Noninvasive Quantification of Cerebral Blood Flow Using ^{99m}Tc-ECD and SPECT

Ikuo Odano, Masaki Ohkubo and Takashi Yokoi

Department of Radiology, Niigata University School of Medicine, Niigata; and Department of Research for Nuclear Medicine, Medical System Division, Shimadzu Corporation, Kyoto, Japan

The aim of this study was to develop a simple, noninvasive method for quantifying regional cerebral blood flow (rCBF) using ^{99m}Tc-ethyl cysteinate dimer (ECD) by a single SPECT scan and single venous sampling. **Methods:** Using a three-compartment model, we introduced the regional brain fractionation index (BFI), $Cb(T_s)/\int_0^{T_s} Ca(\tau)d\tau$ [Ca(t), arterial input; Cb(t), brain activity]. Regional BFI obtained at the optimum time T_s (min) was converted to rCBF using an exponential function, which was obtained by analyzing the relationship between regional BFI and rCBF (= F) obtained by the standard ^{133}Xe inhalation SPECT method. The integral of the concentration of ^{99m}Tc-ECD in arterial blood corrected for physical decay [Ca(t)] in BFI was estimated from a single venous blood sample obtained at the optimum time T_v using the regression line obtained by analyzing the relationship between the integral of Ca(t) and venous sample data. The data come from three groups of patients. The first group of patients (n = 16) underwent a complete ^{99m}Tc-ECD BFI study with measurement of Ca(t) and dynamic SPECT scanning, as well as a ^{133}Xe inhalation study to measure rCBF. The results were used to analyze the relationship between regional BFI and rCBF (obtained with ^{133}Xe) and to determine the optimum time T_s for obtaining BFI. Data from the second group of patients (n = 15) were used to analyze the relationship between the integral of Ca(t) and venous sample data and to determine the optimum time T_v for one-point venous blood sampling. Finally, the third group of patients (8 patients, 10 studies) was used to validate the current method by comparing the results with ^{133}Xe inhalation SPECT. **Results:** Regional BFI obtained at time T_s = 20 min showed good agreement (r = 0.907; a = 0.552, b = 0.962) with rCBF. The venous sample data obtained at time T_v = 6 min showed a good correlation (r = 0.988) with BFI. In comparing rCBF values thus obtained and those obtained by the ^{133}Xe method, we found a good correlation (r = 0.917, slope = 1.01). **Conclusion:** The proposed method has three advantages: (a) accurate quantification of rCBF without underestimation in the high flow range, (b) simplicity and noninvasiveness and (c) the ability to use any type of SPECT camera for the study.

Key Words: ^{99m}Tc-ethyl cysteinate dimer; regional cerebral blood flow measurement; SPECT; three-compartment model

J Nucl Med 1999; 40:1737–1744

Recent advances in various radiolabeled tracers and SPECT devices have made it possible to map cerebral perfusion with high-quality images. The primary clinical interest is in their use as a measure of regional cerebral blood flow (rCBF) in pathologic conditions. ^{99m}Tc-ethyl cysteinate dimer (ECD) has high in vitro stability (*1*) and rapid in vivo blood clearance (*2*); hence, it is reliable for use in quantifying rCBF when the appropriate modeling is introduced. Quantitative approaches with ^{99m}Tc-ECD have been used on experimental animals with an indicator fractionation model (*3*) and attempted on humans using a steady-state influx constant (*4,5*). Graphic plot methods using dynamic planar or SPECT images have been reported (*6,7*). We also reported an invasive method using a steady-state rate of uptake as a preliminary study (*8,9*). However, these procedures have not been firmly established.

In this study, we developed a simple, noninvasive method for quantifying rCBF with ^{99m}Tc-ECD and SPECT based on a three-compartment model. The method requires a single SPECT scan and one-point sampling of venous blood at the optimum time after tracer injection. This method may overcome the laborious procedures for acquiring dynamic planar or SPECT data and the invasiveness and laboriousness for sequential sampling of arterial blood. The one-point sampling method introduced was modified from previous reports (*10,11*). To validate this method, the values of rCBF were compared with those obtained by an independent technique, the standard ^{133}Xe inhalation SPECT method.

MATERIALS AND METHODS

Theory

Regional Cerebral Blood Flow Measured by Regional Brain Fractionation Index. To analyze ^{99m}Tc-ECD kinetics in the brain, the three-compartment model was used in this study as described in previous reports (*5,12*) (Fig. 1). Ca(t) and Cb(t) are the respective concentrations of ^{99m}Tc-ECD in arterial blood and brain tissue corrected for physical decay. K_1 is the influx rate constant, k_2 is the outflux rate constant for washout, k_3 is the lipophilic-to-hydrophilic conversion constant and k_4 is the hydrophilic-to-lipophilic conversion constant. The reverse conversion constant, k_4, is hypothetic,

Received Nov. 24, 1998; revision accepted Mar. 23, 1999.

For correspondence or reprints contact: Ikuo Odano, MD, Department of Radiology, Niigata University School of Medicine, Asahimachi 1-757, Niigata 951-8510, Japan.

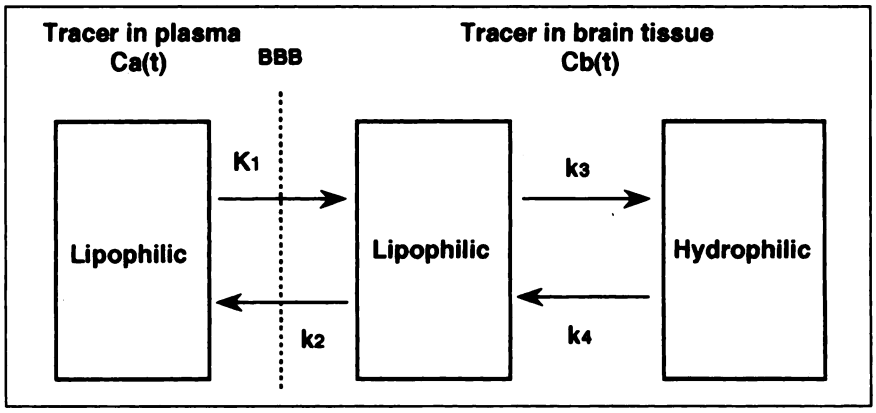

FIGURE 1. Kinetic model of ^{99m}Tc-ECD with three compartments and four parameters. K_1 to k_4 are rate constants for transport of tracer between compartments. Ca(t) and Cb(t) are respective concentrations of ^{99m}Tc-ECD in arterial blood and brain tissue corrected for physical decay. BBB = blood–brain barrier.

