Comparison of Thermal Clearance Measurement of Regional Cerebral Blood Flow With Radiolabelled Microspheres

Paul J. Hoehner, J. Michael Dean, Mark C. Rogers, and Richard J. Traystman

A thermal clearance technique for measuring cerebral blood flow is described and compared with the radiolabelled microsphere technique. The thermal technique involves measurement of the rewarming curve generated after bolus infusion of 4–5 ml of ice-cold saline into the common carotid artery with a subdural thermistor placed on the parietal cortex. Evaluation of the biexponential decay curves obtained with this technique demonstrated a close correlation with total hemispheric, parietal, and parietal gray blood flow determined by simultaneous microsphere measurement. Despite significant correlations ($p < 0.001$), scatter in the data produced a broad 95% confidence interval, thus making interpretation of blood flow with the thermal clearance technique impossible. Furthermore, instrumentation with the thermal probe, which required opening of the dura, blunted the cerebral blood flow response to hypercapnia. We conclude that the major limitations of the thermal clearance technique include 1) nonhomogeneous clearance function, 2) significant variability, and 3) depression of CO$_2$ reactivity. These limitations must be addressed before this technique can be used reliably in the laboratory. (Stroke 1987;18:606–611)

Studies of cerebral blood flow (CBF) have contributed to our increased understanding of normal and abnormal cerebral physiology. In a previous report a technique for quantitative determination of regional cerebral blood flow (rCBF) in experimental animals by thermal clearance was described. This technique involves measuring the heat clearance in cerebral cortical tissue using a subdural thermistor in direct contact with the cortex after a bolus intracarotid injection of ice-cold saline. This technique should not be confused with other heat clearance techniques to measure alterations in CBF. Although this technique has never been validated against any other acceptable technique of measuring CBF, this method has been previously used to generate published data concerning CBF. The purpose of this study was to compare the thermal clearance method with the radiolabelled microsphere technique of measuring CBF under control and hypercapnic conditions.

Materials and Methods

General Procedures

Eleven mongrel dogs (25–33 kg) of either sex were anesthetized with pentobarbital sodium (30 mg/kg i.v. induction, 2 mg/kg/hr i.v. supplemental) and paralyzed with 0.1 mg/kg i.v. pancuronium. The dogs were ventilated with room air through an endotracheal tube with a Harvard Model 607 volume respirator. Tidal volume and respiratory rate were set to maintain end-tidal CO$_2$ at 4%. Pao$_2$ was maintained at > 100 mm Hg throughout the experiment. A catheter was placed in the inferior vena cava for fluid and drug administration. A catheter was placed into the left ventricle for injection of radiolabelled microspheres, and another catheter in the descending aorta was used for arterial pressure monitoring and microsphere reference withdrawal. Arterial blood pressure, left ventricular blood pressure, and heart rate were continuously monitored with Statham 23Db pressure transducers referenced to the level of the right atrium. Arterial and venous PO$_2$, PCO$_2$, and pH were measured with Radiometer BMS 3 electrodes and analyzer. Oxygen saturation and hemoglobin concentration were measured using an Instrumentation Laboratory Model 182 CO-oximeter. All blood samples were analyzed immediately after drawing.

Measurement of Cerebral Blood Flow

Radio-labelled microsphere technique. CBF was measured with radiolabelled microspheres $15 \pm 1.5 \mu$m in diameter (Dupont — New England Nuclear Products). Five or six radiolabels ($^{153}$Gd, $^{51}$Cr, $^{113}$Sn, $^{103}$Ru, $^{95}$Nb, $^{45}$Sc) were injected in a random sequence in each dog. Prior to each injection the vial containing the spheres (suspended in 10% dextran with 0.01% Tween-80 added to prevent aggregation) was shaken vigorously and sonicated for 20 minutes. Approximately $2.4 \times 10^8$ spheres were injected into the left ventricle over a 20-second period followed by a 20-
second flush with 10 ml of saline. The reference blood sample was withdrawn from the aorta using a Harvard withdrawal syringe pump set at 4.94 ml/min beginning 1 minute before the injection and continuing for 3 minutes after the flush. At the end of the experiment the dog was killed with KCl, and the brain was removed and fixed in 10% buffered formalin for 4–7 days. We have found no significant difference between microsphere concentration in 2 simultaneous reference samples regardless of whether the left atrium or left ventricle was used as the injection site.9

