Regional Cerebral Blood Flow and Distribution of $[^{99m}Tc]$Ethyl Cysteinate Dimer in Nonhuman Primates

Cesare Orlandi, MD, Paul D. Crane, PhD, Steven H. Platts, BS, and Richard C. Walovitch, PhD

Increases in regional cerebral blood flow have been described in a variety of cerebral pathologic states, including stroke and seizure disorders. The usefulness of technetium-99m-labeled cysteinate dimer as a marker in the measurement of regional cerebral blood flow was tested in five cynomolgus monkeys. To expand the range of blood flow to beyond the normal limits, 40 mg/kg i.v. of the carbonic anhydrase inhibitor acetazolamide was administered. Regional cerebral blood flow in all five monkeys was measured using radiolabeled microspheres (before and after acetazolamide) and the marker (after acetazolamide) in 60-70 samples from 12 brain regions. Acetazolamide significantly increased the mean±SEM regional cerebral blood flow measured using microspheres from 0.56±0.21 to 1.71±0.9 ml/min/g ($p<0.01$ for each region). A significant positive correlation was found between regional cerebral blood flow values calculated using microspheres and the marker after normalizing the values to those in the cerebellum ($r=0.773$, $p<0.0001$). The mean±SEM regional cerebral blood flow determined using the marker in a single monkey (1.21±0.04 ml/min/g) did not differ significantly from that determined in the same monkey using microspheres (1.13±0.04 ml/min/g). These data support the potential use of this new brain perfusion imaging agent to assessment regional cerebral blood flow over a clinically relevant range of blood flows. (Stroke 1990;21:1059–1063)

Technetium-99m-labeled ethyl cysteinate dimer ($[^{99m}Tc]$ECD) is a new radiochemically stable and pure brain perfusion agent that is being evaluated as a marker of regional cerebral blood flow (rCBF) in stroke and other neurologic diseases. Preliminary work has demonstrated that the distribution of $[^{99m}Tc]$ECD under conditions of normal or reduced perfusion as determined by single-photon emission computed tomography (SPECT) is a function of cerebral blood flow.

It is widely acknowledged that in several pathologic states, such as focal ictal seizures, brain tumors, and stroke during the subacute phase, locally increased metabolic demands increase rCBF to beyond the range observed under normal conditions. Similar increases in rCBF are also pharmacologically induced clinically with agents such as the carbonic anhydrase inhibitor acetazolamide. These pharmacologic stress tests have been extensively used to evaluate cerebral vascular reserve in stroke patients. Brain regions with reduced rCBF responses to acetazolamide indicate perfusion alterations for which surgical interventions may be appropriate.

A linear relation between rCBF and $[^{99m}Tc]$ECD retention over a clinically relevant range of blood flows is needed before we can employ this new radiopharmaceutical agent in high-flow pathologic states and stress studies. We studied the relation between rCBF as measured with radiolabeled microspheres and $[^{99m}Tc]$ECD retention during acetazolamide-induced hyperemia in nonhuman primates to evaluate the cerebral distribution of $[^{99m}Tc]$ECD at blood flow levels beyond the normal range.

Materials and Methods

Five adult, male cynomolgus monkeys weighing 3.6–10.3 kg were food-deprived for approximately 20 hours, and water was removed on the morning of the study. The monkeys were anesthetized with 10 mg/kg i.m. ketamine hydrochloride and 1 mg/kg i.m. acepromazine maleate. Anesthesia was then maintained with a constant infusion of 1.5 mg/ml i.v. thiamylal sodium (Parke-Davis, Morris Plains, N.J.) delivered through a 22-gauge catheter inserted into a saphenous vein at a rate averaging <0.5 ml/min. The monkeys were mechanically ventilated with room air (Harvard Apparatus, South Natick, Mass.). Arterial
blood samples were obtained at regular intervals throughout the experiments to monitor pH, PaCO₂, and Pao₂ (model 178, Corning, Medfield, Mass.), and the respirator was adjusted to maintain arterial blood gases within the normal physiologic range.