and its value is assumed to be zero in this study (*5*). After ^{99m}Tc-ECD is injected, the following equation can be obtained:

$$Cb(t) = \frac{K_1 \times k_3}{k_2 + k_3}\int_0^t Ca(\tau)d\tau + \frac{K_1 \times k_2}{k_2 + k_3} \times e^{-(k_2+k_3)t}\int_0^t Ca(\tau) \times e^{(k_2+k_3)\tau}d\tau \quad \text{Eq. 1}$$

When enough time has passed, Equation 1 is approximated by Gjedde-Patlak graphic plot analysis (*13,14*) as follows:

$$\frac{Cb(t)}{Ca(t)} = \frac{K_1 \times k_3}{k_2 + k_3} \times \frac{\int_0^t Ca(\tau)d\tau}{Ca(t)} + \frac{K_1 \times k_2}{(k_2 + k_3)^2}. \quad \text{Eq. 2}$$

Equation 2 is converted as follows:

$$\frac{K_1 \times k_3}{k_2 + k_3} = \frac{Cb(t)}{\int_0^t Ca(\tau)d\tau} - \frac{K_1 \times k_2}{(k_2 + k_3)^2} \times \frac{Ca(t)}{\int_0^t Ca(\tau)d\tau}. \quad \text{Eq. 3}$$

When more time (t) has passed ($t \geq T_s$), it is assumed that the values of $K_1 \times k_2/(k_2 + k_3)^2 \times Ca(t)$ become small enough in comparison with those of Cb(t). That is, the second part of the right side of Equation 3 is smaller than the first part and can be ignored. Then Equation 3 is

$$\frac{K_1 \times k_3}{k_2 + k_3} = \frac{Cb(t)}{\int_0^t Ca(\tau)d\tau}. \quad \text{Eq. 4}$$

Because the right side of the Equation 4 at time T_s becomes constant, it is defined as the brain fractionation index (BFI), namely,

$$BFI = \frac{Cb(T_s)}{\int_0^{T_s} Ca(\tau)d\tau}. \quad \text{Eq. 5}$$

The left side of Equation 4 is the steady-state rate of uptake into the system (*13*), and BFI is considered to reflect K_1. Time T_s is the minimum time to reach an effective steady state and the optimum time to acquire a single SPECT scan and obtain the integral of arterial input value.

On the other hand, because the value of rCBF, F (mL/g/min), depends on K_1, we assume that F is a function Φ in BFI variables, namely, $F = \Phi(BFI)$. The relationship between F and the extraction fraction of ^{99m}Tc-ECD has been reported to be nonlinear (*5*), and, in general, the extraction fraction decreases exponentially as flow increases (*15,16*). Hence, we assume that BFI increases in the following exponential manner as F increases:

$$BFI = \Phi^{-1}(F) = a \times (1 - e^{-F/b}), \quad \text{Eq. 6}$$

where a and b are different constants. Then the function $\Phi(BFI)$ is obtained as the inverse function of Equation 6:

$$F = \Phi(BFI) = -b \times \ln\left(1 - \frac{BFI}{a}\right). \quad \text{Eq. 7}$$

When Equation 6 is prepared beforehand as a BFI-F regression curve by estimating constants a and b using nonlinear least-squares fitting (NLLSF) analysis, F is obtained from BFI using Equation 7.

One-Point Venous Sampling Method. Because the procedure used for arterial blood sampling is invasive and labor intensive, the noninvasive method that was modified from that previously reported (*10,11*) is introduced, in which the integral of arterial input is estimated from venous blood data obtained at the optimum time T_v. By performing sequential sampling of both arterial blood and venous blood after tracer injection on several subjects, Ca(t) and the venous function, Cv(t), of each subject are obtained. By analyzing the relationship between the values of $\int_0^{T_s} Ca(\tau)d\tau$ and Cv(t) of each subject, a regression line, pCv(t) + q (both p and q are constants), is calculated for each time point, and then $\int_0^{T_s} Ca(\tau)d\tau$ is estimated from Cv(t) using the regression line at time t. Optimum time T_v is determined to minimize the difference between the true values of $\int_0^{T_s} Ca(\tau)d\tau$ and its values estimated from Cv(t). The following cost function $\Psi(t)$ (×100%) is minimized for this estimation:

$$\Psi(t) = \frac{1}{n} \times \sum_{i=1}^{n} \frac{\left|\int_0^{T_s} Ca_i(\tau)d\tau - [p_t Cv_i(t) + q_t]\right|}{\int_0^{T_s} Ca_i(\tau)d\tau}, \quad \text{Eq. 8}$$

where n is number of subjects, $Ca_i(t)$ and $Cv_i(t)$ are the arterial input function and venous function, respectively, of subject i, and p_t and q_t are the coefficient and y-intercept, respectively, of the regression line at time t.

Briefly summarizing this method, the values of regional $Cb(T_s)$ and $\int_0^{T_s} Ca(\tau)d\tau$ in Equation 5 are obtained from a single SPECT

scan at time T_s and from a single venous sample at time T_v, respectively; then rCBF values are calculated by substituting the regional BFI values in Equation 7.

Subjects

The subjects of this study consisted of three groups. Informed consent was obtained from each subject. All subjects were nonsmokers.

