Xenon-Enhanced Computed Tomography Compared With [14C]Iodoantipyrine for Normal and Low Cerebral Blood Flow States in Baboons

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The correlation between the acute, invasive diffusible [14C]iodoantipyrine technique for cerebral blood flow and the noninvasive xenon-enhanced computed tomographic method has been assessed by simultaneous measurements in the baboon. Blood flows in small tissue volumes (about 0.125 cm³) were directly compared in normal and low flow states. These studies demonstrate a statistically significant association between the two methods (p<0.001). Similar correlations were obtained by both the Kendall (τ) and the Spearman (r) methods (r=0.67 to 0.92, n=19 for each study). The problems and limitations of such correlations are discussed. (Stroke 1990;21:751-757)

Techniques have been described in which global, regional, or local cerebral blood flow (CBF) can be assessed in vivo by noninvasive, or minimally invasive, means.1-7 More precise measurements have been made possible by using invasive methods that provide absolute measurements of CBF values in animals,8-11 but these methods cannot be used in large scale or clinical settings. Comparison and validation of absolute values from invasive and noninvasive methods are important when function of normal or abnormal brain tissue is to be inferred12 and especially when flow values from clinical measurements are to be compared with laboratory studies. Absolute flow values measured simultaneously by two different methods in the same tissue rarely correlate well.12-14

The [14C]iodoantipyrine (IAP) technique, used since 1969 to assess blood flow in small tissue volumes,10,11 is based on a diffusible indicator, whose tissue concentration is proportional to local CBF.11,15 The measurement of CBF by nonradioactive xenon-enhanced computed tomography (CT) is an extension of the noninvasive xenon-133 method, in which washin or washout of freely diffusible inert xenon gas is measured in brain tissue and arterial blood. Xenon-enhanced CT has proven to be useful in both laboratory and clinical applications and is especially convenient and widely available because add-on systems are commercially available for several CT scanners, which include those available from General Electric Corporation, Medical Systems Division, Milwaukee, Wisconsin; Siemens Analytical X-Ray Instruments, Inc., Madison, Wisconsin; Picker, Cleveland, Ohio; Philips Electronic Instruments Co., Mahwah, New Jersey; and Toshiba (America Medical Systems, Tustin, California). Because measurement of normal and low flow CBF is most important, the IAP technique was selected as a method in which accuracy is not inherently limited to higher flow values. Cerebral blood flow measurements obtained from an injection of IAP were compared with those from simultaneous, multilevel xenon-enhanced CT studies in nonhuman primates (baboons).

Materials and Methods
All procedures were in accordance with institutional guidelines, the Animal Welfare Act, and the
Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, 1985).

Xenon inhalation and IAP studies were performed on three baboons (Papio cynocephalus/anubis) weighing 9.2, 10.0, and 10.5 kg. After sedation with ketamine hydrochloride (Vetalar, Parke-Davis, Morris Plains, New Jersey), 4% halothane (Fluothane, Ayerst Laboratories, Inc., New York, New York) was administered by mask to allow introduction of auffed endotracheal tube; then halothane was reduced to 0.5–1%. A semiclosed ventilation apparatus (respirator, Harvard Apparatus, South Natick, Massachusetts) also controlled the inhalation of xenon and oxygen when appropriate. Low flow states were produced by combining a reproducible hemorrhagic shock model with selective partial or complete ligation of one or both common carotid arteries. Xenon-enhanced CT blood flow measurements were made at appropriate intervals and just before IAP injection. All skin incisions and vessel catheterizations were made before heparinization (450 IU/kg sodium heparin from beef lung, Upjohn Co., Kalamazoo, Michigan). The aorta was catheterized via both femoral arteries. A high (arch) line was used for central arterial pressure measurement and blood sampling, and a larger (8F) subdiaphragmatic line was used for hemorrhage and reperfusion. This line passed through a bidirectional finger peristaltic pump (model 1410, Harvard Apparatus) operated by a remote speed control and switch box, which were kept out of the radiation exposure area. Hemorrhage and reinfusion of shed blood were under control of the investigators viewing the central arterial pressure and the end-tidal CO₂ concentration (capnograph); this arrangement formed a “human” closed loop. Measurement of central venous pressure and infusion of IAP were by means of femoral vein catheter.

