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Quantitative assessment of cerebral blood flow using Technetium-99m-hexamethyl-propyleneamine oxime: part I, Design of a mathematical model

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To design a mathematical model for quantifying cerebral blood flow using ^{99m}Tc-hexamethyl-propyleneamine oxime (HM-PAO), basic studies were performed in animals and human volunteers. Microautoradiography revealed that HM-PAO crossed the blood-brain barrier. Thin layer chromatographic studies demonstrated the rapid disappearance of free HM-PAO in the brain tissue. Back diffusion from brain to blood was found negligible. From these observations, the familiar microsphere model was employed in the measurements of blood flow with HM-PAO. This, however, resulted in much lower flow values than simultaneously obtained values with the labeled microspheres. This underestimation was ascribed to the high affinity of HM-PAO to blood cells and serum protein. Taking the binding of HM-PAO to blood components into consideration, the following model equation was designed for quantifying cerebral blood flow: $Ce(t)=Ca(t)-kCa(t)*\exp(-kt)$, $Cb(T)=F\int_0^T Ce(t)dt$, where Ce and Ca are the free HM-PAO concentration in the intravascular space and the arterial whole-blood concentration of HM-PAO, respectively, as a function of time (t), Cb is the brain activity concentration, k is the rate constant for the binding of HM-PAO to the blood components, F is the blood flow value, T is time of measurement, and $*$ denotes the operation of convolution. In clinical studies, $Ca(t)$ and $Cb(T)$ are obtainable from a dynamic single photon emission computerized tomographic study of the brain and multiple arterial blood sampling, respectively. The values for F and k can be estimated using a non-linear least squares fitting method.

Key words: ^{99m}Tc-hexamethyl-propyleneamine oxime, Single photon emission computerized tomography, Cerebral blood flow

INVESTIGATION of regional cerebral blood flow has been made easier in the past few years by the development of new radiopharmaceuticals for single photon emission computerized tomography (SPECT) imaging. Of these radiopharmaceuticals, ^{99m}Tc-hexamethyl-propyleneamine oxime (HM-PAO)[1] is the newest, and superior in availability to N-isopropyl-p-(^{123}I) Iodoamphetamine (IMP)[2] because of its kit form. The clinical usefulness of HM-PAO has been already confirmed mainly in Europe.[3–7] In these reports, however, only qualitative evaluation was described. We previously reported the importance of quantitative assessment for diagnosing diffuse cerebral flow reduction in IMP studies.[8] In the present study, we performed several basic studies in animals and human volunteers to design a mathematical model for quantifying cerebral blood flow using HM-PAO.

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MATERIALS AND METHODS

Microautoradiography

To determine whether HM-PAO crosses the blood-

brain barrier, one male Sprague-Dawley rat (250 g) was administered 20 mCi of HM-PAO by intracarotid injection under pentobarbital anesthesia, 60 mg/kg, and sacrificed by decapitation 2 minutes later. The brain was quickly removed, coated with embedding medium, and frozen in dry ice with added hexan chilled to −70°C. The cryostat was set at −20°C and 5 μm sections were cut. These sections of fresh, frozen, unfixed tissue were mounted on emulsion-coated* microscope slides and subsequently dessicated and stored for 2 days at −20°C in a dry, sealed, dark box. The slides were processed at 20°C in developer (5 minutes) and acid fixer (10 minutes), and the sections were stained with hematoxylin-eosin.

Thin layer chromatography

To investigate the change in the radiochemical composition of HM-PAO in the brain tissue, five male Sprague-Dawley rats (180 g–220 g) were administered 10 mCi of HM-PAO by tail-vein injection. At 1 minute, 5 minutes, 10 minutes, 30 minutes, and 60 minutes postinjection, the animals were sacrificed by decapitation. The brains were quickly removed and homogenized with 5 m*l* of 0.32 M sucrose. These homogenized tissues were subsequently extracted using 5 m*l* of methanol. Twenty μl of methanol-extracted aliquots and HM-PAO control samples were applied 2.3 cm from the base of three chromatographic strips,[1] two were ITLC/SG (2.5×20 cm) and the third was Whatman No. 1 (2.5×20 cm). Immediately after the application of the sample, the chromatograms were developed by ascending chromatography in tanks containing fresh solvent to a depth of 1 cm. One ITLC/SG strip was developed in methylethylketone (MEK, system 1) and the other in 0.9% saline (system 2). The Whatman No. 1 strip was developed in 50% aqueous acetonitrile (system 3). After development, the strips were dried, and the radioactivity distribution was determined using a TLC scanner†.

