Ultrasound Microbubble Destruction Imaging in Acute Middle Cerebral Artery Stroke

Rolf Kern, MD; Fabienne Perren, MD; Katrin Schoeneberger; Achim Gass, MD; Michael Hennerici, MD; Stephen Meairs, MD

Background and Purpose—Cerebral perfusion imaging in acute stroke assists in determining the subtype and the severity of ischemia. Recent studies in perfusion models and in healthy volunteers have shown that ultrasound perfusion imaging based on microbubble destruction can be used to assess tissue perfusion. We applied ultrasound microbubble destruction imaging (MDI) to identify perfusion deficits in patients with acute middle cerebral artery (MCA) territory stroke.

Methods—Fifteen acute MCA stroke patients with sufficient transtemporal bone windows were investigated with ultrasound MDI and perfusion-weighted MRI (PWI). MDI was performed using power pulse-inversion contrast harmonic imaging. Thirty seconds after a bolus injection of the echo contrast agent SonoVue, microbubbles were destroyed using a series of high-energy pulses. Local perfusion status was analyzed in selected regions of interest by destruction curves and acoustic intensity differences (\(\Delta I\)) before and after microbubble destruction. Local perfusion status was then compared with perfusion compromise as identified on PWI.

Results—The mean differences of acoustic intensity from the ischemic MCA territory were significantly diminished compared with the normal hemisphere (\(\Delta I=2.52\pm1.75\) versus \(\Delta I=13.79\pm7.31; P<0.001\)), resulting in lower slopes of microbubble destruction. PWI confirmed perfusion changes in the selected anatomical regions on time-to-peak maps in all 15 patients.

Conclusions—MDI is a qualitative method that can rapidly detect perfusion changes in acute stroke. When combined with other ultrasound techniques and PWI, it may well be valuable in the care of stroke unit patients, eg, as a screening method and for follow-up assessments of perfusion deficits. (Stroke. 2004;35:1665-1670.)

Key Words: hemodynamics | magnetic resonance imaging | microbubbles | stroke, acute | ultrasonography | ultrasonography, Doppler, transcranial

There has been growing interest in using cerebral perfusion imaging in acute stroke because it is capable of detecting ischemic lesions earlier than computed tomography (CT) and can assist in determining the stroke subtype and the severity of cerebral ischemia. Moreover, perfusion imaging may be useful for predicting stroke recovery, for differentiating stroke pathogenesis, and for monitoring therapy. A number of techniques are available for cerebral perfusion imaging. These include \([^{99m}Tc]\)hexamethylpropyleneamine oxime–single-photon emission computed tomography (Tc-HMPAO-SPECT), positron-emission tomography (PET), Xenon-CT, perfusion CT, and perfusion-weighted MRI (PWI). Not all of these methods are available for the acute setting; some methods may require radioactive tracers, whereas others are expensive and time-consuming. Most of these methods are intolerable for critically ill or restless patients. There is clearly a need for a noninvasive and easily accessible method to perform monitoring of brain perfusion in stroke patients.

Several studies have demonstrated the value of ultrasound perfusion imaging (UPI) for depiction of blood flow in the microcirculation of the brain. Furthermore, there is mounting evidence that echo contrast harmonic imaging can be used to visualize perfusion deficits in patients with acute stroke. In contrast to both the progress in the technical development of and the rapidly increasing experience in the field of UPI, general guidelines for numerous variables are still not established, including the selection of hardware, echo contrast agent, dosage, application mode (eg, bolus injection versus constant infusion), and data analysis. In particular, transcranial UPI procedures requiring optimal insonation conditions, constant microbubble concentrations, or long acquisition times are demanding or even impossible for restless patients. Therefore, to be widely applicable in acute stroke patients, the UPI procedure should be capable of delivering useful perfusion information very rapidly, and in a simple and efficient manner. We hypothesize that an ap-
TABLE 1. Overview of Technical Modalities for Ultrasound Perfusion Imaging (UPI)

