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A method for the quantification of benzodiazepine receptors by using ^{123}I-iomazenil and SPECT with one scan and one blood sampling

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Iodine-123-iomazenil (Iomazenil) is a ligand of central type benzodiazepine receptors for single photon emission computed tomography (SPECT). Previously we reported a simple, table look-up method for quantification of its binding potential (BP) by using two SPECT scans and calibrated standard input function with one blood sampling. This method is based on a two-compartment model (K_1: influx rate constant; k_2: efflux rate constant; V_d (= K_1/k_2): the total distribution volume corresponding BP), and requires two SPECT scans for calculating both K_1 and V_d values. If the K_1 value in the two-compartment model can be assumed to be constant, the radioactivity of one SPECT scan at 180 min after injection can be considered to tabulate as a function of V_d for a given K_1 value and a given input function, and a table look-up procedure provides the corresponding V_d value. The purpose of this study was to develop a simple, autoradiographic method for quantification of BP by using one SPECT scan and calibrated standard input function with one blood sampling. SPECT studies were performed on 14 patients. A dynamic SPECT scan was initiated following an intravenous bolus injection of Iomazenil. A static SPECT scan was performed at 180 min after the injection. Frequent blood sampling from the brachial artery was performed on all subjects to determine the arterial input function. Simulation studies revealed that errors in calculated V_d values were around ± 10–15% for varied K_1 values. A good correlation was observed between total distribution volume values calculated by three-compartment model analysis and those calculated by the present method (r = 0.90), supporting the validity of this method. The present method is simple and applicable for clinical use, and will be able to provide images of BP.

Key words: iodine-123-iomazenil, SPECT, benzodiazepine receptor, brain

INTRODUCTION

Iodine-123-iomazenil (Iomazenil), a ligand for central type benzodiazepine receptors, is suitable for single photon emission computed tomography (SPECT) measurement due to its high affinity to benzodiazepine receptors in the brain and small non-specific binding.[1–4] Changes in central benzodiazepine receptor binding have been reported in some diseases.[5–15] Quantitative assessment of neuroreceptors using positron emission tomography (PET) and SPECT has been reported for dopamine D_2 receptors[16–20] and central type benzodiazepine receptors.[21–28] Although the binding potential (BP equal to B_{max}/K_D, B_{max}: receptor density; $1/K_D$: affinity to receptors)[16] can be obtained by kinetic analysis with a single bolus injection,[21,22,24,26–30] this method necessitates dynamic scan and frequent arterial blood sampling with separation of unchanged radioligand for each sample, and therefore is not applicable for routine clinical use.

We previously reported a simple, table look-up method for quantification of BP of Iomazenil using two SPECT scans and calibrated standard input function with one blood sampling,[31] which was originally developed as a method of measuring cerebral blood flow (CBF) and distribution volume using N-isopropyl-p-[^{123}I]iodoam-

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phetamine and SPECT.[32–35] This method is based on a two-compartment model (K_1: influx rate constant; k_2: efflux rate constant; V_d (= K_1/k_2): the total distribution volume corresponding BP), and requires two SPECT scans for calculating both K_1 and V_d values, but, limited reproducibility of selecting regions-of-interest in the same location on two SPECT images (early and delayed scan images) might hamper accurate calculation of V_d values. Since the registration between two SPECT images is difficult, it will also be difficult to calculate an image of V_d by the table look-up method.

If the K_1 value can be assumed to be constant, the V_d value can be obtained from one SPECT scan, which will remove the error caused by limited reproducibility in selecting regions-of-interest and allow V_d images to be calculated. In addition, it will improve the throughput of clinical SPECT examinations. The purpose of this study was to develop a simple, autoradiographic method for quantification of BP by using one SPECT scan and calibrated standard input function with one blood sampling.

