Measurement of Regional Cerebral Blood Flow with Copper-62-PTSM and a Three-Compartment Model

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We evaluated quantitatively ^{62}Cu-labeled pyruvaldehyde bis(N^4-methylthiosemicarbazone) copper II (^{62}Cu-PTSM) as a brain perfusion tracer for positron emission tomography (PET). For quantitative measurement, the octanol extraction method is needed to correct for arterial radioactivity in estimating the lipophilic input function, but the procedure is not practical for clinical studies. To measure regional cerebral blood flow (rCBF) by ^{62}Cu-PTSM with simple arterial blood sampling, a standard curve of the octanol extraction ratio and a three-compartment model were applied. **Methods:** We performed both ^{15}O-labeled water PET and ^{62}Cu-PTSM PET with dynamic data acquisition and arterial sampling in six subjects. Data obtained in 10 subjects studied previously were used for the standard octanol extraction curve. Arterial activity was measured and corrected to obtain the true input function using the standard curve. **Results:** Graphical analysis (Gjedde–Patlak plot) with the data for each subject fitted by a straight regression line suggested that ^{62}Cu-PTSM can be analyzed by the three-compartment model with negligible k_4. Using this model, K_1–k_3 were estimated from curve fitting of the cerebral time–activity curve and the corrected input function. The fractional uptake of ^{62}Cu-PTSM was corrected to rCBF with the individual extraction at steady state calculated from K_1–k_3. The influx rates (K_i) obtained from three-compartment model and graphical analyses were compared for the validation of the model. A comparison of rCBF values obtained from ^{62}Cu-PTSM and ^{15}O-water studies demonstrated excellent correlation. **Conclusion:** The results suggest the potential feasibility of quantitation of cerebral perfusion with ^{62}Cu-PTSM accompanied by dynamic PET and simple arterial sampling.

Key Words: PET; copper-62-PTSM; cerebral blood flow; three-compartment modeling

J Nucl Med 1996; 37:1089–1093

After the development of the ^{62}Zn/^{62}Cu positron generator (*1,2*) and the application of copper (II) pyruvaldehyde bis(N^4-methylthiosemicarbazone) labeled with ^{62}Cu (^{62}Cu-PTSM) as a perfusion tracer for positron emission tomography (PET) (*3–6*), several institutions investigated their clinical and quantitative applications (*7–12*). We previously evaluated the feasibility of the ^{62}Zn/^{62}Cu positron generator in clinical PET studies and performed a quantitative analysis of ^{62}Cu-PTSM as a cerebral perfusion tracer (*11,12*). For quantitative measurement of regional cerebral blood flow (rCBF) with this tracer, use of the octanol extraction procedure is necessary to obtain the arterial input function (*10–14*) because most radioactivity in the blood a few minutes after intravenous injection of ^{62}Cu-PTSM is due to components that cannot penetrate the blood-brain barrier (BBB). However, the octanol extraction procedure is not practical for clinical use. Furthermore, correction for the intersubject variation of the net extraction is considered necessary, which makes quantitative estimation of rCBF difficult (*11*).

In the present study, we examined measurement of rCBF by ^{62}Cu-PTSM PET with simple arterial sampling. The radioactivity of the sampled blood was corrected using the curve of the average octanol extraction ratio obtained from 10 subjects studied previously (*11,12*) for estimation of the true input function. A three-compartment model was used for kinetic analysis based on the retention mechanism of ^{62}Cu-PTSM in the brain (*15–17*). The corrected input function and change in global brain activity provided the rate constants and the net extraction at steady state in each subject, which are needed to correct for intersubject variation of the fractional uptake of ^{62}Cu-PTSM. The rCBF values obtained from ^{62}Cu-PTSM were compared with those measured by ^{15}O-water PET. A graphical analysis was to validate the three-compartment analysis with negligible k_4.

MATERIALS AND METHODS

Subjects

We studied six subjects with various neurological diseases (three men, three women; mean age 45.3 ± 15.6 yr, range 30–68 yr) (Table 1) who underwent PET measurement of rCBF with ^{15}O-labeled water, followed by ^{62}Cu-PTSM study with arterial blood sampling. Data for 10 subjects previously studied were used to construct a curve of average octanol extraction ratio (*11*).

