Measurement of Regional Cerebral Blood Flow in the Dog Using Ultrafast Computed Tomography
Experimental Validation

Glenn T. Gobbel, DVM; Christopher E. Cann, PhD; Harriet S. Iwamoto, PhD; and John R. Fike, PhD

The applicability, feasibility, reproducibility, and accuracy of the method of measuring regional cerebral blood flow using ultrafast computed tomography were evaluated in 25 dogs under varying physiological and pathophysiological conditions. Regional cerebral blood flow values were 75.6±29.4 ml/100 g/min (mean±standard deviation) for the hemisphere, 68.4±28.2 ml/100 g/min for the basal ganglia, 41.2±15.0 ml/100 g/min for the internal capsule, and 80.8±37.2 ml/100 g/min for the neocortex. Measurements made 10 minutes apart were significantly (p<0.05) correlated. Simultaneous measurements of regional cerebral blood flow by the microsphere and ultrafast computed tomography methods showed a significant (p<0.05) correlation for the hemisphere (r=0.95), basal ganglia (r=0.95), and neocortex (r=0.94) but not for the internal capsule (r=0.51). Microsphere and ultrafast computed tomography regional cerebral blood flow values were also in agreement in radiation-damaged brain with appreciable blood-brain barrier breakdown, and the two methods demonstrated similar responsiveness of regional cerebral blood flow to alterations in arterial carbon dioxide tension. The accuracy and sensitivity of the ultrafast computed tomography technique suggests that it affords a useful new tool for studying normal and abnormal regional cerebral blood flow.

Present methods of measuring regional cerebral blood flow (rCBF) are useful in the care of patients with cerebrovascular disorders.1-7 Before new methods of rCBF measurement can be employed in the study and management of such disorders, their accuracy under a variety of physiological and pathophysiological conditions must be assessed.

A theoretical approach to using dynamic computed tomography (CT) to quantify rCBF noninvasively has been described.8 Using this technique, measures of fractional blood volume and mean transit time are derived from a series of CT scans acquired following an intravenous bolus of iodinated contrast medium. Unlike previous applications of dynamic CT, these derivations consider the arterial input of contrast into the tissue region of interest (ROI).9-11 Fractional blood volume and mean transit time are calculated by comparing the parameters characterizing the time versus CT number curve taken from the ROI with the parameters of the curve from an artery adjacent to the ROI. The rCBF is then derived from the quotient of the fractional blood volume and the mean transit time.

In the present study, dynamic CT studies of rCBF were carried out on an ultrafast CT scanner. The feasibility, reproducibility, and accuracy of this technique of rCBF measurement were determined in a series of studies in beagle dogs under normal physiological, altered physiological, and pathophysiological conditions.

Materials and Methods

Purebred adult beagle dogs (16 males, nine females) approximately 1 year of age were purchased from a commercial supplier. In accordance with guidelines from the National Institutes of Health for
the care and handling of laboratory animals, all dogs were housed under standardized conditions and given routine veterinary care as described previously. The dogs were assigned to three experimental study groups; some dogs were assigned to more than one study group. Two dogs received brain irradiation before entering study 3.

The dogs were anesthetized for all procedures. Preanesthetics included 0.05 mg/kg atropine sulfate and 0.25 mg/kg acepromazine maleate administered subcutaneously 20-30 minutes before induction. General anesthesia was induced with thiamylal sodium (4% to effect) given intravenously through an 18-gauge catheter placed in a cephalic vein. The dogs were intubated, and general anesthesia was maintained with periodic intravenous bolus injections of thiamylal sodium. For the placement of arterial lines for the microsphere studies, anesthesia was maintained with a mixture of methoxyflurane gas and oxygen as described previously.

Study 1, which included 12 normal dogs, was designed to test the feasibility of using ultrafast CT to measure rCBF. The rCBF was measured in the left and right internal capsule, neocortex, basal ganglia, and whole hemisphere (Figure 1), and results from the various regions were compared. During scanning, the dogs were ventilated mechanically using a tidal volume of 200 ml and a respiratory rate of 25 breaths/min. To determine arterial carbon dioxide tension (Paco2), 1-ml blood samples were collected from the femoral artery immediately after the last scan of the ultrafast CT procedure. To reduce variability in rCBF resulting from alterations in Paco2, only those studies in which Paco2 was 35-40 mm Hg were compared.