MATERIALS AND METHODS

Subjects

SPECT studies were performed on 14 patients with cerebrovascular diseases, dementia or brain tumors (age range, 33–71 yr; mean age ± SD, 56.0 ± 12.2 yr). None of the patients had any heart, renal or liver diseases. All patients received potassium iodide to minimize thyroid uptake of free radioactive iodine. Informed consent was obtained from each subject. The project was approved by the Clinical Test Committee of the Institute of Development, Aging and Cancer, Tohoku University. No side effects were observed in any of the subjects after administration of Iomazenil.

SPECT study

Iomazenil was supplied by Nihon Medi-Physics Co., Ltd. The specific activity of Iomazenil was 92.5 GBq/μmol. The amount of the ligand administered was 0.5 μg (1.2 nmol)/111 MBq. Radiochemical purity was more than 95%.

A dynamic SPECT scan was initiated following an intravenous bolus injection of 111 (one subject) or 167 MBq (13 subjects) of Iomazenil. The dynamic scan sequence consisted of sixteen 200 second scans with 360° continuous rotation of the camera. A static SPECT scan was performed at 180 min after the injection. Frequent blood sampling from the brachial artery was performed on all subjects to determine the arterial input function, and the radioactivity concentrations of the octanol-extracted component from whole blood were measured. In seven subjects, the radioactivity of the octanol-extracted component from plasma were also measured. In this study, the radioactivity concentrations of the lipophilic component extracted by octanol from plasma were considered as representative of true arterial input function.[28,31,36] The arterial input function for each subject was calculated by using mean ratios of the radioactivity of the octanol-extracted component from whole blood to that from plasma in these seven subjects, same as our previous report.[31]

One SPECT scanner (SPECT-2000H, Hitachi Medico Corp., Tokyo, Japan),[37] a four-head rotating gamma camera with in-plane and axial resolutions of 14 mm full width at half maximum (FWHM), was used for all measurements. Image reconstruction was performed by filtered backprojection using a Butterworth filter and attenuation correction was made numerically by assuming the object shape to be an ellipse for each slice and the attenuation coefficient to be uniform (0.08 cm^{-1}).[38,39] Image matrix size was 64 × 64. Correction for scattered photons was not performed. Image slices were set up parallel to the orbitomeatal (OM) line and obtained at 8 mm intervals through the whole brain. A cross-calibration scan was performed using a cylindrical uniform phantom 16 cm in inner diameter to calibrate sensitivity between the SPECT scanner and the well counter system. X-ray CT scans were also obtained with the same slices as those of SPECT images in all subjects.

Image analysis

Data analysis was performed on a UNIX work station (TITAN-750, Kubota computer Corp., Tokyo, Japan). Regions-of-interest in the cerebellum, brain stem, thalamus, basal ganglia, centrum semiovale and cerebral cortex regions including the frontal, temporal, parietal and occipital lobes were outlined on SPECT images, by referring to X-ray computed tomographic images of the patients. The shapes of regions-of-interest were circular with a 20 mm diameter for the brain stem, and elliptic with a short axis of 16–20 mm and a long axis of 28–40 mm for other regions.

Theory (Autoradiographic method)

In the present method, a two-compartment model was employed in line with our previous report.[31] In this model, the following model equation can be expressed:

$$C_b(t) = K_1 \cdot C_a(t) \otimes e^{-(K_1/V_d)\cdot t} \qquad \text{Eq. 1}$$

where $C_b(t)$ = radioactivity in the brain and $C_a(t)$ = arterial input function. ⊗ denotes the convolution integral.