Evaluation of back diffusion

To determine whether HM-PAO is washed out from the brain tissue to the blood, a normal volunteer was administered 20 mCi of HM-PAO by intravenous injection in the resting state with eyes closed. At 3 minutes, 2 hours, 3.5 hours, and 10.6 hours postinjection, tomographic brain images were obtained using a multicrystal transaxial SPECT system‡.[9] All data were corrected for attenuation and the tomographic data were reconstructed using a filtered back projection algorithm.

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† Aloca, JTC-501

‡ Shimadzu, Headtome II

Comparison with labeled microspheres

To see whether the mathematical model equation for the measurement of blood flow with labeled microspheres can be employed in this HM-PAO study, five Sprague-Dawley rats (250–300 g) were administered HM-PAO and strontium-85-microspheres§ simultaneously under pentobarbital anesthesia, 60 mg/kg. The right carotid artery was catheterized with PE-10# polyethylene tubing, and the left femoral artery and vein were catheterized with PE-50#. The catheter in the right carotid was passed retrogradely approximately 4.2 cm into the left ventricle. Strontium-85 labeled microspheres ($15\pm1.5\ \mu$ in diameter) were suspended in 10% dextran with the addition of 0.01% Tween-80. Suspension of microspheres with activities ranging from 1 to 2 μCi (40,000–80,000 microspheres) and HM-PAO with activities ranging from 200 to 400 μCi were simultaneously injected into the left ventricle and left femoral vein, respectively, over a 45-second period. The blood samples were collected in drops from the free-flowing left femoral artery catheter directly in to small tubes. The animals were sacrificed at 2 minutes postinjection by decapitation. The blood samples and rapidly removed brains were weighed and analyzed for both ^{99m}Tc and ^{85}Sr activities in a gamma counter. According to the following equation, the values of whole brain blood flow were calculated for HM-PAO and microspheres.[10–12]

$$Cb(T)=F\int_0^T Ca(t)dt, \qquad (1)$$

where Cb is the brain activity concentration at time of sacrifice (T), F is the blood flow value, Ca is the arterial whole-blood concentration of a tracer, and (t) is time.

Binding of HM-PAO to blood cells and serum protein

To see how much HM-PAO binds to blood cells and serum protein, HM-PAO was incubated with human whole-blood and serum protein *in vitro* at 37°C.

One mCi of HM-PAO was added to heparinized whole-blood and incubated for 1,5,30, and 60 min. Thereafter, serum activity was counted in a well counter after centrifugalization. Binding rates to blood cells were estimated by calculating the ratio of serum activity to whole-blood activity.

After incubation of 1 mCi of HM-PAO with human serum for 1 minute and 15 minutes, cold 10% perchloric acid was added and mixed in an ice bath. The mixture was left for 5 minutes in the ice bath. The precipitate of the mixture was subsequently washed three times with cold 5% perchloric acid.

§ New England Nuclear, NEM-052A

Clay-Adams Inc.

Binding rates to serum protein were estimated by dividing the precipitate activity after washing by the HM-PAO activity which was added initially.

To see whether HM-PAO bound to blood components crosses the blood-brain barrier, the following study was performed in two male normal volunteers. As a control study, 10 mCi of HM-PAO was injected intravenously and the first tomographic image was obtained 3 minutes postinjection. Then an additional 10 mCi of HM-PAO was injected and the second tomographic image was obtained at the same level as the first image. In the other subject, 10 mCi of HM-PAO was injected after preincubation with whole venous blood for 5 minutes and the first tomographic image was obtained. Then an additional 10 mCi of HM-PAO was injected without preincubation and the second tomographic image was obtained in the same way as in the other control subject.

RESULTS

Microautoradiography

The autoradiograms were viewed at 2,000 × magnification. A representative micrograph of the striatum (caudate putamen) is shown in Fig. 1. The nuclei and cytoplasms of neurons are stained darkly and lightly, respectively. Scattered autoradiographic grains were found in the brain parenchyma.

Thin layer chromatography

All three systems showed remaining activity at the origin for the methanol-extracted tissue homogenate from 1 minute to 60 minutes postinjection (Fig. 2). The ITLC/MEK system and the Whatman No. 1/50% aqueous acetonitrile system showed high activity at Rf, 0.9–1.0 for the HM-PAO control samples, indicating a high proportion of primary lipophilic HM-PAO (Fig. 2).