The protocol was applied according to the guidance of the Ethical Committee of the Kyoto University Faculty of Medicine, and all subjects provided written informed consent to participate in the study.

Preparation of Copper-62-PTSM

A ^{62}Zn/^{62}Cu generator was prepared with $^{62}ZnCl_2$ aqueous solution (1.1 GBq, pH 5.0), and PTSM was synthesized by a previously reported method (*2,18*). The ^{62}Cu-PTSM was obtained by simple mixing of generator eluate (^{62}Cu-glycine) and PTSM solution by a ligand-exchange reaction (*18*). The product was acquired in a total volume of 4 ml of ^{62}Cu-PTSM (0.1 mmole PTSM in 5% dimethylsulfoxide) with glycine solution.

Imaging Protocol

We used a PCT-3600W (Hitachi Medical Co., Tokyo, Japan) as the PET imaging device (*11,12*). This system permits the simultaneous acquisition of 15 transverse slices with interslice spacing of 7 mm. Images were reconstructed to a full width at half maximum (FWHM) of 9 mm in the transaxial direction and 6.5 mm in the axial direction. The field of view and pixel size of the reconstructed images were 256 and 2 mm, respectively. Transmission scans were obtained with a standard plate source of ^{68}Ge/^{68}Ga for attenuation correction of the emission images. The tissue activity concentration in the PET images was cross-calibrated against a scintillation counter using a cylindrical phantom filled

Received Jun. 23, 1995; revision accepted Dec. 13, 1995.

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TABLE 1
Patient Characteristics

Patient no.	Age/Sex	Condition	MRI findings	Lesion size (cm)
1	68/M	Deaf	n.p.	—
2	31/M	Cerebral AVM	R frontal AVM	1.5 × 2
3	54/F	Cerebral AVM	L temporal AVM	2.5 × 2
4	54/F	Astrocytoma	L parietal glioma	2 × 2
5	30/F	Deaf	n.p.	—
6	35/F	Cerebral AVM	R temporal AVM	1.5 × 1.5

AVM = arteriovenous malformation; n.p. = nothing particular; R = right; L = left.

with an ^{18}F solution. The subject's head was immobilized with a headholder. A small cannula was placed in the left brachial artery.

For comparison of rCBF obtained by ^{62}Cu-PTSM with that obtained by ^{15}O-water PET, approximately 1110 MBq (30 mCi) of ^{15}O-labeled water in 6 ml saline was injected into the right cubital vein over 5–8 sec, and PET data were acquired for 120 sec. Ten to 15 min after the ^{15}O-water study, ^{62}Cu-PTSM scanning was started. The subjects were injected intravenously with 296–740 MBq (8–20 mCi) of ^{62}Cu-PTSM for a total volume of 4 ml over 5–8 sec. PET data acquisition was started at the time of ^{62}Cu-PTSM injection and continued for 10 min, in 15-sec frames for the first 120 sec and in 60-sec frames for the next 8 min. In addition to the serial dynamic PET images, static images of 2–10 min after injection were reconstructed from the dynamic scan data.

Arterial blood samples were obtained manually from the left brachial artery during each PET scan from the time of intravenous injection to the end of the scan. In the ^{15}O-water study, 1 ml of blood was sampled every 4–5 sec for the first minute and then every 10–15 sec for the rest of the session. In the ^{62}Cu-PTSM study, 1 ml of blood was obtained at the same time as in the ^{15}O-water study for the first 2 min and then sampled at 3, 5 and 10 min after injection. The blood samples thus obtained were immediately measured with the scintillation counter to obtain the arterial radioactivity.