A short right axillary artery catheter was advanced into the subclavian artery for blood sampling during the IAP infusion. Both common carotid arteries were exposed and encircled with tourniquets that could produce reversible partial or total occlusion. Electroencephalogram (EEG) electrodes were applied with collodion in accordance with the international “10-20 System.”24 The standard leads selected did not interfere with CBF measurement; the leads were as follows: frontal (F₃, F₄, and Fz), central (C₃, C₄, and Cz), and parietal (P₃, P₄, and Pz), as well as ear electrodes (A₁ and A₂). Both referential and bipolar derivations were used.

After surgical preparation, halothane was discontinued. Every 2 hours, 0.2 mg/kg morphine SO₄ (Eli Lilly, Indianapolis, Indiana) and 0.2 mg/kg diazepam (Valium, Hoffman LaRoche, Nutley, New Jersey) were administered; every hour, 0.02 mg propranolol hydrochloride (Inderal, Ayerst Laboratories, Inc., New York, New York) and 0.2 mg/kg pancuronium bromide (Pavulon, Organon Pharmaceuticals, West Orange, New Jersey) were administered. This regimen provided sedation and analgesia to minimize pain and stress-related cardiovascular effects and to induce paralysis for total immobilization during xenon-enhanced CT CBF measurements.

Combinations of hemorrhage and carotid occlusion were used to obtain the desired low CBF. Typically, the animal was bled to a mean central arterial pressure of 6.65 kPa (50 mm Hg) and held at that pressure by using the reversible peristaltic pump. The xenon-enhanced CT CBF and EEG served as guides to the degree of cerebral ischemia. Superimposition of carotid obstruction over the reduced perfusion reserve of hemorrhagic shock provided the ability to create different flow level states involving both temporal and anatomic variations. In three pilot experiments shed blood was returned, and the animal was allowed to recover; thus, the reversibility of the procedures was established. For autoradiography, the animal had to be killed very soon after injection of IAP.

A standard GE 9800 scanner (General Electric Corporation) equipped with the xenon inhalation system was used for xenon-enhanced CT CBF measurements. Details of the methodology and its limitations are described elsewhere.22,23 Two CT brain levels were selected for each study, and two nonenhanced baseline scans were obtained at each level. Then the inhaled gas was switched to a 32% xenon/68% oxygen mixture. Rapid serial scanning was initiated 0.3 minutes after the start of xenon inhalation. Six enhanced scans were obtained at each level. The xenon-enhanced CT inhalation lasted 6.5 minutes; then the animal was ventilated with room air for a period of 1 hour. This procedure allowed for xenon washout and establishment of stabilization of the next blood flow state. When IAP was to be injected (last measurement of each experiment), the xenon inhalation was continued for an additional minute to maintain its effect on CBF and to provide more comparable flow for both measurements (xenon-enhanced CT and IAP).

The two baseline images were averaged and subtracted from the xenon-enhanced images. Each voxel was thus defined by a series of enhancement values as a function of time. This series was used in conjunction with end-tidal measurements, assumed to be proportional to xenon concentrations in arterial blood, to solve for a monocompartmental Kety equation:

\[
C(t) = \lambda k \int_{0}^{t} C_0(u) e^{-k(t-u)} du
\]