Evaluation of back diffusion

Regions of interest (ROI) were drawn over the insula area (gray matter) and over the centrum semiovale (white matter) on the tomographic images. The reconstructed counts were almost constant after decay correction from 3 minutes up to 10.6 hours postinjection (Fig. 3a), and the distribution pattern also remained constant (Fig. 3b).

Comparison with labeled microspheres

The flow values obtained for the whole rat brains were 33±3 ml/100 g/min (mean±SEM, N=5) for labeled microspheres and 20±1 ml/100 g/min for HM-PAO.

Binding rates to blood cells and serum protein

Binding rates to blood cells were 17%, 27%, 34%, and 31% of the whole-blood activity for 1 minute, 5 minutes, 30 minutes, and 60 minutes incubation respectively.

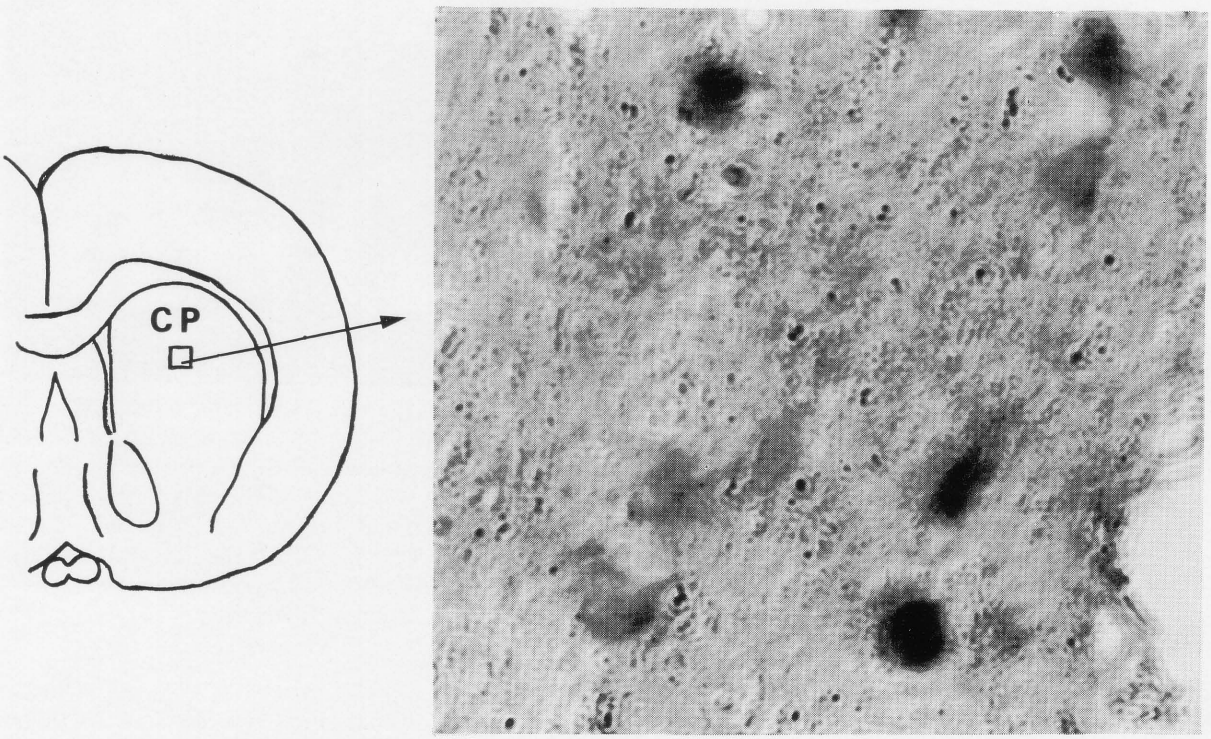

Fig. 1 Microautoradiographic localization of HM-PAO in caudate putamen (CP) of rat brain. Scattered grains were observed in parenchyma (×2,000).