A 4-French Model PC-340 pressure transducer (Millar Instruments Inc., Houston, Tex.) was inserted into a superficial femoral artery through a small incision in the groin for measuring arterial blood pressure. A 5-French polyethylene catheter was placed in the contralateral superficial femoral artery and guided to the aortic arch for sampling arterial blood. One common carotid artery was surgically isolated, and carotid blood flow was monitored with a 2.0–2.5-mm-diam. electromagnetic flow probe connected to a Biotronex Model BL-613 flowmeter (Kensington, Md.). Limb-lead electrocardiographic tracings were also obtained for monitoring heart rate.

The cardiovascular variables blood gases, heart rate, blood pressures, and carotid blood flow were recorded continuously on a Grass Model 7 polygraph (Quincy, Mass.). With the monkey in dorsal recumbency, a left fifth thoracotomy was performed, the lungs were retracted, and the pericardium was opened. A polyethylene tube was placed in the left atrial appendage for injecting radiolabeled microspheres and [⁹⁹mTc]ECD.

Microspheres 10–15 μm in diameter (E.I. du Pont de Nemours & Co., Boston, Mass.) were labeled with scandium-46 or tin-113. These labels provided a minimum peak-to-peak separation of at least 100 keV, thereby assuring statistically accurate spill correction calculations. Aliquots for injection were calibrated to contain approximately 1–2×10⁹ microspheres suspended in 0.9% saline containing 0.01% poloxymethylene sorbitan monooctade and diluted to a final volume of 1 ml. Adequate mixing was obtained by sonication. Microscopic examination of a drop of the microsphere suspension revealed no significant clumping.

We used a kit formulation of [⁹⁹mTc]ECD with a commercial molybdenum-99/technetium-99m generator (Du Pont, North Billerica, Mass.).¹ The radiochemical purity of the [⁹⁹mTc]ECD was determined by reverse-phase thin-layer chromatography (Whatman MKC18 plates [Clifton, NJ], with a 60:40 acetonitrile/0.5 M ammonium acetate [pH 7.0] mobile phase). Plates were quantified with a linear scanner fitted with a NaI detector.¹³ Ethyl cysteinate dimer formed a stable complex of >90% radiochemical purity with technetium-99m within 15 minutes at room temperature.

After obtaining stable baseline recordings of the cardiovascular variables in all five monkeys, the first set of microspheres was injected as a bolus over 5–10 seconds into the left atrium; the injection catheter was then flushed with 10 ml of 0.9% saline. A 6-ml arterial blood sample was withdrawn from the aortic arch catheter using a Sage Model 351 infusion/withdrawal pump (Cambridge, Mass.) for 1 minute after the initiation of the injection. To increase CBF the five monkeys then received 40 mg/kg i.v. acetazolamide. [⁹⁹mTc]ECD was injected 18 minutes after the injection of acetazolamide, and 2 minutes later the second set of microspheres was injected. Arterial blood samples were withdrawn after the injection of both [⁹⁹mTc]ECD and the second set of microspheres. At the end of the experiments the monkeys were killed by the intravenous injection of T61-euthanasia solution (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.).

The brains were removed, rinsed, and prepared for analysis by a modification of the method of Marcus et al.,¹⁴ which quantifies the instantaneous nutrient blood flow at the time of each microsphere injection. The brains were sectioned into 12 anatomic regions, and several samples weighing 1–1.5 g were taken from each region. The resulting brain samples and reference arterial blood samples were weighed and assayed simultaneously for scandium-46, tin-113, and technetium-99m in a Packard Auto Gamma scintillation spectrometer (Sterling, Va). Blood flow in milliliters per minute was calculated as (Cₐ.x.Qr)/Cₐ, where Cₐ=brain sample counts per minute per gram, Q_r=reference arterial blood sample withdrawal rate (milliliters per minute), and Cₐ=reference arterial blood sample counts per minute. rCBF was obtained by dividing blood flow by the weight of the brain sample.

The concentration of [⁹⁹mTc]ECD retained in brain samples of all five monkeys was expressed as a percentage of the injected dose per gram of tissue. Since only unmetabolized [⁹⁹mTc]ECD (parent compound) is available for extraction from the blood by the brain,³ the amount of parent compound in the blood was determined by ethyl acetate/1-octanol extraction. Previous studies have shown this organic solution to extract only the parent compound. rCBF was determined using [⁹⁹mTc]ECD in one monkey by collecting aortic blood at 3 ml/min for 2 minutes into an extraction tube containing 0.5 ml ethyl acetate and 0.5 ml 1-octanol. The blood was acidified with 0.025 ml of 1.0N HCl. The extraction tube was vortexed for 15 seconds and centrifuged for 10 minutes at 650g. The organic layer (containing parent compound) was removed, and the aqueous pellet (containing metabolized [⁹⁹mTc]ECD) was washed twice with ethyl acetate/1-octanol. Both layers were then assayed for technetium-99m content.

