Quantification of Regional Cerebral Blood Flow with Continuous Infusion of Technetium-99m-Ethyl Cysteinate Dimer

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We propose a new method to quantify regional cerebral blood flow (rCBF) with continuous infusion of ^{99m}Tc-ethyl cysteinate dimer (ECD) and dynamic SPECT. **Methods:** Thirteen subjects were studied. Seven subjects had SPECT and PET studies, and the other six subjects were involved in the measurement of blood clearance of ^{99m}Tc-ECD. During constant infusion of ^{99m}Tc-ECD (740 MBq) over 10 min, dynamic SPECT scans were obtained every 1 min by means of a triple-head rotating SPECT camera. Intermittent arterial blood sampling with octanol extraction was performed every 1 min to estimate the arterial input function. Influx constant (Ku) obtained by Gjedde-Patlak graphical plot method was compared with rCBF measured by PET using ^{15}O CO_2 steady state method. In order to simplify the procedure, arterial input function in each subject was estimated by calibration of the arterial blood sampled at the end of the scan to the standard arterial input function estimated from the blood clearance rate in six subjects. **Results:** Ku was linearly correlated with rCBF ($Ku = 0.09 + 0.62$ rCBF, $r = 0.85$, $p < 0.05$). Ku calculated with the estimated input function (Ku') and rCBF also demonstrated a linear relationship ($Ku' = 0.05 + 0.65$ rCBF, $r = 0.84$, $p < 0.05$). **Conclusion:** The proposed method with one-point arterial sampling is a simple, clinically feasible tool for quantitative measurement of rCBF with ^{99m}Tc-ECD.

Key Words: continuous infusion; dynamic SPECT; technetium-99m-ECD; graphical plot cerebral blood flow; quantification

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In recent years, various cerebral perfusion tracers, including ^{123}I-N-isopropyl-*p*-iodoamphetamine (IMP), ^{99m}Tc-d,l-hexamethyl-propyleneamine oxime (HMPAO) and ^{99m}Tc-ethyl cysteinate dimer (ECD) have been introduced in clinical nuclear medicine for measuring regional cerebral blood flow (rCBF) with SPECT. Several methods have been developed for quantifying rCBF with these tracers. In case of IMP, microsphere method with continuous arterial blood sampling (*1*), autoradiographic method (*2*) and fractional uptake method (*3*) were proposed. In case of technetium-labeled tracers, noninvasive graphical plot method was also proposed (*4,5*) using the time-activity curves of the brain and aorta obtained by serial dynamic planar imaging. However, the accuracy of the serial changes in radioactivity obtained from the anterior planar images is limited. Dynamic SPECT data are obviously more reliable than the planar images for measurement of temporal changes in regional tissue activity. For dynamic SPECT imaging, slow infusion of the tracer is more appropriate than bolus injection because SPECT scan requires a longer scanning time than planar imaging. We now propose a new method with continuous infusion of ^{99m}Tc-ECD and dynamic SPECT for quantification of rCBF. We also tried to simplify the method for clinical use using one-point calibration of the standard arterial input function.

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MATERIALS AND METHODS

Subjects

We examined 13 male subjects (age range 28–78 yr), including 11 normal volunteers and two patients who had unilateral cerebrovascular disease. Two patients and five normal volunteers received continuous infusion of ^{99m}Tc-ECD and dynamic SPECT (Group 1). Six other normal subjects (20–36 yr old) were involved in the measurement of blood clearance of ^{99m}Tc-ECD (Group 2).

Preparation of Technetium-99m-ECD

Technetium-99m-ECD was prepared from the commercially supplied kit (Daiichi Radioisotope Lab, Tokyo, Japan). The ^{99m}Tc-ECD preparation kit consisted of two vials, one containing a sterile and nonpyrogenic lyophilized mixture and the other a liquid phosphate buffer. Three milliliters of normal saline were injected into the first vial to dissolve its contents. Technetium-99m generator eluant (1110 MBq) was injected into the second vial, and 1 ml of the contents of the first vial was then transferred into the second vial. The mixture was allowed to stand at room temperature for 30 min. Approximately a 740-MBq ^{99m}Tc-ECD dose was collected in the 20 ml of plastic syringe and diluted with saline (740 MBq/20 ml) for continuous infusion.

