Stable Xenon Versus Radiolabeled Microsphere Cerebral Blood Flow Measurements in Baboons

Douglas S. DeWitt, PhD, Panos P. Fatouros, PhD, Abund O. Wist, PhD, Lauraine M. Stewart, MD, Hermes A. Kontos, MD, PhD, James A. Hall, MD, Pulla R.S. Kishore, MD, Richard L. Keenan, MD, and Anthony Marmarou, PhD

Regional cerebral blood flow was simultaneously determined using the stable xenon computed tomographic and the radioactive microsphere techniques over a wide range of blood flow rates (<10 to 300 ml/100 g/min) in 12 baboons under conditions of normocapnia, hypocapnia, and hypercapnia. A total of 31 pairs of determinations were made. After anesthetic and surgical preparation of the baboons, cerebral blood flow was repeatedly determined using the stable xenon technique during saturation with 50% xenon in oxygen. Concurrently, cerebral blood flow was determined before and during xenon administration using 15-μm microspheres. In Group 1 (n=7), xenon and microsphere determinations were made repeatedly during normocapnia. In Group 2 (n=5), cerebral blood flow was determined using both techniques in each baboon during hypocapnia (Paco₂=20 mm Hg), normocapnia (Paco₂=40 mm Hg), and hypercapnia (Paco₂=60 mm Hg). Xenon and microsphere values in Group 1 were significantly correlated (r=0.69, p<0.01). In Group 2, values from both techniques also correlated closely across all levels of Paco₂ (r=0.92, p<0.001). No significant differences existed between the slopes or y intercepts of the regression lines for either group and the line of identity. Our data indicate that the stable xenon technique yields cerebral blood flow values that correlate well with values determined using radioactive microspheres across a wide range of cerebral blood flow rates. (Stroke 1989;20:1716–1723)
bated, paralyzed with 0.2 mg/kg i.v. pancuronium bromide, and mechanically ventilated. Supplemental anesthetic was administered during the experiment to ensure that the baboons were as free from stress as possible. Arterial blood pressure and heart rate were monitored continuously, and additional supplemental doses of anesthetic were given if either parameter increased significantly.

Following anesthesia, canulas were placed in the left brachial and right femoral arteries for withdrawal of blood for microsphere arterial reference samples. Canulas were also placed in the left femoral artery for measurement of blood pressure and in the right femoral vein for drug infusion. For microsphere injection, a canula with a slightly flared tip was placed in the left atrium via a thoracotomy. Following surgical preparation, baboons were placed in the CT scanner in a special headholder and taped into place. A line corresponding to the plane of the CT slice chosen was marked on the shaved scalp using the aiming lasers in the scanner.

For Xe-CT, the baboons were scanned in a Picker 1200SX CT scanner (Cleveland, Ohio). The CT parameters were 120 kvp, 80 mA, 5-mm slice thickness, scan time of 5.1 seconds, and a 256x256 matrix. The stability of the scanner was evaluated using a water-filled phantom that was scanned using the same protocol used for the experiments. The maximum drift in a large region of interest during the entire period was <1 Hounsfield unit. Before each Xe-CT cycle and during the washout period between cycles, the baboons were ventilated with 100% O2 to displace N2. Two baseline scans were performed. Then, using an anesthesia machine modified to administer xenon instead of N2O, a mixture of 50% xenon and 50% O2 was blended and immediately delivered to the ventilator. An O2 sensor in the inspiratory tubing confirmed that 50% O2 was being delivered to the baboon. Xe-CT scans were performed every 20 seconds for the first 4 minutes, every 30 seconds for the next 10 minutes, and then at 3-minute intervals during the remainder of the 30 minutes of xenon administration. This protocol allowed us to follow the initial sharp rise in brain xenon concentration accurately without accumulating more measurements than the scanner could process in the time available. A minimum of 60 minutes was allowed between xenon administrations to allow complete washout of the xenon.

