Quantification of Cerebral Perfusion With “Real-Time” Contrast-Enhanced Ultrasound

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Background—No noninvasive technique is currently capable of “real-time” assessment and monitoring of cerebral blood flow (CBF). We hypothesized that cerebral perfusion could be accurately measured and monitored in “real time” with contrast-enhanced ultrasound (CEU).

Methods and Results—Cerebral perfusion was assessed in 9 dogs through a craniotomy with CEU at baseline and during hypercapnia and hypocapnia while normoxia was maintained. Cerebral microvascular blood volume (A), microbubble velocity (β), and blood flow (A×β) were calculated from time-versus–acoustic intensity relations. Compared with baseline, hypercapnia and hypocapnia significantly increased and decreased CBF, respectively, as measured by CEU. These changes in blood flow were mediated by changes in both A and β. A good correlation was found between A×β derived from CEU and CBF measured by radiolabeled microspheres (y=0.67x−0.04, r=0.91, P<0.001).

Conclusions—Changes in both cerebral microvascular blood volume and red blood cell velocity can be accurately assessed with CEU. Thus, CEU has the potential for bedside measurement and monitoring of cerebral perfusion in real time in patients with craniotomies or burr holes. (Circulation. 2001;104:2582-2587.)

Key Words: cerebrovascular circulation ▪ contrast media ▪ ultrasonics

In current clinical practice, noninvasive assessment of cerebral blood flow (CBF) can be achieved by use of various techniques, such as CT, MRI, and single photon emission CT (SPECT). These techniques, however, are ill suited for measurement of CBF at the bedside or in the operating room. Furthermore, they cannot be used repeatedly, in rapid succession, to measure changes in CBF that may occur in the natural course of events or in response to treatment. In addition, these accurate, albeit expensive, modalities cannot be used for monitoring regional CBF in patients with acute ischemic stroke or intracranial hemorrhage, or for the serial assessment of global CBF in patients with increased intracranial pressure.

We have previously shown that blood flow to various tissues, including the myocardium, skeletal muscle, and kidney, can be measured by contrast-enhanced ultrasound (CEU). This technique, which uses small gas-filled microbubbles, can provide an assessment of microvascular blood volume (MBV) as well as red blood cell velocity (RBCv). These parameters can be obtained during a continuous intravenous infusion of microbubbles. After steady state is achieved, the microbubbles within tissue are destroyed with high-energy ultrasound, and the rate at which they replenish the ultrasound beam is measured, which reflects RBCv. The concentration of microbubbles when the beam is fully replenished is proportional to MBV.

We therefore hypothesized that CEU can be used to assess CBF noninvasively. We performed experiments in dogs in which CBF was measured with both CEU and radiolabeled microspheres during rest, hypercapnia, and hypocapnia. The aim was to demonstrate proof of principle so that future technical developments could assist in transferring this method to the clinical setting.

Methods

Animal Preparation

The study was approved by the Animal Research Committee at the University of Virginia and conformed to the American Heart Association “Guidelines for the Use of Animals in Research.” Nine adult mongrel dogs (30 to 35 kg) were anesthetized with 30 mg/kg sodium thiopental IV, intubated, and ventilated by a respiratory pump (model 607, Harvard Apparatus). Anesthesia was maintained with inhaled halothane (1.5 vol% in O2), End-tidal PCO2 (PetCO2) was monitored with a capnometer (Datex-Engstrom) placed in the ventilatory circuit. The ECG was monitored continuously, and core body temperature was maintained at 37°C with a heating pad.
Catheters were placed in both femoral veins for administration of intravenous fluids and microbubbles. A catheter was advanced into the left ventricle from the right femoral artery for injection of radiolabeled microspheres. The left femoral artery was cannulated with a catheter for blood pressure monitoring, systemic arterial blood gas analysis, and withdrawal of reference blood samples for blood flow analysis. A craniotomy (4×2.5 cm) was performed in the left parietal bone to provide an acoustic window for imaging of the brain.