<table>
<thead>
<tr>
<th>Modality</th>
<th>Description</th>
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<tbody>
<tr>
<td>CHI</td>
<td>Contrast harmonic imaging, standard technique for UPI</td>
</tr>
<tr>
<td>PICHI</td>
<td>Pulse-inversion contrast harmonic imaging, technological enhancement for UPI</td>
</tr>
<tr>
<td>PPICHI</td>
<td>Power pulse-inversion contrast harmonic imaging, novel UPI technique combining PICHI with motion suppression capabilities of PDI</td>
</tr>
<tr>
<td>PDI</td>
<td>Power Doppler imaging, amplitude-based, flow-sensitive, color-coding technique for duplex ultrasound</td>
</tr>
<tr>
<td>MDI</td>
<td>Microbubble destruction imaging, UPI procedure using high acoustic energy for rapid echo-contrast microbubble destruction</td>
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</table>

Technical Aspects of UPI

Considerable progress has been made in developing ultrasound modalities for the visualization of tissue perfusion with echo contrast agents. Likewise, efforts have been undertaken to produce echo contrast agents with high microbubble stability, providing improved signal-to-noise ratio and increased examination times. Table 1 gives an overview of the imaging modalities for UPI, which are explained below.

Contrast harmonic imaging (CHI) is a contrast-specific technique that allows differentiation of echo contrast agent microbubbles in the vascular compartment from the surrounding tissue. Nonlinear oscillations of microbubbles can produce ultrasound signals at harmonic frequencies. Because the harmonic components from microbubbles are much larger than those scattered from biological tissue, the contrast between microbubbles and tissue is improved. CHI uses transducers with broad bandwidths to transmit ultrasound at one fundamental frequency and receive signals at twice that frequency. With bandpass filters, the fundamental frequency can be separated from the received second harmonic signal. The signal-to-noise ratio becomes strongly enhanced at the presence of microbubble harmonics in vascular beds.

Pulse-inversion contrast harmonic imaging (PICHI) is a technological enhancement for UPI that improves image quality by preserving axial resolution and avoiding harmonic frequency overlaps. PICHI works by sending out 2 successive ultrasound pulses of the same frequency, waveform, and focusing, but with opposite polarities to cancel the effect of transmitted second harmonics on the received signal.

Power Doppler imaging (PDI) is an ultrasound technique that generates color signals from the reflected echo amplitude, depending mainly on the density of intravascular red blood cells. It is commonly used for color-coded duplex ultrasound of larger vessels, eg, the carotid artery.

PICHI and PDI have recently been combined to result in a further contrast-specific application called power pulse-inversion contrast harmonic imaging (PPICHI). PPICHI uses additional pulses of alternating polarity; therefore, more pulses can be used to improve separation between tissue and contrast. This imaging modality allows simultaneous visualization both of tissue vascular space in a colorized, contrast-specific, harmonic mode, and of background tissue in the fundamental mode for orientation and navigation. Additional motion discrimination capabilities at high frame rates are an important gain for real-time measurements. Both low-energy nondestructive UPI and high-energy MDI are feasible.

Ultrasound Studies

Transcranial ultrasound was performed using a Philips HDI 5000 platform with a 2- to 4-MHz sector transducer (Philips Medical Systems). Before UPI, standard transcranial color-coded duplex sonography (TCCD) of both MCAs was performed in order to exhibit sufficient transtemporal bone windows and to demonstrate persistent vascular obstruction. In addition to TCCD, baseline transcranial PPICHI was performed bilaterally before contrast agent application to ensure sufficient and symmetrical insonation conditions. Good visualization of standard landmarks, such as mesencephalon and the contralateral skull, was required for inclusion in this study.

Because of its optimal technical properties, we used PPICHI as an imaging modality for UPI. Hardware settings were as follows: pulse repetition frequency was set at 4000 Hz; wall filter, low; dynamic range, low; frame rate, 14 Hz. The color gain settings were adjusted before injection of the echo contrast agent to avoid background noise and were kept unchanged during measurements of both hemispheres. From the transtemporal approach, ultrasound images were adjusted to a standardized axial plane ~20° tilt from the mesencephalic plane visualizing basal ganglia and subcortical white matter structures of the MCA territory. The anatomical orientation of the ultrasound scan is illustrated in Figure 1 in a T1-weighted MRI image performed with a corresponding tilt angle.