SPECT Imaging

Technetium-99m-ECD was continuously infused with a mechanical syringe pump at a rate of 2 ml/min for 10 min. A triple-head rotating SPECT camera equipped with low-energy, high-resolution, fanbeam collimators was used to acquire dynamic SPECT imaging. The spatial resolution was 8 mm full width with at half maximum (FWHM) in the center of the field of view. Dynamic data acquisition was performed with continuous rotation repeating clockwise (CW) and counterclockwise rotation (CCW) by turns. Images were acquired at 4° intervals in 64 × 64 matrices. The camera rotation of 120° around the brain in 30 sec covered the projections of 360°. A total of 21 frames of data were acquired. The first frame of the scan was not used because of negligible activity in the brain. Paired data of CW and CCW was summed to minimize the asymmetry tissue activity of the brain to generate 10 frames of dynamic data. Acquired dynamic data were reconstructed at the condition as follows: prefilter; Butterworth filter (filtered back-projection method), cutoff frequency; 0.3 order; 8 reconstruction filter; ramp filter. Attenuation correction and scatter correction were not performed.

A small catheter was placed in the brachial artery for blood sampling. Arterial blood sampling of 2 ml was performed every 1 min, and the blood samples were extracted by octanol to obtain the true arterial input function. The blood-to-octanol ratio was set to

1:2. The mixture of blood and octanol was vortexed over 30 sec and then centrifuged. Octanol-extracted activity (lipophilic activity) in each sample was taken out of the tube, and the count was measured by a well counter.

PET Imaging

After the acquisition of dynamic SPECT data, regional cerebral blood flow (rCBF) was measured by the ^{15}O-labeled CO_2 steady-state method (*6*) using a whole-body PET scanner. Physical characteristics of this camera were described previously in detail (*7*). The spatial resolution of the reconstructed clinical PET images is 6 mm in FWHM at the center of the field of view, and axial resolution is 4 mm. Before the emission scan, a 10-min transmission scan was performed using two rotating ^{68}Ge pin sources for attenuation correction. Tissue activity concentration in the images was cross-calibrated against the well counter using a cylindrical phantom filled with ^{18}F solution.

The subject's head was immobilized with a headholder and positioned parallel to the canthomeatal line using a light beam. The subject wore a light, disposable plastic mask and nasal cannula for inhalation of ^{15}O gas produced by a small cyclotron. The steady-state inhalation method for ^{15}O-labeled CO_2 with 5-min data acquisition and intermittent arterial blood sampling was used to calculate rCBF (*6*).

Calibration of the Sampled Blood Count and SPECT Data

A cylindrical phantom of 20 cm diameter was used to obtain the calibration factor between the sampled blood count and the tissue count of SPECT images. The phantom was filled with saline containing approximately 185 MBq ^{99m}Tc-pertechnetate and mixed well with a magnetic stirrer. Dynamic data of the phantom were acquired on the same protocol as the patient study. After the scanning, the content in the phantom was taken out and divided into five tubes, and their counts were measured by a well counter. Dynamic SPECT images were reconstructed under the same conditions as those performed on patient data. In reconstructed images, ROIs were placed with the diameter of about 16 cm. These ROIs were placed in 10 slices. With the mean value of ROIs and counts in the solution, the calibration factor was obtained.

Technetium-99m-ECD Blood Clearance Data

In six normal volunteers, 740 MBq ^{99m}Tc-ECD was continuously infused for 1 min. Arterial blood sampling was performed at 15, 30, 45, 60 and 90 sec and 2, 3, 4, 5, 7 and 10 min after the start of injection. The octanol extracted activity was plotted for the measurement of blood clearance of ^{99m}Tc-ECD.

