Noninvasive Measurement of Cerebral Blood Flow With $^{99m}$Tc-Hexamethylpropyleneamine Oxime Single-Photon Emission Computed Tomography and 1-Point Venous Blood Sampling

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Background and Purpose—The arterial and venous blood concentration of technetium 99m–labeled hexamethylpropyleneamine oxime ($^{99m}$Tc-HMPAO) reaches an equilibration more rapidly than other CBF tracers. We hypothesized that $^{99m}$Tc radioactivity of a venous sample at equilibrium, which is similar to that of an arterial sample, would allow estimation of the integrated input function for the clinical measurement of CBF by use of single-photon emission CT.

Methods—In 53 patients with stable cerebrovascular disease, the radioactivity of a venous sample 5 minutes after injection of $^{99m}$Tc-HMPAO was correlated with 5-minute arterial blood radioactivity and the first 5 minutes of the integrated arterial curves of the lipophilic tracer. The measured CBF values were compared with those of xenon 133.

Results—The radioactivity of 5-minute venous blood was almost equivalent to that of 5-minute arterial blood ($r^2=0.987$; $y=0.993x+1.63$; $P<0.0001$). The correlation between the venous blood radioactivity and the integrated arterial lipophilic fraction was excellent ($r^2=0.935$, $P<0.0001$). A strong correlation was obtained between $^{99m}$Tc-HMPAO and $^{133}$Xe CBF values ($r^2=0.825$, $P<0.0001$). CBF values were reproducible (coefficient of variation, 8.6%).

Conclusions—This approach is fast, simple, and an alternative to continuous blood sampling in clinical quantitative $^{99m}$Tc-HMPAO CBF studies. (Stroke. 2000;31:2203-2207.)

In stroke research, the availability of a quantitative cerebral blood flow (CBF) image is important for interpretation of changes in the brain. Hexamethylpropyleneamine oxime labeled with technetium 99m ($^{99m}$Tc-HMPAO) is useful for evaluating the physiological changes that accompany regional cerebral blood flow (rCBF) abnormalities in acute stroke. The tracer is suitable for the routine determination of CBF, because it is readily available from a freeze-dried kit and is rapidly converted to a hydrophilic form that is retained for many hours.

Several methods of rCBF quantification are reported for $^{99m}$Tc-HMPAO single-photon emission CT (SPECT). Generally, there are 2 ways of measuring CBF by this tracer. One is to measure the brain time-activity curve and to obtain the arterial input curve either directly by arterial blood sampling or indirectly from $^{99m}$Tc-HMPAO angiography. The other way is to use the true CBF of the reference region so that $^{99m}$Tc-HMPAO SPECT yields a quantitative rCBF image relative to a reference CBF. These methods require continuous arterial blood sampling, the dynamic information of the tracer uptake in the brain, or introduction of another diffusible tracer to obtain a reference CBF. Drawbacks to the CBF quantification are that this is time consuming and not readily available in the clinical setting. The present study describes the noninvasive and rapid quantification of CBF by measurement of the steady-state influx constant of $^{99m}$Tc-HMPAO. CBF can be quantified with a single SPECT scan and 1-point venous sampling when equilibrium between arterial and venous radioactivities is reached.

Subjects and Methods

Theory

The kinetic model of blood-brain exchange reported by Neirinckx et al. was used. The $^{99m}$Tc forms a lipophilic complex with HMPAO. After intravenous injection of HMPAO, it passes through the blood-brain barrier, and a fraction $E$ of the lipophilic tracer is extracted into the brain. Inside the brain, the lipophilic tracer is rapidly converted to a hydrophilic form that is retained for many hours, whereas some of lipophilic complexes diffuse back to the blood. When complete brain-blood equilibrium is reached, $t$ minutes after tracer injection, observed brain radioactivity can be expressed as follows:

\[ SPECT = E \cdot R \cdot CBF \cdot \int_0^t CaL(t) \, dt \]

where SPECT is the radioactivity in the whole-brain region of interest (ROI) of SPECT, $E$ is the first-pass extraction of HMPAO by arteriovenous washout, $R$ is the true rCBF at the reference region, $Cbf$ is the true rCBF at the reference region, and $CaL(t)$ is the arterial concentration-time curve of the tracer.
the brain, R is the retained fraction of lipophilic radioactivity in the
brain, and CaL is the lipophilic tracer radioactivity in the arterial
blood.