The 15-μm polystyrene microspheres were labeled with strontium-85, scandium-46 (3M, New Brighton, Minnesota), tin-113, niobium-95, or gadolinium-153 (New England Nuclear, Boston, Massachusetts). Before injection, microspheres in 10% dextran and 0.01% poloxymethylene sorbitan monooleate (Tween 80) were agitated for 4 minutes using a Vortex mixer. The microspheres were then withdrawn into a 3-ml syringe and connected to a three-way stopcock on the canula in the left atrium. The remaining port of the stopcock was connected to another syringe containing saline warmed to 37°C. The saline was mixed vigorously with the microspheres through the stopcock, and the microspheres were then injected into the left atrium over 30 seconds. Before and for 90 seconds after the start of microsphere injection, arterial reference samples were withdrawn at a rate of 1.53 ml/min using Gastight syringes (Hamilton Co., Reno, Nevada) and a Harvard syringe pump (Harvard Apparatus, South Natick, Massachusetts). The number of microspheres in the arterial reference samples was averaged for use in the CBF calculation. For each determination of CBF, 2.7x10^6 microspheres were injected.

At the conclusion of all the experiments, anesthesia was supplemented and the baboons were perfused transcardially using a mixture of aldehydes. The calvaria underlying the marks made on the scalp was scored, the scalp was carefully reflected, and the underlying bone was cut along the score marks. The calvaria was removed, and the brain was marked deep to the score marks. The brain was removed and frozen in crushed dry ice, and a 5-mm slice was made using a sliding microtome. The slice was photographed and dissected into the caudate nucleus, lentiform nucleus, thalamus, anterior and posterior limbs of the internal capsule, and cerebellum. A lumped white matter sample was collected since in some baboons, the internal capsule samples were not large enough to ensure accurate microsphere CBF values. In those baboons, the lumped white matter microsphere CBF values were compared with Xe-CT CBF values for the internal capsule. Each region dissected was marked on the photograph of that slice. These photographs were used to identify corresponding regions on the CT image from which ΔH values (see below) were determined, ensuring that Xe-CT CBF values were determined in the same regions from which microsphere CBF values were calculated.

Seven baboons (Group 1) were subjected to three 30-minute periods of 50% xenon administration, during which CT scans were taken at the intervals described. Paco2 was maintained at approximately 40 mm Hg. Paco2, Pao2, and arterial pH were measured immediately before each xenon administration. Radioactive microspheres were injected 25 minutes after the initiation of each xenon administration and immediately preceding the first and third xenon administrations, for a total of five microsphere injections during the three Xe-CT cycles.

Five baboons (Group 2) underwent three similar 30-minute periods of xenon administration, one at each Paco2 level of 20, 40, and 60 mm Hg. Hypocapnia or hypercapnia was achieved by hyperventilation or by adding CO2 to the inspired gases, respectively. Paco2 was continuously monitored using a capnometer and was maintained constant during the CBF determinations. Radioactive microspheres were injected 5 minutes after the start of each administration and immediately before the first and second administrations, for a total of five microsphere injections during the three Xe-CT cycles.
We employed 50% xenon for the determination of Xe-CT CBF because that concentration provides better CT enhancement and a better signal-to-noise ratio than the concentration (33–35%) most often used clinically. Arterial xenon concentration was computed by subtracting from 100% the measured concentrations of end-tidal O₂, CO₂, and H₂O vapor after each exhalation. N₂ concentrations were assumed to be negligible since the baboons had been ventilated with 100% O₂ before xenon administration. The end-tidal xenon concentrations were fitted by an unweighted least-squares routine to a double-exponential function.18