Data Analysis

Three-compartment analysis with a curve-fitting procedure was used to evaluate the rate constants because our previous study (*11*) and the studies of Fujibayashi et al. (*15,16*) and Taniuchi et al. (*17*) indicated that the retention mechanism of ^{62}Cu-PTSM is consistent with this model. In the three-compartment kinetic model with negligible k_4 (Fig. 1), brain radioactivity $C_b(t)$ can be expressed as follows (*19–22*):

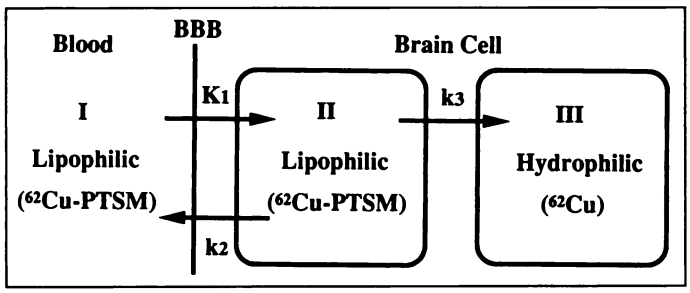

FIGURE 1. Schematic representation of three-compartment model. Reversible compartment (II) freely communicates with plasma. Irreversible compartment (III) communicates with the reversible compartment. The tracer can move from the plasma to compartment (II) freely, and the tracer in compartment (II) can move to compartment (III) but cannot exit. BBB = blood-brain barrier.

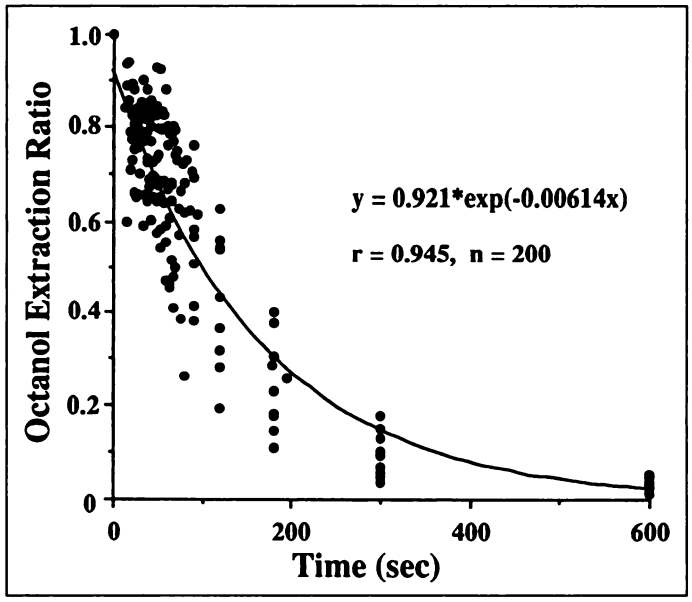

FIGURE 2. Octanol extraction ratio of ^{62}Cu-PTSM with respect to total blood activity as a function of time after intravenous injection of ^{62}Cu-PTSM in the 10 previously studied subjects. The rate of decrease in extraction was fitted monoexponentially.

$$C_b(t) = K_i \int_0^t C_a(\tau)\, d\tau + V_n Ca(t), \qquad \text{Eq. 1}$$

where $C_a(t)$ is the arterial input function; V_n is the initial distribution volume for the tracer; and K_i is the influx rate. Equation 2, derived from Equation 1, demonstrates that K_i is the slope of a straight regression line in a graphical analysis by plotting $C_b(t)/C_a(t)$ versus $\int_0^t C_a(\tau)\, d\tau/C_a(t)$ from each group of data:

$$\frac{C_b(t)}{C_a(t)} = K_i \cdot \frac{\int_0^t C_a(\tau)\, d\tau}{C_a(t)} + V_n. \qquad \text{Eq. 2}$$

The arterial input function and the brain tissue activity as a function of time should be consistent with the three-compartment model, with negligible k_4, when $C_b(t)/C_a(t)$ is plotted versus $\int_0^t C_a(\tau)\, d\tau/C_a(t)$ on a straight line (*20*). Graphical analysis was performed to validate our compartment hypothesis.

The standard octanol extraction curve obtained from data from 10 previously studied subjects was fitted monoexponentially, as shown in Figure 2, and the arterial blood activity was corrected to the lipophilic arterial input function as follows:

$$C_a(t) = 0.921 * \exp(-0.00614t) \cdot A(t), \qquad \text{Eq. 3}$$

where A(t) is the radioactivity of sampled blood as a function of time.

