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A simplified double-injection method to quantify cerebral blood flow and vascular reserve using iodine-123 IMP-SPECT

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We developed and evaluated a simplified double-injection method for iodine-123 *N*-isopropyl-*p*-iodoamphetamine (IMP) to quantify regional cerebral blood flow (rCBF) twice in a single SPECT session. The method enabled rapid calculations of rCBF with five 10-minute SPECT scans, a fixed distribution volume (V_d), and one-point arterial blood sampling to calibrate a standard input function (SIF). ***Methods:*** Sixty neurological patients were examined to measure rCBF twice in a single session of IMP-SPECT. Patients underwent frequent arterial blood sampling with two injections of IMP and acetazolamide challenge. We generated the SIF and determined the optimal V_d and calibration time (t_{cal}) for the SIF in 30 patients. Validities of the fixed t_{cal} and V_d were assessed in the remaining 30 patients. Simulation studies were also performed to evaluate the error sensitivity of the method. ***Results:*** The optimal t_{cal} and V_d were 34 min and 30 m*l*/m*l*, respectively. The method was robust in rCBF calculation with noisy SPECT data and yielded rCBF with negligible bias and acceptable errors compared with those obtained by the double-injection method previously reported. ***Conclusion:*** The method can be applied to measure rCBF twice in a single SPECT session more easily and less invasively.

Key words: iodine-123-IMP, regional cerebral blood flow, SPECT, cerebrovascular reserve capacity

INTRODUCTION

IT IS IMPORTANT to assess cerebral perfusion reserve in patients with an occlusive disease of the major cerebral arteries to understand hemodynamic compromise and select candidates for revascularization surgery.[1–8] The cerebral perfusion reserve can be assessed by testing cerebral vasodilatory capacity or vascular reactivity, which is shown by an increase in regional cerebral blood flow (rCBF) with a cerebral vasodilative agent such as carbon dioxide or acetazolamide (ACZ).[9–15] Two measurements of rCBF at rest and after ACZ challenge are required to quantify cerebral vascular reserve (CVR), and cerebral perfusion SPECT has been commonly used for this purpose and performed twice on separate days to assess CVR. Quantitative assessment is also required in patients with bilateral decrease in rCBF and/or CVR which may be obscure if analyzed qualitatively.

N-isopropyl-[^{123}I]*p*-iodoamphetamine (IMP) is a cerebral perfusion tracer with high first-pass extraction by the brain and good linearity between its uptake and rCBF,[15–17] and has been proved to yield reliable rCBF values.[18–20] Nevertheless, for precise assessment of rCBF change, studies should be performed in succession during a single procedure to ensure the stability of baseline rCBF and reproducibility of the measurement without moving the patient's head. We showed that the double-injection (DI) method for IMP was feasible for two sequential rCBF measurements in a single procedure and applicable for ACZ challenge,[21] whereas the DI method previously

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reported required intricate nonlinear least squares fitting (NLLSF) procedures with sequential arterial blood samplings and a dynamic SPECT scan. This procedural complexity and invasiveness of the DI method are disadvantages for clinical application. Therefore, from the practical view, the simplified and less invasive protocol of the DI method is needed for daily practice. Several simplified methods for quantifying rCBF have been reported, but most of them were for the single measurement of rCBF,[22–32] and only a few studies have been reported on the DI method.[33,34] The purpose of this study was to develop a simplified version of the DI method to measure sequential rCBF in a single session of IMP-SPECT. We devised a simple method for calculation with a fixed distribution volume (V_d) and a standardized input function that was calibrated by the radioactivity of a single blood sample. The accuracy of this proposed method was evaluated by comparing it with the CDI method previously reported.[21]

MATERIALS AND METHODS

Theory for the proposed method

The two-compartment model is generally used to describe the kinetics of IMP in the brain as follows,

$$dC_b(t)/dt = K_1\, C_a(t) - k_2\, C_b(t) \qquad \text{Eq. 1}$$

where $C_b(t)$ denotes the radioactivity concentration in the regional brain tissue at a time t, $C_a(t)$ the arterial input function, K_1 (m*l*/min/m*l*) the wash-in rate constant from the blood to the brain, and k_2 (1/min) the wash-out rate constant from the brain to the blood. Assuming that the first-pass extraction fraction of IMP is unity and the density of the brain tissue is 1.0 g/m*l*, K_1 and k_2 are related to rCBF (f) (m*l*/min/g) and the regional distribution volume of IMP (V_d) (m*l*/m*l*) as follows,

$$K_1 = f \qquad \text{Eq. 2a}$$

$$k_2 = K_1 / V_d = f / V_d \qquad \text{Eq. 2b}$$

Eq. 1 can be described, after being integrated from t_1 to t_2 with Eqs. 2a and 2b as follows,

$$f = \frac{C_b(t_2) - C_b(t_1)}{\int_{t_1}^{t_2} C_a(t)dt - (1/V_d)\int_{t_1}^{t_2} C_b(t)dt} \qquad \text{Eq. 3}$$

