Noninvasive quantitation of rat cerebral blood flow using $^{99m}$Tc-HMPAO—assessment of input function with dynamic chest planar imaging

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Abstract

Background: Cerebral blood flow (CBF) quantitation using technetium-99m hexamethylpropyleneamine oxime ($^{99m}$Tc-HMPAO) generally requires assessment of input function by arterial blood sampling, which would be invasive for small animals. We therefore performed chest dynamic planar imaging, instead of arterial blood sampling, to estimate the input function and establish noninvasive quantitation method of rat CBF using the image-derived input function.

Results: Integrated radioactivity concentration in the heart-blood pool on planar images (AUC Blood-planar) was identical to that in arterial blood samples (AUC Blood-sampling). Radioactivity concentration in the brain determined by SPECT imaging (C_{Brain-SPECT}) was identical to that using brain sampling (C_{Brain-sampling}). Noninvasively calculated CBF obtained by dividing C_{Brain-SPECT} by AUC Blood-planar was well correlated with conventionally estimated CBF obtained by dividing C_{Brain-sampling} by AUC Blood-sampling.

Conclusion: Rat CBF could be noninvasively quantitated using $^{99m}$Tc-HMPAO chest dynamic planar imaging and head SPECT imaging without arterial blood sampling.

Keywords: Cerebral blood flow, Image-derived input function, Planar imaging, Rat, $^{99m}$Tc-HMPAO

Background

Cerebral blood flow (CBF) is associated with brain function in acute and chronic brain disorders, such as cerebrovascular disease, dementia, and epilepsy [1]. CBF levels also influence drugs’ therapeutic efficacy because most drug delivery to the brain is dependent on CBF. For studies using small-animal models of these brain disorders, CBF assessment meaningfully enhances the understanding of pathology in model animals and facilitates evaluation of drug efficacy.

$^{99m}$Tc hexamethylpropyleneamine oxime ($^{99m}$Tc-exametazime, $^{99m}$Tc-HMAO) is a single-photon emission computed tomography (SPECT) radiotracer for CBF assessment [2]. Our previous study reported that rat CBF could be quantified using $^{99m}$Tc-HMPAO [3]. Quantitative assessment of CBF using $^{99m}$Tc-HMPAO requires input function, which is generally assessed by arterial blood sampling. Arterial cannulation would be invasive for small animals and difficult to repeat in the same animal over the long term [4]. In addition, abundant and frequent blood sampling can affect the physiological condition, including blood pressure, blood cell count, and plasma hormone levels [5, 6]. Although blood sampling systems using microfluidic technologies have been developed to reduce the amount of blood sampling from small animals [4, 7], they cannot entirely obviate the need for arterial cannulation and blood sampling. Therefore, evaluation of the input function without blood sampling would be desirable for CBF quantification in small animals.

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Input function assessed by dynamic imaging of a large blood pool is used for quantitative analysis of some SPECT and positron emission tomography (PET) radiotracers in humans [8–10] and small rodents [11]. In the present study, we performed dynamic planar imaging of the heart after intravenous administration of 99mTc-HMPAO to assess image-derived input function. Rat CBF was quantified using the input function obtained from dynamic planar images and compared with the CBF conventionally quantified using arterial blood sampling.

Methods
Sodium [99mTc]pertechnetate was eluted from a 99Mo/99mTc generator (Ultra Techne Kow, Fujifilm RI Pharma Co., Ltd., Tokyo, Japan), which was previously eluted within 24 h. The kit formulation of HMPAO was obtained from Nihon Medi-Physics Co., Ltd. (Tokyo, Japan). The animal experiments in this study were performed in accordance with institutional and national guidelines regarding animal care and approved by the Animal Care and Use Committee of the Hamamatsu University School of Medicine. Male SD rats (8 w) supplied by Japan SLC Co. (Hamamatsu, Japan) were housed under light/dark 12-h cycles and had free access to food and water.

Preparation of 99mTc-HMPAO
Approximately 1.11 GBq freshly eluted sodium [99mTc]pertechnetate (5 mL) was added to the reaction vial of the HMPAO kit, and the reaction vial was gently swirled for a few seconds. Radiochemical purity was determined using a paper chromatography strip (Tec-Control Chromatography Strips for Bicisate and Exametazine; Biodex, Shirley, NY, USA) with ethyl acetate used as the mobile phase. Radioactivity in the fraction of the strips was determined with the auto-well gamma counter. Radiochemical purity of 99mTc-HMPAO just before injection was 90.5% ± 2.2%.

Animal preparation
Rats (n = 13) were fasted for 15 h and then administered pentobarbital (50 mg/kg) intraperitoneally. The subsequent experiments were performed under pentobarbital anesthesia. A polyethylene catheter (i.d. 0.5 mm, o.d. 0.8 mm) was inserted into the femoral artery and filled with heparin saline solution (10 IU/mL) for blood sampling. Acetazolamide (0 mg/kg (n = 8), 25 mg/kg (n = 2), or 50 mg/kg (n = 3)) was then injected intravenously 15 min before 99mTc-HMPAO administration.