The brain was subsequently divided into left and right halves and sectioned into the following areas: cerebellum, medulla, pons, midbrain, diencephalon, caudate nucleus, hippocampus, occipital lobe, temporal lobe, parietal lobe, and frontal lobe. Pure samples of white and gray matter were obtained from the parietal lobe. All tissue samples were weighed, placed in 15-ml poly-Q vials, and counted in a Packard Model 9042 multichannel autogamma scintillation spectrometer with a 3-in. through-hole NaI crystal. The energy levels of the window settings used for 40Sc, 95Nb, 109Ru, 113Sn, 51Cr, and 153Gd isotopes were 840–1200, 710–820, 460–550, 370–440, 280–364, and 70–180 keV, respectively. The overlap of activity among isotopes was subtracted to obtain corrected counts for each isotope by solving simultaneous equations using overlap coefficients from pure isotope standards.8 Blood flow (Qr) was calculated from the equation

\[ Q_r = C_r \times \frac{Q_a}{C_a}, \]

where \( C_r \) is the corrected tissue counts, \( Q_a \) is the reference blood sample withdrawal rate in milliliters per minute, and \( C_a \) is the total corrected counts in the reference arterial blood sample. Flow values were then normalized per 100 grams of tissue.

Thermal Clearance Technique. The superior thyroid branch of the right common carotid artery was cannulated, and a catheter was advanced into the common carotid artery. The scalp was reflected bilaterally drilled over the right parietal area, the dura was opened, and a Janssen Scientific Instruments thermal probe was placed under it in direct contact with the parietal cortex. An identical thermistor was placed in the right femoral artery. This preparation required approximately 45 minutes.

The thermistors on the cortex and in the artery were connected to a Janssen Scientific Instruments Model 0646 thermal transducer that permitted subtraction of the 2 signals to correct for changes in arterial temperature. A bolus of cold (0–4°C) saline was injected into the carotid artery, and the thermal curve generated on the cortex was recorded. The volume of saline injected was approximately 3.0 ml, necessary to achieve a 0.5–0.7°C change in cortical temperature. The rate of thermal clearance is related to tissue blood flow. The analysis of these thermal clearance curves is similar to analyzing tracer clearance curves and has been previously described in detail.1 In this study we compared the negative slope (k) of the line obtained by plotting ln(∆T) vs. t (Figure 1) with the microsphere-derived CBF value, where ∆T is the change in temperature sensed by the cortical thermistor and t is time.

Experimental Protocol

Seven dogs were fully instrumented for simultaneous thermal and microsphere rCBF measurement made at randomized ventilator settings (hyperventilation, normal, and hypoventilation) to obtain a wide range of Paco2 values, thereby providing a wide range of rCBF values (hyperventilation, twice normal ventilatory rate; hypoventilation, half normal ventilatory rate). An average of 4 thermal determinations were attempted per measurement, but curves were excluded from analysis if there were technical problems with the injection or if the curve was of poor quality. Each analyzed thermal curve was compared independently with the microsphere value.

To evaluate the effects of instrumentation on the CBF response to hypercapnia, 4 additional dogs were instrumented for only microsphere rCBF measurement, and rCBF was measured in room air, 5% CO2, and 10% CO2. Craniotomy was then performed, the subdural thermistor was placed, and simultaneous thermal and microsphere measurements of rCBF were made in room air, 5% CO2, and 10% CO2. All thermal determinations were made within 10 minutes of microsphere injections. Paco2, Po2, and pH were measured immediately prior to each microsphere injection. Microsphere-derived rCBF values were used to evaluate vascular responsivity to changes in Paco2 concentration. The ratios of the change in rCBF to the change in Paco2 were compared with the paired Student's t test; p < 0.05 was required for significance.
Table 1. Arterial Blood Gas, pH, and Hemodynamic Values During Control Conditions, Hyperventilation, and Hypoventilation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hyper-ventilation</th>
<th>Hypo-ventilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>33±5</td>
<td>16±2</td>
<td>65±20</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>108±6</td>
<td>100±10</td>
<td>105±8</td>
</tr>
<tr>
<td>pH</td>
<td>7.35±0.04</td>
<td>7.69±0.12</td>
<td>7.25±0.15</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>110±10</td>
<td>110±10</td>
<td>100±15</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>112±15</td>
<td>110±15</td>
<td>115±10</td>
</tr>
</tbody>
</table>

Paco₂, arterial CO₂ tension; PaO₂, arterial O₂ tension; MABP, mean arterial blood pressure; HR, heart rate. Each value is the mean ± SEM of 7 dogs.