Data Analysis

Graphical Analysis Using Measured Input Function. By visual inspection, we selected the slices on PET images that were closest to the corresponding SPECT images. ROIs were manually placed in the frontal, temporal, occipital and parietal cortices, white matter in centrum semiovale and cerebella in bilateral hemispheres, thus avoiding the deep structures such as basal ganglia or thalamus to minimize the under- or overestimation of the radioactivity accumulation at the center of the brain. The Gjedde-Patlak graphical plot method was used for data analysis in this study (*4,8,9*). The method was expressed as:

$$\frac{Cb(t)}{Ca(t)} = Ku \cdot \frac{\int_0^t Ca(\tau)\, d\tau}{Ca(t)} + Vn,$$

where Cb(t) is the tissue activity in the brain, Ca(t) is arterial input function, Ku is influx constant (ml/min/1 g brain tissue) and Vn is the initial distribution volume (ml/ml). Ku was expressed as the product of E by F, where E is the first-pass extraction of the tracer, and F is rCBF (ml/min/1 g brain).

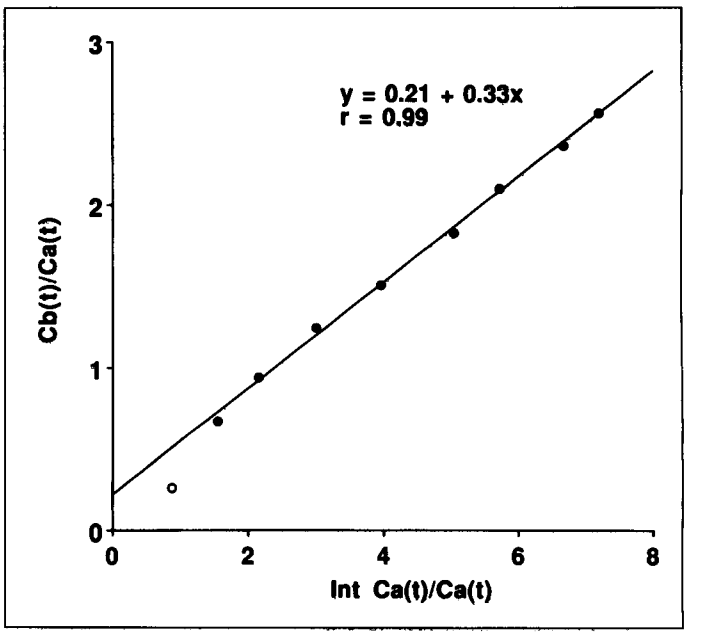

FIGURE 1. Representative graphical plotting using the corrected input function and time-activity data. The slope, showing the influx constant (Ku), was calculated by linear regression analysis of 2–10-min data (closed circle). Int = integral.

Each dataset of Cb(t) calibrated by phantom data, Ca(t) and integration of Ca(t) was plotted. The second to the 10th frames of the datasets were used to calculate Ku. Ku was compared to rCBF in PET images in each region.

Simulation of Input Function Using Clearance Rate. As the method noted above requires intermittent arterial blood sampling, we tried to simplify the method for clinical use. First, we measured the lipophilic activity by 1-min infusion to obtain the blood clearance rate of ^{99m}Tc-ECD. The clearance curve was estimated by monoexponential curve fitting. The lipophilic activity, f(t), is expressed as:

$$f(t) = \alpha \cdot e^{-k(t-1)} (1 < t),$$

where k is the clearance rate and α is the peak count at 1 min.

During continuous infusion, the clearance rate of ^{99m}Tc-ECD should be considered to obtain the arterial input function. The k value was determined from the measured blood clearance data for six subjects. The simulation curve of arterial input function was generated using these values. The equation of simulation curve, g(t), is expressed as:

$$g(t) = \int_0^t e^{-ks}\, ds.$$

The simulation curve was calibrated with the lipophilic activity at 10 min after injection in each individual subject examined with dynamic SPECT. Ku obtained from the simulated input data (Ku') was compared with rCBF measured by PET.

RESULTS

Figure 1 demonstrates an example of graphical analysis plotting. The corrected input function and time-activity data demonstrated an excellent linear regression line. Figure 2 shows a relationship between Ku and rCBF, demonstrating a linear correlation ($Ku = 0.09 + 0.62$ rCBF, $r = 0.85$, $p < 0.05$).