Group 1. To determine time T_s and obtain the BFI-F regression curve, 16 subjects between the ages of 23 and 75 y (8 men, 8 women; mean age 53.8 ± 16.9 y) were chosen. These subjects included 14 patients with cerebrovascular diseases and 2 healthy volunteers. Among these, 3 subjects (1 subject with middle cerebral artery occlusion, 1 subject with moyamoya disease and 1 healthy volunteer) underwent both SPECT studies for the control state and acetazolamide challenge to examine high flow.

Group 2. To develop the one-point venous sampling method, 15 other subjects between the ages of 26 and 71 y (8 men, 7 women; mean age 53.1 ± 13.9 y) were examined. None of the subjects had heart or pulmonary disease. The subjects consisted of 8 patients with cerebrovascular disease, 5 patients with degenerative disease and 2 healthy volunteers.

Group 3. To validate the values of rCBF obtained by this method, 8 patients with cerebrovascular disease between the ages of 45 and 79 y (4 men, 4 women; mean age 60.0 ± 13.3 y) underwent a single SPECT scan and the one-point venous sampling method and ^{133}Xe inhalation SPECT studies. Two patients among them underwent both SPECT studies for the control state and acetazolamide challenge.

Optimum Time T_s

When both sides of Equation 4 are used, K and u(t) have to be described in words as follows:

$$K = \frac{K_1 \times k_3}{k_2 + k_3} \qquad \text{Eq. 9}$$

$$u(t) = \frac{Cb(t)}{\int_0^t Ca(\tau)d\tau} \qquad \text{Eq.10}$$

Optimum time T_s is determined by calculating the time point when u(t) converts to K. In this study, we performed dynamic SPECT scanning and sequential arterial sampling on the subjects described in group 1 and obtained the K value of the entire brain using the Gjedde-Patlak plot graphic analysis (*13,14*) and u(t) values, then calculated the differences.

The arterial input function Ca(t) of each subject was individually obtained as follows. A dose of 200–600 MBq ^{99m}Tc-ECD obtained from Daiichi Radioisotope Laboratories (Tokyo, Japan), which was commercially supplied in its ^{99m}Tc-labeled form, was injected through a cubital vein during 60 s, then flushed with 20 mL physiologic saline solution during 15 s. Arterial blood was simultaneously withdrawn through a catheter inserted into the radial artery of the opposite side. Blood was withdrawn every 15 s from 0 to 3 min and 4, 5, 6, 8, 10, 20 and 30 min after tracer injection. The fraction of true tracer activity in each arterial blood sample was examined by the octanol extraction method and the two-strip thin-layer chromatography (TLC) method (*11,17*). Ca(t) was the product of activity in an entire blood sample obtained at time t and the value of the octanol extraction rate.

Brain activity Cb(t) was obtained by dynamic SPECT scanning from 0 to 30 min after injection as described later. Placing irregularly shaped regions of interest (ROIs) on the entire brain in the slice of the basal ganglia of dynamic transaxial SPECT images in each subject, we analyzed the values of Ca(t) and Cb(t) using the Gjedde-Patlak plot analysis (*13,14*), obtained the slope of the regression line as the K value of each subject and calculated the u(t) values at each time point. We determined optimum time T_s by minimizing the difference between mean K and u(t) values.

Validation of Regional Brain Fractionation Index by Nonlinear Least Squares Fitting Analysis

We calculated the rate constants K_1, k_2, k_3 and k_5 of the entire brain of each subject described in group 1 by NLLSF analysis. The rate constant k_5 assumes the pathway from the hydrophilic compartment to blood (*5,18*). To determine whether $K_1 \times k_3/(k_2 + k_3)$ (=BFI) reflected K_1, we obtained the relevant values and compared them.

Preparation of Brain Fractionation Index-F Regression Curve

To prepare the BFI-F regression curve, we obtained a single SPECT scan followed by dynamic scanning and ^{133}Xe inhalation SPECT studies on the subjects described in group 1. $\int_0^{T_s} Ca(\tau)d\tau$ values in regional BFI were obtained by sequential arterial sampling, and $Cb(T_s)$ values were calculated by placing irregularly shaped ROIs on the cerebellum; frontal, temporal, occipital and parietal cortices; basal ganglia; thalamus; and centrum semiovale (total 28–30 ROIs) of the transaxial SPECT images. rCBF values were obtained by placing similar ROIs on the ^{133}Xe SPECT images with reference to CT images obtained on the same day. Analyzing the relationship between the regional BFI values and rCBF values, we obtained the exponential regression curve by calculating constants a and b in Equations 6 using NLLSF analysis.

One-Point Venous Sampling Method

To develop the one-point venous sampling method, we performed sequential sampling of both arterial and venous blood after tracer injection for group 2 subjects. After the injection of 200–600 MBq ^{99m}Tc-ECD during 60 s with flushing, arterial input Ca(t) was obtained individually as described. Simultaneously, venous blood was withdrawn through a catheter inserted into the median cubital vein of the same side as was used for arterial sampling. To spontaneously obtain venous blood samples, the catheter was positioned against venous flow and a rubber band was not used. Venous blood was withdrawn 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 20 and 30 min after injection. The fraction of true tracer activity in each venous blood sample was examined by the octanol extraction and TLC methods. The venous function, Cv(t), the product of activity in an entire venous blood sample obtained at time t and the octanol extraction rate, was obtained in each subject. We determined optimum time T_v by minimizing cost function $\Phi(t)$ as previously described.

Validation of Regional Cerebral Blood Flow by ^{133}Xe Inhalation SPECT

To validate this method, we obtained a single SPECT scan at time T_s and a single venous sample at time T_v using the subjects in group 3. The high flow range was also examined by adding acetazolamide challenges. rCBF values obtained by this method were compared with those obtained by the standard ^{133}Xe inhalation SPECT method.