For a given K_1 value and a given arterial input function, $C_a(t)$, the radioactivity in the brain, $C_b(t)$, can be considered to tabulate as a function of V_d. For a given radioactivity in the brain, the table look-up procedure then provides a corresponding V_d value (Fig. 1). The V_d value approximates the BP value in case of Iomazenil.[31] In this study, the mid-scan time of SPECT scan was set at 180 min after the injection of Iomazenil. The arterial input function, $C_a(t)$, is obtained by calibration of the standard

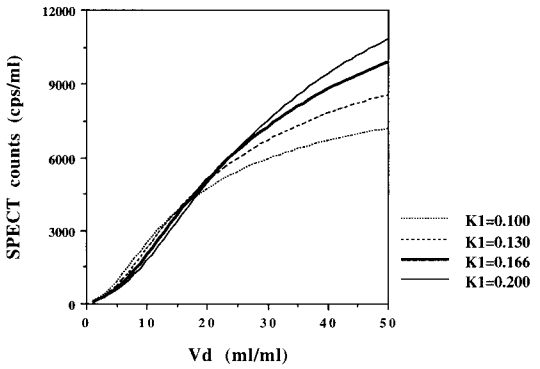

Fig. 1 The relation between brain radioactivity and V_d in which the standard input function is used. The corresponding V_d value is provided by the table look-up procedure. Assumed K_1 values are 0.100, 0.130, 0.166 and 0.200 ml/ml/min.

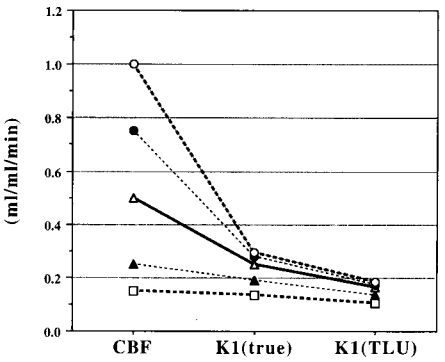

Fig. 2 The range of Iomazenil K_1 calculated by the Renkin-Crone model ($K_{1(true)}$) and that calculated by the table look-up method ($K_{1(TLU)}$) for a CBF range of 0.150–1.000 ml/ml/min. A CBF range of 0.150–1.000 ml/ml/min corresponds to a $K_{1(true)}$ range of 0.135–0.293 ml/ml/min, and to a $K_{1(TLU)}$ range of 0.103–0.183 ml/ml/min.

input function using the arterial whole blood radioactivity gained from the single sampling at 30 min after the injection of Iomazenil, same as our previous report.[31] The standard input function was obtained by averaging arterial input functions of all subjects.[31]

Simulation study of K_1 range of Iomazenil

In order to investigate the range of the K_1 value of Iomazenil, a simulation study was performed. The first-pass extraction fraction of Iomazenil (E) is probably about 0.5.[26,28] First, the first-pass extraction fraction of Iomazenil was assumed to be 0.5 for a CBF of 0.500 ml/ml/min, which corresponds to a capillary permeability-surface area product (PS) value of 0.347 ml/ml/min, and then K_1 values of Iomazenil were calculated by using this PS value according to the Renkin-Crone model ($E = 1 - e^{-(PS/CBF)}$),[40,41] where CBF was varied from 0.150 to 1.000 ml/ml/min. Next, tissue time-activity curves (0–180 min) were generated based on the three-compartment model using these calculated K_1 values and assumed rate constants, where total and nondisplaceable distribution volume values were assumed to be 30 and 3 ml/ml, respectively ($k_3 = 0.180$ min^{-1} and $k_4 = 0.020$ min^{-1}).[26,28,31] Then, the table look-up method previously reported[31] was applied to these tissue data for calculating K_1 and V_d values, and these K_1 values were compared with CBF and K_1 values calculated by the Renkin-Crone model. In the table look-up method, the combination of scan times (early/delayed SPECT scan) used was 30/180 min. In this simulation, the standard input function was used as an input function.

Simulation study of the error of V_d calculated by the present method

In order to estimate the error of V_d values calculated by the present method, another simulation study was also performed. First, tissue time-activity curves (0–180 min) were generated based on the three-compartment model using four assumed rate constants, where K_1 was varied 0.135–0.293 ml/ml/min corresponding to a CBF of 0.150–1.000 ml/ml/min (PS = 0.347 ml/ml/min), total distribution volume values were assumed to be 10, 20, 30 and 40 ml/ml ($k_4 = 0.020$ min^{-1}), and nondisplaceable distribution volume value was assumed to be 3 ml/ml.[26,28,31] Then, the present method was applied to these tissue data for calculating V_d values, and these V_d values were compared with assumed V_d values. In this simulation, the standard input function was used as an input function.