We defined an MDI procedure for the qualitative assessment of cerebral perfusion in both MCA territories after a bolus injection of 2.5 mL of an echo contrast agent containing sulfur hexafluorane gas microbubbles infolded in a phospholipid shell (SonoVue, Bracco). Thirty seconds after injection of SonoVue, nondestructive ultrasound imaging was applied using a low mechanical index (MI). Then, ultrasound emission power was paused for 10 seconds to ensure microbubble replenishment in the beam elevation. When restarting ultrasound emission, 20 repetitive pulses with the maximum MI of 1.2 were used, resulting in full and rapid parenchymal microbubble destruction. These 20 consecutive frames were digitally stored. The experiment was repeated for the contralateral hemisphere with identical hardware settings and contrast agent concentrations.

Analysis of microbubble destruction curves obtained from regions of interest (ROI) was performed with the HDI Laboratory software version 1.91d (Philips Medical Systems). ROIs were drawn over the cerebral hemisphere on the selected scanning plane with a mean area of 1233 mm². Under avoidance of larger vessels, ROIs were adapted to include parts of the insular cortex, basal ganglia with caudate and lentiform nuclei, internal capsule, and subcortical white matter structures of the MCA territory. Ultrasound backscatter was measured within the selected ROI in all frames of the imaging sequence using linear acoustic intensity units (AIU), ie, measurements of acoustic intensity before log compression and postprocessing. For comparison of data, mean AIU were converted into a decibel (dB) scale. The decay of mean acoustic intensity expressed in dB then served as a parameter reflecting the amount of microbubble destruction. The difference of acoustic intensity (ΔI) was calculated accord-
ing to the formula $\Delta I = I(t_0) - I(t_{baseline})$ where $I(t_0)$ represents the intensity in the first frame and $I(t_{baseline})$ represents the intensity at baseline.

**MRI Studies**

MRI was performed on a 1.5 Tesla MR System (Magnetom Vision, Siemens Medical Systems) with echo planar (EP) hardware (gradient power 25 mT/m, rise time 83 ms/85 ms; field of view [FOV], 180×240 mm²; matrix size, 192×256).

2. Proton density-weighted and T₂-weighted images (Turbo Spin Echo; 2620 ms/14 ms/85 ms; field of view [FOV], 180×240 mm²; matrix size, 192×256).

3. Diffusion-weighted EP-images (repetition time [TR] 4000 ms/echo time [TE] 100 ms; b=0/600/40/1000 s/mm²; FOV, 240×240 mm²; matrix size, 96×128; sequential application of 3 separate diffusion-sensitizing gradients in perpendicular directions).

4. Three-dimensional (3D) time-of-flight (TOF) magnetic resonance angiography (MRA) sequence of the intracranial vasculature (circle of Willis) (flip angle, 20°; FOV, 180×240 mm²; matrix size, 165×512; slice thickness, 2 mm).

5. Perfusion-weighted free induction decay echo-planar sequence after the first pass of a contrast bolus through the brain (2000/65/flip angle, 90°; 11 slices; 40 acquisitions; FOV, 240×240 mm²; matrix size, 128×128). Contrast agent was injected manually through a large gauge venous cannula in the antecubital vein.

In all patients, PWI was obtained as the reference method for UPI. Time-to-peak (TTP) images demonstrating the delay of the contrast agent bolus arrival in the brain parenchyma were used to obtain information about hemodynamic alterations. TTP map lesions were identified visually as parenchymal areas of increased signal intensity. MCA infarct size was graded visually from lesion size on diffusion-weighted MRI (DWI) as 1/3, 1/3, or 2/3 of the MCA territory. The local extent of hemodynamic alteration was graded on PWI as 50% of the MCA territory (complete) or 50% (partial).

**Patient Selection**

Patients treated in our Stroke Unit were included if they met the following criteria: acute stroke in the MCA territory demonstrated by DWI, evidence of persistent MCA obstruction (M1 or M2 segment) as identified by TCCD and confirmed by MRA, and sufficient bilateral transtemporal bone windows. The initial National Institute of Health Stroke Scale (NIHSS) score and the assumed stroke etiology was documented in all patients. UPI and PWI studies had to be performed within 24 hours after stroke onset with a maximum delay between UPI and PWI of 6 hours. Recanalization of the MCA detected in TCCD or MRA was considered exclusion criteria. Furthermore, patients with signs of acute bivemispheric or infratentorial stroke, intracranial hemorrhage, coexistent high-grade stenosis or occlusion of the extracranial or intracranial internal carotid arteries, dissection of the extracranial vasculature, known chronic MCA stenosis or occlusion, chronic supratentorial lesions identified in the T₂-weighted MRI, severe cardiac or pulmonary disease, patients with known hypersensitivity to echo contrast agents, and women of childbearing age were excluded. Informed consent was
obtained from all patients to participate in the study. The study was approved by the local ethics committee.