The influx rate (K_i) and K_1 are expressed by Equations 4–6:

$$K_i = \frac{K_1 k_3}{k_2 + k_3}, \qquad \text{Eq. 4}$$

$$K_1 = E \cdot F, \qquad \text{Eq. 5}$$

$$K_i = F \cdot \frac{Ek_3}{k_2 + k_3}, \qquad \text{Eq. 6}$$

where E is the first-pass extraction fraction, and F is rCBF. The net extraction at steady state ($\bar{E}$), explained in our previous report, is expressed by Equation 7 (*11,23*):

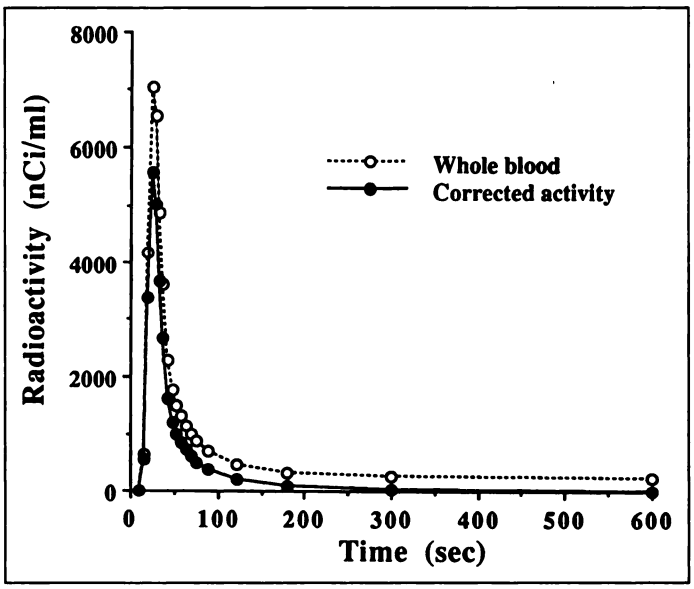

FIGURE 3. Representative time-activity curves of total blood (dashed line) and corrected radioactivity (solid line) of ^{62}Cu-PTSM obtained from the brachial artery. The standard octanol extraction ratio of ^{62}Cu-PTSM obtained from the octanol extraction study in the 10 previously studied subjects was used for the correction of blood activity. The arterial radioactivity showed rapid clearance, and the corrected activity was nearly negligible about 3 min after the injection.

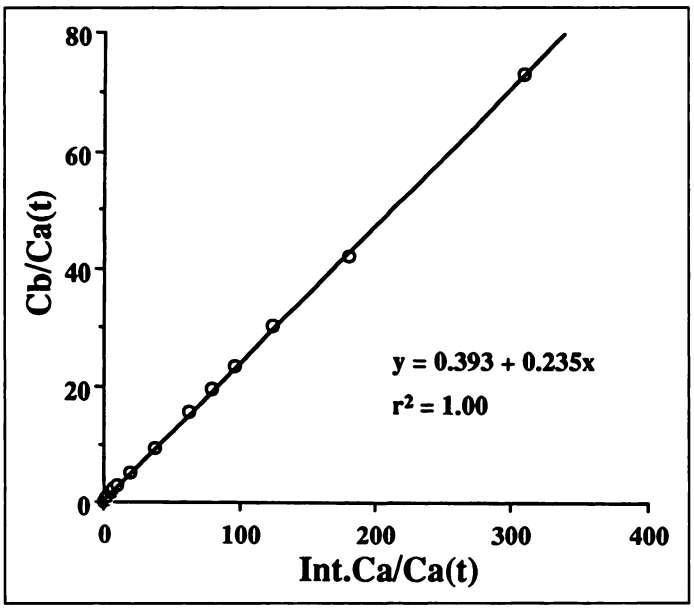

FIGURE 4. Representative graphical plotting using the corrected input function and time-activity data obtained from global cerebral tissue activity. A straight regression line with excellent correlation (r^2 = 1.00) was obtained. Int. = integral.