Results

Comparison of Microsphere and Thermal Clearance Measurements

In all dogs studied, the values (mean ± SEM) for mean arterial blood pressure and heart rate before the first injection of microspheres were 110 ± 10 mm Hg and 112± 15 beats/min, respectively. Arterial pressure and heart rate did not change significantly during the experiment (Table 1).

A total of 70 thermodilution curves were obtained and analyzed, corresponding to 27 microsphere measurements. In 51 of the 70 curves analyzed (73%) a "slow" component (biexponential decay) could be discerned when plotted on a semilogarithmic scale. In all the curves analyzed, only the initial "fast" component was used for comparison with the microsphere-derived rCBF values.

Figure 2 compares the thermodilution and microsphere methods during hypoventilation and hyperventilation of the dogs. The arterial blood gas values are shown in Table 1. Each point in Figure 2 represents a single thermal clearance curve value plotted against a simultaneously obtained radiolabelled microsphere rCBF value. A regression line for the data and a 95% confidence interval (p < 0.05) for the correlation are also shown. The slopes correlated well with right hemispheric, right parietal gray, and right parietal total flows. Despite significant correlation (p < 0.001) between the thermal clearance and microsphere measurements, the 95% confidence limit is large. For instance, a value of k = 10 sec⁻¹ would correspond to a range of right hemisphere CBF values of 18–65 ml/min/100 g obtained by the microsphere technique.

Effect on Hypercapnic Response

Figure 3 shows the hypercapnic response of the 4 regions before and after instrumentation for the ther-
The hypercapnic response is severely blunted after instrumentation. To evaluate whether this was a local or general effect on the cerebral vasculature, Figure 4 shows the hypercapnic response on left-sided regions (side opposite the thermistor). The hypercapnic response was not significantly affected in the left parietal lobe or in the entire left hemisphere. There was also no change in the hypercapnic response of brainstem structures.

The hypercapnic response was also blunted in the 7 original dogs in which Paco2 was altered by hypoventilation and hyperventilation. Figure 5 shows CBF values obtained by the microsphere technique as a function of Paco2 in the 4 regions studied. The results were similar to those shown in Figure 3.

**Discussion**

In this study we compared a thermal clearance technique for measuring CBF with the radiolabelled microsphere technique. Our data show a close correlation between the negative slope \(-\Delta \ln(\Delta T)/\Delta t\) of the thermal clearance curve and blood flow in structures underlying the thermistor. However, the precision with which the slope predicts the actual blood flow measured by radiolabelled microspheres is poor. Furthermore, the instrumentation for this technique blunts the hypercapnic response of the cerebral vasculature.

The radiolabelled microsphere technique has been validated under a variety of conditions. It has been shown that microspheres are well mixed when injected into the left ventricle,11,12 are distributed in proportion to regional flow,13,14 that shunting of 15-µm spheres is minimal,11,13,14 and that the circulation is cleared of spheres within 3 minutes of injection.15,16 We have used the radiolabelled microsphere technique in our laboratory for a number of years and have found, as have other investigators, that injection of large boluses of 15-µm spheres does not affect hemodynamic variables,14 neurologic scoring,13,14 or the distribution of a second injection,15 nor does it distort normal flow distribution in small regions of the brain.13 Microspheres have also been shown to accurately reflect CBF at both high and low flow.19