Statistical Analysis
Mean $\Delta I$, $I(t_0)$, and $I(t_{baseline})$ values were compared between ischemic and normal MCA territories using a $t$ test for independent variables. Statistical analysis was performed with SPSS for Windows (version 11.5). A value of $P<0.05$ was considered statistically significant.

Results
Fifteen acute MCA stroke patients (7 women, 8 men; mean age 60.1 years, range 26 to 79 years) with a mean initial NIHSS score of 10 were included in the study. None of the patients experienced side effects from UPI or PWI examinations. Table 2 illustrates the demographic, clinical, and neuroradiological characteristics of the study population. In 9 patients, the right MCA territory was affected; the left side in 6 patients. Four patients (cases 1, 3, 7, and 9) experienced large infarcts of $>2/3$ of the MCA territory; the M2 segment was affected in 7 cases. PWI lesion size was found persistent obstruction of the M1 segment; the M2 territory compared with the normal side ($\Delta I=2.52 \pm 1.75$ versus $\Delta I=13.79 \pm 7.31$; $P<0.001$). In all patients, the MCA territory with lower $\Delta I$ values corresponded to the MCA territory showing signs of compromised perfusion in PWI. The acoustic intensities before and after microbubble destruction $I(t_0)$ and $I(t_{baseline})$ were also significantly lower in the ischemic MCA territory, all resulting in lower slopes of the microbubble destruction curves than in those of the contralateral side. Figure 3 shows an example of a patient (case 10) with right MCA territory stroke with typical behavior of acoustic intensity related to microbubble destruction.

TABLE 3. Results of MDI: Acoustic Intensities from Ischemic and Normal MCA Territory

<table>
<thead>
<tr>
<th>Patient</th>
<th>Ischemic MCA Territory</th>
<th>Normal MCA Territory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$I(t_0)$</td>
<td>$I(t_{baseline})$</td>
</tr>
<tr>
<td>1</td>
<td>4.47</td>
<td>2.77</td>
</tr>
<tr>
<td>2</td>
<td>4.37</td>
<td>2.57</td>
</tr>
<tr>
<td>3</td>
<td>5.79</td>
<td>4.21</td>
</tr>
<tr>
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<td>7.03</td>
<td>4.62</td>
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<td>5</td>
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<td>3.89</td>
</tr>
<tr>
<td>6</td>
<td>8.54</td>
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<td>7</td>
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<tr>
<td>8</td>
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<tr>
<td>10</td>
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<td>3.63</td>
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<tr>
<td>12</td>
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<td>2.75</td>
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<tr>
<td>13</td>
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<td>1.77</td>
</tr>
<tr>
<td>14</td>
<td>8.64</td>
<td>7.72</td>
</tr>
<tr>
<td>15</td>
<td>9.44</td>
<td>3.7</td>
</tr>
<tr>
<td>Mean</td>
<td>6.37</td>
<td>3.85</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>1.96</td>
<td>1.61</td>
</tr>
</tbody>
</table>

All measurements are in decibels (dB). MDI indicates microbubble destruction imaging; dB, decibel; MCA, middle cerebral artery.
All intensity values, however, showed a high interindividual variation both in normal and ischemic parenchyma. This may be because of the differences of acoustic attenuation from the human skull. Furthermore, the acoustic intensities of MCA territories with partial lesion size in PWI were not significantly different from those with complete lesion ($\Delta I = 2.79 \pm 2.01$ versus $\Delta I = 2.22 \pm 1.44$; $P = 0.55$). Likewise, differences of $\Delta I$ values in patients with M1 and M2 obstructions were not statistically significant ($\Delta I = 2.10 \pm 1.38$ versus $\Delta I = 3.01 \pm 2.09$; $P = 0.33$).