In our new method, we used Eq. 3 and assumed that a fixed value for V_d and a standard input function (SIF), $C_a^{std}(t)$, calibrated with a single blood sample at an appropriate time (t_{cal}) can be used to estimate rCBF without any significant error. In a double-injection (DI) protocol where the tracer is injected at 0 and 30 min and a dynamic SPECT acquisition is performed for 50 min (Fig. 1), the first rCBF from 0 to 25 min and second rCBF from 25 to 45 min (f^{1st} and f^{2nd}, respectively) can be expressed with $C_a^{std}(t)$ calibrated at a given time t_{cal} as follows,

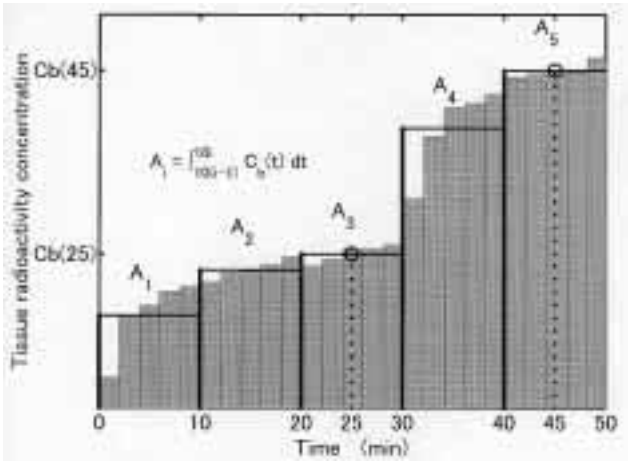


Fig. 1 The schema of procedures of the present method. For calculation of rCBF by the new method, five 10-min SPECT scan data were used, which were obtained by adding every five consecutive frames from 25 dynamic frames. Tissue radioactivity concentrations at mid-scan times of 25 and 45 min were obtained from A_3 and A_5 as average values.

$$f^{1st} = \frac{C_b(25) - C_b(0)}{C_a(t_{cal})\int_0^{25} C_a^{std}(t)dt / C_a^{std}(t_{cal}) - (1/V_d^{1st})\int_0^{25} C_b(t)dt} \qquad \text{Eq. 4a}$$

$$f^{2nd} = \frac{C_b(45) - C_b(25)}{C_a(t_{cal})\int_{25}^{45} C_a^{std}(t)dt / C_a^{std}(t_{cal}) - (1/V_d^{2nd})\int_{25}^{45} C_b(t)dt} \qquad \text{Eq. 4b}$$

where $C_a(t_{cal})$ is an individual radioactivity concentration obtained from a single arterial blood sample at t_{cal}, and the terms, $\int_0^{25} C_a^{std}(t)dt / C_a^{std}(t_{cal})$ and $\int_{25}^{45} C_a^{std}(t)dt / C_a^{std}(t_{cal})$, are constant ($I^{1st}$ and I^{2nd}, respectively). As the terms which contained the tissue radioactivity concentration can be approximately determined from five consecutive 10-minute SPECT frames (A_i, i from 1 to 5), which are summed up from dynamic SPECT frames (Fig. 1), f^{1st} and f^{2nd} are approximated as follows,

$$f^{1st} = \frac{A_3/10}{C_a(t_{cal})I^{1st} - (1/V_d^{1st})\,(A_1 + A_2 + A_3/2)} \qquad \text{Eq. 5a}$$

$$f^{2nd} = \frac{(A_5 - A_3)/10}{C_a(t_{cal})I^{2nd} - (1/V_d^{2nd})\,(A_3/2 + A_4 + A_5/2)} \qquad \text{Eq. 5b}$$

Subjects

We studied 60 patients (mean age 61.3 years old, M : F = 46 : 14) with cerebrovascular disease by the conventional double-injection (CDI) method of IMP previously described.[21] We divided these 60 patients into two groups of 30 (Group 1 and Group 2). We derived the standard input function and optimized a fixed value for V_d and a time t_{cal} for calibration of the SIF in the proposed method from the 30 patients of Group 1 (age: 59.9 ± 12.8 years old). Group 2, including the residual 30 patients (age: 60.0 ± 11.7

years old), served to validate the method with the SIF, the fixed value for V_d and the time t_{cal} for calibration of the SIF.

The study was performed according to the guidelines of the ethical committee of Kyoto University Hospital for human studies, and all subjects gave informed consent.