Figure 3 shows the relative lipophilic activity of ^{99m}Tc-ECD with 1-min infusion. After 1-min infuson, the activity was

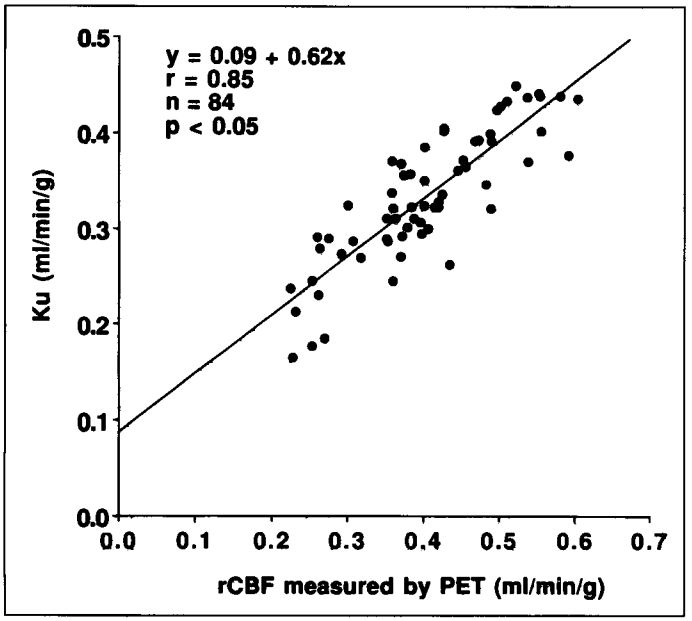

FIGURE 2. Comparison of Ku and rCBF measured by PET. Significant relationship was observed.

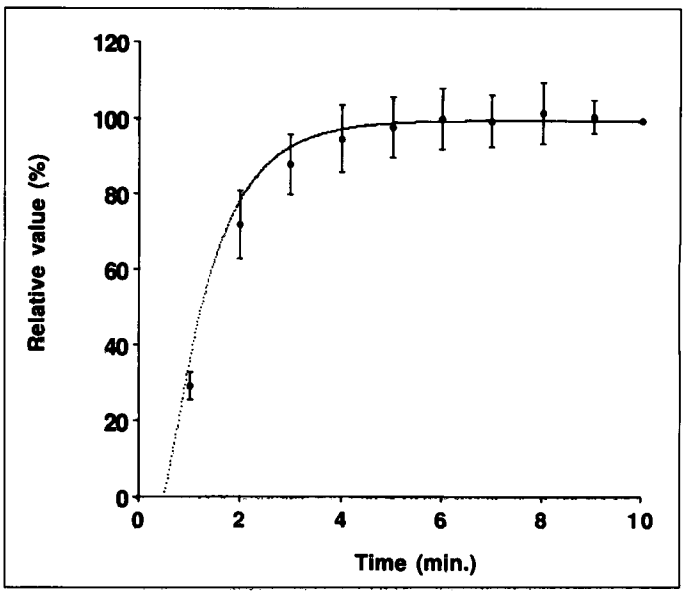

FIGURE 4. Simulation curve of lipophilic activity during continuous infusion (dotted line), measured lipophilic activity normalized with the value at 10 min in each subject (mean; closed circle, s.d.; error bar). In simulation data, liophilic activity reached plateau at 5 min after the start of infusion.

reduced rapidly. The average clearance rate in six subjects (unit; min^{-1}) was 1.095 ± 0.114. Figure 4 shows the standard arterial input curve and the blood ^{99m}Tc-ECD data that were normalized to the lipophilic activity at 10 min for each subject. Figure 5 shows the relationship between Ku' and rCBF, which demonstrates a significant correlation (Ku' = 0.05 + 0.65 rCBF, r = 0.84, $p < 0.05$).

