± 0.330	0.936 ± 0.034
48–60	1.099 ± 0.195	0.975 ± 0.429	0.891 ± 0.050
2–24	0.759 ± 0.116	−0.225 ± 0.164	0.931 ± 0.026
6–28	0.769 ± 0.110	−0.043 ± 0.118	0.966 ± 0.018
20–40	0.911 ± 0.127	0.278 ± 0.137	0.978 ± 0.014
28–48	1.011 ± 0.143	0.394 ± 0.187	0.971 ± 0.016
40–60	1.159 ± 0.176	0.593 ± 0.281	0.947 ± 0.027
2–36	0.814 ± 0.120	−0.232 ± 0.117	0.956 ± 0.019
14–48	0.952 ± 0.134	0.177 ± 0.085	0.988 ± 0.007
28–60	1.124 ± 0.160	0.331 ± 0.137	0.980 ± 0.010

Regression of DV on "static scan" images (SUV). Values are mean ± standard deviation of 10 subjects.

In our injection protocol, the input function reaches a sharp peak during the first three minutes after injection followed by a gradual decrease (Fig. 1A). This peak in the input brings about a tissue uptake proportional to K_1, which is subsequently washed out at the rate k_2. Since k_2 is about 0.1/min, the washout time constant is about 10 minutes in the cerebral cortex. Therefore the initial peak has only a limited influence on the tissue radioactivity after 20 minutes, so that the tissue FMZ concentration is roughly determined by DV after 20 minutes, whereas it reflects K_1 until 20 minutes. If the radioactivity distribution is correlated with the DV, it is more likely to be so at a later stage than at an earlier stage, but due to physical decay, late images are affected by extreme statistical noise. Accordingly there should be an optimum scan time for the static image to be most strongly correlated with the DV.

The pixel correlation analyses in the present study demonstrated that the "static scan" image for 14–48 minutes had the strongest linear correlation with the DV image. The images for 20–40 minutes, 28–48 minutes and 28–60 minutes also had a strong linear correlation with the DV image. Later images showed a weaker correlation because of noise due to poorer statistics. One of the purposes of this study is to reduce the burden imposed on the subjects during the examinations. Our results show that both 20-minute data and 30-minute data had a strong linear correlation with the DV. Therefore, 20-minute data collection is considered preferable for the subjects. Considering these results, the optimum time for [^{11}C]FMZ PET static scan as semi-quantitative imaging of BZR distribution is from 20 to 40 minutes after [^{11}C]FMZ injection. The scan time for the delayed static image reported by Millet et al.[11] was similar to our conclusion.

In this study, all subjects were healthy. Further studies should investigate the pathologic condition with decreased K_1 and preserved DV which is observed in such patients as those with cerebral infarction and Alzheimer's disease. The near-proportional relationship between the optimal "static scan" images and the DV images raises the possibility that we can get obtain images of BZR distribution from the static scan if we know the slope. This involves the question of input normalization and is now under investigation.[14]

In conclusion, static scan images for 14–48 minutes, 20–40 minutes, 28–48 minutes and 28–60 minutes had a strong linear correlation with the DV image. We recommend that the time for [^{11}C]FMZ PET static scan as semi-quantitative images of BZR distribution is from 20 to 40 minutes after injection, which is thought to be almost proportional to BZR binding capacity and is sufficiently useful for clinical studies.

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REFERENCES

1. Savic I, Persson A, Roland P, Pauli S, Sedvall G, Widen L. *In-vivo* demonstration of reduced benzodiazepine receptor binding in human epileptic foci. *Lancet* 2: 863–866, 1988.
2. Holthoff VA, Koeppe RA, Frey KA, Penney JB, Markel DS, Kuhl DE, et al. Positron emission tomography measures of benzodiazepine receptors in Huntington's disease. *Ann Neurol* 34: 76–81, 1993.
3. Gilman S, Koeppe RA, Junck L, Kluin KJ, Lohman M, St Laurent RT. Benzodiazepine receptor binding in cerebellar degenerations studied with positron emission tomography. *Ann Neurol* 38: 176–185, 1995.
4. Meyer M, Koeppe RA, Frey KA, Foster NL, Kuhl DE. Positron emission tomography measures of benzodiazepine binding in Alzheimer's disease. *Arch Neurol* 52: 314–317, 1995.
5. Gilman S, Koeppe RA, Adams K, Johnson-Greene D, Junck L, Kluin KJ, et al. Positron emission tomographic studies of cerebral benzodiazepine-receptor binding in chronic alcoholics. *Ann Neurol* 40: 163–171, 1996.
6. Kitamura S, Koshi Y, Komiyama T, Sakayori O, Komaba Y, Ohyama M, et al. Benzodiazepine receptor and cerebral blood flow in early Alzheimer's disease—SPECT study using ^{123}I-iomazenil and ^{123}I-IMP. *KAKU IGAKU (Jpn J Nucl Med)* 33: 49–56, 1996.
7. Ohyama M, Senda M, Ishiwata K, Kitamura S, Mishina M, Ishii K, et al. Preserved benzodiazepine receptors in Alzheimer's disease measured with C-11 flumazenil PET and I-123 iomazenil SPECT in comparison with CBF. *Ann Nucl Med* 13: 309–315, 1999.
8. Frey KA, Holthoff VA, Koeppe RA, Jewett DM, Kilbourn MR, Kuhl DE. Parametric *in vivo* imaging of benzodiazepine receptor distribution in human brain. *Ann Neurol* 30:

663–672, 1991.
9. Koeppe RA, Holthoff VA, Frey KA, Kilbourn MR, Kuhl DE. Compartmental analysis of [^{11}C]flumazenil kinetics for the estimation of ligand transport rate and receptor distribution using positron emission tomography. *J Cereb Blood Flow Metab* 11: 735–744, 1991.
10. Price JC, Mayberg HS, Dannals RF, Wilson AA, Ravert HT, Sadzot B, et al. Measurement of benzodiazepine receptor number and affinity in humans using tracer kinetic modeling, positron emission tomography, and [^{11}C]flumazenil. *J Cereb Blood Flow Metab* 13: 656–667, 1993.
11. Millet P, Delforge J, Mauguiere F, Pappata S, Cinotti L, Frouin V, et al. Parameter and index images of benzodiazepine receptor concentration in the brain. *J Nucl Med* 36: 1462–1471, 1995.
12. Iida H, Miura S, Kanno I, Murakami M, Takahashi K, Uemura K. Design and evaluation of HEADTOME-IV, a whole-body positron emission tomograph. *IEEE Trans Nucl Sci* NS-36: 1006–1010, 1989.
13. Ishiwata K, Ito T, Ohyama M, Yamada T, Mishina M, Ishii K, et al. Metabolite analysis of [^{11}C]flumazenil in human plasma: Assessment as the standardized value for quantitative PET study. *Ann Nucl Med* 12: 55–59, 1998.
14. Toyama H, Yamada T, Uemura K, Kimura Y, Ishii K, Ohyama M, et al. Quantitative mapping of benzodiazepine receptor concentration in a static PET study. *NeuroImage* 5: A34, 1997.