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The goal of this study was to develop a simple method for quantification of regional cerebral blood flow (rCBF) with $^{99m}$Tc-ethyl cysteinate dimer (ECD) SPECT. Following an intravenous constant infusion of ECD for one minute, serial dynamic SPECT imaging was performed for 40 minutes in 6 healthy male volunteers with intermittent arterial blood sampling. PET scan with $^{15}$O-water was performed on the same day before the SPECT study for measurement of rCBF. Arterial blood data demonstrated rapid conversion of ECD to the hydrophilic metabolites, and most of the arterial input to the brain was completed within 5 minutes after the injection. Brain activity reached a peak value soon after the cessation of infusion, and was stable thereafter with very little washout. Net extraction of ECD in the brain calculated by arterial input of ECD and rCBF demonstrated a rapid decrease within a few minutes, reaching 42.7% at 5 minutes. The simulation study suggested that the arterial blood activity obtained by continuous drawing for 5 minutes and a single SPECT scan would provide a reasonable estimate of rCBF under the assumption of constant net extraction in the brain.

**Key words:** $^{99m}$Tc-ethyl cysteinate dimer (ECD), regional cerebral blood flow (rCBF), SPECT, quantification, arterial sampling

## INTRODUCTION

$^{99m}$Tc-ETHYL CYSTEINATE DIMER (ECD) was developed as a brain retained tracer for evaluation of cerebral perfusion with SPECT.[1] Compared with $^{99m}$Tc-d,l-hexamethylpropyleneamine oxime (HMPAO), which has already been widely used in clinical practice, *in vitro* chemical stability and less soft tissue background activity make it possible to obtain excellent quality brain perfusion SPECT images.[2] The regional distribution of ECD was reported to be well correlated with regional cerebral blood flow (rCBF) except for cerebrovascular disease with luxury perfusion.[3–5] Absolute quantification of rCBF with ECD was also attempted by kinetic analysis of serial dynamic SPECT scan data based on the compartment model,[6] but this method requires frequent intermittent arterial sampling and complicated kinetic analysis, which make it difficult to carry out in routine clinical studies.

The kinetic behavior of ECD after intracarotid injection demonstrated stable brain activity after the initial fall within a few minutes, which was probably due to the back-diffusion of ECD.[7] As the washout of the radioactivity from the brain was very slow in the later phase, the quantification of rCBF with ECD may be possible with the SPECT images obtained during the period of stable brain activity, if the arterial input function and extraction of the tracer are known. Friberg et al. measured the net extraction of ECD by the intracarotid injection technique. We applied the microsphere model for quantification of rCBF with ECD SPECT by using the fixed net extraction value.

Our final goal is to develop a simple method for the measurement of rCBF by means of a single SPECT scan of ECD. In this report, we describe the characteristics of arterial input function and brain activity after the intravenous injection of ECD obtained in normal subjects, and then demonstrate the feasibility of rCBF measurement with continuous arterial sampling in simulation studies.

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## MATERIAL AND METHODS

*Subjects*

Six normal healthy male volunteers (age: 20–36 year) were studied. None of them had a history of neurological disorders or psychiatric diseases. Informed consent was obtained from all subjects.

A small catheter was placed in the cubital vein of the subject's right arm for administration of the tracer and in the brachial artery of the left arm for intermittent arterial blood sampling. The subject lay in a resting state with eyes open, and the head was immobilized with a head holder.

*Radiotracers*

$^{15}$O-water was synthesized in a small cyclotron (CYPRIS-325; Sumitomo Heavy Industries, Tokyo) and an automated synthesizer installed at Kyoto University Hospital. Approximately 740 to 1100 MBq (20 to 30 mCi) of $^{15}$O-water was administered.

$^{99m}$Tc-ethyl cysteinate dimer (ECD) was prepared in a commercially supplied cold kit (Daiichi Radioisotope Lab., Tokyo). Each subject received 740 MBq (20 mCi) of ECD diluted in 10 m*l* saline solution at a constant speed for one minute by means of an infusion pump.