where \(C_0(u)\) is the concentration of xenon in cerebral arterial blood, \(C(t)\) is its concentration in cerebral tissue, and \(\lambda\) and \(k\) are, respectively, the partition coefficient and rate constant for the flow compartment. A nonweighted, least-squares routine was used to derive the estimates of two parameters, \(\lambda\) and \(f\) (where \(f = \lambda k\)). The flow values \(f\) were used to generate a flow image. Pixel-to-pixel variation was reduced using a 3 pixel by 3 pixel bell-shaped filter in preanalysis and postanalysis smoothing routines.
Immediately after the last xenon-enhanced CT measurement and while the animal was still inhaling the Xe-O₂ mixture, 1 mCi IAP (4-iodo[N-methyl-14C]antipyrine, Amersham Corp., Arlington Heights, Illinois) was infused continuously over 80 seconds into the inferior vena cava through a femoral venous catheter using an infusion pump (model 341, Sage Instrument, Cambridge, Massachusetts). Blood samples were obtained every 2–3 seconds from the subclavian arterial catheter by touching capillary tubes (Unopette, Becton Dickinson Labware, Rutherford, New Jersey) to the shortened end of the freely flowing arterial catheter (cleared >2 times/sec). The animal was immediately decapitated by guillotine, the scalp was reflected, and the upper third of the skull was removed with an oscillating bone saw (Stryker Corp., Kalamazoo, Michigan). The brain was dissected free of dura mater, the cranial nerves were drilled along the inked line and marked the center of each slice identified; indelible ink in organic solvent was used. The brain was returned to the isopentane (Fisher Sci. Co., Pittsburgh, Pennsylvania) and dry ice mixture at -50° C. This sequence was repeated until the original scan plane was located.

The CT laser guide was used to mark the midline of each slice identified; indelible ink in organic solvent was used. The brain was repositioned in the inverted skull cap precoated with a silicone releasing agent (Silicone 7 Compound, Dow Corning, Midland, Michigan) and lowered into an isopentane bath after each 5-minute scan session. Eight 1 mm diameter by 5 mm deep holes were drilled along the inked line and marked the center of each slice. After insertion of carbon black, these holes would permanently identify the center of the slice during serial cryosection. Since the xenon-enhanced CT image represented a tissue slice through the selected level, the exact time of the CBF measurement could be determined. The CT flow of the same 5-mm thick region. The sections were dried at 55° C for 5 minutes on a hot plate and placed onto x-ray film (SB-5, Eastman Kodak) for 10 days along with calibrated [14C]methyl methacrylate standards. The blood samples in the capillary tubes were placed into liquid scintillation vials with 1 ml water and 10 ml scintillation fluid (American Chemical Society grade II, Amersham) and counted (model 4530, Packard Instrument Co., Downers Grove, Illinois) for quantitative densitometric evaluation of the autoradiographic images by using a computerized scanning image processor (Grinnell Systems Corporation, San Jose, California) attached to a DEC 11/34 computer. The slices were analyzed in such a way that the mean flow of these 11 sections or of the regions of interest (ROIs) projected through them, as measured by autoradiography, would be roughly equivalent to the xenon-enhanced CT flow of the same 5-mm thick region. The sections were stained for histologic verification of structure identification. The blood IAP concentration was determined by quantitative densitometric evaluation of the autoradiographic images by using a computerized scanning image processor (Grinnell Systems Corporation, San Jose, California) attached to a DEC 11/34 computer.

The slice angle and levels for each section were fixed during the first preliminary xenon-enhanced CT CBF measurements and thus had to apply to both xenon-enhanced CT and IAP measurements. Levels were chosen to contain representative regions of gray and white matter, including deep and superficial structures. The 5-mm CT slices provided reasonably good reproducibility while retaining the ability to obtain good approximation by selection of representative samples of the much thinner autoradiographic sections. The tissue slice autoradiographic
images were then compared with the CT images. ROIs (5×5 mm²) were marked on the center autoradiographs, projected onto the other autoradiographs as described above, and superimposed on the CT and xenon flow images. The size of the projected autoradiograph was optically adjusted to allow for best visual correspondence of the autoradiographic and CT images (Figure 1). Flow values in 11 IAP maps were averaged to yield a representative value for the 5×5×5-mm³ tissue volume that was used in the correlations with the corresponding xenon-enhanced CT ROI data.

