Measurement of Cerebral Blood Flow During Xenon Inhalation as Measured by the Microspheres Method

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SUMMARY Measurements of cerebral blood flow (CBF) were performed using the microsphere technique in non-human primates (baboons) to assess the effect of non-radioactive xenon gas inhalation on CBF. Blood flows in small tissue volumes (~1 cm³) were directly measured before and during the inhalation of xenon/oxygen gas mixtures. The results of these studies demonstrated that when inhaled in relatively high concentrations, xenon gas does increase CBF, but the changes are more global than tissue-specific. The problems and limitations of such evaluations are discussed.

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A NUMBER OF TECHNIQUES have been investigated in recent years in which global, regional, or focal cerebral blood flow can be assessed in vivo by noninvasive or minimally invasive means.¹⁻³ Noninvasive measurement techniques yield absolute flow values which are of great importance when these are related to the functional state of a specific normal or abnormal brain tissue volume.⁴ The effect of a given technique on the measured values becomes of great interest if the derived information is used for diagnostic purposes or for assessment of the efficacy of therapeutic procedures.

Since 1967 the radiolabelled microsphere technique has been used to assess blood flow and cardiac output.⁵⁻⁶ Measurement by this technique is based on the injection of a non-diffusible indicator, the concentration of which in tissue samples is measured in reference to known concentrations in reference blood samples.⁷ Another method, which uses xenon-133, is based on the measurement of build-up or washout of the freely diffusible xenon gas in brain tissue and in arterial blood.¹⁻³, ⁸⁻⁹

In recent years the latter method has been extended to the use of non-radioactive xenon in measuring CBF by serial CT imaging.¹⁰⁻¹⁸ Unfortunately, the non-radioactive xenon method necessitates a relatively prolonged inhalation (4 to 6 minutes) of relatively high concentrations (30–35%) of xenon gas. The effect such inhalation may have on measured CBF has not yet been thoroughly examined in an effort to maximize the potential benefits or recognize the limitations of blood flow information obtainable by this method.

In this study we investigated this effect. We obtained CBF measurements by the microsphere technique before and during xenon gas inhalation in a series of blood flow studies in non-human primates (baboons). Measurements from multi-injection microsphere studies were compared.

Methods

Prestudy Animal Preparation

Four xenon inhalation studies were performed on three baboons weighing 10, 12, and 12.5 kg. After sedation with phencyclidine hydrochloride (Sernyl®, Bio Centric Labs, St. Joseph, MO), a cuffed endotracheal tube was inserted and connected to a semi-closed ventilation apparatus (Harvard respirator) which controlled the inhalation of xenon and oxygen. At prescribed intervals during the experiment, we administered intravenously propanol hydrochloride (Injeral®, Ayerst Laboratories, Inc., NY) to minimize cardiovascular instability, and pancuronium bromide (Pavulon®, Organon Pharmaceuticals, West Orange, NJ) to keep the animal immobile. In addition, 1 mg dosages of morphine sulphate were administered at prescribed intervals to minimize discomfort. Continuous cardiac and respiratory monitoring were routine. The injection of microspheres was carried out through a 7 French pigtail catheter placed in the left ventricle, and reference arterial blood samples were obtained through a similar catheter inserted into the descending aorta. Local anesthesia (.05% lidocaine hydrochloride) was administered to all sites in which catheters were inserted. Concentrations of stable xenon in expired gas were monitored with a calibrated Gow-Mac thermoductivity gas leak detector (Gow Mac Instrument Co., Bound Brook, NJ). The studies were carried out during inhalation of a 35–42% xenon/oxygen mixture. P&CO₂ levels were directly measured (table 1).

Microsphere injections were performed according to the schedule in table 1. Injections were performed before and during the time the animal was breathing xenon/oxygen mixtures. After completion of the inhalation protocol, the animal was returned to room air for a period of one hour before the next experiment was begun.

