Second Harmonic Imaging of the Human Brain
The Practicability of Coronal Insonation Planes and Alternative Perfusion Parameters

Judith U. Harrer, MD; Christof Klötzsch, MD

Background and Purpose—Second harmonic imaging (SHI) is a novel ultrasound technique that allows the evaluation of brain tissue perfusion. The purpose of this study was to assess normal cerebral echo contrast characteristics in 3 regions of interest (ROIs) in the transverse axial and coronal insonation planes through the temporal bone window.

Materials and Methods—SHI examinations were performed in 25 patients without cerebrovascular disease (aged 50±19 years) in a transverse axial and a coronal diencephalic insonation plane through the temporal bone window. After intravenous administration of 2.5 g (400 mg/mL) of a galactose-based echo contrast agent, 62 time-triggered images with a transmission rate of 1 frame per 2.5 seconds were recorded for offline analysis. Time-intensity curves, including peak intensity (PI) (dB) and positive gradient (PG) (dB/s), were calculated to quantify ultrasound intensity in 3 different ROIs in both planes of the following sections: the thalamus (ROI_thal), the lentiform nucleus (ROI_ncl), and the area supplied by the middle cerebral artery (ROI_mca).

Results—Characteristic time-intensity curves with high PIs and steep PGs were recorded in each ROI. Statistical analysis of the aforementioned parameters showed no significant difference for comparison of the 3 ROIs in the transverse axial versus the coronal insonation plane. Comparison of different ROIs in the transverse axial insonation plane revealed that PI was significantly higher in ROI_thal than in ROI_mca (7.8 versus 5.5 dB; P<0.05) and significantly higher in ROI_ncl than in ROI_thal (9.3 versus 7.8 dB; P<0.05). In contrast, PG was comparable in ROI_thal and in ROI_mca (0.21 versus 0.25 dB/s; P=0.42).

Conclusions—SHI is a promising technique for the evaluation of cerebral parenchymal perfusion. Comparison of the transverse axial and coronal insonation planes shows similar time-intensity curves with comparable values for PIs and PGs. Coronal insonation allows the evaluation of perfusion abnormalities near the vertex and skull base, areas that cannot be depicted in the transverse axial plane. Comparison of the different ROIs indicates that the PG is a more robust and reliable parameter than the PI. (Stroke. 2002;33:1530-1535.)

Key Words: brain ■ contrast media ■ image interpretation ■ perfusion ■ ultrasonography

Until recently, perfusion imaging of the brain has been an unapproachable diagnostic field for transcranial ultrasound. Validated methods for perfusion studies of the brain are perfusion MRI, positron emission tomography, and 99mTc–hexamethylpropyleneamine oxime and 99mTc–ethylcysteinate dimer single-photon emission CT.1–10 The disadvantages of these techniques are that they either require the application of radioactive substances, are stressful or even intolerable for the critically ill patient, and are time-consuming and expensive. Therefore a noninvasive, bedside, nonstressful, easily applied, and frequently repeatable method to assess brain perfusion is needed.

Second harmonic imaging (SHI) is a novel ultrasound technique that enables visualization and measurement of tissue perfusion. SHI is based on the nonlinear properties of ultrasound contrast agents.11 While insonated tissue responds primarily at the transmitted or so-called fundamental frequency, the microbubbles of the contrast agents respond at the fundamental frequency and multiples of this frequency, the so-called harmonic frequencies. SHI is named after the first of these multiples because this is usually the strongest. By the use of a filter, the SHI system insonates at 1 frequency, the fundamental frequency, and receives only at twice that frequency, the second harmonic frequency. Thus, the unwanted fundamental frequency is removed, and the signal-to-clutter ratio is dramatically increased.12–15 As a result, microbubbles in the capillary bed can be distinguished from the avascular tissue, and capillary blood flow becomes visible and measurable by the calculation of time-intensity curves in user-specified regions of interest (ROIs).

Previous studies of myocardial, hepatic, and renal perfusion with the use of SHI have shown the high potential of this...
technique to reliably evaluate normal and abnormal perfusion.\textsuperscript{16–22} SHI can be used as an additional diagnostic tool for the differential diagnosis of perfusion disturbances, such as in acute myocardial infarction, or of space-occupying lesions of the liver.\textsuperscript{23–25}

Neurosonological SHI studies by Federlein, Postert, and Seidel et al\textsuperscript{26–32} have already shown the ability of SHI to evaluate human brain perfusion in healthy volunteers as well as in acute ischemic stroke.