Results

The xenon-enhanced CT CBF method yielded flow maps with good anatomic correlation and tissue specificity, with a typical spatial resolution of approximately 5 mm, full width, and half max (Figure 1). Flow maps can be displayed adjacent to the baseline CT images, or ROIs may be defined on the CT scan and then deposited directly on the map and flow-averaged by computer summation, which provides direct correlation between anatomy and blood flow. With the noise levels associated with the scanner used, the xenon concentration inhaled in these studies, and the computational methodology used, the overall errors of derived average flow in an ROI of approximately 5×5 pixels were approximately ±14%. Similar estimates have been reported elsewhere.23,26,27-30

The IAP method yielded high-resolution flow maps that were summed over the tissue investigated so that they could be correlated directly with the flow maps generated by the xenon-enhanced CT method. The major sources of error in this technique include errors in the evaluation of the tissue concentration and the sample timing, as well as uncertainties in the value of the partition coefficient.15 Previous experience in the laboratory of one of the authors has shown that this error is approximately 8–10%. The greatest source of sample timing errors arises from the circulation time difference between the site of blood sampling and the brain. This error is 1 second

FIGURE 1. Computed tomography (CT) scan, flow map, and autoradiograph showing establishment of coincidence of tissue regions between the xenon-enhanced CT method and the [14C]iodoantipyrine method. CT slices of interest were taken during the experiment (panel a); after processing the data from panel a, a flow map (panel b) was generated; the cryosectioning of frozen brain resulted in autoradiographs (panel c). These were optically adjusted to make the overall sizes of panel b and panel c equivalent. Regions of interest (D) were visually selected on panel c and projected onto the display to identify them on the CT slice (panel a) and xenon flow map (panel b). The images are shown to illustrate methodology only. Serial photographic reproductions have lowered the quality substantially from the original. Quality of images is demonstrated in earlier reports.23,26
at most. Since gross pathology was not present, it is unlikely that the partition coefficient is altered from that obtained in normal tissue. From the error analysis plots of Patlak et al \(^{15}\) and the relatively low blood flows obtained in this study, the error of blood flow measurement can be calculated to be approximately 15–20%.

Individual studies and those combined across all strata (70 paired observations) were evaluated by nonparametric statistics (Kendall \(\tau\)). \(^{31}\) All individual experiments showed statistical significance at \(p<0.01\); values across all strata were highly significant (\(p<0.001\)). Kendall \(\tau\) correlation coefficients ranged from 0.50 to 0.75 (Table 1). Similar observations were made when Spearman rank-correlation coefficient \(r\) was used to demonstrate correlation between the two methods. The association between the techniques was significant at \(p<0.01\) in all of the studies (Figure 2). Similar to the Kendall \(\tau\) test, the Spearman correlation coefficients ranged from 0.67 to 0.92; these values indicate that much of the difference can be explained based on statistical variation alone. Thus, the study demonstrated a significant correlation between the methods over the range of low to normal values (Table 1). The strength of the association, which is indicated by the correlation coefficient values, suggests that approximately 40% of the variation cannot be explained based on statistical consideration alone. Some of this limited strength of association may be attributable to the problems associated with the study (see "Discussion").

The administration of xenon, up to 30% in inspired gas mixture, did have repeatable effects on the EEG. Both animals had increased theta activity as xenon concentration increased; one also had occasional delta activity as well. Severe ischemia alone also caused EEG changes in both animals, but the combination of low blood flow and 20–30% xenon produced marked changes with prominent delta activity and subsequent suppression of activity. In one animal, there was a further reduction of mean flow at the time of the most marked EEG changes. The EEG was thus an indispensible guide to the degree of hemorrhage or carotid obstruction that was needed and could be tolerated.

### Discussion

Direct comparison between the two methods investigated here, or for that matter between any two methods with reasonable resolution, is a most difficult task. This is particularly true when absolute values of low flow states are compared. Similar studies in the past, in which the nondiffusible-microsphere technique was compared with regional (mostly cortical) flow estimates from diffusible indicators (e.g., xenon-133), seem to demonstrate varying degrees of association between the methods. However, most of these studies emphasize the difficulties in comparing results, particularly when absolute flow values in small tissue volumes (as opposed to relative regional changes) are considered.\(^{29,30}\) In the present study, there are several limitations of such comparisons.