Microsphere Injections and Computational Methodology

In each study, flow was determined by injecting 2
The injections schedule was performed according to the protocol noted in table 1. Heart rate and arterial pressure were continuously monitored. Blood samples drawn just after collection of the reference sample were free of radioactivity. The injections schedule was performed according to the protocol noted in table 1.

After the studies were completed, the animal was killed with a massive overdose of thiopental and then perfused with formalin delivered by gravity to the arterial catheter. The brain was then removed and suspended in formalin solution for 72 hours. The same axial plane (petrosal apex to orbital roof) was then approximated in each animal with a specially designed cutting board for 2-mm thick sectioning. Other organs such as the liver, kidney, and spleen were sampled as well. Cortical gray and white matter sampling was performed by visual separation with a #11 scalpel blade.

A fine wire grid was used to cut the brain tissue into 9.6 × 9.6 mm² samples after the 2-mm thick sections were stacked into groups of five. Individual tissue samples were homogenized in a blender, and triplicate aliquots of each homogenate were transferred into preweighed counting vials to a height of 2 cm from the bottom of the vial. Sample weight was determined and the tissue fixed by adding 3 ml of 10% buffered formalin to each vial. The red blood cells in the arterial reference sample were hemolyzed with detergent, and the spheres were centrifuged and resuspended in liquid agar to a height of 2 cm to achieve the same geometry as the tissue samples. The sphere-agar suspension was quickly solidified by rapidly cooling the sample to 0°C.

All tissue and reference samples were counted for 10 min in a three-channel gamma counter (Beckman model 300). Energy windows were set to encompass 85% of the major emission photopeak for each isotope. An additional 2 keV were added to each side of the 85% window to minimize counting variability introduced by electronic window drift. Window drift was quantitated by dividing the 85% window for a ¹³⁷Cs standard into two half windows and determining the variation in the ratio of counts per minute in the two half windows from 1.0. Then coincidence limitations of the gamma counter were determined by calculating the experimentally measured resolving time, using the method of paired sources. No sample counts exceeded these limitations.

Radioactivity due to each isotope in a tissue sample which contained multiple isotopes was estimated from the distribution of counts obtained from pure isotope samples and the tissue sample over all specified energy windows. If \( [f_wi] \) is the matrix whose \( w^{th} \) component, \( f_{wi} \), is the estimated fraction of isotope i in energy window \( w \), and \( [C_w] \) is the column vector whose \( w^{th} \) component, \( C_w \), is the total counts of all isotopes in window \( w \), then the estimated distribution of counts for isotope i over all counting windows is given by the column vector:

\[
[f_wi] \times [C_w]
\]

where \( [f_wi]^{-1} \) is the inverse of matrix \( [f_wi] \). The matrix computations were performed on a computer (DEC-PDP-11/45). Tissue blood during injection of i-labeled microspheres, \( Q_i(i) \), was calculated from the relation:

\[
Q_i(i) = \frac{C_i(i)}{C_R(i)} \times Q_R
\]

where \( C_R(i) \) is the counts per minute of isotope i in the
arterial reference sample, \( Q_R \) is the reference sample withdrawal rate, and \( C_T(i) \) is the counts per minute of isotope \( i \) in the tissue sample.

Assessment of CBF Changes

The assessment of CBF changes was carried out by comparing average and tissue-specific flows before and during xenon inhalation. The correlations between the data sets and across all strata were tested by non-parametric statistics (Kendall \( \tau \)). The Pearson \( (r) \) correlation coefficients were computed for each data set as well. The significance of tissue specific or global changes due to xenon inhalation was tested by the Student T test (two-sided).

Results

Microsphere Results

Derived flow values for tissue samples counted by the multi-injection multi-window microsphere method yield only an average flow value for each sample for each injected radionuclide. The results of multi-sample analyses for each animal were arrayed in a manner similar to that of a flow map so that they could be analyzed in relationship to specific anatomic regions.

The errors associated with this method have been assessed in a comprehensive study reported elsewhere. Such errors depend on tissue weight and the specific activity of radionuclides in the sample at the time of counting. Typical errors associated with single samples in our study have been estimated by error propagation computation to be \( \pm 11\% \) and \( \pm 15\% \) for accuracy and reproducibility, respectively.