After each experiment, the baseline Hounsfield units for each region were determined from an average of the two baseline scans. The enhanced Hounsfield units for each brain region were collected using a microcomputer and subtracted from the baseline Hounsfield units, and a modified Kety equation was used to calculate the rate constant $k^{19,20}$:

$$\Delta H_{br}(T)/\Delta H_{br}(sat) = k \int_0^T \frac{C_a(t)}{C_a(sat)} e^{-k(T-t)} dt$$

where $\Delta H_{br}(T)$ and $\Delta H_{br}(sat)$ are local CT enhancement in Hounsfield units at time T and at saturation, respectively, and $C_a(t)$ and $C_a(sat)$ are the xenon concentrations at time t and at saturation, respectively. The parameters for the previously determined double-exponential function were inserted into the Kety equation. By fitting the equation with an unweighted least-squares routine to the enhanced Hounsfield numbers, $k$ and $\Delta H_{br}(sat)$ were estimated for each pixel. The partition coefficient $A$ for each pixel was calculated from $\Delta H_{br}(sat)$ using the equation$^{1-18}$:

$$\lambda = \frac{\Delta H_{br}(sat) \times (\mu^w_{\mu^w} / \mu^w_{\mu^w})}{0.015 \times C} \times \frac{1}{0.0011 \times (Hct) + 0.1}$$

where $\mu^w_{\mu^w}$ and $\mu^w_{\mu^w}$ are the mass attenuation coefficients for water and xenon, respectively, C is the concentration of xenon (50%), and Hct is the hematocrit (approximately 40%). The ratio $\mu^w_{\mu^w}/\mu^w_{\mu^w}$ (0.029) was determined for the scanner using water and iodine phantoms as described by Kelcz et al.$^1$ CBF was calculated as $\lambda \times k$. The Xe-CT protocol and the calculations used in these studies have been described in greater detail elsewhere.$^{18,21,22}$

At the conclusion of all the experiments, the tissue and arterial reference samples were counted using an LKB Compugamma (LKB Instruments, Inc., Rockville, Maryland). The matrix inversion and curve stripping required for isotope separation were performed by a microprocessor in the gamma counter.$^{23}$ CBF was calculated as $\mu^w_{\mu^w} (C_a \times RBF)/(C_t \times W_b)$, where $C_a$ is counts in the tissue sample, $C_t$ is counts in the arterial reference sample, RBF is the arterial reference withdrawal rate, and $W_b$ is the weight of the tissue sample.

Values for CBF determined by the two techniques were compared using linear regression analysis for each group separately and for each Pao₂ level in Group 2 separately. Linear regression analysis was also used to compare Xe-CT CBF values calculated using data from only the five scans during the first 5 minutes of xenon administration with values calculated using data from scans during the entire 30 minutes of xenon administration for all regions in one baboon and for only the white matter regions in all Group 2 baboons. All values are expressed as mean±SEM.

**Results**

In Group 1, CBF values determined using both techniques were significantly correlated ($r=0.69$, $p<0.01$) (Table 1, Equation 1; Figure 1). The linear regression line describing the relation between the two techniques did not differ significantly from the line of identity. Thus, within the variability of Group 1, the two techniques yielded identical CBF values.

In Group 2, CBF values determined using the two techniques also correlated closely over the entire Pao₂ range ($r=0.92$, $p<0.001$) (Table 1, Equation 4).

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**Table 1. Linear Regression Equations Describing Relations Between Cerebral Blood Flow Determined Using Stable Xenon-Computed Tomographic Y and Radioactive Microsphere X Techniques**

<table>
<thead>
<tr>
<th>Pao₂</th>
<th>n</th>
<th>Equation</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−40 mm Hg</td>
<td>7</td>
<td>1) $Y = −8.29 + 1.04X$</td>
<td>0.69</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–60 mm Hg</td>
<td>5</td>
<td>2) $Y = −0.99 + 0.99X$</td>
<td>0.92</td>
</tr>
<tr>
<td>21.3±0.6 mm Hg</td>
<td>3</td>
<td>3) $Y = −3.50 + 0.94X$</td>
<td>0.83</td>
</tr>
<tr>
<td>40.6±0.8 mm Hg</td>
<td>5</td>
<td>4) $Y = −1.70 + 0.93X$</td>
<td>0.93</td>
</tr>
<tr>
<td>60.8±0.9 mm Hg</td>
<td>5</td>
<td>5) $Y = −9.50 + 1.03X$</td>
<td>0.83</td>
</tr>
</tbody>
</table>