Non-linear least squares fitting analysis (NLLSF)

In order to confirm the validity of the present method, NLLSF based on the three-compartment model was performed to determine four rate constants,[42] and then total distribution volume (V_d ($= (K_1/k_2)\cdot(1 + k_3/k_4)$)) values were calculated.[26,28,31] In these analyses, dynamic SPECT data and static SPECT scan data at 180 min were combined and used. However, first frame data (0–200 sec) of dynamic SPECT were not used for removing the effects of cerebral blood volume.[43] The individually measured arterial input function was used in these analyses.

RESULS

Figure 2 shows the range of Iomazenil K_1 calculated by the Renkin-Crone model ($K_{1(true)}$) and that calculated by the table look-up method ($K_{1(TLU)}$) for a CBF range of 0.150–1.000 ml/ml/min. A CBF range of 0.150–1.000 ml/ml/min corresponded to a $K_{1(true)}$ range of 0.135–0.293 ml/ml/min, and this $K_{1(true)}$ range corresponded to a $K_{1(TLU)}$ range of 0.103–0.183 ml/ml/min. A CBF of 0.500 ml/ml/min corresponded to a $K_{1(true)}$ of 0.250 ml/ml/min and to a $K_{1(TLU)}$ of 0.164 ml/ml/min. Mean K_1 value obtained by NLLSF in cerebral cortex regions, which showed no abnormalities on X-ray CT, was 0.270 ± 0.053 ml/ml/min ($\pm$ SD). Mean K_1 value obtained by the table look-up method[31] with individually measured input function and

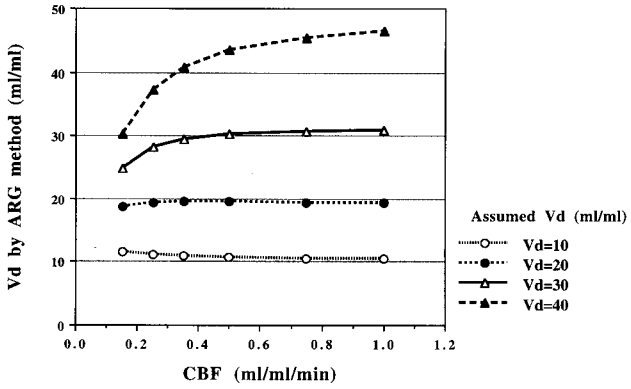

Fig. 3 The error of V_d values calculated by the present method (ARG method). The error is not significant when CBF values are 0.250–1.000 ml/ml/min. However, significant underestimation is observed when CBF value is 0.150 ml/ml/min and V_d values are 30–40 ml/ml.

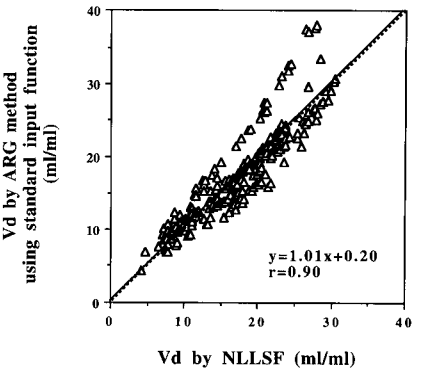

Fig. 6 The correlation between V_d values obtained by NLLSF and those obtained by the present method (ARG method), in which the K_1 value is assumed to be 0.166 ml/ml/min. A good correlation is observed. The dot line is identity.