$$\bar{E} = \frac{Ek_3}{k_2 + k_3}. \qquad \text{Eq. 7}$$

A region of interest (ROI) was selected to include a whole-brain slice in serial dynamic PET images, and the time-activity curve of the global brain tissue was obtained for three-compartment analysis. In the global analysis, the brain lesions were excluded in the setting of the ROI to prevent interference with the radioactivity in the ROI. The rate constants K_1–k_3 in the three-compartment model were estimated individually by nonlinear least-squares fitting, and the influx rate (K_i) derived from the rate constants was compared with that obtained from the slope of graphical analysis. In a global brain analysis, we assumed that the global value of first-pass extraction of ^{62}Cu-PTSM could be represented by the mean for the 10 subjects in the previous study (0.78) (*11*), and the extraction fraction at steady state was ultimately calculated as follows:

$$\bar{E} = \frac{0.78 * k_3}{k_2 + k_3}. \qquad \text{Eq. 8}$$

The fractional uptake image of ^{62}Cu-PTSM was obtained from the cerebral tissue count at steady state and the integral of the corrected input function. These tissue activity images and blood activity were corrected for physical decay of ^{62}Cu (half-life, 9.74 min). Furthermore, the fractional uptake image was divided by $\bar{E}$ obtained from Equation 8 to acquire the rCBF image in each subject (*11*). Seventy-seven small square ROIs (12 × 12 mm) were placed on the various regions in the cerebral hemispheres and cerebellum. One slice for the cerebellum and three slices for the cerebrum were selected to place ROIs in which the counts were not considered to be affected by the activity of the brain lesions. The same ROIs were transferred to the rCBF image obtained from the ^{15}O-water study to compare the rCBF values in both PET images (*11*).

RESULTS

Graphical Analysis

Figure 3 shows the time-activity curves for ^{62}Cu-PTSM for total arterial blood and corrected radioactivity obtained by the standard octanol extraction curve (Eq. 3). The corrected activity was almost negligible at 3 min after the injection, even though total blood radioactivity remained. A graphical analysis plotting the data for Subject 1 is demonstrated in Figure 4; the analysis used the corrected input function and time-activity data obtained from the cerebral tissue counts with a global ROI. A straight regression line with a high correlation coefficient was observed for each subject (Table 2).

Three-Compartment Analysis and Correction of Extraction

Table 2 summarizes K_i values obtained from the individual graphical analysis and those for K_1 and K_i obtained from the three-compartment analysis. The K_i values with graphical analysis were consistent with those obtained from the three-compartment analysis. The extraction at steady state ($\bar{E}$) was calculated according to Equation 8 using the k_2 and k_3 values from the three-compartment analysis.

Figure 5 shows the relation between the fractional uptake of ^{62}Cu-PTSM without correction of the individual $\bar{E}$ and rCBF values measured by ^{15}O-water PET obtained from multiple ROIs. A large intersubject variation was observed in the relation between ^{62}Cu-PTSM uptake and rCBF. By means of individual

TABLE 2
Graphical Versus Compartmental Analysis

Patient no.	Graphical analysis		Three-compartment model		
	K_i	r^2	K_1	K_i	Net E
1	0.235	1.000	0.362	0.231	0.50
2	0.157	1.000	0.260	0.164	0.49
3	0.100	0.999	0.414	0.107	0.20
4	0.092	1.000	0.267	0.097	0.28
5	0.110	0.999	0.278	0.109	0.31
6	0.064	0.999	0.348	0.077	0.17

K_i = influx rate of ^{62}Cu-PTSM; r^2 = correlation coefficient of graphical analysis; Net E = extraction ratio at steady state.