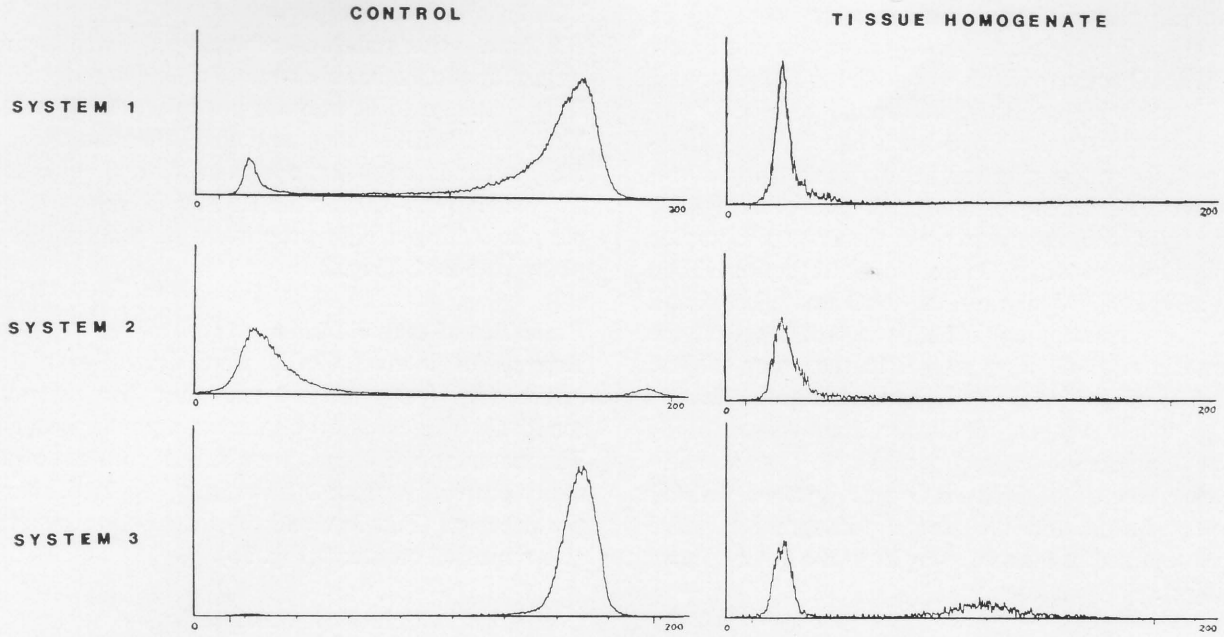

Fig. 2 TLC studies of tissue homogenate of rat brain one minute after injection of HM-PAO and its control sample. All three systems showed remaining activities at origin for tissue homogenate up to 60 minutes postinjection. system 1: ITLC/methylethylketone, system 2: ITLC/0.9% saline, system 3: Whatman No. 1/50% aqueous acetonitrile.

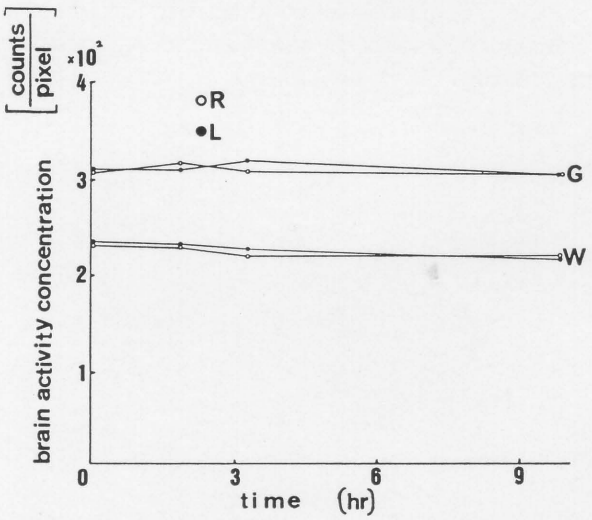

Fig. 3a Time course of brain activity concentrations for gray and white matters in normal volunteer showing almost constant activities (R: Right hemisphere, L: Left hemisphere, G: Gray matter, W: White matter).