Data Acquisition

SPECT images were acquired with a three-head rotating gamma camera (PRISM 3000, Picker Inc., Bedford Heights, OH) equipped with low-energy high-resolution fan-beam collimators. A dynamic SPECT scan with continuous acquisition for 50 min (50 one-minute frames) was performed in all subjects. The raw data from 50 one-minute frames were reconstructed to obtain SPECT images of 25 two-minute frames by adding two serial raw data to compensate for radioactivity changes during clockwise and counter-clockwise rotations of the detectors. The images were reconstructed with a filtered back projection algorithm with a ramp filter after prefiltering with a Butterworth filter. The attenuation correction was performed by Chang's method. The SPECT scanner and the well counter were cross-calibrated by scanning a cylindrical phantom containing iodine-123 solution of known concentration. Patients were injected with 111 MBq of IMP (Nihon Medi-Physics Co., Ltd.) in one minute into the right antecubital vein twice at 0 and 30 min after the start of the scan. We used an infusion pump for constant infusions. Patients received a dose of ACZ (1 g/60 kg Body weight) intravenously between 18 and 20 min. Frequent blood sampling was performed manually from the brachial artery in all subjects. The sampling time was every 10 seconds for the first 2 min after each injection of IMP, and then the interval was gradually prolonged. In order to generate an individual arterial input function, a correction for unmetabolized IMP in the blood was performed by using a standard time course of the lipophilic fraction of IMP previously determined in 15 other subjects.

For each patient, one region of interest (ROI) was manually drawn in the right frontal cortex on a selected image slice and a time-activity curve of the brain was obtained. We estimated the first and second rCBF by the CDI method using an individual input function and a time-activity curve of the brain with the NLLSF method according to the following equations,

$$C_b(t) = f^{1st}\exp(-k_2 t)\int_0^t C_a(s)\exp(k_2 s)ds \quad (0 < t < 25) \qquad \text{Eq. 6a}$$

$$C_b(t) = \exp(-k_2(t-25))\left\{C_b(25) + f^{2nd}\int_{25}^t C_a(s)\exp(k_2 s)ds\right\} \quad (25 < t < 50) \qquad \text{Eq. 6b}$$

The ROI data of every 5 consecutive frames from 25 dynamic frames were added to make a new set of data of 10-minute duration (A_i, i from 1 to 5, Fig. 1), which were used to evaluate the proposed method.

Optimization of the fixed V_d and the calibration time for the SIF

The SIF was generated by the numerical average of individual input functions obtained from 30 patients in Group 1. The proposed method assumed a fixed V_d and a given time t_{cal} to calibrate the SIF. Therefore, those values were optimized in Group 1. The optimal t_{cal} was chosen to minimize errors in rCBF calculated by the SDI method with the SIF compared with that obtained by the CDI method with an individual input function. We used three cost functions to optimize the t_{cal}, as follows,

$$\Omega_1(t_{cal}) = (1/30)\sum_{i=1}^{30}\left| f_{SDI}^{1st}(t_{cal})/f_{CDI}^{1st} - 1\right| \qquad \text{Eq. 7a}$$

$$\Omega_2(t_{cal}) = (1/30)\sum_{i=1}^{30}\left| f_{SDI}^{2nd}(t_{cal})/f_{CDI}^{2nd} - 1\right| \qquad \text{Eq. 7b}$$

$$\Omega_3(t_{cal}) = (1/30)\sum_{i=1}^{30}\left\{\left(f_{SDI}^{1st}(t_{cal})/f_{CDI}^{1st} - 1\right)^2 + \left(f_{SDI}^{2nd}(t_{cal})/f_{CDI}^{2nd} - 1\right)^2\right\}^{1/2} \qquad \text{Eq. 7c}$$

where $f_{SDI}^{1st}(t_{cal})$ and $f_{SDI}^{2nd}(t_{cal})$ were the first and the second rCBF obtained by the SDI method, and f_{CDI}^{1st} and f_{CDI}^{2nd} were the first and second rCBF obtained by the CDI method.

The V_d value was also optimized to minimize errors in calculated rCBF compared with that obtained by the CDI method according to cost functions ($\Omega_4(V_d^{1st})$ and $\Omega_5(V_d^{2nd})$) as follows,

$$\Omega_4(V_d^{1st}) = (1/30)\sum_{i=1}^{30}\left| f_{SDI}^{1st}(V_d^{1st})/f_{SDI}^{1st} - 1\right| \qquad \text{Eq. 8a}$$

$$\Omega_5(V_d^{2nd}) = (1/30)\sum_{i=1}^{30}\left| f_{SDI}^{2nd}(V_d^{2nd})/f_{SDI}^{2nd} - 1\right| \qquad \text{Eq. 8b}$$

where V_d^{1st} and V_d^{2nd} were for the first and second parts of the measurement, respectively.