Contrast-Enhanced Ultrasound

CEU was performed with an HDI-5000 system (ATL-Phillips) capable of performing “real-time” pulse inversion imaging. The behavior of microbubbles exposed to ultrasound depends on the acoustic energy (measured as mechanical index [MI]) to which they are subjected. At a high MI, the bubbles are destroyed, resulting in a high-amplitude signal. Because of microbubble destruction, however, imaging has to be performed intermittently to allow the ultrasound beam to become replenished with bubbles. At a low MI, the bubbles can be made to oscillate nonlinearly with minimal destruction, allowing for real-time imaging.

Unlike B-mode imaging, pulse-inversion imaging is a multipulse technique using a low MI. Two pulses of opposite polarities are transmitted sequentially along the same sector line. When these pulses interrogate tissue, the returning signals do not undergo any transformation because the tissue is incompressible. Conversely, the bubbles, being compressible, produce signals that are distorted. When these signals are added, the tissue signals of opposite polarities, being mirror images of each other, are canceled. The nonlinear signals from bubbles, not being mirror images of each other, are not canceled. In this manner, tissue signal is suppressed, whereas bubble signal is still seen (Figure 1). The bubble signal, however, is still not as strong as during bubble destruction. The dynamic range of this method is also low (20 dB) compared with B-mode imaging. Because multiple pulses are used along each line, the frame rate is lower for the same pulse repetition frequency than with B-mode imaging.

The ultrasound transducer was fixed in position, and a saline bath served as an acoustic interface between the transducer and the dural surface of the brain. Imaging was performed in a coronal plane at the level of the parietal lobe with an ultrasound transmit frequency of 1.6 MHz and a receive frequency of 3.3 MHz. A pulse-repetition frequency of 2500 Hz was used, with a resulting frame rate of 15 Hz. The image depth (usually 7 cm), focus (usually 3.5 cm), and gains (set to minimize tissue signal) were optimized at the beginning of each experiment and were held constant for individual dogs. Baseline images were recorded before contrast infusion.

A suspension of 2 mL of Sonazoid (Nycomed-Amersham) in 48 mL of normal saline was infused via the femoral venous catheter at a constant rate of 5 mL/min. Sonazoid is a perfluorocarbon-containing lipid microbubble with a mean diameter of 3 μm and a mean concentration of 10^9/mL. Imaging was initiated after a steady-state systemic concentration of microbubbles was reached (~2 minutes). For each imaging sequence, 4 high-power destructive pulses at an MI of 1.1 were delivered to destroy the bubbles within the beam elevation. This was followed by real-time (15-Hz) low-MI (0.1) imaging to measure the rate of microbubble replenishment. Digitally acquired images (for ≥10 seconds after the destructive pulses) were stored on magnetic optical disk.

Data were transferred to HDI laboratory (ATL-Phillips). A large region of interest (ROI) was drawn over the cerebral hemisphere, avoiding the larger vessels seen immediately after bubble destruction (Figure 2A). Ultrasound backscatter was measured within this ROI in all frames within the imaging sequence and was expressed in acoustic intensity (AI) units. These are arbitrary units in a range of 0 to 255 and, unlike video intensity units, have a linear relation with actual backscatter because the measurement is performed before log compression and postprocessing. The relation between microbubble concentration and AI is linear in the range of microbubble doses used in our study, and the microbubble concentration in the blood pool remains constant during the period of infusion. The same ROI was used for all stages within each experiment.

Figure 1. Diagram of real-time pulse-inversion imaging. See text for details.

Time-versus-AI plots generated beginning immediately after high-power destructive frames were fit to the function $y = A(1 - e^{-\beta t})$, where $y$ is the AI at time $t$, $A$ is the plateau AI reflecting MBV, and the rate constant $\beta$ represents the rate of microbubble (or red blood cell) replenishment (RBC). The product $A \times \beta$ reflects CBF. We have previously demonstrated a close linear relation between MCE- and radiolabeled microsphere–derived MBF ($r=0.92$). Our interobserver and intraobserver variabilities for the MCE-derived parameters are good ($r=0.88$ and $r=0.91$ for $A$, $r=0.91$ and $r=0.92$ for $\beta$).

Figure 2A illustrates a set of images obtained immediately after microbubble destruction and 0.5, 1, 3, and 8 seconds later, showing greater microbubble replenishment with time. Figure 2B shows that AI increases with time and finally reaches a plateau. The fit to the AI values is also shown.