Experimental protocol
99mTc-HMPAO (~ 185 MBq) was injected intravenously into the rats. Just after the 99mTc-HMPAO injection, dynamic chest coronal planar scans were performed dorsally for 1 min using a small animal PET/SPECT/CT system (FLEX; Gamma Medica Ideas, Northridge, CA, USA) equipped with a single pinhole collimator (1 mm). Arterial blood (approximately 100 μL per sample) was collected at 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, and 58 s post-injection of 99mTc-HMPAO. After a CT scan of the head, SPECT scan was performed using the small animal FLEX system equipped with multiple pinhole collimators (1 mm) from 15 to 57 min post-injection of 99mTc-HMPAO, since radioactivity concentration in the brain is steady from 14 s to 60 min post-injection of 99mTc-HMPAO [3]. SPECT data acquisition was performed at 30 s per projection with stepwise rotation of 64 projections over 360°. SPECT images were reconstructed using three-dimensional-ordered subset expectation maximization algorithms with 8 subsets and 10 iterations. After SPECT scanning, the rats were immediately sacrificed by decapitation, and the brain was removed and weighed. 99mTc radioactivity concentrations in the arterial blood samples (C_{blood-sampling}) and brain (C_{brain-sampling}) were measured with the auto-well gamma counter.

Phantom studies were performed using a 14.1 mm diameter cylindrical phantom with known 99mTc concentration (~ 11.1 MBq/mL) to mimic the size of rat heart and brain. Planar and SPECT imagings of the phantom were performed under the same conditions as already described rat heart and brain imagings. The region of interest (ROI) on planar images and the volume of interest (VOI) were manually drawn over the whole phantom to cross-calibrate between the radioactivity concentrations obtained from the planar or SPECT images, and auto-well gamma counter.

**CBF calculation using arterial blood and brain sampling (conventional procedure)**
CBF was calculated using the microsphere method, as previously described [3], with slight modification. Briefly, C_{blood-sampling} at 14–60 s was fitted to a biexponential curve by the least-squares method to estimate the arterial blood radioactivity concentration at 19 s post-injection according to previous results [3]. C_{blood-sampling} was integrated using the trapezoidal approximation to obtain the area under the curve (AUC) until 19 s post-injection of 99mTc-HMPAO (AUC_{blood-sampling}). C_{brain-sampling} quantified by the auto-well gamma counter was divided by the AUC_{blood-sampling} value to calculate the CBF using arterial blood and brain sampling (CBF-sampling).

**CBF calculation using planar and SPECT imaging**
The ROI was manually drawn on the heart in the chest planar image using the radiographic planar image of the same rat as a reference (Fig. 1a), and the mean radioactivity concentration in the ROI was quantified (C_{blood-planar}).
CBlood-planar was integrated using the trapezoidal approximation to obtain the AUC until 19 s post-injection of 99mTc-HMPAO (AUCBlood-planar). Head CT and SPECT images were combined, and the VOI was manually drawn over the whole brain (Fig. 2a). CBrain was quantified as the mean concentration in the VOI (CBrain-SPECT). CBrain-SPECT was divided by AUCBlood-planar to obtain CBF-imaging. CBF-imaging was also compared with the CBF calculated from CBrain-SPECT and AUCBlood-sampling to estimate the effects on CBF calculation between AUCBlood-planar and AUCBlood-sampling.

**Statistical analysis**

The associations between AUCBlood-sampling and AUCBlood-planar, CBrain-sampling and CBrain-SPECT, and CBF-sampling and CBF-imaging were calculated using Spearman’s rank correlation coefficients.

**Results**

**Input function measured from dynamic planar images vs. blood sampling**

The time–activity curves for the heart measured from dynamic chest planar images and in arterial blood of the same rat measured from blood samples are shown in Fig. 1b. Both time–activity curves showed the highest concentration of 99mTc at 5–10 s post-injection and followed time-dependent decreased radioactivity. AUCBlood-planar showed a good correlation with AUCBlood-sampling (Fig. 1c) ($n = 13$, $\rho = 0.868$, $p < 0.001$), and the regression line was nearly $y = x$ (slope = 1.09).

**Brain radioactivity measured from SPECT images vs. resected samples**

Figure 2b compares CBrain obtained from SPECT images and resected brain samples. CBrain-SPECT was well correlated with CBrain-sampling ($n = 13$, $\rho = 0.929$, $p < 0.001$), and the regression line was nearly $y = x$ (slope = 1.02).

**Noninvasively quantified CBF vs. conventionally quantified CBF**

Figure 3a shows the correlation between CBF-imaging, which is noninvasively quantified using AUCBlood-planar and CBrain-SPECT, and CBF-sampling, which is conventionally quantified using AUCBlood-sampling and CBrain-sampling. CBF-imaging showed a good correlation with CBF-sampling. ($n = 13$, $\rho = 0.918$, $p < 0.001$). The regression line was nearly $y = x$ (slope = 0.94).