*PET measurement*

A wholebody PET scanner (PCT-3600; Hitachi Medical Co., Tokyo) was employed for PET scanning.[8] This scanner simultaneously acquires 15 slices with a center-to-center interslice distance of 7 mm. All scans were performed at a resolution of 9 mm full width at half maximum (FWHM) in the transaxial plane and 6.5 mm in the axial direction. The field of view and pixel size of the reconstructed images were 256 mm and 2 mm, respectively. Prior to the administration of $^{15}$O-water, transmission scan was performed with a standard $^{68}$Ge/$^{68}$Ga plate source for attenuation correction.

Emission PET data were acquired for 2 minutes starting at the tracer administration. Arterial blood samples were obtained manually from the left brachial artery every 5 seconds for the first minute and then every 10 seconds until scanning was completed. These blood samples were quickly measured in a scintillation counter to obtain the arterial input curve for each subject. Functional images of rCBF were calculated from the PET images and the individual arterial input curve.[9]

*SPECT measurement*

Serial dynamic SPECT scan was performed for 40 min by means of a triple-head SPECT scanner (PRISM3000; Picker International Inc., Ohio) with high resolution fan beam collimators and a 140 keV ± 10% photo window. The SPECT data were acquired every 30 sec by continuously rotating detectors 120 degrees (40 steps/120 degrees/30 sec). The raw projection data were added to make a total of 20 dynamic SPECT images (1 min × 10 frames, 2 min × 6 frames, 4 min × 4 frames). In addition, static SPECT images were obtained by totaling 20 to 40 min data. All SPECT images were reconstructed by a filtered back-projection algorithm after preprocessing with a Butterworth filter (cut-off frequency 0.25, power factor 4) and displayed on a 64 × 64 matrix. The pixel size was 4.5 × 4.5 mm, and the slice thickness was 7.1 mm. Attenuation correction was performed by assuming the elliptical outline of the head in each slice and uniform attenuation with an attenuation coefficient of 0.1 cm$^{-1}$.

Arterial blood samples were drawn manually every 15 seconds for the first 90 seconds and subsequently at 2, 3, 5, 7, 10, 20 and 40 minutes. In each sample both total radioactivity and lipophilic radioactivity which was estimated by octanol extraction (blood : octanol = 1 : 2) were measured.

*Data analysis*

Calibration of PET or SPECT images with arterial blood data was carried out by cross-calibration of the PET or SPECT counts and the activity measured in a scintillation counter. Cross calibration of PET images was performed with $^{18}$F solution, and that of SPECT images was done with $^{99m}$Tc solution and a cylindrical phantom.

By comparing PET and SPECT images visually, three tomographic slices, corresponding to the levels of centrum semiovale (slice 3), basal ganglia (slice 2) and cerebellum (slice 1), were selected. Irregularly shaped regions of interest (ROIs) were placed manually to cover the whole slice of the brain, cortical gray matter and white matter in slice 3, basal ganglia and thalamus in slice 2, and cerebellum in slice 1 for both PET and SPECT images.

If one can assume complete microsphere-like behavior of ECD, brain activity measured by SPECT ($Cb(t)$) can be expressed by the following simple equation:

$$Cb(t) = F \cdot \int_0^t Ca(\tau)d\tau \qquad (1)$$

where, $F$ is rCBF and $Ca(t)$ denotes arterial input function. When the tracer is not completely trapped in the brain, the net extraction ($Enet(t)$) of ECD is calculated in each frame of dynamic SPECT scan data by using rCBF ($F$) measured by PET as a reference:

$$Enet(t) = \frac{Cb(t)}{F \cdot \int_0^t Ca(\tau)d\tau}, \qquad (2)$$

where $Cb(t)$ is brain activity measured by SPECT.[10] To calculate serial changes in $Enet(t)$, the average brain activity and rCBF in the slice of centrum semiovale were used.