Study 2, which included 12 normal dogs, investigated the reproducibility of the ultrafast CT technique. For each dog, two rCBF measurements were made 10 minutes apart and the resulting rCBF values were compared. No mechanical ventilation was used, and arterial blood samples were collected for PacO2 determination immediately after the last scan of each ultrafast CT procedure. In addition, to establish interobserver reproducibility, 12 of the examinations were arbitrarily selected and the rCBF values within the left and right hemispheric ROIs were determined independently by two observers. To examine intraobserver reproducibility, five of these studies were evaluated twice, 1-2 weeks apart, by a single observer. Reproducibility was defined as the standard deviation (SD) of the difference in two measurements expressed as a percentage of the mean of the two measurements.

Study 3 was used to validate the ultrafast CT technique against the radiolabeled microsphere method in both normal and abnormal brain. Three normal dogs, one dog with a focal radiation lesion caused by an iodine-125 brain implant, and one dog that had previously received a single radiation dose of 15 Gy to the right hemisphere were included. Both radiation treatments produce significant and well-characterized increases in permeability of the blood–brain barrier (BBB). Up to five simultaneous ultrafast CT/microsphere studies were done in each dog, with an interval of 10-15 minutes between studies. To alter PacO2, the dogs were ventilated mechanically using a tidal volume of 200 ml and respiratory rates of 10, 25, or 50 breaths/min. Arterial blood samples were collected for PacO2 determination after each ultrafast CT/microsphere study.

Ultrafast CT studies were done using a C-100XL CT scanner (Imatron Inc., South San Francisco, Calif.). The dog was placed in sternal recumbency with the head extended onto a plastic head rest within the scanner. Based on a lateral computed radiograph of the skull followed by scout transverse images, the scan level just rostral to the thalamus was chosen for the rCBF study.

A 15-ml bolus of iodinated contrast agent (Na/meglumine iothalamate, iodine content 400 mg/ml)
was injected into a cephalic vein at a rate of 5 ml/sec using a mechanical injector (Medrad, Mark IV, Pittsburgh, Pa.). A series of 20 CT scans was initiated at the beginning of the injection using interscan delays of 2.0 seconds for scans 1–3 and 0.8 seconds for scans 4–20. Scan duration was 0.1 second, and scan thickness was 6 mm. The scanner was operated at 130 kV (peak) and 63 mA. Images were reconstructed on a 360×360 matrix, and pixel size was 0.5 mm.

For the microsphere studies, a 14-gauge pigtail catheter was placed in the femoral artery and advanced to the left ventricle. Arterial pressure at the catheter tip was monitored to confirm proper placement. A second 14-gauge catheter was placed in a brachial artery. Between 1.5 and 2.0×10⁶ microspheres, 15 μm in diameter, radiolabeled with gadolinium-153, cobalt-57, strontium-85, niobium-95, manganese-54, or zinc-65 were injected into the left ventricle beginning with the start of administration of the iodinated contrast agent. A reference blood sample was withdrawn from the brachial artery at a rate of 7.5–8.0 ml/min for 15 seconds before and 55 seconds after microsphere injection.

After the last study, the skull was marked at the level of the CT scan plane and the dog was killed with an overdose of pentobarbital. The left ventricle of the heart was opened to confirm proper catheter placement. The brain was removed, sectioned at the level of the CT scan plane, and fixed in formalin. Sections of the brain corresponding to the internal capsule, neocortex, and basal ganglia were dissected and weighed. Additional samples of the left and right frontal lobes were taken to verify adequate mixing of the microspheres and uniform distribution to the two sides of the brain. The activity of each isotope within each section was determined as described previously.15

A manually directed trackball-guided cursor was used to outline ROIs within the CT images. The ROIs used included the left and right internal capsule, neocortex, basal ganglia, and hemisphere and were well-defined anatomically on the CT images, thus minimizing operator influence on the choice of the boundary (Figure 1). The average CT number of the pixels within the outlined ROIs was determined for each of the 20 scans and plotted versus time (Figure 2). The arterial CT number versus time data was determined from an artery visualized in the same scan. Because changes in the CT number after the administration of contrast agent are linearly related to the amount of contrast agent within the tissue,10,16 the CT number versus time data corresponded directly to the contrast concentration versus time data.