Measurement of CBF With Radiolabeled Microspheres

Measurement of CBF was performed with the following 15-μm-diameter microspheres: 47Ce, 125INb, 99mTc, 103Ru, and 113Sn (Dupont Medical Products) injected into the left ventricle over 20 seconds. Reference blood samples were simultaneously withdrawn from the femoral artery at 4.12 mL/min for a total of 130 seconds with a withdrawal pump (model 944, Harvard Apparatus). At the end of the experiment, the cerebral hemisphere was removed and divided into small pieces weighing 0.5 to 1 g. The tissue and blood reference samples were counted with a gamma-well scintillation counter with a multichannel analyzer (model 1282, LKB Wallac), and corrections were made for activity spilling from one energy window to another with custom-designed software. Flow per gram of tissue sample was calculated from the equation $Q = (C_t \times Q_r) / C_r$, where $Q$ is blood flow to cerebral sample (mL/min), $C_t$ is tissue counts (per gram), $Q_r$ is the rate of withdrawal of the arterial reference sample (mL/min), and $C_r$ is arterial reference sample counts (per gram).

CBF to the brain was calculated as the quotient of the summed flows to the individual pieces and their combined weight.

Experimental Protocol

At baseline, hemodynamic parameters and P_ETCO2 were measured, and arterial blood was sampled for blood gas analysis (model 288, Ciba-Corning). CEU was performed, and radiolabeled microspheres were injected. These measurements were repeated after CBF had been altered by adjustment of the respiratory rate and tidal volume to produce either hypercapnia (P_TiCO2 >60 mm Hg) or hypocapnia (P_TiCO2 <30 mm Hg) in random order. At least 15 minutes was allowed after each ventilatory adjustment for the establishment of steady-state PaCO2. O2 saturation was maintained by administration of 100% O2. At the conclusion of the experiment, the dog was euthanized with an overdose of sodium pentobarbital and KCl, and the brain was excised for radiolabeled microsphere–derived CBF analysis.

Statistical Methods

Data are expressed as mean±SD. Comparisons between stages were made by repeated-measures ANOVA. Correlation was performed by
use of linear regression analysis. Differences were considered significant at a value of $P<0.05$ (2-sided).

**Results**

**Blood Gas and Hemodynamic Data**
The hemodynamic, arterial blood gas, and ventilatory PCO$_2$ data during different stages are shown in Table 1. Heart rate did not change between stages, and mean arterial pressure declined slightly, but not significantly, during hypoventilation. At baseline, PETCO$_2$ was maintained within 35 to 45 mm Hg. It increased and decreased significantly with hypoventilation and hyperventilation, respectively. Accordingly, arterial pH also changed significantly with hypocapnia and hypercapnia, whereas systemic O$_2$ saturation remained unchanged.

**CBF Measurements by Use of CEU**
Figure 3 illustrates 3 different time-AI plots obtained during baseline as well as hypoventilation and hyperventilation from a single dog. A, representing MBV, was highest during hypoventilation and lowest during hyperventilation. Similarly, $\beta$, representing RBC, was also highest during hypoven-
tilation and lowest during hyperventilation. In this example, both A and $\beta$ changed by the same degree. The fluctuations in AI seen in each plot were not associated with either the cardiac or respiratory cycles, and there was no associated motion of the cranium or the ultrasound transducer.

CEU-derived parameters from all 9 dogs and the corresponding radiolabeled microsphere–derived CBF values are listed in Table 2. Compared with baseline, hypercapnia produced a significant increase and hypocapnia caused a significant decrease in CBF. CEU-derived A$\times$B showed similar trends. Whereas alterations in CEU-derived CBF were attributable to changes in both A (MBV) and $\beta$ (RBC), the change in A was almost twice as great as that in $\beta$ ($P<0.01$), indicating that during these conditions, capillary recruitment or derecruitment played a greater role in CBF alterations than did changes in RBC. Figure 4 shows an excellent linear relation between radiolabeled microsphere–derived CBF and CEU-derived A$\times$B values.