**The effects on CBF quantification between dynamic planar images and blood sampling**

Figure 3b shows the correlation between CBF-imaging and CBF quantified using AUCBlood-sampling and CBrain-SPECT (CBF-SPECT/sampling). CBF-imaging showed a good correlation with CBF-SPECT/sampling ($n = 13$, $\rho = 0.945$, $p < 0.001$). The regression line was nearly $y = x$ (slope = 0.90).
Discussion

Rat CBF could be quantified by a microsphere method in which $C_{\text{Brain}}$ at 5 min post-injection is divided by the integrated arterial blood radioactivity concentration until 19 s post-injection of $^{99m}$Tc-HMPAO (AUC$_{\text{Blood}}$) [3]. In the present study, AUC$_{\text{Blood}}$ and $C_{\text{Brain}}$ were noninvasively estimated using planar imaging and SPECT, respectively. Using the noninvasively estimated AUC$_{\text{Blood}}$-planar and $C_{\text{Brain}}$-SPECT, rat CBF was quantified (CBF-imaging) and compared with conventionally quantified rat CBF (CBF-sampling).

$C_{\text{Blood}}$ was estimated using dynamic planar imaging of the heart-blood pool. To quantify the radioactivity concentration in arterial blood as an input function via chest dynamic planar images, the ROI should be drawn only on the left ventricle cavity. However, in this study, we drew the ROI over the whole heart to calculate the blood radioactivity concentration in the heart (C$_{\text{Blood}}$-planar) as an input function, because rat left ventricle cavity is too small and too complicated to confirm by two-dimensional planar images. Hence, there could be some differences between C$_{\text{Blood}}$-sampling and C$_{\text{Blood}}$-planar values. In addition,
planar imaging could underestimate the radioactivity concentration because of scatter and attenuation of gamma rays [12]. Despite these differences, C\textsubscript{Blood-planar} was identical to C\textsubscript{Blood-sampling} until 19 s post-injection of \textsuperscript{99m}Tc-HMPAO (Fig. 1b). AUC\textsubscript{Blood-planar} was well correlated with AUC\textsubscript{Blood-sampling} (Fig. 1c), suggesting that dynamic chest planar imaging could estimate the AUC\textsubscript{Blood} until 19 s post-injection, which is required to quantify the rat CBF using \textsuperscript{99m}Tc-HMPAO [3]. These results indicate that the differences between arterial blood sampling and chest planar imaging might be negligible for estimating AUC\textsubscript{Blood} until 19 s post-injection under the experimental conditions of the present study.

\(C_{\text{Brain}}\) was determined by SPECT. Because our previous study showed that \(C_{\text{Brain}}\) was quite steady from 14 s to 60 min post-injection of \textsuperscript{99m}Tc-HMPAO [3], SPECT would be well suited for quantitative evaluation of \(C_{\text{Brain}}\) within this period. Figure 2b shows that \(C_{\text{Brain-SPECT}}\) at 15–57 min post-injection was identical to \(C_{\text{Brain-sampling}}\) determined just after SPECT imaging. This result suggests that scatter and attenuation of gamma rays would be negligible for \(C_{\text{Brain}}\) quantitation by SPECT under these conditions, and SPECT could determine \(C_{\text{Brain}}\) after \textsuperscript{99m}Tc-HMPAO injection. Considering that \(C_{\text{Brain}}\) is steady from 14 s to 60 min post-injection of \textsuperscript{99m}Tc-HMPAO [3], \(C_{\text{Brain-SPECT}}\) at 15–57 min post-injection of \textsuperscript{99m}Tc-HMPAO could be equal to \(C_{\text{Brain}}\) at 5 min post-injection, which is required for quantification of rat CBF by the \textsuperscript{99m}Tc-HMPAO microsphere method [3].

Thus, rat CBF was calculated using a microsphere method in which \(C_{\text{Brain}}\) was divided by AUC\textsubscript{Blood} until 19 s post-injection. CBF-imaging, which is obtained by dividing \(C_{\text{Brain-SPECT}}\) by AUC\textsubscript{Blood-planar}, was identical to conventionally estimated CBF-sampling using \(C_{\text{Brain-sampling}}\) and AUC\textsubscript{Blood-sampling}, suggesting that rat CBF could be quantitatively estimated using \textsuperscript{99m}Tc-HMPAO-planar and SPECT imaging without sampling arterial blood. Thus, noninvasive quantification of rat CBF could enhance the repeatable CBF assessment without the physiological damage due to arterial cannulation and blood sampling.

Conclusions

We propose a noninvasive procedure for quantitating rat CBF using \textsuperscript{99m}Tc-HMPAO. Rat CBF values, which were noninvasively quantified using chest dynamic planar imaging and head SPECT, were nearly identical to conventionally determined rat CBF using arterial blood and brain sampling. These findings indicate that rat CBF could be noninvasively and quantitatively assessed by combining \textsuperscript{99m}Tc-HMPAO-planar imaging and SPECT, thereby avoiding arterial blood sampling.