The purpose of our study was to assess human brain perfusion with the use of SHI in patients without cerebrovascular diseases. In comparison to previous studies, 2 insonation planes, the transverse axial and the coronal planes, were investigated. This approach was adopted because certain parts of the brain, such as the temporoparietal region and regions near the skull, can only be visualized by the coronal insonation plane and moreover because these 2 planes are both regularly used in other imaging techniques. Furthermore, we evaluated a supplementary, user-defined ROI, the middle cerebral artery territory, because this area is the most frequently affected in stroke. Finally, we analyzed an additional parameter of the time-intensity curve, the positive gradient (PG), which is the peak intensity (PI) divided by the time to peak intensity (TP), because the PI is known from previous studies to be highly variable intrindividually and interindividually and therefore is an unreliable parameter for the evaluation of brain tissue perfusion.\textsuperscript{26–29}

Subjects and Methods

Patients

The study included 25 patients (mean age, 50 years; range, 26 to 79 years; 17 men, 8 women). For inclusion, patients needed to have an adequate acoustic bone window enabling the depiction of the brain stem and third ventricle in ordinary B-mode imaging. Exclusion criteria were as follows: cerebrovascular disease (excluded by extracranial and transcranial Doppler studies); uncontrolled hypertension; repetitive systolic blood pressure of $\geq$140 mm Hg or diastolic blood pressure of $\geq$85 mm Hg during admission or previously known; severe heart failure (New York Heart Association grade III to IV); galactosemia; pregnancy or lactation; history of alcohol or drug abuse; and previous allergic reactions. All patients had a complete physical and neurological examination, routine blood tests, and a 12-lead ECG. Informed consent to ultrasound examination was given by all subjects.

Technical Equipment and Ultrasound Contrast Agent

The technical equipment consisted of the Hewlett Packard Sonos 5500 duplex device, which was connected to a 1.8/3.6-MHz sector transducer (S3 probe, Hewlett Packard) capable of fundamental and harmonic imaging. All harmonic imaging studies were stored on a magneto-optical disc and analyzed offline. For harmonic imaging, 2.5 g of a galactose-based ultrasound contrast agent (Levovist, Schering AG) was used in a concentration of 400 mg/mL. The ultrasound contrast agent was applied as a bolus by an infusion pump (Pulsar, Medrad) at a rate of 2.5 mL/s into the antecubital vein, which was cannulated with an 18-gauge catheter. The second injection for investigation of the coronal plane was given after a 30-minute interval.

Harmonic Imaging Studies

Each examination was started in B-mode with the use of the transaxial approach. After depiction of the hypointense butterfly-shaped brain stem, the probe was shifted 10 degrees upward in transverse axial examination or 10 degrees forward in coronal examination to depict the diencephalic plane of section, with the third ventricle in the middle and the thalami on its sides. These anatomic structures are easily recognized because of their different echogenities: the lumen of the third ventricle is very hypointense, whereas its margins are clearly hyperintense, and the flanking elliptical thalami are hypointense again. To rule out the inclusion of larger vessels in the investigated plane, an angio mode image was obtained, and the investigated plane was adjusted, if needed, before the quickset was switched to the acoustic quantification mode. This mode provides an integrated online capability to measure the average acoustic image intensity within a user-specified ROI and is based on the integrated backscatter technology. Integrated backscatter is a relative measure of the total ultrasonic energy backscattered by a small volume of the interrogated tissue. To enable offline generation of time-intensity curves, the acoustic densitometry time-intensity study type was used. Harmonic imaging studies were performed at a low insonation depth of 10 cm to increase spatial resolution. Depth gain compensation was optimized before the start of the harmonic examination. In each examination 62 time-triggered images were recorded at a rate of 1 frame every 2.5 seconds, so that the whole examination lasted roughly 2.5 minutes. The ultrasound contrast agent was applied after 3 baseline images.

For offline analysis of the data, the acoustic densitometry unit was used to quantify contrast intensity of the integrated backscatter, calculating time-intensity curves in 3 different ROIs. Analyzed ROIs were the ipsilateral thalamus (ROI\textsubscript{th})\textsuperscript{1} the lentiform nucleus (ROI\textsubscript{LN})\textsuperscript{2} (circular ROIs with a sample area of 21×21 pixels, which have been established in previous studies by Postert et al\textsuperscript{26} and Seidel et al\textsuperscript{27,29}); and the middle cerebral artery territory (ROI\textsubscript{MCA}, a user-specified ROI that includes the whole brain region that is supplied by this artery. Figures 1 and 2 give an overview of the ROIs in both insonation planes. For a better anatomic overview, these images are in B-mode and at a depth of 14 cm.

Using a predefined ROI that includes the whole brain region that is supplied by this artery. Figures 1 and 2 give an overview of the ROIs in both insonation planes. For a better anatomic overview, these images are in B-mode and at a depth of 14 cm.