**Discussion**
The new information from this study is that CEU can be used to measure regional cerebral perfusion in real time over a

| TABLE 1. Hemodynamic and Arterial Blood Gas Results |
|-----------------------------------|----------------|----------------|----------------|---|
|                                    | Baseline       | Hyperventilation| Hypoventilation| $P$ |
| Heart rate, bpm                    | 106±14         | 107±12          | 112±25         | 0.34 |
| Mean arterial pressure, mm Hg      | 87±11          | 85±9            | 77±16          | 0.23 |
| Minute ventilation, L/min          | 5.4±1.3        | 9.5±1.9         | 1.6±1.0        | <0.001 |
| PETCO$_2$, mm Hg                   | 36.9±2.6       | 24.7±2.9        | 76.3±13.2      | <0.001 |
| Paco$_2$, mm Hg                    | 37.0±4.0       | 26.0±3.4        | 79.2±12.7      | <0.001 |
| pH                                | 7.337±0.037    | 7.467±0.057     | 7.075±0.063    | <0.001 |
| HCO$_3$, mmol/L                    | 20.3±2.5       | 20.1±4.2        | 23.3±2.8       | 0.09 |
| Paco$_2$, mm Hg                    | 456.4±20.8     | 469.4±56.2      | 430.5±40.3     | 0.42 |
| % O$_2$ saturation                 | 99.7±0.3       | 99.8±0.3        | 99.6±0.3       | 0.77 |
A wide range of CBFs. The CEU-derived measurements were validated by use of radiolabeled microspheres. This study also shows the relative contributions of MBV and RBC, in CBF alterations during hypercapnia and hypocapnia. In addition, they also indicate spontaneously occurring alterations in MBV during all stages. Thus, with future technical developments, this technique has the potential to measure changes in CBF, MBV, and RBC.

Although it is important to know the total volumetric flow to an organ, it is equally important to have a measure of microvascular perfusion. CEU is unique in that it provides 2 measures of microvascular perfusion: MBV and RBC. CBF may change as a result of alterations in one or both of these parameters. For example, in this study, we found that during the wide range of CBFs induced by hypocapnia and hypercapnia, the change in MBV was almost twice that in RBC. Intravital microscopic studies have demonstrated that these manipulations can result in significant changes in MBV, whereas most other causes of changes in CBF result predominantly from changes in RBC.

MBV is composed of blood present in arterioles, venules, and capillaries. As in the heart, the majority of microvessels in the brain are capillaries (≈1000/mm³). Thus, any changes in MBV reflect changes in either capillary number (recruitment or derecruitment) or capillary diameter. This is particularly true if the larger vessels in the brain (which replenish much faster and can be seen soon after microbubble destruction compared with the capillaries (Figure 2A), which fill later) are excluded from the ROI.

Other noninvasive techniques, such as CT and MRI, measure blood in all the vessels in the brain. Therefore, they measure total cerebral blood volume (CBV) and not MBV. Total CBV can change without any alterations in MBV. Microbubbles also have a microvascular rheology similar to that of red blood cells and remain entirely within the vascular space. Thus, they reflect blood volume more accurately than CT and MRI contrast agents, which extravasate into the interstitial space.

CBF calculations with CT and MRI are performed by measuring the transit time of a tracer through the brain. This transit time is influenced by the total CBV. Thus, even if ROIs are placed over a specific location, this calculation provides as much an estimate of total organ flow as regional flow, especially if the macrovascular compartment is as large as or larger than the microvascular compartment. CEU, conversely, provides an accurate estimation of microvascular RBC and MBV. Microvascular RBC, and MBV may behave differently from global CBF and CBV under different physiological and pathological conditions. There is evidence that cerebral microvascular flow heterogeneities may become smaller or greater under different conditions, despite there being no change in total CBF. Thus, microvascular tracer kinetics may provide earlier evidence of regional perfusion abnormalities than global tracer kinetics.