Validation of the new method

The validity of the proposed method with the fixed V_d and the SIF calibrated from the single arterial blood sample drawn at t_{cal} was evaluated in 30 patients in Group 2. We compared the rCBF obtained by the proposed method with the fixed V_d value and the SIF calibrated at the time t_{cal} determined in the previous section with that obtained by the CDI method. Linear-regression analyses were performed to correlate rCBF values obtained by the two methods. The mean and standard deviation (s.d.) of percent differences in rCBF values between the SDI and CDI methods were calculated and plotted.[35]

Simulation study

We also performed simulation studies to validate the accuracy of our method and to know how errors caused by the simplification propagated to the calculated rCBF. First, we evaluated the effects of errors caused by the fixed

V_d. Time-radioactivity curves of the tissue were generated according to Eqs. 6a and 6b with the SIF and various combinations of rCBF (20, 40, 60 m*l*/min/100 g) and V_d (21–39 m*l*/m*l*). rCBF values were then calculated by means of Eqs. 5a and 5b with a fixed value of V_d (30 m*l*/m*l*) and the SIF. Percent errors in the calculated rCBF compared with the actual rCBF were plotted as a function of percent errors in a fixed V_d compared with the actual V_d.

Effects of individual differences in the arterial input function on the rCBF estimates in the present method were investigated with a simulation study. Tissue radioactivity concentration curves were generated with 30 individual input functions (Group 2) for various rCBF values (20, 40, 60 m*l*/min/100 g), and then rCBF values were calculated by the proposed method referring to the standard input function calibrated by the one-point radioactivity concentration at t_{cal}. V_d values for the first and second measurements were also assumed to be 30 m*l*/m*l*. Then the mean and s.d. of the difference in calculated rCBF were plotted as a function of rCBF values.

Another simulation study was performed to evaluate the effects of errors in time integrated input functions on the calculated rCBF in the proposed method. Simulation curves of regional brain time-radioactivity were generated according to Eqs. 6a and 6b with the SIF for various rCBF values (20, 40, 60 m*l*/min/100 g). We used the V_d value of 30 m*l*/m*l* for both the first and second measurements in this simulation. For these simulation curves, rCBF values were calculated according to the SDI method (Eqs. 5a and 5b) by using the SIF with various errors (−10 to +10%). Percent errors in calculated rCBF compared with actual rCBF were plotted as a function of percent error in the time integrated SIF.

We also investigated the error sensitivity of calculated rCBF to the noise level of SPECT data. Simulated noisy curves of tissue radioactivity concentrations were generated by adding Gaussian noise (the mean of 0 and the s.d. of 1, 3, 5 and 10%) to the noiseless curves that were generated according to Eqs. 6a and 6b with the SIF, the V_d value of 30 m*l*/m*l* and rCBF of 20, 40 and 60 m*l*/100 g/min. For these noisy curves, the first and the second rCBF values were calculated by using both the SDI method and the CDI method. Percent errors in calculated rCBF compared to actual rCBF were plotted as a function of the noise level added to SPECT data.

RESULTS

Optimal V_d and t_{cal} for the proposed method

The standard input function (SIF) generated from 30 patients in Group 1 is shown in Figure 2. Individual variations in input functions were large around the peaks. The cost functions (Eqs. 7a, 7b, and 7c) were minimized when the SIF was calibrated at 4 min for the first rCBF (7.3% as expressed by the absolute percent error), 34 min for the second rCBF (11.0%) and 34 min both for the first and second rCBF (13.7%) after the start of the scan (Fig. 3). Because the absolute error for the first rCBF at 34 min was 8.2%, which was not so different from the 7.3% seen at 4 min, we determined the optimal t_{cal} to be 34 min and used it for analyses thereafter.

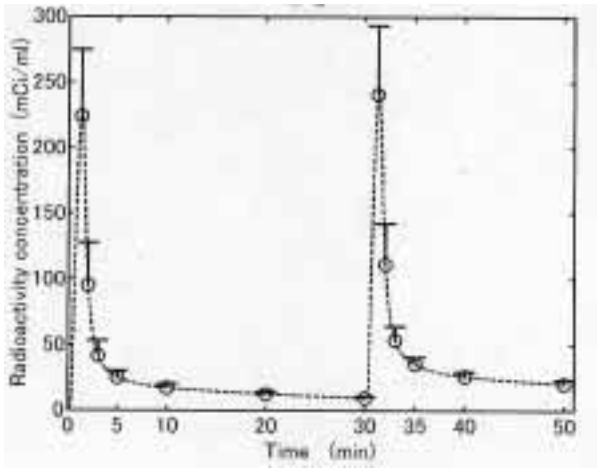


Fig. 2 The standard input function (SIF) was obtained as the average of individual input functions (n = 30, Group 1). Error bars show the s.d. of individual input functions at each time point.