DISCUSSION

In this study, we developed a method to calculate rCBF with a continuous infusion of ^{99m}Tc-ECD and dynamic SPECT imaging. To calculate Ku using a Gjedde-Patlak plot, the input should be obtained during dynamic data acquisition with a sufficient number of frames. Continuous infusion is essential because the clearance of the lipophilic component of ^{99m}Tc-ECD from the blood is quite rapid. To obtain enough linear portion in Gjedde-Patlak plot analysis for stable estimation of Ku, we have chosen 10 time point sampling. Using SPECT, at least 1-min data acquisition per each frame is necessary to obtain a sufficient signal to noise ratio. Hence, we chose a 10-min infusion. Continuous infusion is suitable for the dynamic SPECT as the changes in tissue activity can be accurately measured, and busy arterial sampling is not required. As shown in our results with continuous infusion, arterial input function was easily obtained by intermittent arterial blood sampling every 1 min.

Technetium-99m-ECD is more suitable than IMP or HMPAO for continuous infusion. IMP, which is initially trapped in the lungs and then gradually released to the circulatory blood and delivered to the brain (*10*), may not provide a predictable input function. HMPAO is not suitable for continuous infusion either because of its chemical instability (*11*). Technetium-99m-ECD has the same character as HMPAO of first-pass distribution in the brain and is stable for several hours after it is labeled (*12*). Therefore, ^{99m}Tc-ECD is the most suitable tracer for continuous infusion.

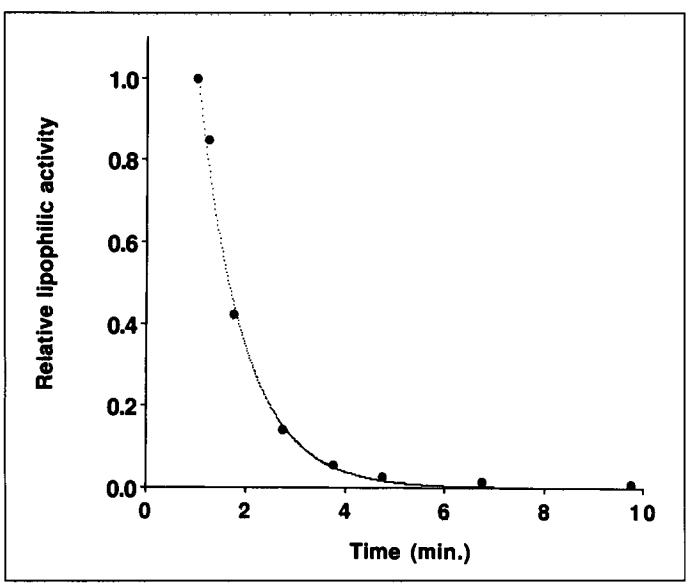

FIGURE 3. An example of relative lipophilic activity of ^{99m}Tc-ECD with 1-min infusion. The clearance of averaged lipophilic activity in six normal volunteers was estimated with monoexponential fitting.

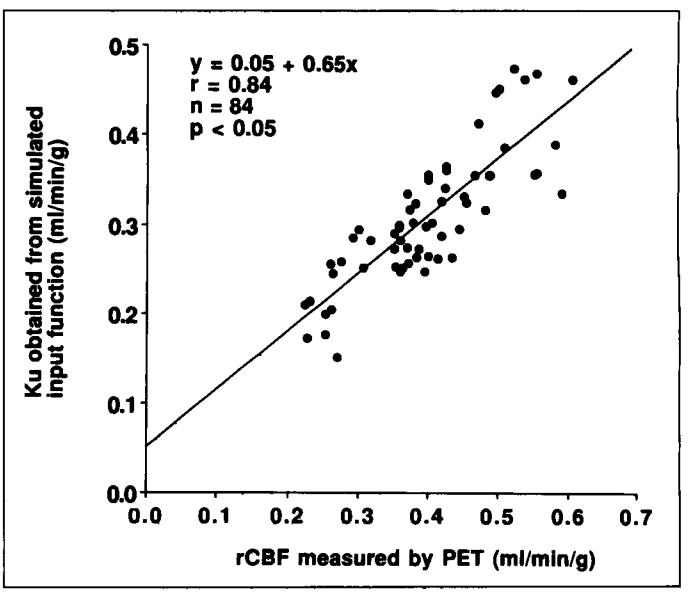

FIGURE 5. Relationship between Ku' and rCBF. Significant correlation also was observed.