Correlation between Data Sets

Individual studies and those combined across all strata (116 paired observations) were evaluated by nonparametric statistics (Kendall \( \tau \)). All individual sets showed statistical significance at \( p < 0.01 \); \( p \) values across all strata were highly significant \( (p < 0.001) \). Kendall \( \tau \) correlation coefficients ranged from 0.58 to 0.74. Similar observations were made when the Spearman Rank correlation coefficient \( (r) \) was used to demonstrate correlation between the experiments. The association between the data sets was significant at \( \alpha = 0.01 \) in all of the studies. Similar to the Kendall \( \tau \) test, the correlation coefficients \( r \)'s ranged from 0.69 to 0.87, indicating that much of the difference can be explained based on statistical variation alone. A typical data set of paired observations from one study is shown in figure 1.

Further investigations in which we compared average flow in cortical gray matter and white matter separately (16 samples) demonstrated a similar and significant association \( (p < 0.01) \) between the data sets. The Student T test demonstrated clearly that blood flow significantly increased while xenon was inhaled at the high concentrations used in these studies. A summary of our results is given in table 2.

Discussion

The results of these studies indicate that prolonged xenon inhalation periods (\( \geq 2 \) minutes) at concentrations somewhat higher than, yet similar to, those used

<table>
<thead>
<tr>
<th>Animal</th>
<th>Study #</th>
<th># of observations</th>
<th>Average flow ml/100 cc/min</th>
<th>Kendall ( \tau )</th>
<th>Spearman ( r )</th>
<th>Student test ( T )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>baseline</td>
<td>22</td>
<td>31.9</td>
<td>(&lt;0.01)</td>
<td>0.58</td>
<td>(&lt;0.01)</td>
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<td></td>
<td>vs. 4.5 min</td>
<td></td>
<td>37.8</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>baseline</td>
<td>26</td>
<td>48.1</td>
<td>(&lt;0.01)</td>
<td>0.74</td>
<td>(&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>vs. 4.0 min</td>
<td></td>
<td>49.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>baseline</td>
<td>34</td>
<td>34.0</td>
<td>(&lt;0.01)</td>
<td>0.70</td>
<td>(&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>vs. 2.0 min</td>
<td></td>
<td>38.1</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>baseline</td>
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<td>(&lt;0.01)</td>
<td>0.61</td>
<td>(&lt;0.01)</td>
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<tr>
<td></td>
<td>vs. 4.5 min</td>
<td></td>
<td>39.2</td>
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<td></td>
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</tr>
</tbody>
</table>

*Not statistically different than 0; but if corrected for \( P_2CO_2 \), it is significant at \( p < 0.01 \).
in clinical studies do affect CBF. We measured a statistically significant increase in blood flow (~17%). For the most part, this effect was global rather than site- or tissue-specific. This effect was found to be consistent, and from our limited data, it seems to be relatively constant as a function of duration of inhalation. Therefore, while this phenomenon should be considered in the interpretation of CBF maps generated by the xenon/CT method, it should not diminish significantly the validity of the method in most clinical applications. Because we compared pairs of measurements before and during xenon inhalation in the same tissue and under identical physiological conditions (e.g., drugs, PaCO₂) the validity of these measurements should hold even if the drugs had some effect on CBF. Similar results have been observed in a preliminary clinical study where the xenon-133 inhalation method was employed to determine blood flow before and during 35 percent xenon inhalation in patients (personal communication, WD Obrist, 1984).

While our measurements have demonstrated a statistically significant increase in CBF during xenon inhalation, such an increase may present only a moderate stress which could aid in the recognition of tissue with flow reserve compromise. Further investigations are needed to study the effects of various concentrations of xenon gas with and without stress (e.g., CO₂ challenge) on CBF. In addition, work should be carried out to examine the tissue specificity of these changes under various physiological conditions.

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