If E and R are known and steady-state radioactivity of the venous
blood has close associations with CaL and \( j_0 \), CaL (t)dt, Equation 1
can be solved simply in terms of the tracer concentrations in the
venous blood and in the brain as follows:

\[
\text{CBF [mL/100 g/min]} = \frac{\text{SPECT [\mu Ci/100 g]}}{E \cdot R \cdot k \cdot \text{Cv}(5) [\mu Ci/min/mL]}
\]

where \( E = 0.72, R = 0.54, \) Cv(5) is the radioactivity of the venous
blood at steady state (5 minutes), and k is the slope of the regression
line of

\[
\int_0^5 \text{CaL}(t) dt
\]

with Cv(5)=4.62.

Patients

CBF was measured in 53 patients with stable cerebrovascular disease
(CVD) (28 men and 25 women; mean age 70.6 [range 47 to 87]
years). No patients had a renal insufficiency. Diagnosis was based on
criteria from the National Institute of Neurological Disorders and
Stroke. Six patients had had a transient ischemic attack, 33 a
lacunar infarction, 1 an atherothrombotic infarction, 2 a cerebral
hemorrhage, and 11 a poststroke dementia. All patients gave in-
formed consent for participation in the study.

CBF Studies

\(^{99}\text{Tc}\)-HMPAO was formed by reconstituting a commercial vial of
HMPAO (Cerebrotec, Amersham Health Care) with 5 mL of 15 to
30 mCi (555 to 1110 MBq) fresh \(^{99}\text{Tc}\) pertechnetate. Arterial blood
samples were obtained from a small catheter placed in the brachial
artery. The sampling was performed every 15 seconds for the first 2
minutes and every 30 seconds for the next 3 minutes after intrave-
nous injection of 10 mCi \(^{99}\text{Tc}\)-HMPAO. Arterial blood was col-
llected in vials containing 1 mL of octanol, and the arterial concen-
tration of lipophilic tracer was measured by the rapid octanol
extraction technique. Venous blood also was sampled 5 minutes after
injection.

SPECT scanning was then started with a single-head rotating
camera (GCA-901A, Toshiba) with a resolution of 17 mm full-width
half-maximum, using a low-energy, high-resolution collimator. Sixty
views, 20-second frames collected over 360°, were recorded into a
parallel 32-detector system (BF 1400, Valmet). Sixteen detectors
were symmetrically placed in each hemisphere. Approximately 20
mCi of \(^{133}\text{Xe}\)-labeled saline was injected into the antecubital vein.
The clearance of the head curve was recorded over 15 minutes from
each head detector as well as from a separate detector that monitored
the radioactivity in expired air. The clearance curve was fitted by a
2-compartment deconvolution, with end-tidal \(^{133}\text{Xe}\) counts as an
input function.

Integrated lipophilic activity was determined by summing the area
under the measured concentration curve CaL(t) between 0 and 5
minutes and was calibrated and converted to units of microcuries per
minute per millilitre. The time course of the ratio of lipophilic to
nonlipophilic radioactivity was expressed as a percentage of the total
radioactivity in each arterial sample. Venous blood radioactivity 5
minutes after \(^{99}\text{Tc}\)-HMPAO injection was compared with that of
arterial blood and the integrated lipophilic tracer activity up to 5
minutes after injection. Eight subjects were scanned twice, 1 week
apart, to determine the reproducibility of CBF values. An ROI in the
whole brain was defined by incorporating all pixels that were >30% of
the maximum counts per pixel on a single SPECT section
containing the basal ganglia. Tracer concentration measured within
the whole brain was expressed as \( \mu Ci \) (37 kBq)/100 g, assuming a
brain weight of 1270 g. A mean whole-brain \(^{133}\text{Xe}\) CBF value was
calculated for each subject from fast flow (f1), slow flow (f2), and
the obtained weight ratio between gray and white matter (w1/w2). Results were analyzed by using the Pearson equation and linear
regression. Data were presented as mean±SD. Statistical signifi-
cance was set at P<0.05.

Results

As shown in Figure 1, the 5-minute tracer counts of venous
blood sample were almost equivalent to those of the arterial
blood sample: \( 36.4±137.1\times10^4 \) counts per minute per millilitre
(cpm/mL) versus \( 567±137.2\times10^4 \) cpm/mL (\( r^2 = 0.987, P<0.0001; \) \( y = 0.993x + 1.63 \)). The table shows the
time course of the concentration of lipophilic tracer in the
arterial blood. The percentage of the lipophilic radioactivity
was highest after 15 seconds, then decreased rapidly within