$PI$ (decibels) and $PG$ (decibels per second), which is the PI divided by the TP, were obtained from the calculated time-intensity curve and used for statistical analysis. To match the transverse axial and the coronal insonation planes, these parameters were compared between corresponding ROIs in the 2 insonation planes. To evaluate the reliability of PI and PG, both parameters were compared between the 3 different ROIs in the transverse axial plane. Baseline for each calculated time-intensity curve was the mean acoustic intensity of the first 3 images before ultrasound contrast agent application.

The Friedman test and the Wilcoxon signed rank test were used for statistical analysis. Differences were considered statistically significant at values of $P<0.05$.

Results

General

No adverse effects were observed after contrast medium administration. Anatomic structures could be identified easily in B mode. Signal intensity decreased in harmonic mode, but image quality was well preserved. However, in integrated backscatter mode, spatial resolution was usually lower. Strong contrast enhancement was detected, particularly in the basal ganglia and, to a lesser extent, in the thalami in all patients (Figures 3 and 4). In most patients the time-intensity curve had not reached the baseline at the end of the examination.

Transverse Axial Versus Coronal Scan

No significant differences in PI and PG were found when the insonation planes were compared. Both parameters were lower in the coronal plane. Mean PI in the 3 ROIs ranged from 5.5 to 9.3 dB in the transverse axial plane and from 4.5 to 8.6 dB in the coronal plane, while mean PG varied between 0.21 and 0.42 dB/s and between 0.15 and 0.47 dB/s, respec-
Quantitative data of mean PI and PG for both in-sonation planes are displayed in Table 1.

**Comparison Between Different ROIs in the Transverse Axial Plane**

There were considerable interindividual differences of PI and PG, as shown by the wide ranges (Table 1). Intraindividual PIs also varied to a large extent, but there was much less variation when PGs in the 3 different ROIs were compared. The mean PG was comparable in ROI_{thal} and ROI_{mca} ($P=0.42$), although it was significantly lower in ROI_{thal} than in ROI_{mca} ($P=0.001$). Detailed data of these parameters are displayed in Table 2. Figure 4 demonstrates the time-intensity curves of the 3 ROIs from a transverse axial examination.
While the PIs differ considerably, PGs clearly have approximately the same grade of inclination.

**Middle Cerebral Artery Territory**

The analysis of the newly introduced, comparatively large ROI demonstrated time-intensity curves comparable to those of the established ROIs, with smaller PIs (Tables 1 and 2). Placement of this ROI was simple and allowed fewer options for variation than the placement of the smaller ROIs.

**Discussion**

A number of recent studies have shown that the transcranial application of SHI enables relative quantification of normal brain perfusion as well as the disturbance of cerebral perfusion in acute ischemic stroke. Additional investigation of the coronal insonation plane, as performed in other methods for cerebral perfusion studies, is of particular importance because ultrasonic application on the transverse axial plane only enables partial depiction of the brain: planes from the base of the skull up to the lateral ventricles are well visualized, but any apical region near the top of the skull or the temporobasal region can only be depicted with the use of the coronal plane. We conclude that the coronal plane is suitable for use on a regular basis since the analysis of both insonation planes showed no significant differences between the PI and PG of corresponding ROIs. Moreover, perfusion studies in 2 planes may enable the quantitative analysis of the dimension of a perfusion disturbed area of the brain. SHI studies on myocardial perfusion in multiple views have shown that this technique can accurately identify regional myocardial perfusion abnormalities. Thus, for example, the size of an ischemic infarct might be predicted more precisely than if only 1 plane was assessed.

Analysis of time-intensity curves of the contrast enhancement after ultrasound contrast agent application showed wide interindividual and intraindividual variation in PIs, which depend on the frequency, insonation depth, and thickness of the temporal bone. We observed >10-fold differences in decibels between different patients as well as wide-ranging values in different ROIs of the same patient. Thus, the PI is not a sufficient parameter for either the absolute or the relative quantification of brain tissue perfusion. Previous studies have suggested the area under the curve of the time-intensity curve as the most robust parameter for the
In conclusion, this study demonstrates that SHI enables visualization and measurement of echo enhancement in perfused areas of the human brain. Additional application of the coronal insonation plane allows evaluation of brain regions that cannot be depicted in the transverse axial plane, such as the temporobasal region or the region near the skull. A second insonation plane also facilitates detection of focal perfusion abnormalities and more reliable prediction of size. In our opinion, in comparison to the PI and the area under the curve, the PG is a more reliable and the most robust parameter for relative evaluation of brain tissue perfusion. Examination of the newly introduced ROI

\[ \text{ROI}_{\text{mca}} \] may provide more reliable data of ROIs of a transverse axial examination. Each point along the horizontal axis represents 1 frame at a rate of 1 frame every 2.5 seconds. The vertical axis represents the relative intensity in decibels. thal indicates thalamus; mca, middle cerebral artery territory; and ncl, lentiform nucleus. The 3 curves show comparable PGs (dB/s) and washout curves, but the PI (dB) is considerably higher in the lentiform nucleus and middle cerebral artery territory than in the thalamus.