In one monkey rCBF was calculated using [⁹⁹mTc]ECD as with microspheres. This method assumes that 100% of the marker is extracted and that there is no washout during measurement. Previous studies³ have shown a 77% extraction of [⁹⁹mTc]ECD with essentially no washout. Hence, blood flow was calculated using [⁹⁹mTc]ECD data by correcting for extraction as (Cₐ.x.Qr)/(Cₐ.x.0.77), and rCBF was obtained by dividing blood flow by the weight of the brain sample.

Cardiovascular variables and rCBF were compared among and within monkeys across paired (left and right) brain regions using repeated-measures analysis of variance followed by the paired t test. Changes in rCBF within each monkey were analyzed using the paired t test. Linear regression analysis was used to compare rCBF and [⁹⁹mTc]ECD retention in individual monkeys. Values are expressed as mean±SEM.
TABLE 1. Cardiovascular Variables Before and After Injection of 40 mg/kg i.v. in Five Cynomolgus Monkeys

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.41±0.03</td>
<td>7.38±0.02</td>
</tr>
<tr>
<td>Paco2</td>
<td>44.68±5.25</td>
<td>48.55±2.07</td>
</tr>
<tr>
<td>Pao2</td>
<td>102.40±5.18</td>
<td>101.43±3.93</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>87.00±5.6</td>
<td>93.00±6.60</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>113±14</td>
<td>108±11</td>
</tr>
<tr>
<td>Carotid blood flow (ml/min)</td>
<td>55±6</td>
<td>74±8*</td>
</tr>
</tbody>
</table>

Data are mean±SEM. *p<0.05 different from before by repeated-measures ANOVA and paired t test.

The paired t test was used to analyze differences in rCBF calculated from $[^{99mTc}]$ECD distribution and microspheres in a single monkey. The null hypothesis was rejected at p<0.05.

Results

The cardiovascular variables before and 20 minutes after the acetazolamide injection are summarized in Table 1. No significant changes in arterial blood gases, mean arterial blood pressure, or heart rate were produced by acetazolamide. Carotid blood flow increased significantly. rCBF in paired brain regions did not differ significantly before or after acetazolamide injection (data not shown).

Data for rCBF calculated using microspheres and for $[^{99mTc}]$ECD retention are summarized in Table 2. Acetazolamide significantly (p<0.01) increased rCBF in all 12 brain regions, by a mean±SEM of 207.6±62.5%. A cortical gray matter to cortical white matter ratio of approximately 2:1 was observed for both rCBF and $[^{99mTc}]$ECD retention.

Significant positive correlations between rCBF and $[^{99mTc}]$ECD retention after acetazolamide were found in each monkey (Figure 1). When data from the five monkeys were pooled and normalized to values in the cerebellum we observed a significant positive correlation between rCBF and $[^{99mTc}]$ECD retention after acetazolamide (Figure 2). Linearity was maintained for rCBF values up to approximately that in the cerebellum. Indeed, in monkey 1, in which rCBF after acetazolamide remained below the upper limit of the normal clinical range (<1.2 ml/min/g), the correlation was linear (r=0.901). Beyond a normalized rCBF value of 1.0 the relation appears to

FIGURE 1. Scatterplot of relation between $[^{99mTc}]$ethyl cysteinate dimer (ECD) retention and regional cerebral blood flow measured after acetazolamide using radioactive microspheres (rCBF) in five cynomolgus monkeys. For monkey 1 r=0.901, for monkey 2 r=0.686, for monkey 3 r=0.724, for monkey 4 r=0.781, and for monkey 5 r=0.679.