In spite of the care in marking the excised tissue at the same levels and angles as those at which the CT images were obtained, some minor differences were observed. Alteration of the tissue volumes during treatment and cutting may have occurred. Yet, visual comparison between tissue radiographs and the CT images indicates that the correspondence is good within approximately 1 mm. Although such correspondence would be more than adequate in studies with large brains and poor resolution, it does present

### Table 1. Correlations of Blood Flow Values Obtained With Xenon-Enhanced Computed Tomography and \([14C]\)Iodoantipyrine

<table>
<thead>
<tr>
<th>Animal</th>
<th>Paco(_2) (mm Hg)</th>
<th>Blood pressure (mm Hg)</th>
<th>Number of observations</th>
<th>Xenon-enhanced CT average flow (ml/[100 cm(^3) • min])</th>
<th>([14C])Iodoantipyrine average flow (ml/[100 cm(^3) • min])</th>
<th>Kendall (p)</th>
<th>Spearman (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>50</td>
<td>21</td>
<td>30.2</td>
<td>31.6</td>
<td>&lt;0.01</td>
<td>0.50</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>45</td>
<td>30</td>
<td>22.4</td>
<td>20.4</td>
<td>&lt;0.01</td>
<td>0.64</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>140</td>
<td>19</td>
<td>45.8</td>
<td>60.6</td>
<td>&lt;0.01</td>
<td>0.75</td>
</tr>
</tbody>
</table>

### Figure 2. Regression line fitted to paired observations in one animal. The correlation between paired observations is clearly demonstrated. The square represents the average flow of those regions of interest that have flows <16 ml/(100 cm\(^3\) • min). Xe/CT, xenon-enhanced computed tomography; \(R_s\), correlation by Spearman \(\tau\) method; \(\tau\), correlation by Kendall \(\tau\) method.
some problem in our study. Difficulties with placement of the ROI resulted in significant reduction in the association between the methods. In particular, this is true in regions near ventricles or in regions with rapidly varying flow, such as nuclear regions with adjacent white matter tracts where, in either method, 1-mm misplacement of the tissue investigated can result in large changes of the average flow value for the region.

Reports of stable xenon effects on EEG before 1960 were concerned with anesthetic use of xenon and dealt with higher concentrations. With renewed interest in stable xenon inhalation for CBF measurement, several investigators have reported EEG effects. Hartmann et al., using 35% xenon in baboons, found an increase in slow activity (theta and delta) that is consistent with our results. Two studies in humans breathing 25% and 35% xenon reported an increase in beta and alpha power, respectively. Thus, several studies have shown EEG changes at stable xenon concentrations used to measure cerebral flow. Our result is suggestive that the depressive effects of xenon inhalation and of ischemia may be additive and can produce changes that are greater than those of ischemia alone. Recovery was rapid on removal of xenon and especially on relief of ischemia. In spite of the limited observations, this result should not be overlooked because of the paucity of data in the literature and the conflicting statements that have been put forth.

All of the problems discussed above have the potential for reducing the strength of a direct correlation between the xenon-enhanced CT and the IAP methods. The complexity of the experiment has necessarily limited the number of animals studied to six: three pilot methodology experiments and three definitive comparisons. The goal of comparing methods is easily achieved with this number because of the relatively large number of observations that could be analyzed (≥19 for each animal) and because of variability of flow within each animal. When comparing ROIs with low absolute flow values of ≤15 ml/(100 cm³·min), the within-ROI heterogeneity and small errors in registration make the correlation for individual observations a much more difficult task. This difficulty could be overcome by averaging many different ROIs with low absolute flow values, as was done in Figure 2. In spite of these limitations, we have been able to obtain a series of simultaneous flow measurements by the two methods that demonstrates a most significant association between the results in small tissue volumes at normal as well as the more important low flow states, which are the most difficult ones to validate. These correlations are better than those reported by us previously using the microsphere technique at normal and high flow values. Although the correlation in high flow values is high, there are noticeable and statistically significant differences in absolute values between the xenon-enhanced CT and postmortem tissue autoradio-

graphic methods that have been previously discussed.29

In spite of the direct CBF effects of xenon, this study lends support to the reliability of the flows measured and minimizes concerns that a changing flow during measurement (caused by xenon uptake) has violated the Kety-Schmidt equation assumption of constant flow. Although there was an increase in arterial xenon concentration during the xenon-enhanced CT measurement, it was virtually constant at the time of IAP measurement, which was 7 minutes after the onset of xenon inhalation.

References


KEY WORDS • tomography, emission computed • xenon • cerebral blood flow • baboons
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