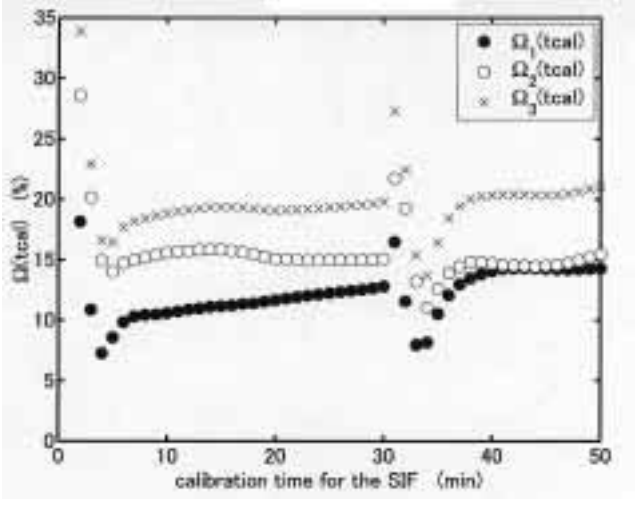


Fig. 3 The plots of results obtained from the cost functions defined by Eqs. 7a, 7b and 7c are shown. The minimum errors of 7.3% and 11.0% in rCBF are obtained at the calibration time of 4 min for the first measurement and 34 min for the second measurement, respectively, after the scan start.

The optimal values for V_d for the first and second measurements were both in the range from 30 to 35 m*l*/m*l* (Fig. 4), where a change in the cost function was very small. We therefore used 30 m*l*/m*l* as the optimal V_d value thereafter. The effect of errors in a fixed V_d on rCBF was slightly larger for the second measurement (approximately 11%) than for the first one (approximately 8%).

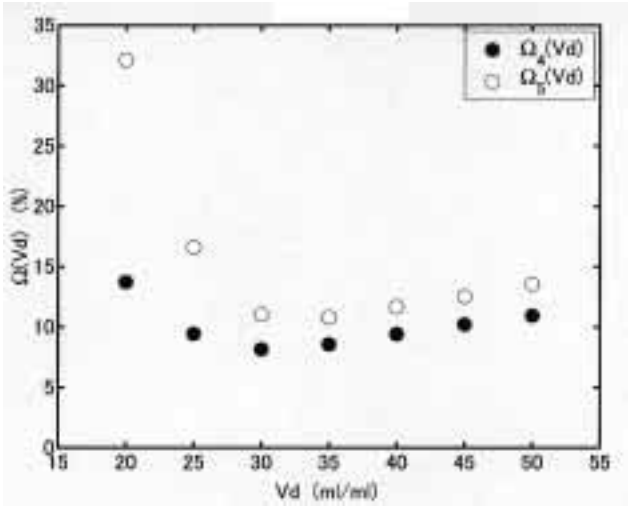


Fig. 4 The plot of the cost function defined by Eqs. 8a and 8b as a function of V_d (n = 30, Group 1). The optimal values of V_d for the first and second measurement were both 30–35 m*l*/m*l*.

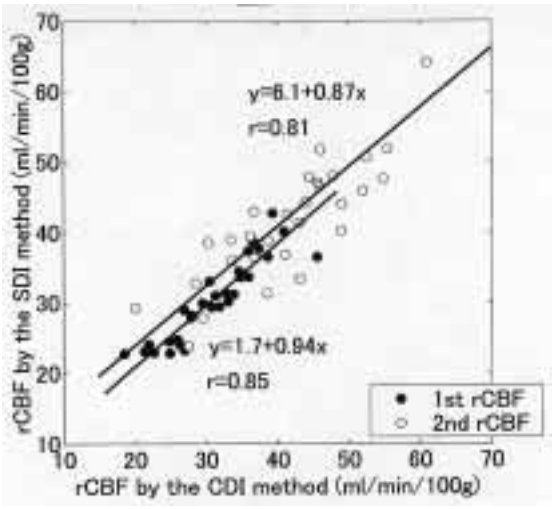


Fig. 5 Comparison of rCBF values obtained by the proposed method with those by the conventional double injection (CDI) method. Data were obtained from 30 patients of Group 2. Each mark corresponds to each subject. The SDI method used the fixed V_d value (30 m*l*/m*l*) and used the SIF calibrated by one blood sample (at 34 min). The solid line denotes the linear-regression line. Significant correlation was confirmed in each pear of comparison between the two methods.

Validation study with patients data

In Group 2 (n = 30), the mean and the s.d. of rCBF values calculated by the proposed method (30.5 ± 6.5, 43.0 ± 11.4 m*l*/min/100 g, for the first and second measurements, respectively) with the SIF, t_{cal} (34 min) and V_d (30 m*l*/m*l*) were approximately equivalent to those obtained by the CDI method (30.8 ± 5.9, 42.4 ± 0.6 m*l*/min/100 g, for the first and second measurements, respectively). Significant linear correlations were observed between the SDI method and the CDI method for both the first and second rCBF (Fig. 5). The mean and the s.d. of differences between rCBF calculated with the SDI method and that with the CDI method plotted against the average rCBF of the two methods (Fig. 6) were −0.22 ± 3.42 m*l*/min/100 g for the first rCBF, and +0.61 ± 6.90 m*l*/min/100 g for the second rCBF. It should be noted that negligible biases and the acceptable errors were observed.