The thermal clearance technique makes four funda-
mental assumptions. The first is that the hydrostatic heat transfer in cerebral tissue is nearly instantaneous. Support for this assumption comes from the large capillary surface area and large diffusion coefficient for heat transfer. Secondly, it is assumed that the rate of cerebral heat production is small with respect to the caloric load of the inflowing arterial blood. The general rate of heat production per mole of O₂ consumed is 2 cal/mol O₂. Using a cerebral flow rate of 40 ml/min/100 g, a cerebral arterial-to-venous O₂ difference of 0.2 mol O₂/100 ml blood gives a rate of cerebral O₂ consumption (CMRO₂) of approximately 0.09 mol O₂/min/100 g. Therefore, the total endogenous cerebral heat production during the approximately 3 minutes for the thermal curves to equilibrate is calculated to be 0.54 cal/100 g. Using the same flow value of 40 ml/min/100 g, at 37° C blood temperature the total caloric load of the inflowing blood is 1,480 cal/min/100 g. These calculations argue that the error induced by endogenous cerebral heat production on the rewarming curve is negligible. However, this argument is complicated by the presence of adjacent tissues with unequal rates of rewarming, blood flow, and/or metabolism. Areas adjacent to the thermistor, such as the dura or skull, may act as heat sinks, thereby altering the rate of rewarming. Furthermore, adjacent areas of different flow (e.g., white and gray matter) may influence the rate of rewarming of each other. These limitations occur with H₂ clearance when there is an adjacent low flow area (white matter or infarct) or when actual flow is high (high Paco₂) and the difference between gray matter and skull flows is greatest.

The third assumption is that the temperature of the inflowing arterial blood is constant. For this reason a dual thermistor was employed to adjust for baseline temperature changes. It is possible, however, that bolus injections of microspheres in room temperature diluent may attenuate the clearance curves by cooling the tissue even if the arterial temperature is subtracted. The fourth assumption is that the volume of tissue influencing the clearance curve is known and constant. Given the above theoretical limitations in the assumptions of this technique, it is difficult to precisely analyze the volume of tissue (intravascular and extravascular space) influencing the clearance curve. Even if the anatomic volume could be determined, the relative contribution from different tissue sources would be difficult to assess. For this reason the negative slopes of the semilog transformed thermal clearance curves \[-\Delta \ln(\Delta T)/\Delta t\] are reported in seconds⁻¹ instead of converting this value to an actual flow. The biexponential decay of most of the clearance curves is problematic and represents either the inhomogeneity of the tissue in which the thermistor is recording or the influence of adjacent tissues acting as heat sinks. The correlations found in this study imply only that the slopes of the rewarming curves in some way depend on the blood flow to a large volume of tissue, not that they measure the actual flow in any particular tissue.

Probably the greatest limitation of this technique is its effect on CO₂ reactivity. In this study, high flow values generated by high Paco₂ were blunted after placement of the cortical thermistor. This apparently is a local effect as the blunting was not seen with flow measurements recorded simultaneously in corresponding structures of the opposite hemisphere. The most likely explanation is that the opening of the dura and/or the physical contact of the thermistor damaged the cortex, although no gross damage was observed at autopsy. An alternative explanation, that the cold bolus affected reactivity by changing the vessel temperature, was not supported by our microsphere measurements, which demonstrated a blunted hypercapnic response merely in the presence of the required instrumentation; no cold injections were made in that group of dogs. These results raise the possibility that other invasive techniques, particularly ones utilizing cortical probes, may also alter the physiologic response of rCBF to Paco₂. This study did not address the possible influence of this technique on the hypoxic or autoregulatory responses of the cerebral vasculature.

Despite the strong correlations between the thermal rewarming curves and the microsphere-derived flow values, the accuracy of this technique, when compared
to microspheres, is not good. Because of the large scatter in repeated measurements using the thermal technique, the 95% confidence intervals, limits which seem necessary to generate reliable data, are too large for practical use. For instance, a $k$ value of 10 sec$^{-1}$ generated by the thermodilution technique with a thermostimulator directly over the parietal cortex may correspond

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Quantification of blood flow to the brain is important for studies of cerebral physiology and pathophysiology. The thermal clearance technique was originally described as a technique allowing repeated measurements of local CBF with a minimum of equipment and expense and a possible clinical applicability. The results of this study, however, indicate that this technique has severe limitations in its accuracy and alters the CBF response to hypercapnia. A further limitation of this technique is the nonregional nature of the data obtained due to the inhomogeneous clearance function. These theoretical and practical problems must be addressed before this technique can be reliably used.

References


Key Words • cerebral blood flow • cerebral circulation • radiolabelled microspheres • thermal clearance
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