Discussion

In this study we examined whether the detection of microbubble destruction in the brain could provide useful information on cerebral tissue perfusion in MCA stroke. We hypothesized that the absence or reduction of such contrast agent destruction may serve as a parameter for identification of low perfusion states. Pohl et al were the first to propose that the phenomenon of microbubble destruction could be used to characterize cerebral perfusion in the human brain. They used color Doppler imaging with a high transmitting power first to destroy contrast agent bubbles, then to depict a transient high-amplitude broadband response caused by the microbubble destruction. Because ultrasound Doppler systems correlate the signals backscattered from a target within a number of successive pulses, the loss of signal correlation caused by the transient bubble collapse is interpreted by the machine as a random Doppler shift, resulting in a mosaic of colors at the location of the microbubbles. Pohl et al postulated that the color Doppler signals resulting from stimulated acoustic emission (SAE) may represent cerebral tissue perfusion. Indeed, further in vitro experiments demonstrated that the intensity of SAE signals correlated with the concentration of microbubbles. However, as the SAE technique is based on complex artifacts that arise from color or power Doppler systems in response to bubble destruction and are also very sensitive to motion artifacts, there has been limited clinical utility of this technique in patients with cerebrovascular disease.

Advanced contrast-specific harmonic imaging modalities for visualization of tissue perfusion now allow direct visualization and quantitative assessment of microbubble destruction. Meyer and Seidel have demonstrated that a series of high-energy pulses applied to human cerebral tissue that use different contrast agent infusion rates results in characteristic exponential decreases in acoustic intensity levels in different regions of the brain. Likewise, Eyding et al have examined the value of destruction imaging for characterization of brain perfusion. Assuming a constant microbubble concentration at a specific time after bolus application, they used a high acoustic energy (mechanical index 1.8) at a low frame rate (1 Hz) to destroy microbubbles through the transcranial bone window in healthy volunteers. Their approach allowed calculation of a perfusion coefficient, which provides a semi-quantitative parameter for blood flow velocity and shows little interindividual variation in the basal ganglia, thalamus, and subcortical white matter.

In this study we investigated the value of MDI using transcranial PPICHI after bolus injection of SonoVue to assess cerebral perfusion in a selected population with MCA stroke. We chose a rapid, real-time pulse sequence with high acoustic energy that destroys all microbubbles within a field of view in < 0.5 seconds. This ultrafast method fosters the use of a contrast-specific imaging modality for emergency evaluation of stroke, because it can be used even in restless patients. Our results demonstrate that perfusion deficits are reliably detectable with MDI. All patients with MCA infarction show markedly reduced microbubble destruction, as compared with the nonaffected MCA territory. These findings correlate well to those of PWI that demonstrate a delayed contrast bolus arrival in corresponding brain regions. However, MDI only provides qualitative information on tissue perfusion with interindividual variation. An additional limitation is that MDI can only be performed in patients with sufficient temporal bone windows.

Unexpectedly, our results show that after complete microbubble destruction, baseline values of acoustic intensity are lower in hypoperfused brain parenchyma. Although the underlying physical principles of such intensity differences are unclear, we suspect that they may be caused by differences in tissue harmonics of the infarcted brain. Further systematic studies are necessary to validate this finding.

MDI is a fast and simple technique that can be of use for a quick reference of brain perfusion in MCA stroke. When combined with MRI and other ultrasound techniques, it may well be valuable in the care of stroke unit patients, eg, as a
screening method and for follow-up assessments of perfusion deficits. Although based on different principles, perfusion abnormalities are detectable with both MDI and PWI and may provide complementary information in brain ischemia. Because of its short acquisition time as well as its reliance on destruction of microbubbles in a specified brain region, this technique provides an ideal framework for examination of multiple planes, which has not been feasible in previous approaches to ultrasound brain perfusion imaging. This extension may enable analysis of different areas of cerebral tissue with variable grades of hemodynamic impairment, thus providing progress toward differentiation between the ischemic core and tissue at risk.

Because PPICH provides both low-energy nondestructive UPI and high-energy MDI, another promising future application may be the combination of our technique with that of real-time microbubble replenishment kinetics to enable quantification of regional cerebral blood flow. Rim et al have applied real-time replenishment kinetics to study brain perfusion in dogs. They measured in well-defined regions of interest ipsilateral to a craniotomy compared well with those of global cerebral blood flow obtained using radioactive microspheres. As they noted, however, considerable work is necessary before this method migrates to the clinical setting.

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References
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