TABLE 2. rCBF Before and After and $[^{99mTc}]$ECD Retention After Acetazolamide Administration in Brain Regions of Five Cynomolgus Monkeys

<table>
<thead>
<tr>
<th>Region</th>
<th>Before</th>
<th>After</th>
<th>% Increase</th>
<th>$[^{99mTc}]$ECD retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medulla</td>
<td>0.630±0.16</td>
<td>1.72±0.4*</td>
<td>173.0±65.2</td>
<td>0.077±0.012</td>
</tr>
<tr>
<td>Pons</td>
<td>0.567±0.12</td>
<td>1.58±0.58*</td>
<td>178.7±92.8</td>
<td>0.076±0.014</td>
</tr>
<tr>
<td>Mesencephalus</td>
<td>0.525±0.12</td>
<td>1.89±0.5*</td>
<td>260.0±85.5</td>
<td>0.094±0.009</td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>0.325±0.08</td>
<td>1.09±0.41*</td>
<td>235.4±57.0</td>
<td>0.044±0.007</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.567±0.12</td>
<td>1.72±0.38*</td>
<td>203.1±46.7</td>
<td>0.088±0.013</td>
</tr>
<tr>
<td>Caudate</td>
<td>0.615±0.14</td>
<td>2.08±0.55*</td>
<td>238.2±95.6</td>
<td>0.097±0.014</td>
</tr>
<tr>
<td>Frontal pole</td>
<td>0.487±0.10</td>
<td>1.49±0.37*</td>
<td>205.9±71.9</td>
<td>0.073±0.019</td>
</tr>
<tr>
<td>Occipital pole</td>
<td>0.554±0.12</td>
<td>1.48±0.36*</td>
<td>167.2±51.1</td>
<td>0.120±0.019</td>
</tr>
<tr>
<td>Superior temporal gyrus</td>
<td>0.706±0.14</td>
<td>1.84±0.45*</td>
<td>160.6±59.1</td>
<td>0.097±0.016</td>
</tr>
<tr>
<td>Parietal lobe (posterior central gyrus)</td>
<td>0.568±0.09</td>
<td>1.74±0.49*</td>
<td>206.1±105.7</td>
<td>0.099±0.020</td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vermis</td>
<td>0.619±0.11</td>
<td>1.96±0.48*</td>
<td>216.6±93.3</td>
<td>0.104±0.017</td>
</tr>
<tr>
<td>Hemisphere</td>
<td>0.524±0.11</td>
<td>1.89±0.55*</td>
<td>260.7±62.1</td>
<td>0.087±0.017</td>
</tr>
</tbody>
</table>

*p<0.01 different from before by repeated-measures ANOVA and paired t test.

rCBF, regional cerebral blood flow measured using radiolabeled microspheres; $[^{99mTc}]$ECD, technetium-99m-labeled ethyl cysteinate dimer (retention as percentage of injected dose per gram of tissue). Data are mean±SEM.
FIGURE 2. Scatterplot of relation between $[^{99m}Tc]$ethyl cysteinate dimer (ECD) retention and regional cerebral blood flow (rCBF) (both values normalized to cerebellum) after acetazolamide in pooled data from five cynomolgus monkeys ($r=0.773$, $p=0.0001$).

deviate from linearity extrapolated from low normalized values of rCBF.

We also calculated rCBF from the $[^{99m}Tc]$ECD activity in a single monkey. Mean±SEM rCBF in this monkey (1.21±0.04 ml/min/g) did not differ significantly from that in the same monkey calculated using microspheres (1.13±0.04 ml/min/g). rCBF values calculated from the $[^{99m}Tc]$ECD activity and from microspheres in selected brain regions are compared in Figure 3.

Discussion

The use of $[^{99m}Tc]$ECD as a marker of rCBF has been evaluated under conditions of normal and reduced perfusion in both animals using autoradiography and in humans using SPECT. These reports suggest that $[^{99m}Tc]$ECD may be useful for SPECT imaging of rCBF patterns in the brain. We evaluated how the distribution of this tracer correlates with rCBF under conditions of hyperemia.