SPECT Data Acquisition

Dynamic scanning was performed at a scan duration of 20 s from 0 to 30 min, and single-SPECT scanning followed at a scan duration of 15 min. A three-head rotating gamma camera

(GCA9300A/HG; Toshiba, Tokyo, Japan) equipped with low-energy super-high-resolution fanbeam collimators was used. The spatial resolution of the system was 8.0 mm full width at half maximum (FWHM) in the center of the field of view. SPECT data were obtained parallel to the orbitomeatal line. Dynamic data were acquired in 64 × 64 matrices at continuous mode, and the single-SPECT data were acquired in 128 × 128 matrices using a step mode of 4 rotating degrees. Images of the former were reconstructed in 64 × 64 matrices—and those of the latter, in 128 × 128 matrices—with 20 simultaneous slices at 5.2-mm intervals using a filtered backprojection algorithm with a RAMP and Butterworth filter. By analyzing the single-SPECT data, both absorption correction by Chang's method (*19*) and scatter correction by the triple energy window method could be performed, but with the dynamic data, only absorption correction was performed.

Acetazolamide Challenges

To examine data in the high flow range, a few days later we performed acetazolamide challenges on 2 patients and 1 healthy volunteer in group 1 and 2 patients in group 3. Acetazolamide (1 g/60 kg body weight) was injected intravenously 10 min before ^{99m}Tc-ECD injection or ^{133}Xe inhalation.

Cross Calibration

To analyze the dynamic and static SPECT counts and arterial blood activity, cross calibration was performed using a series of uniform cylindric phantoms (16 cm in inner diameter × 15 cm in height) composed of water with 1 of 11 concentrations of ^{99m}Tc-pertechnetate. The activity of the SPECT images on the computer was linearly related to the activity concentration in the phantom measured with the well-scintillation counter.

^{133}Xe Inhalation SPECT

Approximately 60 min before ^{99m}Tc-ECD SPECT studies (except for the acetazolamide challenge), ^{133}Xe inhalation SPECT studies were performed with a ring-type SPECT scanner, Headtome SET-050 (Shimadzu, Kyoto, Japan), equipped with a high-sensitivity collimator. The spatial resolution of the system was 19.0 mm FWHM. SPECT data were acquired in 64 × 64 matrices. SPECT images were reconstructed at a 10-mm slice thickness with a filtered backprojection algorithm. Referring to both ^{99m}Tc-ECD SPECT and CT images obtained on the same day, we visually minimized anatomic error by placing ROIs that were irregular in shape and position and were as alike as possible, and we measured rCBF values by the sequential picture method. In the acetazolamide challenge, the studies were performed a few days before or after ^{99m}Tc-ECD SPECT studies using the 1-d protocol method.

RESULTS

Determination of Optimum Time T_s

Figure 2 shows the mean ± SD of the percentage difference between u(t) values obtained at each time point and the K value obtained from the Gjedde-Patlak plot graphic analysis (*13,14*). The difference was 8.1% ± 3.2% at 5 min, 4.1% ± 3.9% at 10 min, 2.4% ± 2.5% at 14 min, 0.34% ± 2.2% at 20 min and −0.50% ± 2.1% at 24 min. The mean difference reached almost zero at approximately 20 min after injection. According to these results, we determined the optimum time T_s to be 20 min and calculated the value of BFI in Equation 5.

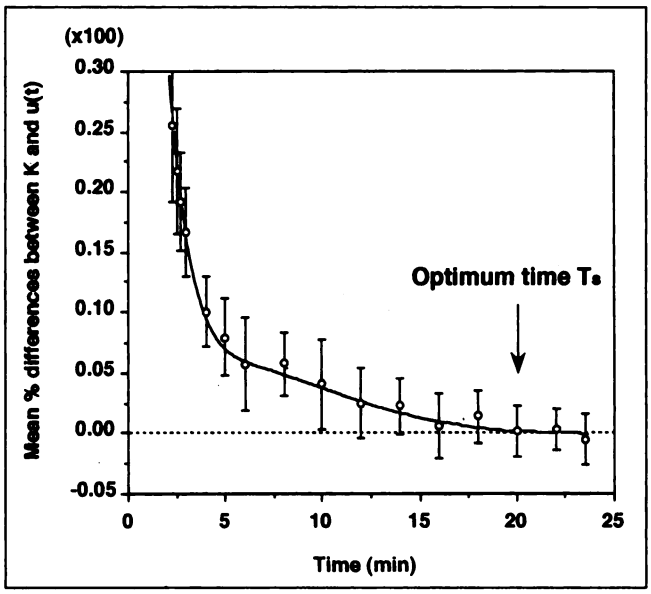

FIGURE 2. Optimum time T_s was graphically determined to minimize difference between K and u(t) in each of 19 examinations of 16 subjects in group 1. Mean % differences ± SD at each time point and curve obtained by triexponential curve fitting analysis in mean values are shown.

Rate Constant Values and Brain Fractionation Index

The means ± SDs of rate constants K_1, k_2, k_3 and k_5 of the subjects in group 1 obtained by NLLSF analysis were 0.254 ± 0.054, 0.121 ± 0.042, 0.367 ± 0.049 and 0.00473 ± 0.00098, respectively. Figure 3 shows a good correlation (r = 0.978) between the values of K_1 and $K_1 \times k_3/(k_2 + k_3)$ (=BFI); thus, we confirmed that BFI represents K_1.

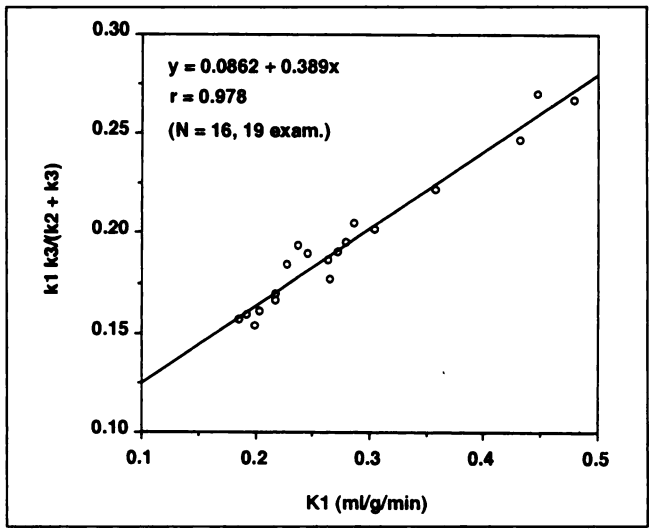

FIGURE 3. Correlation between K_1 and $K_1 \times k_3/(k_2 + k_3)$ obtained from entire brain in group 1 subjects using NLLSF analysis. Because of good agreement, BFI is considered to reflect K_1. K_1 to k_3 are rate constants for transport of tracer between compartments; exam. = examinations.