It is important to notice which process Ku reflects, blood flow or metabolism. In three-compartment analysis, Ku is expressed as $k1 \times k3/(k2 + k3)$. Ishizu et al. (*13*) reported that in normal subjects the retention fraction, which is expressed as $k3/(k2 + k3\text{-}k5)$, does not change significantly in normal flow range. As k5 is small enough to be negligible, the retention fraction, $k3/(k2 + k3)$, is constant. This means that Ku reflects k1, which in turn reflects blood flow, assuming that the extraction fraction is constant. In pathological states, Ku would be affected not only by the change in rCBF, but also by k2 and k3. Hence, careful interpretation is necessary.

We previously reported that the ^{99m}Tc-ECD uptake ratio to the reference region was nonlinearly related to the relative regional cerebral blood flow (rCBF), not against absolute CBF values, and that the PS value and, thus, the extraction fraction were obtained by the least-squares fitting method using group data (*14*). In this study, we tried to evaluate Ku with reference to absolute rCBF, without extraction fraction correction, as an extraction fraction of each individual cannot be directly measured by our method. Although nonlinearity was observed in the high-flow range in Figure 2, the tendency was relatively subtle, and the overall linearity between rCBF and Ku was well shown. This is partly due to the limitation of the rCBF measurement by PET. The method does not include the extraction fraction of water (*6,15,16*). Thus, from a practical point of view, Ku can be regarded as a rCBF measure. In certain conditions with the PS product available, nonlinear correction using the PS product could be a choice.

Though Ku is well correlated with rCBF, the slope is far from unity, and positive intercept was observed. This is due to the underestimation in the high-flow range caused by backdiffusion (*17*), limited first-pass extraction (*14,18,19*) and scatter radiation. The underestimation of rCBF was less than the previous report with static ^{99m}Tc-ECD SPECT and continuous arterial blood sampling based on the microsphere model (*20*). Scatter correction, such as the triple-energy window method (*21*), may improve the results by eliminating the positive offset in the low-flow range caused by scatter radiation.

To simplify the procedure for clinical use, we adopted standardized arterial input function and one-point sampling for calibration. By continuous infusion, measured input function reached plateau at 5 min after the start of infusion, which was well fitted by the simulation study using a blood clearance rate of ^{99m}Tc-ECD. A longer time interval allows better input function by using standardized input function with one-point arterial sampling. Theoretically, blood sampling could be done at any time once plateau is reached. We have chosen 10 min because of the safety of margin. The simulation curve generated from the ^{99m}Tc-ECD blood clearance data was close to the measured input function. The slope between Ku and rCBF was almost identical to that of Ku'. These data demonstrate that one-point arterial sampling can replace intermittent multiple-blood sampling in the continuous infusion method.

The conventional graphical plot method, acquiring the arterial input data from the aortic arch on planar images, is noninvasive but inaccurate in estimating the input function. One-point arterial blood sampling (*22*) is a clinically acceptable procedure, if accurate quantitative values of rCBF can be obtained, that depends on the accuracy of the calibration of the standard input function. As the constant infusion provides the stable lipophilic activity within a few minutes, the calibration of the blood activity in each study is not sensitive to the time of blood sampling. This stable ^{99m}Tc-ECD activity in arterial blood is due to the equilibration of increasing activity by constant infusion and the clearance of ^{99m}Tc-ECD. This method requires fast dynamic SPECT imaging, which may not be practical for the clinical situation. To further simplify the method, the dynamic scan could be omitted, and a single, static SPECT scan after cessation of input function also would provide a reasonable estimate of rCBF. This requires further investigation.

CONCLUSION

Dynamic SPECT during continuous infusion in ^{99m}Tc-ECD is a promising method for quantification of regional cerebral blood flow.

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