We determined rCBF using radiolabeled microspheres. The method has been validated and is used widely. Baseline rCBF values obtained in our study (32–71 ml/min/100 g) are similar to those reported in the literature (24–50 ml/min/100 g). We used the carbonic anhydrase inhibitor acetazolamide to extend the rCBF range. Acetazolamide is a cerebral vasodilator used clinically to evaluate brain perfusion in patients with suspected cerebrovascular disease. rCBF increases of approximately 60% from baseline are obtained in normal cerebral regions during the intravenous administration of this compound. Studies have also demonstrated that within the area of a vascular lesion acetazolamide does not produce rCBF increases similar to those seen in normal portions of the brain. On the contrary, in many instances a "steal" phenomenon may actually decrease rCBF in these regions. In our studies, acetazolamide produced rCBF increases approximately three times those reported in patients. However, these rCBF increases were not sufficient to produce any systemic effect, and all the cardiovascular variables monitored remained stable. Acetazolamide increased carotid blood flow less than it increased rCBF. This unexpected finding may be related to shunting from the external to the internal carotid artery or to the anatomic characteristics of the monkey vasculature.

Substantial differences in the rCBF response to acetazolamide was observed among the monkeys (Figure 1). Several factors, including individual sensitivity to the agent and the type and depth of anesthesia, may play a role in this phenomenon. However, evaluation of the rCBF responses to acetazolamide in nonhuman primates was beyond the scope of our study and would require further experiments.

The relation between rCBF measured with microspheres and that measured using $[^{99m}Tc]$ECD activity (both values normalized to those in the cerebellum) appeared to be linear for rCBF up to approximately that in the cerebellum (1.2 ml/min/g), above which the relation deviated from linearity extrapolated from low values. A similar nonlinear correlation was also described by Yonekura et al between technetium-99m–labeled hexamethylpropyleneamine oxime ($[^{99m}Tc]$HM-PAO) SPECT ratio and rCBF positron emission tomographic (PET) data and by Marcus et al between xenon-133 clearance and microsphere estimates of rCBF. However, $[^{99m}Tc]$HM-PAO versus rCBF data appears to deviate from linearity at values above 50% of that in the cerebellum. This upper portion of the range includes values that are commonly seen under conditions of normal and luxury perfusion. To compensate for the lack of linearity, a correction algorithm has been developed for $[^{99m}Tc]$ HM-PAO data by Lassen et al. We demonstrated that the distribution of $[^{99m}Tc]$ECD was linearly related to
rCBF at values beyond those achievable during pathologic conditions. However, it is still possible that an algorithm similar to that for \([^{99m}Tc]HM-PAO\) will be needed for \([^{99m}Tc]ECD\) under clinical conditions to accurately determine rCBF in patients.

In the brains of rats under pentobarbital anesthesia10 and in normal patients,20 Matsuda et al also demonstrated rCBF values determined with \([^{99m}Tc]HM-PAO\) significantly lower than those obtained with radiolabeled microspheres. These data also suggest that \([^{99m}Tc]HM-PAO\) acts like a chemical microsphere over only a limited range of blood flows.

Using radiolabeled microspheres, we evaluated the increases in rCBF induced by acetazolamide. Calculation of both baseline and postacetazolamide concentrations of \([^{99m}Tc]ECD\) is not technically possible in the same animals. Sequential, double-injection imaging studies may be necessary to assess changes in the regional concentrations of \([^{99m}Tc]ECD\) following pharmacologic stimulation.

We calculated rCBF with \([^{99m}Tc]ECD\) in only a single monkey. A significant positive correlation was demonstrated between rCBF calculated with \([^{99m}Tc]ECD\) and that calculated with microspheres. While a single animal is insufficient for estimating the sensitivity of this agent as a quantitative marker of rCBF, these promising data indicate the potential clinical use of \([^{99m}Tc]ECD\) for calculating rCBF under hyperemic conditions. This information could prompt human crossover studies using xenon-133 or PET.

We demonstrated a cortical gray matter to cortical white matter ratio of approximately 2:1 for both radiolabeled microspheres and \([^{99m}Tc]ECD\). These data are in contrast to those previously reported by Walovitch et al.3 A greater gray-to-white matter ratio (4.5:1) was observed by those authors using dual-label autoradiography in a similar animal model to compare the distributions of \([^{99m}Tc]ECD\) and \([^{14}C]iodoantipyrene\). Our lower gray-to-white matter ratio should be attributable to the partial volume averaging inherent when using microspheres for measuring rCBF. Indeed, \([^{99m}Tc]ECD\) data have been consistent with those obtained with either \([^{14}C]\) iodoantipyrine or microspheres.

Our results, together with preliminary clinical evidence, seem to indicate that rCBF may be calculated using \([^{99m}Tc]ECD\). However, this technique needs further validation under clinical conditions.

References
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