### Time Course of the Arterial Lipophilic and Nonlipophilic Radioactivities

<table>
<thead>
<tr>
<th>Time, s</th>
<th>Lipophilic%</th>
<th>Nonlipophilic%</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>85.9</td>
<td>14.1</td>
</tr>
<tr>
<td>30</td>
<td>73.6</td>
<td>26.4</td>
</tr>
<tr>
<td>45</td>
<td>42.9</td>
<td>57.1</td>
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<tr>
<td>60</td>
<td>19.4</td>
<td>80.6</td>
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<tr>
<td>75</td>
<td>15.3</td>
<td>84.7</td>
</tr>
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<td>90</td>
<td>5.5</td>
<td>94.5</td>
</tr>
<tr>
<td>105</td>
<td>7.8</td>
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<tr>
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<td>2.7</td>
<td>97.3</td>
</tr>
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<td>150</td>
<td>4</td>
<td>96</td>
</tr>
<tr>
<td>180</td>
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<td>93.5</td>
</tr>
<tr>
<td>210</td>
<td>1.8</td>
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</tr>
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<td>240</td>
<td>1.3</td>
<td>98.7</td>
</tr>
<tr>
<td>270</td>
<td>0.1</td>
<td>99.9</td>
</tr>
<tr>
<td>300</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Lipophilic% indicates the percentage of total radioactivity extracted by
octanol; nonlipophilic%, the percentage of radioactivity extracted, primarily in
the hydrophilic form.
the subsequent 1 minute, and reached nearly zero after 4 minutes. There was a strong correlation between the 5-minute radioactivity of venous blood and the integrated arterial lipophilic tracer activity up to 5 minutes after injection \((r^2=0.935, P<0.0001; y=4.62x-0.14)\) (Figure 2). The y intercept of this regression line was sufficiently small compared with the magnitude of integrated arterial lipophilic tracer activity.

Average values of \(^{99m}\)Tc-HMPAO CBF and \(^{133}\)Xe CBF in the whole brain were 35.6±7.3 and 37.0±8.3 mL·100 g⁻¹·min⁻¹, respectively. A close correlation was observed for \(^{99m}\)Tc-HMPAO CBF versus \(^{133}\)Xe CBF \((r^2=0.825, P<0.0001; y=0.804x+5.84)\) (Figure 3).

Mean whole-brain CBF values obtained in the second measurement \((33.5±7.8 \text{ mL}·100 \text{ g}^{-1}·\text{min}^{-1})\) did not differ significantly from those obtained in the first \((34.2±4.7 \text{ mL}·100 \text{ g}^{-1}·\text{min}^{-1})\). The reproducibility of CBF values was good \((r^2=0.622, P<0.005; y=0.987x+0.9)\). The coefficient of variation for CBF was 8.6%.

**Discussion**

We found that the radioactivity of the 5-minute venous blood sample was almost identical to that of the 5-minute arterial blood sample and was proportional to the integrated arterial lipophilic radioactivity of \(^{99m}\)Tc-HMPAO. At 5 minutes, equilibration between brain and blood is fully achieved, because the ratio of radioactivity in the arterial blood versus that in the venous blood reaches a plateau. This rapid decline of lipophilic \(^{99m}\)Tc-HMPAO from the blood can be caused by rapid conversion to hydrophilic metabolites and binding to some blood component. In rat biodistribution data, \(^{a}\) a high proportion of the radioactivity remaining in the blood appears to be trapped within red blood cells. It is possible that the mechanism for entrapment within these cells is similar to that of brain retention. This may explain why radioactivity of the 5-minute blood sample is strongly related to the integrated lipophilic radioactivity from the plasma input curve. Pupi et al.\(^{b}\) found that fractional brain uptake derived from the injected dose was not a reliable indicator of \(^{99m}\)Tc-bicisate CBF, suggesting that intracellular and extracellular radioactivities may vary from individual to individual. Considering the intersubject variability of kinetic parameters in the blood and in the brain, the need of blood sampling and counting for CBF determination was indicated.

We were able to measure CBF with a shorter examination period than those in the previous methods that used continuous arterial blood sampling and/or kinetic analysis of the tracer with a high-performance gamma camera system. This was made possible through the substitution of 1-point venous blood sampling for arterial blood sampling to obtain an input and no need for dynamic SPECT data acquisition. SPECT counts/integrated lipophilic activity reflects essentially the steady-state influx constant of Patlak and Blasberg,\(^{15}\) which can be measured with a single SPECT scanning. In other words, net SPECT counts/integrated lipophilic activity is expected to be a quantitative index of CBF. Data acquisition time can be reduced further by using a multi-ring SPECT camera. CBF measurement can be completed within 25 minutes, and thus the use of the present method as a tool for brain function would not delay acute stroke therapy.