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**Figure 1. Scatterplot. Correlation of cerebral blood flow (CBF) values determined using stable xenon-computed tomographic technique (Xe/CT) with those determined using radioactive microsphere technique in seven baboons in Group 1 under conditions of normocapnia.**
DeWitt et al. Comparison of Xe-CT and Microsphere CBF

Comparison of Xe-CT and Microsphere CBF

Figure 2. Scatterplot. Correlation of cerebral blood flow (CBF) values determined using stable xenon-computed tomographic technique (Xe/CT) with those values determined using radioactive microsphere technique for five baboons in Group 2 at three levels of PaCO2 (r=0.93, p<0.001) (Table 1, Equation 4; Figure 4). The linear regression equations at the three levels of PaCO2 did not differ significantly from one another nor from the overall equation (Table 1, Equation 2). Thus, over the entire range of CBF values, the two techniques yielded statistically identical values for CBF.

Global microsphere CBF values determined during hypocapnia were 62.7% lower than those determined during normocapnia. This represents a decrease of 3.2%/mm Hg PaCO2 during xenon administration.

Xe-CT CBF values calculated from five scans during the first 5 minutes of xenon administration correlated well with those calculated from multiple scans over the entire 30 minutes in both gray and white matter regions in one normocapnic baboon from Group 2 (Figure 6). The same correlation was also calculated for the white matter regions (i.e., the anterior and posterior limbs of the internal capsule) for all the baboons in Group 2. While the values were significantly correlated at all levels of PaCO2, the higher the blood flow, the closer the regression line describing the relation was to the line of identity (Table 2).

Discussion

Our investigations demonstrate a very close correlation between CBF values determined using the Xe-CT and microsphere techniques at all blood flow levels studied. A fundamental assumption of the latter technique is that each tissue sample receives enough microspheres to ensure random distribution of them with the blood flowing to that region. Each sample must contain at least 384 microspheres to achieve 10% precision at the 95% confidence level. The baboon brain receives approximately 4.4% of cardiac output, or approximately 119,000 microspheres when 2.7x10^6 microspheres are injected.
Figure 5. Scatterplot. Correlation of cerebral blood flow (CBF) values determined using stable xenon-computed tomographic (Xe/CT) and radioactive microspheres techniques for five baboons in Group 2 during hypercapnia (Paco₂ = 60.8 ± 0.9 mm Hg).

Figure 6. Scatterplot. Correlation between cerebral blood flow (CBF) values determined from five stable xenon-computed tomographic scans made during 5 minutes (CBF₅) with those calculated from multiple (>25) scans made during 30 minutes (CBF₃₀) in one normocapnic baboon from Group 2.

Table 2. Linear Regression Equations Describing Relation Between Cerebral Blood Flow Calculated Using Limited Data (CBF₅) and Complete Dataset (CBF₃₀) for White Matter Regions in Group 2 Baboons

<table>
<thead>
<tr>
<th>Paco₂</th>
<th>CBF₅ = Equation (CBF₃₀)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mm Hg</td>
<td>CBF₅ = -14.04 + 2.19 x CBF₃₀</td>
<td>0.60</td>
</tr>
<tr>
<td>40 mm Hg</td>
<td>CBF₅ = 8.15 + 0.70 x CBF₃₀</td>
<td>0.69</td>
</tr>
<tr>
<td>60 mm Hg</td>
<td>CBF₅ = 4.64 + 1.00 x CBF₃₀</td>
<td>0.84</td>
</tr>
</tbody>
</table>

The two techniques were significantly correlated in Group 1 (r = 0.69, p < 0.01; Figure 1), but there was a certain amount of variability. This may be due, in part, to differences in the time during which CBF was calculated. Xe-CT CBF was calculated from data collected during 30 minutes of xenon administration. Microsphere CBF measurements, requiring approximately 1 minute, were performed immediately before and 25 minutes after xenon administration was begun. Any transient change in physiologic parameters, such as Pao₂ and Paco₂, that occurred between microsphere injections would have affected Xe-CT CBF but not microsphere CBF values. In addition, as noted by Gur et al.₁₆ it is difficult to ensure that CBF was calculated from exactly the same tissue regions using the two techniques. Improvements in our tissue sampling methods may have contributed to the better correlation in Group 2.