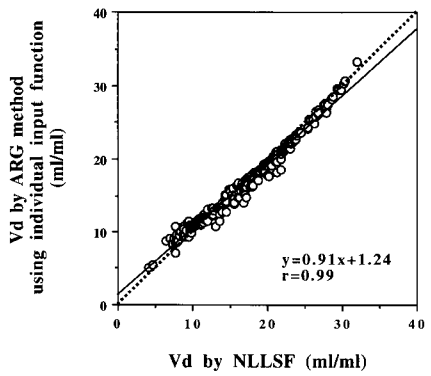

Fig. 4 The correlation between V_d values obtained by NLLSF and those obtained by the autoradiographic method (ARG method) using individually measured input function, in which the K_1 value is assumed to be 0.166 ml/ml/min. A good correlation is observed. The dot line is identity.

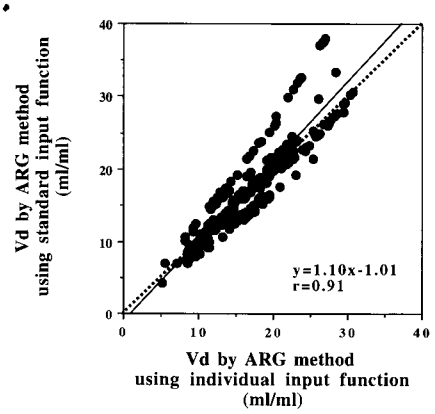

Fig. 5 The correlation between V_d values obtained by the autoradiographic method (ARG method) using individually measured input function and those obtained by the autoradiographic method (ARG method) using calibrated standard input function, that is the present method, in which the K_1 value is assumed to be 0.166 ml/ml/min. A good correlation is observed. The dot line is identity.

calibrated standard input function (scan times combination: 30/180 min) in cerebral cortex regions were 0.166 ± 0.030 and 0.166 ± 0.039 ml/ml/min (± SD), respectively.

Figure 3 shows the error of V_d values calculated by the present method in which the K_1 value was assumed to be 0.166 ml/ml/min. When CBF values were 0.250–1.000 ml/ml/min corresponding to a $K_{1(true)}$ range of 0.188–0.293 ml/ml/min, the error in calculated V_d values were around ± 10% and ± 15% for assumed V_d of 10–30 ml/ml and 40 ml/ml, respectively. However, when CBF value was 0.150 ml/ml/min corresponding to a $K_{1(true)}$ of 0.135 ml/ml/min, the error in calculated V_d values were about −20% for assumed V_d of 30 and 40 ml/ml, while they were around ±10–15% for assumed V_d of 10 and 20 ml/ml. Mean V_d value obtained by NLLSF in cerebral cortex regions, which showed no abnormalities on X-ray CT, was 22.09 ± 3.95 ml/ml (± SD).

Figure 4 shows the correlation between V_d values obtained by NLLSF and those obtained by the autoradiographic method using individually measured input function, in which the K_1 value was assumed to be 0.166 ml/ml/min ($y = 0.91x + 1.24$; r = 0.99; x, NLLSF; y, autoradiographic method). A good correlation was observed between the two methods.

Figure 5 shows the correlation between V_d values obtained by the autoradiographic method using individually measured input function and those obtained by the autoradiographic method using calibrated standard input function, that is, the present method in which the K_1 value was assumed to be 0.166 ml/ml/min ($y = 1.10x - 1.01$; r = 0.91; x, using individual input function; y, using standard input function). A good correlation was observed between the two methods.