*Simulation study*

In order to evaluate the validity of applying the continu-

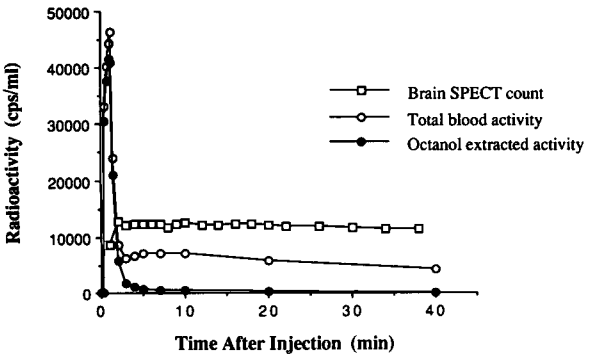


**Fig. 1** Typical time-activity curves of brain SPECT count (open squares), total blood activity (open circles) and octanol extracted blood activity (solid circles) after injection of ECD. The arterial blood activity showed a rapid decrease and the lipophilic activity was almost negligible at 5 min after injection.

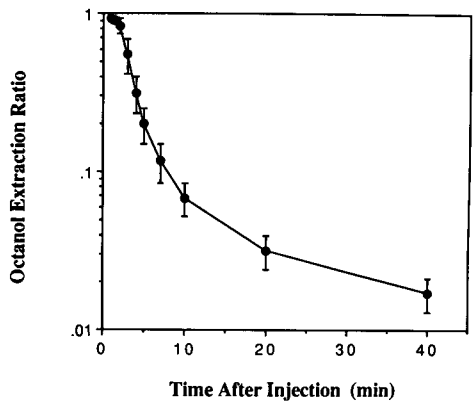


**Fig. 2** Octanol extraction ratio in the arterial blood samples (mean ± s.d. of 6 subjects).

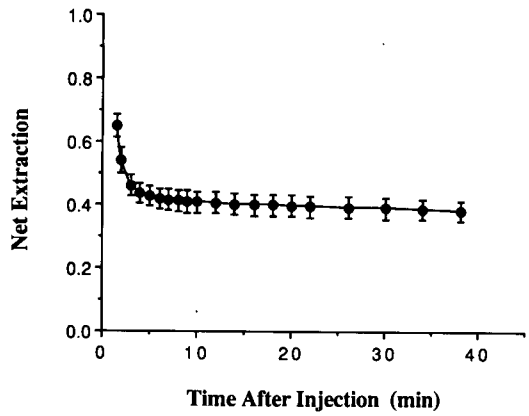


**Fig. 3** Temporal changes of net extraction calculated by brain SPECT counts and arterial input function using rCBF measured by PET as a reference (mean ± s.d. of 6 subjects). The net extraction showed a rapid decrease within 5 min but the value was stable thereafter.

ous drawing method to estimate rCBF with ECD SPECT, a simulation study was performed by using the arterial blood activity obtained in each subject. The total arterial input to the mid-time of SPECT scan was calculated by integration of the serial octanol extracted activities. On the other hand, assuming continuous drawing of the arterial blood by means of a mechanical pump, the total radioactivity during the arterial sampling period was also calculated. The activity ratio of the arterial input to the total blood was obtained for various sampling times. From these data, the optimum duration of the arterial sampling with minimum variation among the 6 normal subjects was evaluated. Finally, rCBF was estimated by assuming a fixed value of 0.44 for the net extraction of ECD reported by Friberg et al.[7] using the brain activity in each ROI of the static SPECT images of 20 to 40 min and the arterial blood data.

## RESULTS

Figure 1 shows typical time activity curves of the brain and the blood. Brain activity reached its maximum just after the completion of tracer infusion and was stable during measurement. On the other hand, the blood activity showed a rapid decrease after cessation of infusion and a slight increase around 5 to 10 min, but the octanol extracted activity did not show this second phase increase, and almost negligible activity remained after 5 min, suggesting the rapid conversion of the parent lipophilic compound to the hydrophilic metabolites. These data suggested that most of the arterial input to the brain was completed within 5 min after the injection. Figure 2 shows the octanol extraction ratio obtained from the 6 subjects. The ratio was greater than 90% during the initial 90 sec, but then rapidly fell to 20.2 ± 5.1% at 5 min, 6.9 ± 1.6% at 10 min, and 1.7 ± 0.4% at 40 min.