Figure 4. Example of the time-intensity curves of the 3 analyzed ROIs of a transverse axial examination. Each point along the horizontal axis represents 1 frame at a rate of 1 frame every 2.5 seconds. The vertical axis represents the relative intensity in decibels. thal indicates thalamus; mca, middle cerebral artery territory; and ncl, lentiform nucleus. The 3 curves show comparable PGs (dB/s) and washout curves, but the PI (dB) is considerably higher in the lentiform nucleus and middle cerebral artery territory than in the thalamus.

TABLE 1. Transverse Axial vs Coronal Insonation Plane: PI and PG

<table>
<thead>
<tr>
<th></th>
<th>Transverse Axial</th>
<th>Coronal</th>
<th>( P^* )</th>
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<tbody>
<tr>
<td>PI, dB (ROI_{thal})</td>
<td>7.8±3.9 (2.0–21.2)</td>
<td>5.9±3.2 (2.0–14.7)</td>
<td>NS</td>
</tr>
<tr>
<td>PG, dB/s (ROI_{thal})</td>
<td>0.21±0.16 (0.04–0.71)</td>
<td>0.17±0.15 (0.02–0.49)</td>
<td>NS</td>
</tr>
<tr>
<td>PI, dB (ROI_{ncl})</td>
<td>9.3±3.2 (4.2–17.1)</td>
<td>8.6±4.5 (3.2–21.6)</td>
<td>NS</td>
</tr>
<tr>
<td>PG, dB/s (ROI_{ncl})</td>
<td>0.42±0.44 (0.04–0.81)</td>
<td>0.47±0.92 (0.04–0.93)</td>
<td>NS</td>
</tr>
<tr>
<td>PI, dB (ROI_{mca})</td>
<td>5.5±2.3 (1.8–9.1)</td>
<td>4.5±2.7 (0.9–12.9)</td>
<td>NS</td>
</tr>
<tr>
<td>PG, dB/s (ROI_{mca})</td>
<td>0.25±0.19 (0.02–0.48)</td>
<td>0.15±0.12 (0.02–0.42)</td>
<td>NS</td>
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Values are mean±SD (range). *Wilcoxon signed rank test.

TABLE 2. Comparison of PI and PG of Different ROIs (Thalamus, Lentiform Nucleus, and Middle Cerebral Artery Territory) in the Transverse Axial Insonation Plane

<table>
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ment of ROI_{mca} is comparatively user dependent, and because of the absence of any striking echogenous structure, the position relies mainly on the instructions for the placement of this ROI. In contrast, the placement of ROI_{mca} is less variable not only because of its size but especially because its margins are well defined by the adjacent anatomic structures. We could show that analysis of the contrast enhancement in this ROI resulted in equivalent time-intensity curves compared with those of the established ROI_{thal}. In addition, we suggest that the examination of this newly introduced ROI possesses additional advantages. First, the enclosure of large vessels in the analyzed ROI has less impact than if this occurred during analysis of a small ROI, where an accidentally enclosed vessel segment could, in the worst case, constitute the whole ROI. Enclosure of a large vessel hinders the accurate analysis of parenchymal perfusion because the contrast enhancement of a large vessel appears earlier and has stronger contrast intensities than parenchymal contrast. Second, minor shifts of the probe and subsequently of the insonated plane, which are aggravated by the relatively long duration of the examination, have less influence on the contrast curve if a large ROI is assessed than if a small ROI is assessed, in which case a slight shift of the insonated plane might alter the entire ROI.

Another limitation of this new technique is the attenuation of the ultrasound beam, which depends on the insonation depth, the thickness of the temporal bone, and the second harmonic receiving frequency (3.6 MHz in our study). The variability of the temporal bone thickness provokes additional asymmetrical attenuation and may cause ambiguous absolute data. According to previous studies, we presume that in view of the depth dependency of the enhancing effect, adequate interpretation of SHI studies is only feasible ipsilateral to the examined side because analyses of contralateral ROIs result in misleading time-intensity curves that are not comparable to those of the ipsilateral side.77,29 An independent examination of each side is therefore recommended.
in comparison to other ROIs because its placement is less subjective and accidental inclusion of larger vessels or slight shifts of the insonated plane have less effect on the contrast curve than in a smaller ROI. This novel, noninvasive technique, which is easily applied and can be repeated as often as needed, is not only comparatively inexpensive but is also applicable to critically ill, bedridden patients. In any patient in whom focal cerebral perfusion abnormalities are suspected, SHI might provide additional as well as alternative information concerning alterations of brain tissue perfusion.

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References
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