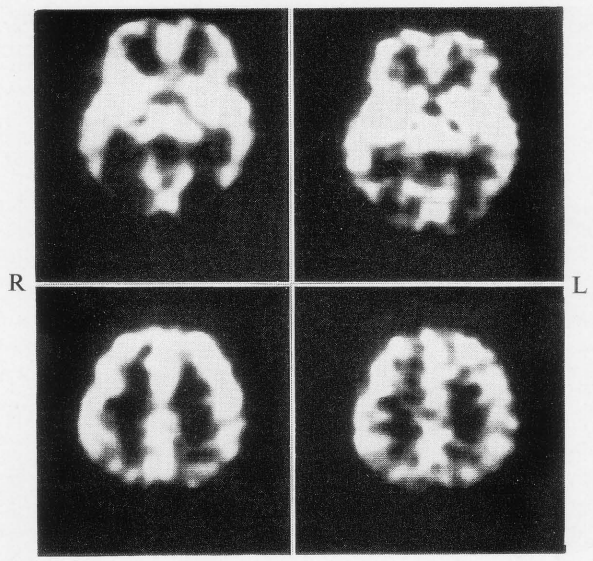

Fig. 3b Tomographic brain images of normal volunteer 3 minutes and 10.6 hours after injection of HM-PAO showing quite constant distribution patterns (Top row: 3 minutes and 10.6 hours postinjection at level of 4.5 cm above orbitomeatal line for left and right columns, respectively. Bottom row: 3 minutes and 10.6 hours postinjection at level of 8 cm above orbitomeatal line for left and right columns, respectively).

Binding rates to serum protein were 30% and 33% of the whole activity for 1 minute and 15 minutes incubation, respectively.

In the control human study, the reconstructed counts in the insular portion doubled in the second tomographic image. On the other hand, preincubation with whole-blood for 5 minutes reduced the accumulation of HM-PAO in the brain to 34% of the expected value (Fig. 4a). Marked activity was observed in the sinus after preincubation with whole-blood (Fig. 4b).

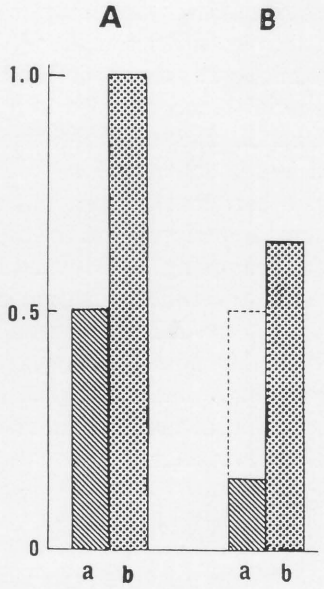

Fig. 4a Influence on brain activity of preincubation of HM-PAO with blood for 5 minutes. In control study without preincubation (A), two-step administration (a: first, b: second) of equal doses of HM-PAO doubled brain activity. Brain activity after second administration was normalized to 1.0. On other hand, in preincubation study (B, a), brain activity was reduced to 34% of expected value (dotted column). Column B, b showed brain activity after administration of additional same dose without preincubation.

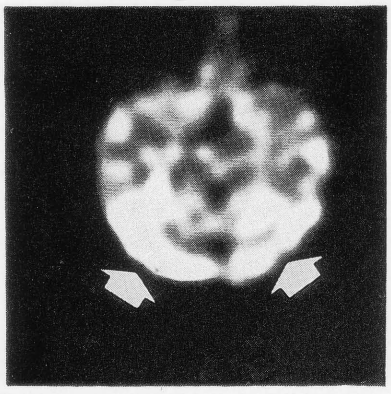

Fig. 4b Tomographic images obtained after preincubation of HM-PAO with blood at 3 cm above orbitomeatal line showing marked activities in transverse sinus (arrows).

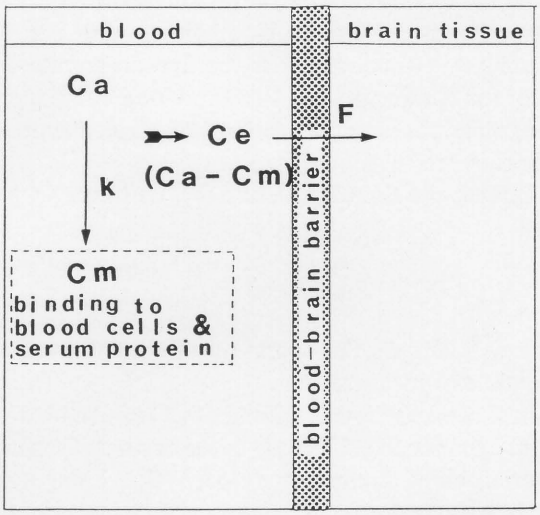

Fig. 5 Diagrammatic representation of theoretical model for quantifying cerebral blood flow using HM-PAO. *Ca*, *Cm*, and *Ce* represent arterial whole-blood concentration of HM-PAO, arterial activity concentration bound to blood cells and serum protein, and free HM-PAO activity concentration which can cross blood-brain barrier, respectively. *F* and *k* represent blood flow value and first-order rate constant for binding of HM-PAO to blood components.