For radiolabeled microspheres, the extraction fraction is \(\approx 100\%\) in the brain tissue of humans, and tracer uptake versus CBF have a linear relationship.\(^{16}\) However, this method is not suitable for human use because of its invasiveness. Most of the limitations of CBF tracers, including \(^{133}\)Xe-, \(^{99m}\)Tc-, or \(^{125}\)I-labeled tracer tracers and \(^{18}\)O arise from the nonlinear relationship between true CBF and measured radiotracer concentration.\(^{17-19}\) Over a CBF range of 20 to 120 mL·100 g⁻¹·min⁻¹, \(^{133}\)Xe CBF correlates linearly with true CBF.\(^{17}\) At a CBF level that corresponds to normal regional CBF for human cortex, 50 mL·100 g⁻¹·min⁻¹, \(^{99m}\)Tc-HMPAO has a first-pass extraction of approximately \(\geq 70\%\).\(^{18}\) The underestimation of CBF in the present method appeared to be less at whole-brain CBF levels of up to 50 mL·100 g⁻¹·min⁻¹. When CBF exceeded 50 mL·100 g⁻¹·min⁻¹, CBF was underestimated because of the limitation of brain permeability to \(^{99m}\)Tc-HMPAO; an estimated \(^{99m}\)Tc-HMPAO CBF was 86.2 mL·100 g⁻¹·min⁻¹ at the \(^{133}\)Xe CBF level of 100 mL·100 g⁻¹·min⁻¹. We measured CBF by using fixed values of E and R that were obtained from the whole brain after an intracarotid bolus injection of the tracer, at a mean CBF level of 59 mL·100 g⁻¹·min⁻¹.\(^{19}\) Calculating the permeability surface area product of brain capillaries\(^{20}\) or the regression between E and \(^{99m}\)Tc-HMPAO CBF,\(^{6}\) we can correct low extraction of \(^{99m}\)Tc-HMPAO CBF SPECT.

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**Figure 2.** Relationship between 5-minute venous blood radioactivity and the first 5 minutes of the integrated arterial curves of the lipophilic radioactivity. Values are expressed as μCi/mL.

**Figure 3.** Relationship between \(^{99m}\)Tc-HMPAO CBF and the \(^{133}\)Xe CBF. Solid line represents regression line; dotted line, identical line.
The rational approach to linearize 99mTc-HMPAO CBF is to correct flow-dependent backdiffusion of the tracer by an equation described by Andersen et al.\(^1\) and Lassen et al.\(^2\) The HMPAO conversion/clearance ratio (α) is different in each individual case, and this assumption is not true in specific diseased regions of the brain. The clinical relevance of the use of correction equations for E and R remains to be clarified. Further studies are necessary to verify the accuracy of the assumptions and to determine the optimal correction method to linearize brain uptake of CBF tracers versus blood-flow relationship.

The accuracy of the estimation of CBF is influenced by multiple sources of variation, such as the difference in E, variation in R, shape and height of the input function, and errors in the measurement of blood flow. The possibility of unreliable CBF estimates arises from propagation of errors. The whole-brain 99mTc-HMPAO CBF value of 35.6±5.3 mL · 100 g\(^{-1}\) · min\(^{-1}\) in patients with chronic CVD is in agreement with the values previously reported in the literature.\(^{21–23}\) As for the reproducibility of the measurement of CBF, coefficients of variation of the 133Xe clearance method\(^24\) and the C\(^{15}\)O\(^2\) inhalation method\(^25\) are 6.5% and 5%, respectively. Our coefficient of variation of 8.6% is thought to be acceptable for the measurement method of CBF.

Early assessment of patient characteristics that predict outcome after acute ischemic stroke is essential in therapeutic trials and clinical practice.\(^{26,27}\) At present, CBF SPECT analysis of the effects of acute stroke therapy with tissue plasminogen activator is under study.\(^28\) The clinical significance of knowing severity, size, and location of ischemia in CVD has not yet been fully determined.\(^29\) Some question remains about whether the theory for CBF quantification is true in a particular tissue of the brain, because many brain regions contain a variety of disease and pathological states. Functional tissue heterogeneity (i.e., inclusion of tissues with different rates of E, R, flow, and metabolism within a single ROI) is an unavoidable problem with functional imaging modality. Focal alteration in E and R in pathological tissue may contribute to the error in calculated CBF, but presently it is impossible to separate these effects from global estimates of E and R. Calculation of true CBF is essentially difficult in most of CBF tracers as long as E is not complete and backdiffusion exits. Furthermore, the hyperfixation of HMPAO in infarct reperfusion may limit the estimation accuracy.\(^30\) In patients with CVD, not only the degree of neurological impairment but also age, gender, risk factors, and severity of carotid atherosclerosis can influence CBF.\(^31\) We have focused on the design and methodology of a simpler, noninvasive method for 99mTc-HMPAO CBF quantification. Our method is noninvasive, computationally fast, and effective for measuring CBF in patients with CVD. Future studies are needed to determine whether the use of 99mTc-HMPAO-SPECT in the evaluation of CVD promises better differentiation between areas of potentially viable and irreversibly injured tissue than that possible by conventional neuroimaging methods alone.

Acknowledgments

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References


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