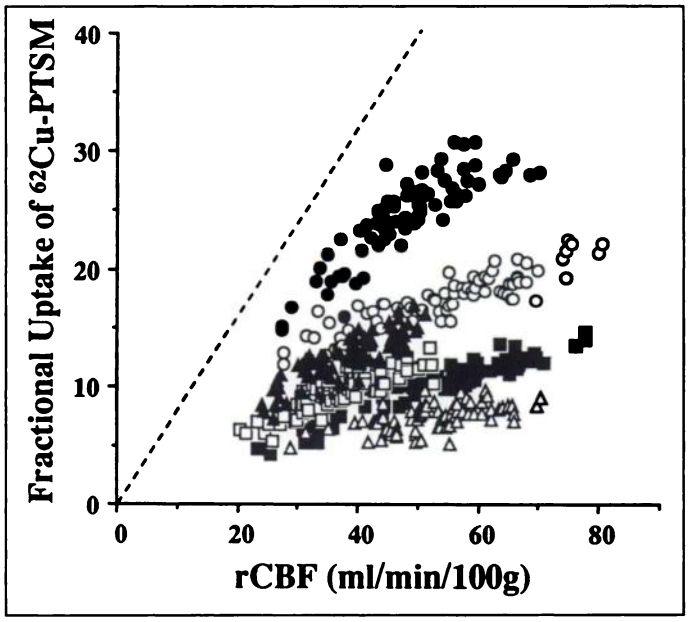

FIGURE 5. Comparison of fractional uptake of ^{62}Cu-PTSM with rCBF measured by ^{15}O-water PET. Datasets for each subject shown by different symbols demonstrate the intersubject variation. The dashed line is the line of identity.

correction with $\bar{E}$, the intersubject variation was diminished, and excellent correlation was obtained (Fig. 6).

DISCUSSION

Copper-62-PTSM derived from a ^{62}Zn/^{62}Cu positron generator has been anticipated as a perfusion tracer for PET, especially in PET centers without an in-house cyclotron, and quantitative studies of its use in myocardium and brain have been performed (*9–12*). For measurement of rCBF, however, the intersubject variation of the net extraction at steady state ($\bar{E}$) has made it difficult to measure the absolute rCBF, even if the true input function is established from octanol-extractable radioactivity (*11*). Further, the octanol extraction procedure is too complicated for use in a clinical study. The objective of the present study was to estimate the net extraction at steady state

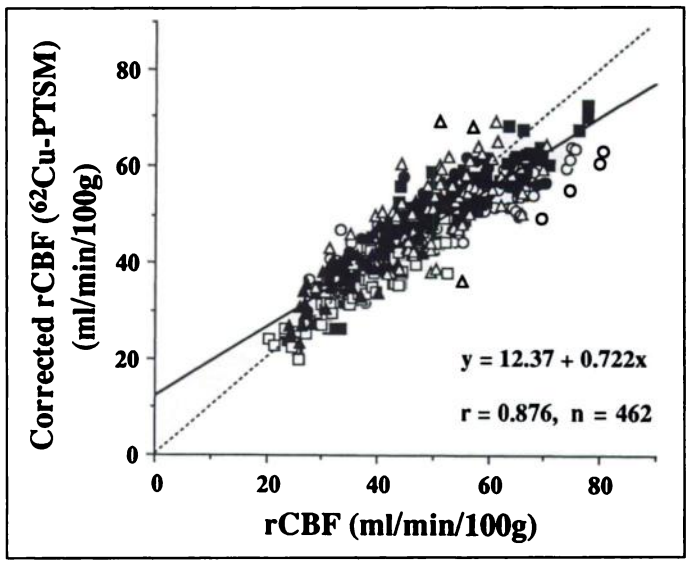

FIGURE 6. Correlation between rCBF values corrected with steady-state extraction ($\bar{E}$) of ^{62}Cu-PTSM in each subject and rCBF values obtained by ^{15}O-water PET. Note that intersubject variation is absent, in contrast to Figure 4. The linear regression analysis (solid line) disclosed excellent correlation. The dashed line is the line of identity.

individually and obtain the rCBF image with ^{62}Cu-PTSM and simple arterial sampling.