DISCUSSION

From microautoradiography and TLC studies, it was revealed that HM-PAO crossed the blood-brain barrier and free HM-PAO rapidly disappeared in the brain tissue. There would be two possibilities regarding the findings obtained from the TLC study. One possibility is that the chemical composition of HM-PAO rapidly changed from lipophilic to hydrophilic in the brain tissue. The other possibility is that HM-PAO was rapidly bound to some brain components like protein in a similar fashion as in blood, consequently it could not ascend in the TLC system.

Back diffusion from brain to blood was found to be almost negligible. In the human volunteer study, brain activity was almost constant throughout the first 10.6 hours postinjection in both gray and white matters.

From the above findings, it is postulated that HM-PAO acts like a chemical microembolus. However, direct quantitative comparison with labeled microspheres resulted in erroneous underestimation of flow values in HM-PAO.

In the present study, this underestimation was ascribed to the high affinity of HM-PAO to blood components, blood cells and serum protein. Though the high affinity to blood cells has been reported,[1,4] the high affinity to serum protein has not been to date. Total binding rates to these components were 47% and approximately 60% for 1 minute and 5 minutes incubation, respectively. Furthermore, it was demonstrated that HM-PAO bound to these components could not cross the intact blood-brain barrier. Preincubation with blood for 5-minutes reduced the accumulation rate of HM-PAO in the brain to 34% of the expected value. In other words, 66% of the HM-PAO did not permeate the blood-brain barrier. The approximate 6% difference in the binding rates to blood components could be explained by taking into consideration the radiochemical change of primary HM-PAO to a more hydrophilic meta-

bolite during the 5-minutes preincubation. However, this factor of radiochemical change is relatively much smaller than the factor of binding to blood components.

Taking the binding of HM-PAO to blood components into consideration, the following simple equations were proposed for quantification of blood flow using HM-PAO.

$$dCm/dt = k \cdot (Ca - Cm) \quad (2)$$

$$dCb/dt = F \cdot Ce \quad (3)$$

$$Ca = Cm + Ce \quad (4)$$

where, Ca is the arterial whole-blood concentration of HM-PAO, Cm is the arterial activity concentration bound to blood components, Ce is the arterial activity concentration of free HM-PAO which can cross the blood-brain barrier, Cb is the brain activity concentration, F is the blood flow value per unit volume, t is time, and k is the first-order rate constant for binding of HM-PAO to blood components. Diagrammatic representation of the theoretical model is presented in Fig. 5.

Solving the Eq. (2), (3), and (4), we have,

$$Ce(t) = Ca(t) - k\ Ca(t) * \exp(-kt) \quad (5)$$

$$Cb(T) = F \int_0^T Ce(t)dt \quad (6)$$

where, * denotes the operation of convolution, and T is time of measurement.

In clinical studies, $Ca(t)$ and $Cb(T)$ can be obtained from dynamic SPECT study of the brain and multiple arterial blood sampling, respectively. Using these data, the values for F and k are estimated using nonlinear least squares fitting. This work has been performed in a normal volunteer and patients with cerebral diseases, and the results will be described in out next report.

There are still two points left to be pursued further. One point is how much the single-pass extraction ratio of free HM-PAO is. In Eq. (6), this ratio is an assumed to be one. If it were under one, the flow value would become higher. The other point is the influence of the radioactivity in the vascular compartment on brain activity. The influence is especially high in the initial period after injection. Eq. (6) would become complex by taking these two factors as follows:

$$Cb(t) = \alpha \cdot Ca(t) + (1 - \alpha) F \int_0^T m\ Ce(t)dt, \quad (7)$$

where α is the fraction of radioactivity attributable to the vascular compartment, and m is the single-pass extraction ratio. The value for m can be determined by animal experiments,[13,14] while the value for α has to be determined as an unknown value, as well as the values for F and k, using nonlinear least squares fitting.

As another method for obtaining free and lipophilic tracer activity in arterial blood, the octanol extraction technique was reported.[12] In this method, withdrawn arterial blood samples were rapidly extracted by octanol, and octanol-extracted activity was regarded as the activity which could permeate the blood-brain barrier. However, this technique is very troublesome to perform, and the time difference between blood sampling and octanol extraction could give rise to the underestimation of free HM-PAO activity since free HM-PAO readily decreases in blood. From this point of view, mathematical analysis is thought to be better than the octanol extraction technique in determining free HM-PAO activity in the intravascular space.

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