Effects of an error in the fixed V_d value

Results of the simulation study assessing the effects of error in the fixed V_d value compared with an actual V_d on the calculated rCBF are shown in Figure 7. Errors in the calculated rCBF increased in a high rCBF range, particularly for the second measurement (Fig. 7b).

Effect of individual differences in input function on rCBF

Effects of individual difference in the arterial input function on the rCBF estimates in the presented method were estimated. The mean ± s.d of percent error for the first and second rCBF were −0.01 ± 8.57 (%) and 1.27 ± 7.15 (%) at a rCBF of 20 m*l*/min/100 g, 0.01 ± 9.62 (%) and 1.50 ± 8.47 (%) at a rCBF of 40 m*l*/min/100 g, and 0.01 ± 10.76 and 1.76 ± 9.88 at a rCBF of 60 m*l*/min/100 g, respectively. The percent errors increased slightly in a higher range of rCBF.

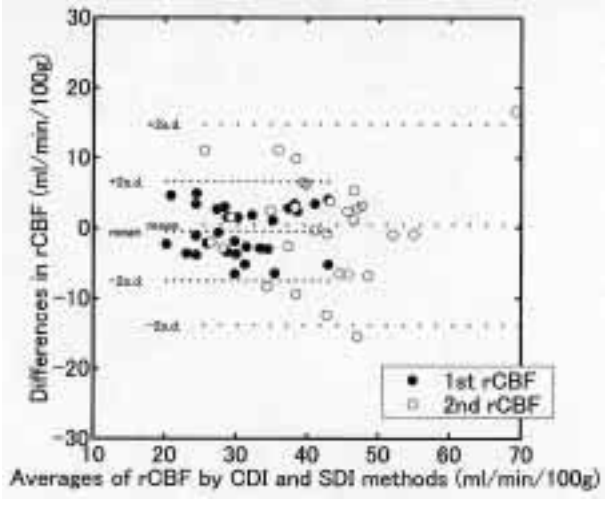
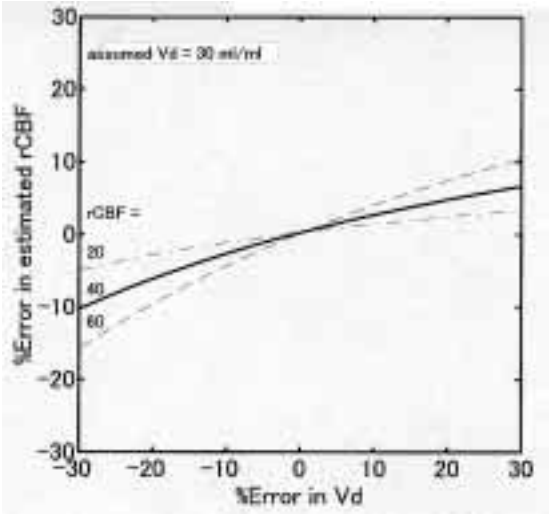


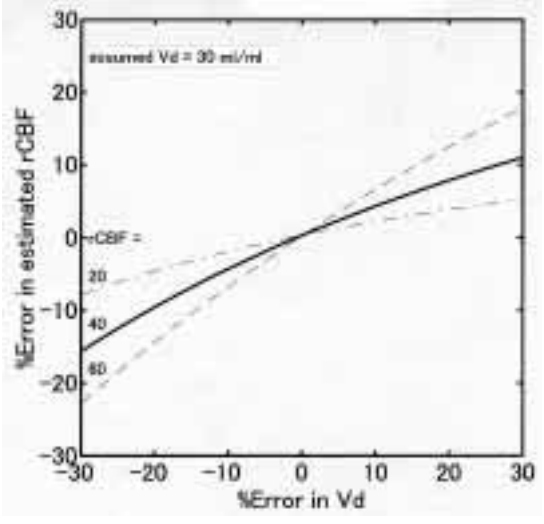
Fig. 6 Differences between the rCBF values obtained by the SDI method and the CDI method are plotted against average rCBF of the two methods (Blant-Altman's plot).[35] The SDI method used the fixed V_d value (30 m*l*/m*l*) and used the SIF calibrated by one blood sample (at 34 min). It should be noted that the negligible bias and the acceptable error were observed.