Although the brain activity was stable and very little washout was observed during the serial SPECT measurement, the net extraction of ECD in the brain showed a rapid change during the first few minutes after the injection. Figure 3 demonstrates the serial changes in the net extraction of ECD calculated from the brain activity measured by serial dynamic SPECT data and the octanol extracted arterial blood activity. The initial net extraction estimated by the SPECT images for 1 to 2 min was 0.650 ± 0.036, but decreased to 0.461 ± 0.035 at 3 min and 0.427 ± 0.033 at 5 min, and the values were more stable thereafter reaching 0.383 ± 0.031 at the end of the study.

Figure 4a shows the integral of arterial input activity as a function of time, which was normalized by the total input for 40 min in each individual subject. The shape of the normalized curve was quite similar for all subjects. On the other hand, as shown in Figure 4b, the integral of total blood activity normalized by the arterial input in each subject demonstrated a variation among the subjects, especially in the later period, but the variation was small for 5 min after the injection.

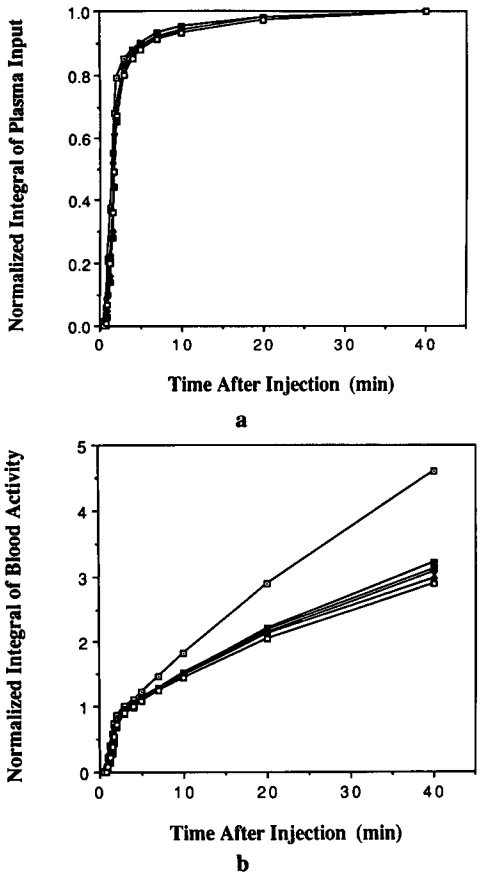


**Fig. 4** a. Temporal changes of the integral of arterial input function in 6 subjects. The value was normalized by the total input activity for 40 min in each subject. The shape was quite similar among the subjects. b. Temporal changes of the integral of total blood activity in 6 subjects. The value was also normalized by the total input activity for 40 min in each subject. The variation among the subjects may be due to the different clearance of hydrophilic metabolites.

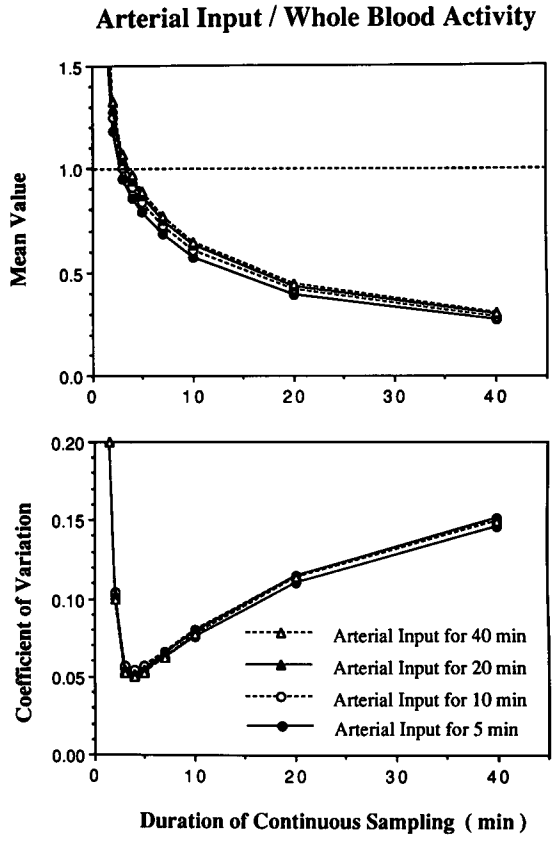


**Fig. 5** The results of simulation study for estimation of arterial input function from the total blood activity. The ratio of the arterial input to the activity in the total arterial blood sample (upper figure) decreased with time due to the increment of hydrophilic metabolites in blood. The coefficient of variation (lower figure) showed minimum values (approximately 5%) between 3 and 5 min.