The gamma-variate curve has been shown to approximate closely an indicator-dilution curve without recirculation.17,18 A gamma-variate curve of the form

\[ \Delta C(t) = C(t - t_q) e^{-k(t - t_q)} \]

where \( \Delta C(t) \) is the change in CT number from baseline at time \( t \) and \( A, B, C, \) and \( t_q \) are parameters obtained from the curve fit, was therefore fit to the data using a routine employed by the scanner software. The curve-fitting routine calculates the time of arrival of the bolus \( (t_0) \) from three successive approximations to the arrival time. The approximations are based on the average of the time of the first point used in the fit (see below) and the time of a preceding point. The differences between the measured and fitted data are calculated for three gamma-variate fits using these approximated arrival times. The arrival time that minimizes these differences is determined based on a parabolic error function fit to these differences and used in the final gamma-variate fit. The fitting routine uses a weighted least-squares procedure in which the weights are proportional to the square of the change in CT number from baseline. The data included in the fitting procedure can be chosen by the operator, and for this study the gamma-variate function was fit to the data starting at the point when the CT number increased to 15% (for neocortex, hemisphere, and arterial ROIs) or 30% (for basal ganglia and internal capsule ROIs) of the maximum CT number and ending at the point when the CT number fell back to 50% of the maximum. The curve fit beyond this 50% point was extrapolated to baseline to eliminate the influence of indicator recirculation.

The errors in the curve fits for study 1 were calculated as

\[
\sum_{i=1}^{n} \left( \frac{M_i - C_i}{(\pi - 1)} \right)^{1/2} \times \left( \frac{n}{\sigma M_i} \right)
\]

in which \( M_i \) and \( C_i \) are the measured and computed values at time \( i \), and \( n \) is the number of fitted data points.

For use in the actual calculation of rCBF, the area under the fitted curve (AUC) and the center of gravity of the curve \( (t_0) \) were automatically displayed adjacent to the CT image. To minimize averaging of the arterial volume with that of the surrounding parenchyma, the single pixel within the left or right...
TABLE 2. Comparison of Percentage Differences in Ultrafast Computed Tomography-Measured Regional Cerebral Blood Flow Values Obtained 10 Minutes Apart With Left-Right Differences in 12 Dogs

<table>
<thead>
<tr>
<th>Region</th>
<th>10-minute difference (%)</th>
<th>Left-right difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemisphere</td>
<td>14.0±27.0</td>
<td>18.2±17.3</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>22.2±28.5</td>
<td>1.3±13.0</td>
</tr>
<tr>
<td>Internal capsule</td>
<td>16.8±26.9</td>
<td>-9.4±21.5</td>
</tr>
<tr>
<td>Neocortex</td>
<td>12.6±33.6</td>
<td>5.2±9.6</td>
</tr>
</tbody>
</table>

Percentage difference defined as (value 1−value 2)/(average of values 1 and 2)×100%, in which value 1 is measurement from first of two measurements separated by 10 minutes or left measurement. Values from left and right regions of interest were averaged for computation of 10-minute percentage differences, and values from two measurements were averaged for computation of left-right percentage differences. Values are mean±SD.

the hemisphere (r=0.95), basal ganglia (r=0.95), and neocortex (r=0.94) but not for the internal capsule (r=0.51). There were no significant differences between the rCBF values obtained using the microsphere method and those obtained using ultrafast CT for any region considered (p>0.10). Both methods typically demonstrated differences of <20% between homologous ROIs on contralateral sides of the brain. In the dog with a focal radiation lesion, rCBF in the irradiated region was reduced relative to that in a homologous contralateral region by 76.4±7.9% (mean±SEM) for the measurements made using the microsphere technique and 70.7±4.7% (mean±SEM) for the measurements made using the ultrafast CT technique. These values are much greater than the left-right differences in normal dogs (Table 2). In contrast, mean±SEM left-right differences for the dog that had undergone hemibrain irradiation were 10.4±3.2% for the hemisphere, 2.3±12.5% for the basal ganglia, 32.3±11.1% for the internal capsule, and 5.9±9.6% for the neocortex when determined by the microsphere technique and 8.6±10.1%, -4.5±17.2%, -6.9±41.3%, and 9.2±10.9%, respectively, when determined by the ultrafast CT method.