An interesting observation was the spontaneously occurring fluctuations in MBV that were not related to either the respiratory or cardiac cycles. Intravital microscopic studies have shown that capillary perfusion of the brain is heterogeneous and that this heterogeneity is both spatial and tempo-

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**TABLE 2. Radiolabeled Microspheres and CEU Results**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hyperventilation</th>
<th>Hypoventilation</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CBF, mL·g⁻¹·min⁻¹</strong></td>
<td>0.93±0.27</td>
<td>0.67±0.26</td>
<td>2.01±0.78</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>A</strong></td>
<td>7.56±3.19</td>
<td>5.37±2.87</td>
<td>13.34±7.26</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>β</strong></td>
<td>0.63±0.26</td>
<td>0.45±0.20</td>
<td>0.79±0.30</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>A×β</strong></td>
<td>4.79±3.06</td>
<td>2.60±2.14</td>
<td>10.29±7.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Derived by use of radiolabeled microspheres.*
Furthermore, this heterogeneity is associated with changes in capillary volume rather than RBCs. Our results indicate that the real-time and high-resolution attributes of CEU can assist in measuring these changes, which may contain physiological and pathological information. Other techniques, such as MRI and CT, are unlikely to measure these changes, because they are probably not seen when total CBV is measured.

Previous reports of CEU for the assessment of cerebral perfusion have been mostly descriptive, without a sound theoretical basis underlying use of the CEU-derived parameters calculated to measure CBF. No independent validation (such as radiolabeled microspheres) was used. We believe that our study provides a strong theoretical as well as experimental basis for using CEU to measure regional cerebral perfusion. The methods used previously were also not real time, which is a recent technological development. Another advantage of the real-time method is that because a low MI is used, potential bioeffects seen at high MIs may be prevented. This is particularly true for an organ such as the brain, where safety is of paramount importance.

Limitations of the Study

The microbubble signal from tissue during their steady-state infusion reflects MBV. If this signal can be normalized to that from blood (such as within the left ventricular cavity), then MBV fraction can be calculated. If the tissue density, size of the ROI, and ultrasound beam width are known, then CBF can be calculated per gram of tissue. We did not perform such an analysis in this study.

Acoustic power is heterogeneous in the ultrasound field, being less along the sides and far field. In this situation, bubbles may not receive enough energy to oscillate, and the returning signal could be poor. Adjusting gain does not solve the problem. Thus, artifacts can occur that need to be recognized. Although these artifacts will affect the value of A, they should not affect β significantly.

Microbubbles themselves can cause far-field shadowing. Although this is a major issue during bolus injections, it is not so common for continuous infusions, for which the infusion rate can be titrated to achieve optimal opacification with minimal or no far-field shadowing. In the same vein, microbubble infusion rate or microbubble concentration can be increased when signal is poor, as might occur through an intact skull. Because the skull will attenuate ultrasound, the MI may also need to be increased to make the bubbles oscillate. One of the limitations of our study is that we did not image transcranially.

Although we measured AI within a small ROI within the cerebral hemisphere, we averaged radiolabeled microsphere–derived CBF from the entire cerebral hemisphere. Because our manipulations altered CBF to the entire brain and not to any one particular region, our results are unlikely to have been influenced by this. ROIs can be placed in different regions of the brain to derive regional CBF to the white and gray matter as well as other specific locations. This would have required validation with radiolabeled microsphere flow to these regions and proper registration between microsphere and CEU data. We have previously shown that we can measure the distribution of perfusion in the entire thickness of the heart muscle.

Finally, much work—technical development, feasibility studies, clinical validation—has to be performed before this method migrates to the clinical setting. The present study offers proof of principle and demonstrates the potential for CEU for measuring cerebral perfusion in real time. It could lead to the use of this technique for the bedside measurement and monitoring of cerebral perfusion in patients with craniotomies or burr holes.

Acknowledgments

This study was supported in part by a grant from the National Institutes of Health (3RO1-HL-48890), Bethesda, Md. Dr Leong-Poi is the recipient of a Fellowship training grant from the Canadian Heart Association and the Heart and Stroke Foundation of Canada, Ottawa. Dr Lindner is the recipient of a Mentored Clinical Investigator Award (K08-HL-03810) from the National Institutes of Health. Dupont Medical Products, Wilmington, Del, provided the radiolabeled microspheres; Nycomed Amersham, Amersham, UK, provided the microbubbles; and ATL-Phillips, Bothell, Wash, provided the ultrasound equipment.

References