Figure 6 shows the correlation between V_d values obtained by NLLSF and those obtained by the autoradiographic method using calibrated standard input function, that is, the present method in which the K_1 value was assumed to be 0.166 ml/ml/min ($y = 1.01x + 0.20$; r = 0.90;

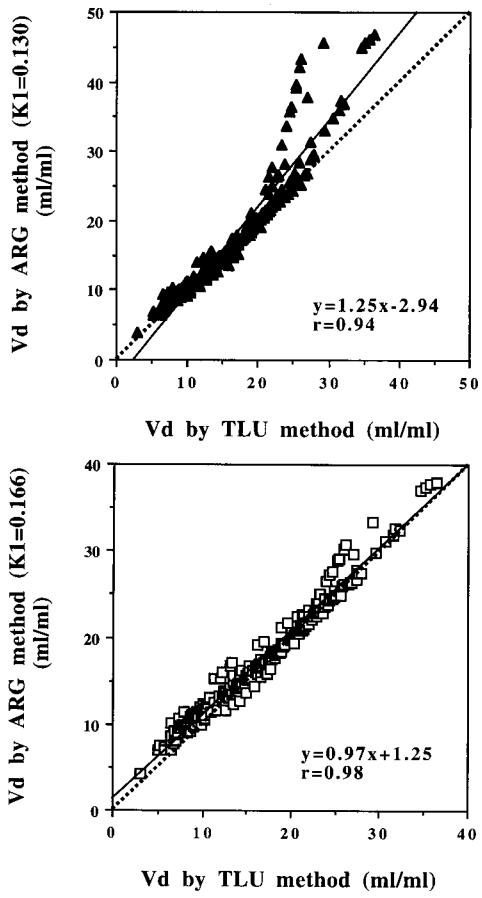

Fig. 7 The correlations between V_d values obtained by the table look-up method (TLU method) and those obtained by the present method (ARG method), in which the K_1 values are assumed to be 0.130 (a), 0.166 (b) or 0.200 (c) ml/ml/min. The best correlation is observed when K_1 value is assumed to be 0.166 ml/ml/min. The dot line is identity.

x, NLLSF; y, autoradiographic method). A good correlation was observed between the two methods.

Figure 7 shows the correlations between V_d values obtained by the table look-up method previously reported[31] and those obtained by the autoradiographic method, in which the K_1 values were assumed to be 0.130, 0.166 or 0.200 ml/ml/min. In the table look-up method, the combination of scan times (early/delayed) used was 30/180 min. In each method, calibrated standard input function was used. Good correlations were observed between the two methods for all assumed K_1 values (K_1 = 0.130: $y = 1.25x - 2.94$, r = 0.94; $K_1 = 0.166$: $y = 0.97x + 1.25$, r = 0.98; $K_1 = 0.200$: $y = 0.87x + 3.08$, r = 0.98; x, table look-up method; y, autoradiographic method). However, the best correlation between the two methods was observed when the K_1 value was assumed to be 0.166 ml/ml/min.

DISCUSSION

Validation of the present method

It has been shown that the two-compartment model analysis, which is employed in the table look-up method[31] and the present method, caused underestimation of K_1 values compared with the three-compartment model analysis in case of Iomazenil.[28,31] In the simulation study, the $K_{1(TLU)}$ values were smaller than $K_{1(true)}$, and the range of $K_{1(TLU)}$ was narrow compared with $K_{1(true)}$ (Fig. 2). This indicates that assuming K_1 values in the two-compartment model analysis might not reveal significant errors in the calculation of V_d. In this study, mean K_1 value obtained by the table look-up method was 0.166 ml/ml/min which was smaller than that obtained by NLLSF (0.270 ml/ml/min). Therefore the K_1 value was assumed to be 0.166 ml/ml/min in the present method.

The simulation study revealed that the error of V_d values calculated by the present method was not significant when CBF values were 0.250–1.000 ml/ml/min (Fig. 3). However, significant underestimation was observed when the CBF value was 0.150 ml/ml/min and assumed V_d values were 30–40 ml/ml. This indicates that the present method can provide accurate V_d values except in the regions which show very low CBF and normal or high V_d. Mean V_d value obtained by NLLSF was 22.09 ± 3.95 ml/ml (± SD), and those previously reported were 27–31 ml/ml in healthy subjects.[27]