We found an excellent correlation between the two techniques over the wide range of CBF studied in Group 2 (Table 1, Equation 2; Figure 2). Xe-CT and microsphere CBF values correlated well under...
hypocapnic (Figure 3), normocapnic (Figure 4), and hypercapnic (Figure 5) conditions.

Whereas the overall correlation was very good, examination of the correlation during hypercapnia revealed that while the lower (predominantly white matter) CBF values correlated well between the two techniques, the higher CBF values exhibited more scatter (Figure 5). This may be due, in part, to technical differences between the techniques. Single-compartment analysis of multicompartmental brain regions may lead to underestimations of Xe-CT CBF at high flow rates. In addition, Xe-CT calculations are based on the rate of change and the saturation values for CT enhancement with xenon. The enhancement values describing the rising part of the curve contribute most to the estimate of k (Figure 7). The greater the number of scans during the first few minutes of xenon administration the better the estimate of k. When CBF is very high (i.e., during hypercapnia), the CT enhancement curve approaches saturation very rapidly, with only a few points describing its rising portion. This could lead to greater uncertainty in the estimate of k. Thus, as CBF becomes very high, the Xe-CT technique becomes less accurate. Conversely, since a greater number of microspheres reach each brain region at higher flow rates and since the accuracy of the technique is related to the number of microspheres in each tissue sample, the microsphere technique tends to become less variable as CBF increases. Increased arteriovenous shunting of microspheres at high flow rates would decrease the accuracy of the microsphere techniques. However, investigations of high-CBF states (including hypercapnia, hypoxia, and postischemic and posttraumatic hyperemia) indicate that shunting of 15-μm microspheres is minimal in the brain. These differences between the two CBF techniques may have contributed to the increased scatter observed at higher CBF rates.

Our studies confirm reports that CO2 reactivity is not affected by the administration of xenon, CBF decreased 3.2%/mm Hg decrease in PacO2, which is consistent with CO2 reactivity reported in cats, monkeys, and humans. We examined whether Xe-CT CBF values calculated from five scans during the first 5 minutes differed from those calculated from the entire series of scans made during the 30 minutes of xenon administration. Preliminary analysis suggested that the correlation is good (Figure 6). Further evaluation of white matter regions (i.e., the anterior and posterior limbs of the internal capsule) from five baboons supported these observations since the two values were significantly correlated at all PacO2 levels (Table 2). The best correlation was achieved at the highest PacO2 (and CBF) levels, perhaps because white matter saturation is slower than gray matter saturation (Figure 7) and because a limited number of data points collected during the first 5 minutes of saturation may yield more variable values at lower CBF levels. These observations suggest that the Xe-CT technique may have correlated well with the microsphere technique had we used a more limited, clinically relevant scanning protocol.

In summary, we reported a close correlation between CBF values determined using the Xe-CT and radioactive microsphere techniques. The two techniques yielded statistically identical values over a wide range of CBF. It is important to note that these studies were performed under "ideal" conditions, including the use of 50% xenon concentrations, long periods of xenon administration, and repeated (approximately 30) CT scans. We realize that these conditions are not practical during the normal clinical application of the technique. However, our preliminary analysis in a limited number of baboons suggests that the correlation between the two techniques would have been close had we used a shorter time and a more limited scanning protocol. We believe that our investigations demonstrate that the Xe-CT technique is accurate over a wide range of CBF values.

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References


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