Using a standard curve of the octanol extraction ratio, fitted monoexponentially with Equation 3, the whole-blood activity sampled from the brachial artery was corrected to acquire the input function. Correction of the sampled blood provided the lipophilic blood activity, which is considered to be the PTSM-associated radioactivity (*10–14*). The rapid clearance of the corrected input function was similar to the octanol-extractable activity observed in our previous study (*11*), and the radioactivity was nearly negligible about 3 min after the injection. Mathias et al. (*13,24*) reported the stronger binding of ^{62}Cu-PTSM with plasma protein in humans than in dogs, which appears to account for some of the problems with using animal data to predict ^{62}Cu-PTSM kinetic behavior in humans. They recommended the octanol extraction procedure for determining the ^{62}Cu-PTSM content of arterial blood samples to establish a true input function (*13*). Although we did not confirm the accuracy of correction in the estimation of the octanol-extractable activity as the lipophilic input function, the standard curve was obtained from human studies, and the radioactivity extracted in the lipophilic part is considered to have penetrated the BBB. This correction of blood activity with the standard curve seemed to be a reasonable means of estimating the true input function, and the method was deemed to be feasible in clinical studies.

The graphical analysis was previously developed to obtain the influx constant of the tracer across the blood-brain barrier and has mainly been used to analyze the metabolic rate of glucose with [^{18}F]2-fluoro-2-deoxy-D-glucose (*19–21*). Matsuda et al. (*22*) used this method for the quantitative analysis of rCBF with [^{99m}Tc]*d,l*-hexamethylpropyleneamine-oxime in a SPECT study (*22*). In that study, a straight line was fitted in a plot of $C_b(t)/C_a(t)$ versus $\int_0^t C_a(\tau)\, d\tau/C_a(t)$ within the first 30 sec after injection of the tracer, followed by divergence from the straight line. The divergence after 30 sec is considered to be caused by overestimation of the input function because the radioactivity of arterial input was the count for the aortic arch without correction for the lipophilic radioactivity. In the present study, graphical plotting was on a straight line until the end of the scanning time because the arterial blood activity was corrected to the lipophilic input function. An excellent correlation between $C_b(t)/C_a(t)$ and $\int_0^t C_a(\tau)\, d\tau/C_a(t)$ suggests that the kinetics of ^{62}Cu-PTSM retention corresponds to that of the three-compartment model and that k_4 is negligible. Furthermore, the initial distribution volume for the tracer (V_n) was also negligible, supporting our previous assumption that this tracer could be analyzed by a microspheric model (*11*) because Equation 1 is rearranged as follows:

$$C_b(t) = F \cdot \bar{E} \int_0^t C_a(\tau)\, d\tau.$$

The influx rates (K_i) obtained from graphical analysis were consistent with those obtained from three-compartment analysis. Although the values of K_i varied individually, and the first-pass extraction (E) might be different in the various areas of high and low rCBF, the first-pass extraction in the whole-brain ROI did not show much intersubject difference. Therefore, the mean value of the first-pass extraction for the 10 subjects previously studied (*11*) was used for calculating the rCBF. This value is similar to results obtained in the rat brain (*25*). The rCBF values corrected with the individual extraction at steady state ($\bar{E}$) in the ^{62}Cu-PTSM study showed good

correlation with rCBF measured by the ^{15}O-water study, indicating that the correction used in the present study is essential for this tracer. By means of three-compartment analysis, the ^{62}Cu-PTSM images can be corrected with the individual extraction at steady state.

The retention mechanism of ^{62}Cu-PTSM is reported to rely on the irreversible reduction of copper (II) to copper (I), and the detachment of radioactive copper from the lipophilic complex results in radioactivity remaining in the cell (*13,26*). Fujibayashi et al. (*15,16*) reported that the reduction was specifically initiated by the mitochondrial enzymatic system in the murine brain, although the reduction was mainly observed in the cytosol in the case of the Ehrlich ascites tumor cells. This result suggested that the retention mechanism of ^{62}Cu-PTSM in the brain might be different from that in other organs. The difference of influx rate might be dependent on the capacity for reduction in the mitochondrial electron transport system (*17*). By means of our correction method, however, the association of the radioactivity in the brain with rCBF could be demonstrated.

CONCLUSION

The application of ^{62}Cu-PTSM with compartment analysis is a feasible method of quantitative measurement of rCBF. Combination of simple arterial blood sampling and correction of metabolites with standard octanol extraction could be used clinically for this purpose.

ACKNOWLEDGMENTS

We thank Mr. H. Kitano and Mr. H. Taniuchi for their excellent technical assistance.

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