As the major fraction of arterial input was completed within a few minutes after the injection, the continuous arterial sampling method may be applied for the estimation of arterial input. In order to evaluate the validity of this approach, the optimum duration of arterial sampling was evaluated by a simulation study. Figure 5 shows the results of simulation on the accuracy of the estimation of arterial input from the total blood activity. The ratio of arterial input to total blood activity decreased with time due to the hydrophilic metabolites which contaminated the blood. The total arterial input activities for 5, 10, 20 and 40 min after injection were used for this simulation, but the effect was quite small because the arterial lipophilic activity was almost negligible after 5 min. The data shown in Figure 5 suggested that if one tried to use the whole blood activity of the continuously drawn arterial blood sample as an estimate of the true arterial input, the duration of sampling should not be less than 3 min, which obviously underestimates the arterial input because the lipophilic activity still remains in blood. On the other hand, longer sampling duration causes overestimation because of the large amount of metabolites in blood. The coefficient of variation was minimum between 3 and 5 min, suggesting that the arterial input activity could be estimated from the total blood activity of the continuously drawn arterial blood sample with small errors.

Figure 6 shows ECD SPECT and rCBF PET images in three tomographic slices for comparison. In order to validate the method of rCBF measurement with continuously drawn arterial samples, we calculated rCBF by using the simulated values for total arterial blood activity during the first 5 min. Table 1 shows the rCBF values estimated from the static ECD SPECT images in 6 normal subjects. For this calculation, we used either the individual value for net extraction obtained from the dynamic SPECT or the fixed value of 0.44. Arterial input activity was obtained from the measured octanol extracted activity for 5 min in each subject, or it was estimated from the whole blood counts for 5 min based on assuming the octanol extraction value to be 0.79. Not only the octanol

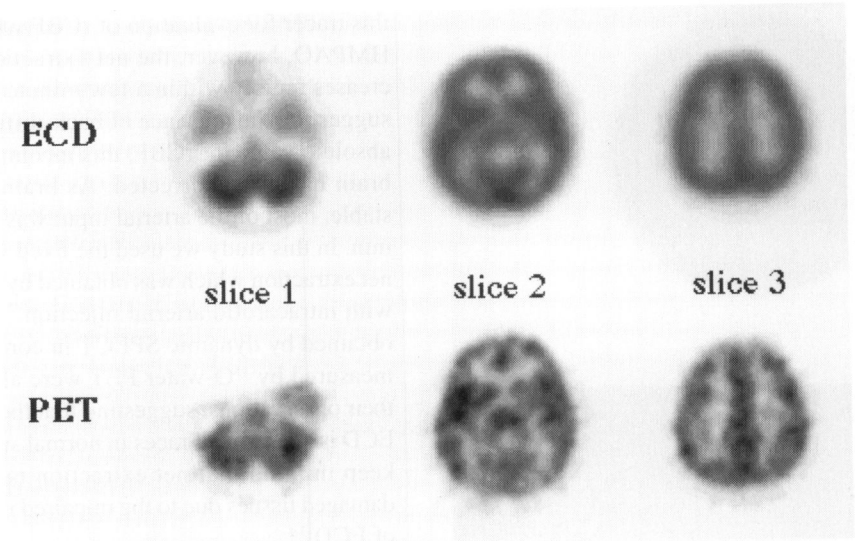


**Fig. 6** Three tomographic slices of the brain used for calculation of rCBF in ECD SPECT and PET.

**Table 1** Regional blood flow (rCBF)[a] calculated by ECD SPECT and continuous arterial sampling in comparison with PET measurement (mean ± standard deviation of 6 normal subject)