Regression Curve for Regional Cerebral Blood Flow Measurement

Figure 4 shows the relationship between regional BFI and rCBF (=F) values obtained by the ^{133}Xe inhalation method. Analyzing the correlation by exponential approximation, we found good agreement ($r = 0.907$). Substituting the obtained values a = 0.552 and b = 0.962 in Equation 7, we obtained the following equation:

$$F = -0.962 \times \ln\left(1 - \frac{BFI}{0.552}\right). \qquad \text{Eq. 11}$$

One-Point Venous Sampling Method

Figure 5 shows the correlation between the Ca(t) and Cv(t) values of 15 subjects from 2 to 30 min after injection. Although there was poor agreement for the data obtained at 2 or 3 min, we found good agreement ($r = 0.992$) from 4 to 30 min. Cv(t) was proportional to Ca(t) from 4 to 30 min; thus, Ca(t) could be estimated from Cv(t) in this interval, suggesting the reliability to estimate $\int_0^{T_s = 20\ min} Ca(\tau)d\tau$ from Cv(t) instead of Ca(t).

Examining the relationship between $\int_0^{T_s = 20\ min} Ca(\tau)d\tau$ and Cv(t) at each time point, we obtained good correlations at 4, 5, 6, 7 and 8 min (r = 0.951, 0.982, 0.988, 0.985 and 0.975, respectively). Figure 6A shows the best correlation, obtained at 6 min, as an example. By using the regression line for each time point, the cost function in Equation 8 was calculated. Figure 6B shows the values of the cost function; 11.5% ± 7.9% at 4 min, 7.2% ± 4.2% at 5 min, 6.1% ± 4.7% at 6 min, 6.7% ± 5.3% at 7 min, 8.7% ± 6.2% at 8 min, 9.2% ± 5.4% at 9 min and 11.0% ± 6.7% at 10 min.

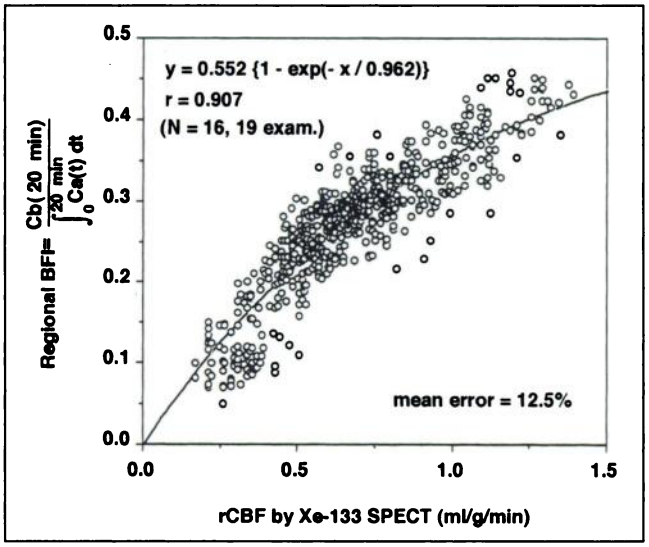

FIGURE 4. Correlation between regional brain fractionation index (BFI) and regional cerebral blood flow (rCBF) (F: mL/g/min) obtained by ^{133}Xe inhalation SPECT in 522 ROIs of group 1. Relation is approximated by exponential curve fitting in Equation 6, calculating constants a (0.552) and b (0.962). Ca(t) and Cb are respective concentrations of ^{99m}Tc-ECD in arterial blood and brain tissue corrected for physical decay. Exam. = examinations; dt = article of integration.

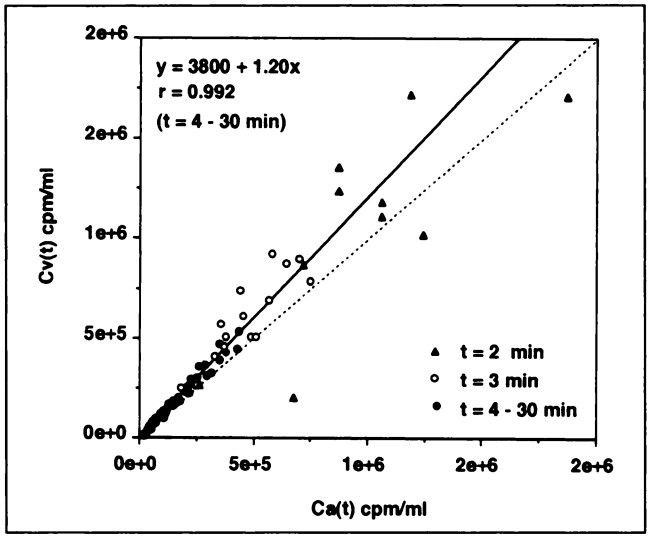

FIGURE 5. Correlation between data of Ca(t) and Cv(t) from 0 to 30 min for 15 subjects in group 2. Good agreement in data is observed from 4 to 30 min, and solid and broken lines show regression line and line of identity, respectively. Cv(t) and Ca(t) are respective concentrations of ^{99m}Tc-ECD in venous blood and arterial blood corrected for physical decay. t = time.

Choosing the lowest cost, we determined optimum time T_v to be 6 min for the one-point venous sampling method. In the following studies, we obtained a venous blood sample at 6 min after injection and calculated $\int_0^{20\ min} Ca(\tau)d\tau$ using the regression line shown in Figure 6A.