Regression analysis showed that, for both the microsphere and ultrafast CT techniques, there were significant positive correlations between rCBF and PaCO₂ for all brain ROIs considered (Table 3). The response of rCBF within the basal ganglia to PaCO₂ alteration determined by the microsphere technique was significantly greater than the response determined by the ultrafast CT technique (p<0.05), and the rCBF response within the internal capsule determined by the microsphere approach achieved significantly greater (p<0.10). However, the trends in regional responses determined by the two techniques were similar, with the neocortex showing the greatest responsiveness, the internal capsule the least, and the basal ganglia an intermediate level (Table 3).

Discussion

The present study shows that rCBF can be quantified noninvasively by CT. Combining a theoretical approach based on indicator dilution theory and the unique technological advances provided by the ultrafast CT scanner, it is possible to obtain CT number versus time curves from which parameters relating to blood flow can be derived. A relatively simple equation can then be used to calculate rCBF.

Absolute rCBF values have been shown to have a wide range in humans and anesthetized animals, and our data support this (Table 1). Furthermore, in agreement with others, our data suggest that left-right differences in rCBF in normal brain tend to be small. This characteristic of rCBF should assist in evaluating pathological changes in rCBF confined to one hemisphere. The fact that the left-right differences observed using ultrafast CT were similar to
those observed using the microsphere method suggests that the small variation in side-to-side differences was not an artifact of the ultrafast CT method.

Although there was good overall agreement of the results obtained with ultrafast CT and the microsphere method over a wide range of rCBF values (Figure 3), there were instances in which the two techniques appeared to differ. In the case of the left–right differences in rCBF in the internal capsule of the dog that had undergone hemibrain irradiation, the microsphere method detected a greater difference than ultrafast CT. In addition, rCBF seemed to be somewhat less responsive to alterations in PaCO₂ than the 10–15% reproducibility obtained with invasive methods such as the microsphere technique.12-22 However, based on our 10% interobserver and intraobserver reproducibility, about one third of the short-term reproducibility is accounted for by differences in how the ROIs are drawn for each measurement. The poorer reproducibility could also be related to hemodynamic alterations such as arterial vasodilatation23-25 induced by hyperosmotic contrast agents. However, such effects generally do not appear until 3–4 seconds after exposure of arteries to the contrast,23 so it seems unlikely that the time–concentration data used for ultrafast CT rCBF calculations would be significantly affected by these agents. Consequently, a single measurement should be valid, but the contrast agent could modify blood flow in such a way that subsequent measurements would be altered relative to the first measurement.23

The use of nonionic and low-osmolar contrast agents might help to reduce these effects and perhaps improve the reproducibility of the ultrafast CT method.

Another potential source for the poorer reproducibility of ultrafast CT is the use of a single value for Pₐ in Equation 2. The parameters that comprise Pₐ and might change between measurements are m and HctPₐ. The factor m could be altered due to changes in the arrival time of the bolus at the peripheral artery used for computation of rCBF relative to the actual disagreement between the methods. Furthermore, some differences between the methods used here can probably be attributed to an unavoidable margin for error in sampling exactly the same tissues with the two techniques and to some imprecision in the measurement of rCBF using the microsphere method.22

The most apparent deviations between the microsphere and ultrafast CT rCBF values arise at flow values greater than 120 ml/100 g/min (Figure 3). Because blood flow is proportional to the inverse of the mean transit time,8 underestimation of the very short mean transit times that accompany high blood flow can result in significant overestimation of blood flow. In certain clinical conditions such as stroke, measurement of low rCBF is generally of greater concern. Nonetheless, to avoid the overestimation of rCBF, one could potentially establish a minimal acceptable mean transit time for blood flow calculation. For example, in our study, all rCBF values of >120 ml/100 g/min had a corresponding mean transit time of 0.8-0.9 seconds, which was >2 standard deviations below the average mean transit time for all ROIs combined. Based on this, 1 second would seem to be a reasonable minimal acceptable mean transit time, and less confidence would be placed in rCBF values corresponding to mean transit times shorter than this.