V_d values obtained by the autoradiographic method using individually measured input function were consistent with those obtained by NLLSF (Fig. 4). This indicates that the statistical noise of the table between SPECT counts and V_d in the autoradiographic method (Fig. 1) was low. A good correlation was observed between V_d values obtained by the autoradiographic method using individually measured input function and those obtained by the autoradiographic method using calibrated standard input function (i.e., the present method) (Fig. 5). This indicates that the individual difference in input function was small, and that calibrated standard input function can be used as an input function. A good correlation was observed between V_d values obtained by NLLSF and those obtained by the autoradiographic method using calibrated standard

input function (i.e., the present method) (Fig. 6), supporting its validity. Since the present method requires only one SPECT scan at 180 min after injection and one blood sampling, it is applicable for routine clinical use, and will be able to provide images of BP.

Furthermore, venous blood sampling can be substituted for invasive arterial sampling, thus simplifying the approach.[31,34,44] In addition, the separation of lipophilic component in a blood sample for calibrating the standard input function might be able to improve the error of individual difference in input function. In this study, the blood sampling time was set at 30 min after injection, however, it can be set at 20–40 min.[31]

In the present method, SPECT data at 180 min after injection were used. If the time of SPECT scan can be set later than 180 min, e.g., 300 or 360 min, the error of V_d values calculated by the present method should be smaller. Further studies will be required.

Mean V_d value obtained by NLLSF in the present study (22.09 m*l*/m*l*) was smaller than those previously reported in healthy subjects (27–31 m*l*/m*l*).[27] The main reason for this must be that all subjects in the present study were patients, not healthy subjects. Further studies for determining normal V_d values in a major healthy subject series are required.

Limitations

The following factors need to be considered as sources of error in the present method.

1. Error in the regions with low CBF and normal or high V_d.
2. Assumed K_1 value
3. Physical accuracy of SPECT scanner system
4. Individual differences of input function

Simulation study revealed that the present method caused underestimation of V_d values in the regions with very low CBF and normal or high V_d (Fig. 3), while a good correlation was observed between V_d values obtained by NLLSF and those obtained by the present method (Fig. 6). Hatazawa et al. reported that normal Iomazenil uptake was observed in remote areas with hypoperfusion in a patient with cerebral infarction.[15] The present method might cause underestimation of V_d values in such regions. Further studies are required. In addition, the table look-up method previously reported may also not be able to calculate accurate V_d values in such regions, because the normal V_d regions with hypoperfusion will show very small k_2 values, hampering accurate calculation.[31]

While good correlations were observed between V_d values obtained by the table look-up method and those obtained by the present method for all assumed K_1 values, the best correlation between the two methods was observed when the assumed K_1 value was 0.166 m*l*/m*l*/min (Fig. 7). This K_1 value was the mean K_1 value obtained by the table look-up method. Since the differences in the reconstruction algorithm, including the scattered photon correction, the attenuation correction and the filter for each SPECT scanner system, might cause the differences in calculated K_1 values, the assumed K_1 value in the present method should be determined for each SPECT scanner system by using the table look-up method.[45,46]

The scattered photons not removed in this study can cause errors in V_d estimation. Incomplete correction for the attenuation of gamma rays is also a problem for the calculation of accurate V_d values. The differences in the reconstruction algorithm for each SPECT scanner system might cause errors in calculating V_d values. The limited spatial resolution of SPECT scanners cause gray-white matter mixture in regions-of-interest, and this might give rise to non-negligible errors. Further studies are required on these subjects.[31]

In this study, none of the patients had any heart, renal or liver diseases. Since the shape of the arterial input function in patients with these disorders will differ from the standard input function,[32] accurate V_d values may not be obtained for such patients by the present method.[31]

CONCLUSION

We have developed a simple method for quantification of Iomazenil binding, requiring one SPECT scan, one blood sampling and an assumed K_1 value. A good correlation was observed between V_d values obtained by NLLSF and those obtained by the present method, supporting its validity. The present method is simple and applicable for routine clinical use, and it will be able to provide images of BP. However, it does have some limitations, and further studies are required.

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