Comparison of Regional Cerebral Blood Flow Values

Figure 7 compares rCBF values calculated from regional BFI using Equation 11 and the one-point venous sampling method with those obtained by ^{133}Xe inhalation SPECT in the subjects in group 3. Agreement was good ($r = 0.917$), and the regression line was near to y = x (slope = 1.01), but dispersion was slightly larger in the high flow range. No underestimation of rCBF values was observed in the high flow range. Figure 8 compares rCBF SPECT images obtained with this method and original images before quantification, as an example.

DISCUSSION

We propose a new simple, noninvasive method for accurately quantifying rCBF with ^{99m}Tc-ECD and SPECT based on the three-compartment model. This method has four characteristics: (a) a single SPECT scan is required instead of dynamic planar or SPECT scanning; (b) optimum time T_s for single-SPECT scanning and acquiring arterial input data is determined graphically; (c) the one-point venous sampling method is used to obtain integrals of arterial input instead of sequential or continuous withdrawal of arterial blood; and (d) the parameter regional BFI is accurately transformed to rCBF values using an exponential regression curve, eliminating underestimation of rCBF values in the high flow range. The values of rCBF obtained are

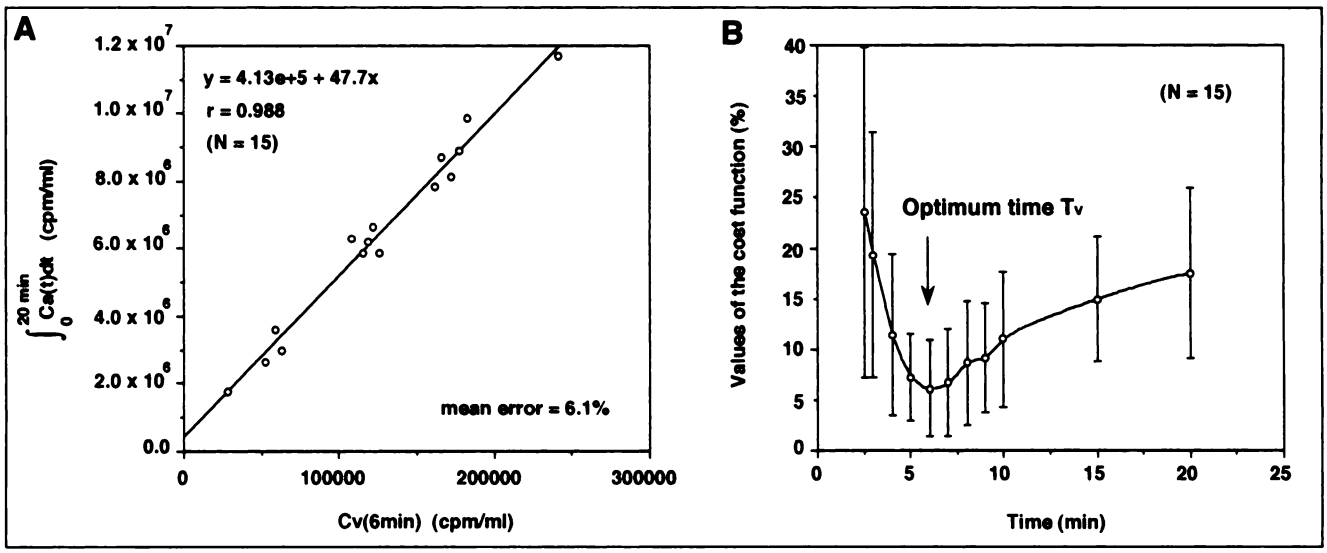

FIGURE 6. (A) Regression line to estimate integral of arterial input from venous sample obtained at 6 min. Good agreement is shown. (B) Mean values ± SD of cost function at each time point. Minimum value had mean error of 6.1% at 6 min, which was optimum time to obtain venous sample. Ca(t) and Cv are the respective concentrations of ^{99m}Tc-ECD in arterial blood and venous blood corrected for physical decay. dt = article of integration.

in good agreement with those obtained by ^{133}Xe inhalation SPECT, indicating the validity of this method.

In this study, we determined optimum time T_s to be 20 min after injection using the graphic method (Fig. 2). Time T_s is the minimum time required to reach an effective steady state (Eqs. 4 and 5). Theoretically, a steady state is obtained when tracer uptake in brain tissue reaches a plateau and the blood plasma concentration is assumed to be zero (*13*). In fact, however, it is not easy to determine the actual time point. By analyzing the data of the entire brain using the graphic method, although regional analysis was still used, we could determine it accurately. Pupi et al. (*4,18*), by analyzing octanol extraction of arterial input, suggested that the time of steady state was 10–20 min after injection, which may confirm our result.

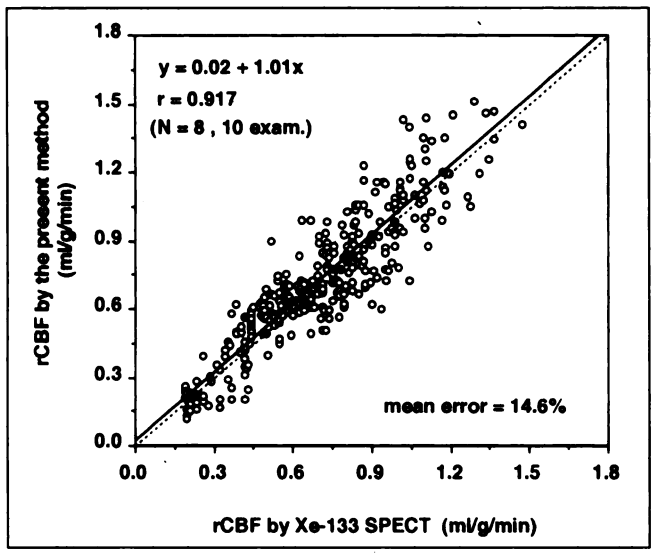

FIGURE 7. Correlation between regional cerebral blood flow (rCBF) values (mL/g/min) obtained by this method and by ^{133}Xe inhalation SPECT method in 280 ROIs of 8 subjects (10 examinations, including 2 acetazolamide challenges). There is good agreement, and slope = 1, showing no underestimation in high flow range. Broken line shows line of identity. Exam. = examinations.