Based on the standard deviation for percentage differences of ultrafast CT studies done 10 minutes apart (Table 2), short-term reproducibility in our study was approximately 30%. This value is poorer than the 10–15% reproducibility obtained with invasive methods such as the microsphere technique.12,22 However, based on our 10% interobserver and intraobserver reproducibility, about one third of the short-term reproducibility is accounted for by differences in how the ROIs are drawn for each measurement. The poorer reproducibility could also be related to hemodynamic alterations such as arterial vasodilatation induced by hyperosmotic contrast agents. However, such effects generally do not appear until 3–4 seconds after exposure of arteries to the contrast, so it seems unlikely that the time–concentration data used for ultrafast CT rCBF calculations would be significantly affected by these agents. Consequently, a single measurement should be valid, but the contrast agent could modify blood flow in such a way that subsequent measurements would be altered relative to the first measurement.
actual arrival time of the bolus at the ROI, and $Ht_{PA}$ could change due to changes in cardiac output resulting in differing degrees of dilution of the hemotocrit by the contrast agent. However, when changes in $m$ and $Ht_{PA}$ were accounted for on a measurement-by-measurement and ROI-by-ROI basis, there was no appreciable change in reproducibility, suggesting that the use of a single value for $n$ was warranted in our study. On the other hand, there are factors, such as pathology (arteriosclerosis), choice of artery for the determination of $AUC_{PA}$ and $t_{PA}$, and species, that might affect the factor $m$ and might require recalculation of $m$ and $n$ in Equation 2. These details need to be considered before our method can be used clinically or in other experimental settings.

The ultrafast CT method described here should be useful clinically, and our results indicate that rCBF can be measured accurately in ROIs as small as 0.3 cm$^3$. Furthermore, up to four different levels in the brain can be studied simultaneously, and repeated studies can be carried out with additional boluses of contrast. Of course, radiation dose (8–9 cGy/20 scans) and amount of contrast may limited the number of studies, and these factors have to be weighed against the clinical benefits of repeated rCBF evaluations. Another factor that may affect the clinical implementation of this method is the presence of a damaged BBB, a common finding in certain types of brain injury. Our results indicate that disruption of the BBB does not adversely affect rCBF measurement, at least in terms of radiation-induced damage, and based on theoretical considerations we have shown that even if BBB breakdown is large, as defined by blood-to-brain transfer constants, rCBF measurements based on first-pass kinetics are minimally affected. However, the clinical usefulness of this technique under a variety of pathological conditions, particularly at very low flows (0–20 ml/100 g/min), will have to be determined.

It is not clear if the ultrafast CT technique as described here could be implemented on a slower CT scanner. Prolonged acquisition times for each scan result in measured contrast concentrations that correspond to the concentration averaged over the period of acquisition rather than an instantaneous concentration. Averaging during nonlinear changes in contrast concentration reduces the accuracy of contrast measurements. However, if these limitations were taken into consideration, the data collected on a slower scanner might be sufficient to provide clinically useful measures of rCBF.

Our results indicate that ultrafast CT is useful in the measurement of rCBF in both normal and pathological tissues. Unlike some methods of rCBF measurement, ultrafast CT is relatively noninvasive, requiring only the placement of a single venous catheter. Moreover, the studies are short and can be repeated many times at multiple levels in the brain. Besides providing measurements of rCBF, ultrafast CT also can be used to evaluate the fractional vascular volume and mean transit time within an ROI. Ultrafast CT has the potential of being quite useful in the diagnosis and management of cerebrovascular disorders.

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