| ROI | $^{15}$O-water PET | ECD SPECT | | | |
|---|---|---|---|---|---|
| | | Octanol extracted input function[b] | | Whole blood activity[c] | |
| | | Measured Enet[d] | Enet = 0.44 | Measured Enet | Enet = 0.44 |
| Whole slice | 42.5 ± 4.5 | 42.8 ± 4.4 | 41.4 ± 4.1 | 43.1 ± 5.5 | 41.6 ± 4.4 |
| Cerebral cortex | 49.5 ± 5.1 | 50.6 ± 5.2 | 48.9 ± 4.3 | 50.9 ± 6.2 | 49.1 ± 4.4 |
| Basal ganglia | 49.0 ± 6.1 | 48.4 ± 3.4 | 46.9 ± 3.8 | 48.7 ± 5.2 | 47.1 ± 4.6 |
| Thalamus | 51.9 ± 5.9 | 41.6 ± 3.7 | 40.3 ± 3.6 | 41.8 ± 4.3 | 40.4 ± 3.4 |
| Cerebral white matter | 22.3 ± 4.0 | 25.1 ± 3.0 | 24.3 ± 2.9 | 25.2 ± 3.5 | 24.4 ± 3.0 |
| Cerebellum | 51.6 ± 6.4 | 51.3 ± 4.7 | 49.8 ± 6.2 | 51.5 ± 4.7 | 49.9 ± 5.2 |

[a]: m*l*/min/100 m*l*, [b]: octanol extracted blood activity during initial 5 min was used for calculation, [c]: whole blood activity during initial 5 min multiplied by 0.79 was used for calculation, [d]: net extraction

extracted input function and individual net extraction measured in this study, but also the fixed values for net extraction and octanol extraction provided an excellent estimate of rCBF values in the cerebral cortex and the cerebellum. On the other hand, rCBF was underestimated in the deep gray matter, especially in the thalamus, and overestimated in the cerebral white matter.

## DISCUSSION

Accurate quantification of rCBF is important for clinical evaluation of cerebrovascular disease as well as other neurological diseases. The method of single SPECT scan and continuous arterial blood sampling was designed to obtain rCBF values with a simple procedure which can be performed in clinical practice. The present results also suggested that rCBF can be estimated by this approach with sufficient accuracy by using the fixed value for the net extraction of ECD if the arterial input function can be estimated properly in each individual subject.

Although SPECT imaging of the brain retained tracer provides relative distribution of cerebral perfusion, absolute quantification of rCBF has been limited. This is partly because of the physical limitation of SPECT imaging in quantitative measurement. The most important factor is the attenuation of photons. Compensation for photon attenuation by using the theoretical value for the attenuation coefficient (0.15 $cm^{-1}$ for 140 keV photons) overestimates the count rate due to the large amount of scattered photons.[11] Therefore, in most clinical SPECT studies, the

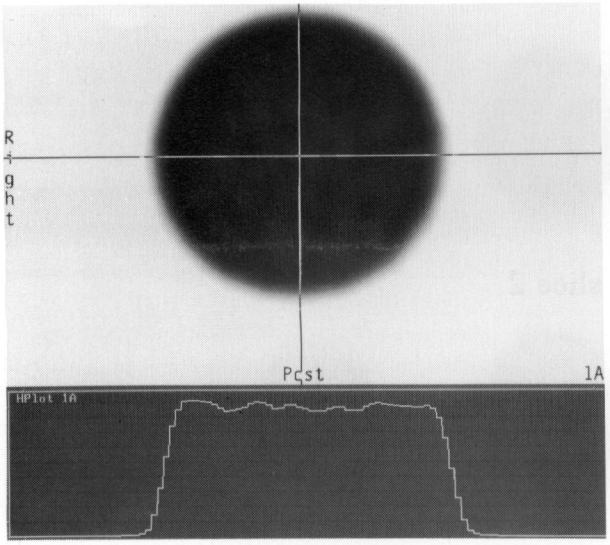


**Fig. 7** SPECT image of cylindrical phantom filled with $^{99m}$Tc solution. The attenuation correction using the attenuation coefficient of 0.1 $cm^{-1}$ provided uniform count rate distribution.

attenuation correction is performed with a smaller value for the attenuation coefficient (0.1 $cm^{-1}$ in this study) to obtain a uniform count rate. Figure 7 shows the SPECT image of the uniform cylindrical phantom, which is used for cross calibration in this study, giving a flat count rate throughout the phantom. However, the accuracy obtained with this simple correction is obviously limited, especially in the deep structures, as shown in this study. Recently developed more sophisticated methods for attenuation and scatter correction would improve the accuracy of SPECT measurement.[11]