Using the three-compartment model, we assumed that rate constant k_4 was zero. A kinetic model has been reported that considers rate constant k_5 and assumes the pathway from the hydrophilic compartment to blood (*5,20*), but its values were not large in this study. Further, the use of k_5 is still controversial (*7*). Hence, we considered three rate constants, K_1 to k_3. BFI is represented by $K_1 \times k_3/(k_2 + k_3)$ (Eqs. 4 and 5). $K_3/(k_2 + k_3)$ represents the retention fraction, the value of which was reported to be constant in normal tissue (*20*). Because there was a good correlation between K_1 and $K_1 \times k_3/(k_2 + k_3)$ (Fig. 3), although there remains an argument about values in the pathologic tissue, we consider that BFI is proportional to K_1 and that regional BFI values reflect rCBF values.

Regional BFI varies as K_1, but K_1 is rCBF × the extraction fraction of ^{99m}Tc-ECD. Hence, it is necessary to consider the extraction fraction. It is reported that the ^{99m}Tc-ECD extraction fraction depends on rCBF and decreases exponentially as flow increases (*5*), resulting in underestimation of ^{99m}Tc-ECD uptake in brain in the high flow range. Therefore, having considered a means to solve the problem, we introduced an exponential function. When we analyzed the relationship between the values of regional BFI and rCBF obtained by ^{133}Xe inhalation SPECT by approximation with an exponential function (Eq. 6), a good

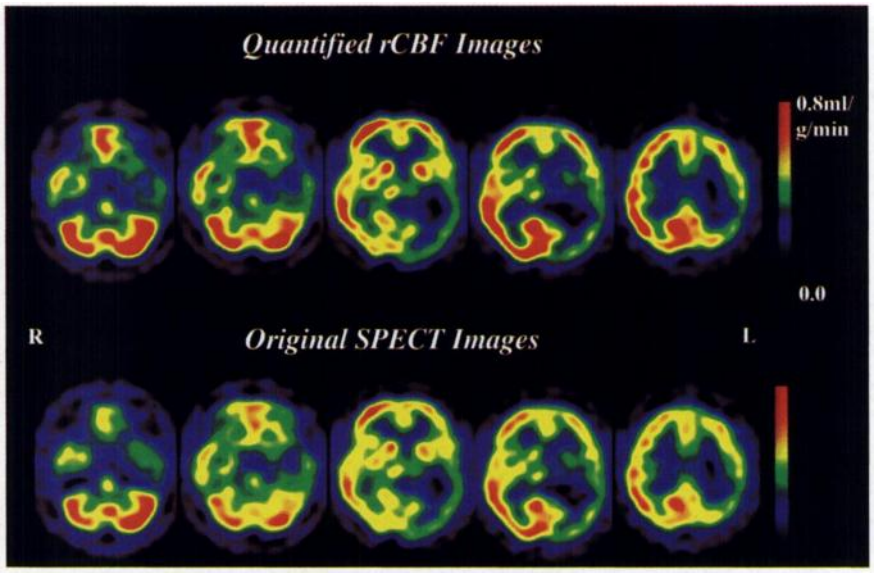

FIGURE 8. Quantified regional cerebral blood flow (rCBF) images obtained by this method (top row) and original SPECT images before quantification (bottom row) of 31-y-old woman with cerebral infarction caused by moyamoya disease after acetazolamide challenge. Note image contrast that is almost same in low perfusion area of left temporoparietal lobe, but is improved in high flow area of cerebellum and right hemisphere.

correlation was obtained (r = 0.907). Using the inverse function derived from it (Eq. 7) as a BFI-F regression curve, we obtained accurate values of rCBF with slightly large dispersion but without underestimation of rCBF in the high flow range (Fig. 7).

To correct the underestimation in the high flow range, we did not use the Renkin-Crone model equation (*15,16*) but introduced a new equation (Eq. 6). The BFI is equal to $K_1 \times k_3/(k_2 + k_3)$. If we assume that the retention fraction, $\alpha = k_3/(k_2 + k_3)$, is constant, we then have BFI proportional to $K_1 = F \times E$, where F is rCBF and E is the extraction fraction of ^{99m}Tc-ECD. When the Renkin-Crone model equation is used, E is described as $E = (1 - e^{-PS/F})$, where PS is the permeability surface area product. Therefore, using the Renkin-Crone model, BFI is described as follows: $BFI = \alpha \times K_1 = \alpha \times F \times E = \alpha \times F(1 - e^{-PS/F})$. This is similar to Equation 6, $BFI = a \times (1 - e^{-F/b})$. Thus, it would be mathematically possible to use the Renkin-Crone model equation in this study. However, it is difficult to obtain the inverse of the Renkin-Crone model equation and to calculate rCBF analytically. Various errors in K_1 caused by several assumptions—$k_4 = k_5 = 0$ and α = constant—and other unknown factors in the compartment model could not be absorbed in PS and α, because PS and α have their own physiologic meanings in the Renkin-Crone model equation. On the other hand, when Equation 6 is used, it is easy to obtain the inverse of function and, thus, to calculate rCBF and to map rCBF images (Fig. 8). Various errors and other unknown factors in the model may be absorbed in constants a and b in the equation. Thus, the equation proposed is more practical than that of the Renkin-Crone model. The detail previously described will be reported soon. In brief, the exponential equation proposed has three advantages: (a) eliminating underestimation of rCBF values in the high flow range, (b) absorbing various errors and (c) easily obtaining the inverse of function and, thus, calculating rCBF.

To estimate the integral of arterial input from venous-blood, we developed the one-point venous sampling method and determined that the optimum time for venous sampling, T_v, was 6 min after injection. Although mean errors for estimation would increase slightly, the data obtained at 5 or 7 min can be used. When preparing regression lines for these time points beforehand, we can calculate the integral of arterial input, even if the time for venous sampling is early or delayed.