Effects of an error in the SIF

Effects of an error in the time-integrated input function on the calculated rCBF were shown for the first and the second measurements in Figures 8a and 8b, respectively. A ten % error in the time-integrated input function corresponds to −13 and −15% errors in the first and second rCBF values, respectively, for a rCBF of 40 m*l*/min/100 g.



a

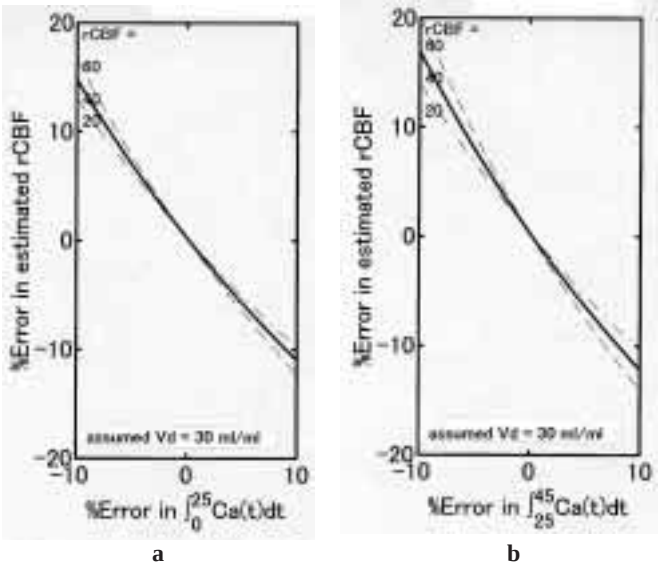


b

Fig. 7 Percent errors in rCBF values calculated by the SDI method with a fixed V_d are plotted for various rCBF values (20, 40, and 60 m*l*/min/100 g) as a function of percent errors in a fixed value from the actual V_d value. (a) for the first rCBF. (b) for the second rCBF. Errors in rCBF increase in a high rCBF range, particularly for the second measurement (b).

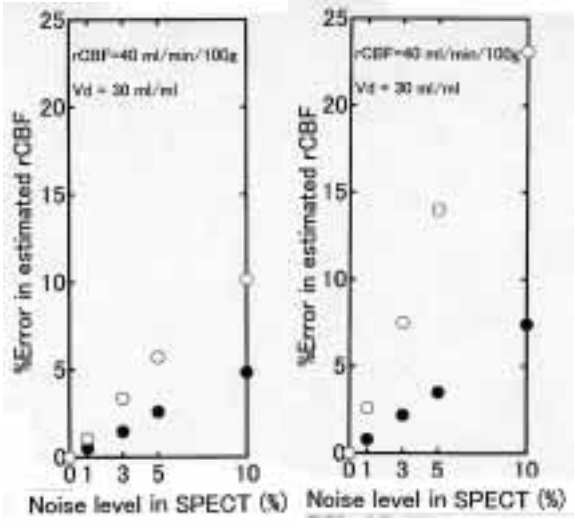
Effects of a noise level on the tissue curve

Figure 9 shows the results of the simulation study that demonstrate the effects of SPECT noise on the calculated rCBF values for the proposed method (a) and the CDI method (b). The fluctuations in calculated rCBF values vary depending on the noise level in SPECT data. In the SDI method, a 10% error in the SPECT data corresponds to approximately 5% and 10% errors in the first and second rCBF values, and corresponds to 7% and 23% errors, respectively, in the CDI method. The proposed method yielded rCBF more robustly than the CDI method when noisy data were analyzed.



a b

Fig. 8 Effects of an error in time-integrated input function on the calculated rCBF in the proposed method are shown for rCBF values of 20, 40 and 60 m*l*/min/100 g. V_d value was assumed to be 30 m*l*/m*l*. A 10% error in the time-integrated input function corresponds to –13 and –15% errors in the first and second rCBF values, respectively, for a rCBF of 40 m*l*/min/100 g.



a b

Fig. 9 Effects of the noise level in SPECT data on the estimated rCBF values are shown for the proposed method (a) and the CDI method (b). V_d value was assumed to be 30 m*l*/m*l*. In the SDI method, a 10% error in the SPECT data corresponds to approximately 5% and 10% errors in the first and second rCBF values, and corresponds to approximately 7% and 23% errors, respectively, in the CDI method.

DISCUSSION

We developed a simplified version of the DI method to avoid drawbacks and to preserve benefits of the CDI method.[21] The advantages of the proposed method are

summarized as follows: (i) yielding robust values for rCBF even with noisy SPECT data, (ii) enabling easy and rapid calculation of rCBF with considering the washout effect of IMP from brain tissues with a fixed V_d value, (iii) reducing invasiveness by using the SIF calibrated with just one arterial blood sample for quantification of two consecutive rCBF values in different conditions. Nevertheless, there are also some drawbacks affecting the accuracy of calculated rCBF caused by simplifications.

Effect of individual difference in the input function

Measurement of the individual input function is essential for the accurate quantification of rCBF. Continuous or frequent arterial blood sampling is usually performed for this purpose, which requires complicated and invasive procedures. A simplified and less invasive method with one-point or, if possible, no blood sampling is preferable for routine clinical use.[31,32] But in general the simplification is achieved at the sacrifice of accuracy in calculating rCBF.