In addition to these physical limitations, the complicated kinetic behavior of the tracer and difficulty in obtaining the arterial input function also make rCBF quantification difficult. The brain activity of HMPAO has excellent characteristics as the brain retained tracer and the brain activity does not change with time. Unfortunately the compound is extremely unstable in blood due to the rapid conversion of the parent HMPAO to the hydrophilic metabolites, which makes it difficult to measure the arterial input activity. Several methods have been proposed for measurement of rCBF with $^{123}$I labeled N-isopropyl-*p*-iodoamphetamine (IMP) by taking advantage of its excellent brain extraction and the chemical stability of the compound in blood, but the temporal changes in brain activity due to the long lasting arterial input and slow but significant washout from the brain require some corrections.[12–14] Moreover, the commercial supply of IMP is currently limited in some countries.

The brain activity of ECD is also stable although it shows some washout in the later period. The chemical stability of ECD in blood is far better than HMPAO, and the arterial concentration of parent ECD can be measured by octanol extraction.[15] These advantages allow us to use this tracer for evaluation of rCBF with SPECT. As with HMPAO, however, the net extraction of ECD also decreases rapidly within a few minutes after the injection, suggesting the presence of back-diffusion. To obtain the absolute value for rCBF, this incomplete trapping in the brain has to be corrected. As brain activity was quite stable, most of the arterial input was completed within 5 min. In this study we used the fixed value of 0.44 for the net extraction which was obtained by direct measurement with intracarotid arterial injection.[7] Our present results obtained by dynamic SPECT in comparison with rCBF measured by $^{15}$O-water PET were also compatible with their observations, suggesting that the kinetic behavior of ECD is stable in all races in normal subjects, but we must keep in mind that net extraction may be decreased in damaged tissues due to the impaired retention mechanism of ECD.[3,4]

Incomplete extraction of the brain retained tracer can be explained by two factors: limited first pass extraction and back diffusion of the tracer. Net extraction should therefore also be related to rCBF, and may be different among regions as well as among subjects. Although more complicated methods introducing flow dependent changes in net extraction could also be applied, we believe that the present simple approach will be sufficient for clinical use.

The initial extraction of ECD was reported to be slightly lower than HMPAO[6,16] but the final retention is not so different and the relationship between rCBF and brain uptake showed better linearity.[3] In general, both limited first pass extraction and back diffusion cause underestimation of rCBF in the high flow range, but Tanada et al. demonstrated that the retention fraction of ECD is not directly related to rCBF.[6] This could explain the better linearity obtained with ECD, and the lesser importance of so-called linearization correction although it could provide a better linear relationship with rCBF.[4,17,18]

A more important factor in the quantification of rCBF is the accuracy of the arterial input function. Arterial blood sampling is usually necessary for the brain retained tracer. Although non-invasive methods by means of serial dynamic imaging without arterial sampling were also proposed,[19] accurate quantification of radioactivity is limited with planar gamma camera imaging. The count rate of radioactivity is significantly influenced by the photon attenuation and scattering in planar imaging, which cannot be corrected accurately.

Continuous arterial blood sampling for a short period is a simple technique and can be applied to clinical studies. Moreover, if one could avoid the octanol extraction procedure, the method would become simpler. Our data as well as others demonstrated a rapid increase in hydrophilic metabolites in blood,[5,15,20] but this mainly occurred in liver, brain and other tissues and the conversion rate in blood is relatively low in humans. The effect of conversion is therefore small during the continuous drawing of arterial blood for a few minutes. In the present study, the

shape of the arterial input function was quite similar in all the normal subjects, but it may be different in patients with impaired renal or liver function.

In conclusion, the present method of single SPECT scan and continuous arterial blood sampling is a simple procedure for quantitative measurement of rCBF with ECD. It is expected to play a role in clinical practice for the evaluation of cerebrovascular disease and other neurological diseases, but further investigation is necessary to validate the method in a larger population to examine the variation in net extraction and the input function.

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