Accuracy of Measured Values

The accuracy of measured rCBF values depends on statistical variations in the BFI-F regression curve (Fig. 4) and the one-point venous sampling method (Fig. 6). The mean statistical variation estimated in the former was 12.5%, and in the latter, 6.1%. Hence, the statistical error of rCBF obtained by the current method is estimated at $\sqrt{12.5^2 + 6.1^2} = 13.9\%$. This value was confirmed by the mean percentage error of 14.6% obtained independently by comparison with rCBF from ^{133}Xe inhalation SPECT (Fig. 7). These values of mean error are not small but may be permissible in clinical studies given the simplicity and noninvasiveness of this method.

Limitations

Because the data used for developing the one-point venous sampling method were obtained from subjects who were without heart or pulmonary disease and were nonsmokers, further studies need to be performed in patients with those diseases and who are smokers. The BFI-F regression curve was obtained from the data of 19 examinations of only 16 subjects, including three with acetazolamide challenges with a slightly large dispersion in the high flow range. More examinations are required to more firmly establish the accuracy of the method in the high flow range.

CONCLUSION

The proposed method is able to provide accurate quantification of rCBF by a single SPECT scan and a single venous sampling. The method can be used with any type of SPECT scanner and is useful in routine clinical studies.

REFERENCES

1. Walovitch RC, Hill TC, Garrity ST, et al. Characterization of technetium-99m-L,L-ECD for brain perfusion imaging: part 1. Pharmacology of technetium-99m ECD in nonhuman primates. *J Nucl Med.* 1989;30:1892–1901.
2. Vallabhajosula S, Zimmerman RE, Picard M, et al. Technetium-99m ECD: a new brain imaging agent—in vivo kinetics and biodistribution studies in normal human subjects. *J Nucl Med.* 1989;30:599–604.
3. Greenberg JH, Araki N, Karp A. Correlation between ^{99m}Tc-bicisate and regional CBF measured with iodo-[^{14}C]antipyrine in a primate focal ischemia model. *J Cereb Blood Flow Metab.* 1994;14(suppl 1):S36–S43.
4. Pupi A, Castagnoli A, Teresa RM, Cristofaro D, Bacciottini L, Petti AR. Quantitative comparison between ^{99m}Tc-HMPAO and ^{99m}Tc-ECD: measurement of arterial input and brain retention. *Eur J Nucl Med.* 1994;21:124–130.
5. Friberg L, Andersen AR, Lassen NA, Holm S, Dam M. Retention of ^{99m}Tc-bicisate in the human brain after intracarotid injection. *J Cereb Blood Flow Metab.* 1994;14(suppl 1):S19–S27.
6. Matsuda H, Yagishita A, Tsuji S, Hisada K. A quantitative approach to technetium-99m ethyl cysteinate dimer: a comparison with technetium-99m hexamethyl propylene amine oxime. *Eur J Nucl Med.* 1995;22:633–637.
7. Tsuchida T, Sadato N, Yonekura Y, et al. Quantification of regional cerebral blood flow with continuous infusion of technetium-99m-ethyl cysteinate dimer. *J Nucl Med.* 1997;38:1699–1702.
8. Odano I, Ohkubo M, Noguchi E, Ohtaki H, Shibaki M, Dobashi S. Quantification of cerebral blood flow with ^{99m}Tc-ECD SPECT based on a 3-compartment model. [in Japanese]. *Kaku Igaku.* 1998;35:697–703.
9. Odano I, Ohkubo M. Quantification of cerebral blood flow using technetium-99m ethyl cysteinate dimer and single-photon emission tomography. *Eur J Nucl Med.* 1999;26;246–252.
10. Odano I, Ohkubo M, Takahashi N, Higuchi T. A new method of regional cerebral blood flow measurement using one-point arterial sampling based on the microsphere model with N-isopropyl-p-[^{123}I]-iodoamphetamine SPECT. *Nucl Med Commun.* 1994;15:560–564.
11. Odano I, Ohkubo M, Takahashi M. Quantification of cerebral blood flow and partition coefficient using iodine-123-iodoamphetamine. *J Nucl Med.* 1997;38: 1248–1253.
12. Sokoloff L, Reivich M, Kennedy C, et al. The [^{14}C] deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J Neurochem.* 1977;28:897–916.
13. Patlak CS, Blasberg RG. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data: generalizations. *J Cereb Blood Flow Metab.* 1985;5:584–590.
14. Gjedde A, Wienhard K, Heiss WD, et al. Comparative regional analysis of 2-fluorodeoxyglucose and methylglucose uptake in brain of four stroke patients-with special reference to the regional estimation of the lumped constant. *J Cereb Blood Flow Metab.* 1985;5:163–178.
15. Renkin EM. Transport of potassium-42 from blood to tissue in isolated mammalian skeletal muscles. *Am J Physiol.* 1959;197:1205–1210.
16. Crone C. The permeability of capillaries in various organs as determined by use of the indicator diffusion method. *Acta Physiol Scand.* 1963;58:292–305.
17. Verbeke K, Boonen C, Verbruggen A. Usefulness of residual fractions of L,L-ethylcysteinate dimer (Neurolite) for the preparation of ^{99}Tc^{m}-L,L-ethylcysteinate dimer. *Nucl Med Commun.* 1997;18:535–539.
18. Pupi A, De Cristofaro MTR, Passeri A, et al. Quantification of brain perfusion with ^{99m}Tc-bicisate and single SPECT scan: comparison with microsphere measurements. *J Cereb Blood Flow Metab.* 1994;14(suppl 1):S28–S35.
19. Chang LT. A method for attenuation correction in radionuclide computed tomography. *IEEE Trans Nucl Sci.* 1978;25:638–643.
20. Ishizu K, Yonekura Y, Magata Y, et al. Extraction and retention of technetium-99m-ECD in human brain: dynamic SPECT and oxygen-15-water PET studies. *J Nucl Med.* 1996;37:1600–1604.