The results of the simulation study suggested that two-point blood samplings at 4 and 34 min might be theoretically the best protocol for the SDI method. But requiring the two arterial punctures in a study is thought not to be less invasive and one-point sampling is more preferable in a clinical setting. The results demonstrated that the SDI method with one-point arterial sampling at 34 min approximately preserved the accuracy of that with two-point samplings at 4 and 34 min (absolute error; 13.7% vs.13.2%). Therefore, one-point calibration at 34 min seems to be practical in a routine clinical setting. Even if the sampling is performed at 33 or 35 min (sub-optimal timing), the difference in the absolute error in rCBF is 1.7 or 2.7%, respectively, which is thought to be negligible, so that the actual time of the blood sampling can be delayed for up to one minute.

In our study, the error caused by the use of the SIF was estimated by the simulation study to be 9.6% for the first rCBF and 8.5% for the second rCBF. These errors were comparable to those in previous reports.[22] It was reported that an approximately 10% error was caused by the difference in individual input function from the SIF and was the most dominant factor in limiting the accuracy of the method.[22]

We used the standard time-course of the octanol extraction fraction of IMP in the blood instead of the individually measured timecourse of it in this study to generate an individual input function from the radioactivity timecourse for the whole blood. The inter-subject variances in the lipophilic fraction were neglected in this study. The personal difference in the lipophilic fraction may cause variation in the shape of true input function, and therefore may cause an error. Another cause of variation in the shape of the input function could be the difference of the lung clearance rate of IMP, particularly in elderly and diseased populations.[36]

Effect of variation in measured V_d

The variance of measured V_d was found in the CDI method among subjects of this study and also in a previous report.[21] Possible causes of the variation in measured V_d in the CDI method are (i) physiological variation among subjects in normal brain tissues, (ii) difference in the regional V_d of pathological tissues from the normal one, and (iii) fitting error due to noisy SPECT data with short frame duration. In a previous report, the physiological variation in V_d value was estimated to be about 10%[22] and the clinical significance of pathological tissues was also suggested in the difference in the regional V_d value.[24] In addition to these factors, the total scan duration of the DI method may be too short to fit k_2 robustly, and noisy tissue data resulting from limited scan duration of each frame may also cause an error in k_2 estimates. In the previous report, the accuracy of k_2 estimates was shown to become worse as the scan duration became short.[21] Since the K_1 value is much greater than k_2 in the kinetics of IMP, the variation in V_d may be predominantly affected by the variation in k_2. The proposed method with the optimal V_d value may provide more robust calculation of K_1 despite the short scan duration, but in the SDI method, the simulation showed that the effect of the error in V_d on the rCBF value for the second measurement was greater than that for the first one. Further study would be needed to investigate whether V_d should be fixed or not.

Fluctuation in tissue activity

The degree of fluctuation in the tissue radioactivity concentration in the dynamic scan with short frame duration of 2 min/frame was estimated in the previous report by our group.[37] Even if a large ROI is selected for the estimation of rCBF, a fluctuation of the tissue radioactivity concentration due to short frame duration would not be negligible, which may lead to substantial error in the calculated rCBF value.

It was shown in the simulation study of the proposed method that an error of 10% in the tissue time-radioactivity curve caused an error in the first and second rCBFs of approximately 5% and 10%, respectively at rCBF = 40 ml/min/100 g, which were smaller than those of the CDI method (approximately 7% and 23%, respectively). This may be explained by the robust character of the SDI method that utilizes summed SPECT data with better statistics than the noisy dynamic SPECT data used in the CDI method.

The advantage of simplification of the DI method

To determine the tissue radioactivity concentration immediately before the second injection of IMP is essential for the calculation of rCBF in the second part of the DI method. In the proposed method, we used the 10-min SPECT data with a mid-frame time of 25 min, which therefore was used for calculating both the first and second measurements of rCBF. This multipurpose use of

the data like this was incorporated into the proposed method in order to simplify the calculation and shorten the total scan duration.

Various methods have been reported for the quantification of rCBF by means of IMP SPECT with fast calculation techniques, such as the table lookup method[24–26] and the graphical method,[27,38] etc. The proposed method uses the fast calculation techniques for rCBF, which are totally different from and simpler than those previously reported. The microsphere method is also simple and enables easy and fast calculation of rCBF, which is achieved by the assumption that the washout of IMP from brain tissues is negligible in the early phase after the injection of IMP.[33] In a recent study of the DI method to which the microsphere model was applied, the washout effect was optionally considered by using a fixed V_d value,[34] but the rCBF estimates with the microsphere model in which the washout effect has been incorporated becomes no longer very simple. The proposed method overcame the drawbacks of this complexity in calculating rCBF values while preserving the accuracy of the method by incorporating the effects of washout with a fixed V_d. The calculation of rCBF with the present method is so simple that it does not require specific software for the analysis, such as least squares fitting and table lookup systems.

In conclusion, the simplified version of the DI method, which is proposed as a practical alternative to the conventional DI method, is easy to perform in the clinical setting and may be useful for quantifying both rCBF and cerebrovascular reserve.

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