Transient Response Harmonic Imaging
An Ultrasound Technique Related to Brain Perfusion

Thomas Postert, MD; Andrea Muhs, MD; Saskia Meves, MD; Jens Federlein, MD; Horst Przuntek, MD; Thomas Büttner, MD

Background and Purpose—Gray-scale harmonic imaging is the first method to visualize blood perfusion and capillary blood flow with ultrasound after intravenous contrast agent application. The purpose of the present study was to evaluate the potential of transient response second harmonic imaging (TRsHI) to assess normal echo contrast characteristics in different brain areas by transcranial ultrasound.

Methods—In 18 patients without cerebrovascular diseases, TRsHI examinations were performed bilaterally with the use of the transtemporal approach after application of 6.5 mL of a galactose-based microbubble suspension (400 mg/mL). The transmission rate was once every 4 cardiac cycles. Regional cerebral contrast was visually assessed and then quantified off-line with the use of time-intensity curves. In 4 different regions of interest (ROI) (posterior part of the thalamus [ROIa], anterior part of the thalamus [ROIb], lentiform nucleus [ROIc], and white matter [ROId]), the following parameters were evaluated: peak intensity, area under the curve (AUC), and time to peak intensity. AUC ratios for ROIc/a, d/a, c/b, and d/b were calculated.

Results—In all patients parenchymal contrast enhancement was visually detectable. One hundred thirty-one characteristic time-intensity curves (baseline phase, peak contrast intensity, slow washout phase) were demonstrable in 144 ROIs. In ROIc and ROId, characteristic contrast curves could be observed most frequently (68/72 examinations), whereas time-intensity curves in ROIa and ROIb could not be evaluated because of inadequate contrast enhancement in 9 of 72 examinations. Time to peak intensity varied between 20 and 52 cardiac cycles; in 1 patient it was 88 cardiac cycles. In all individuals AUCs and in 16 of 18 subjects peak intensity in ROIc and ROId showed a 2- to 10-fold increase compared with ROIa and ROIb. In no examination did AUC ratios show a 2-fold side difference irrespective of the ROI.

Conclusions—The present study demonstrates for the first time that TRsHI produces accurate contrast in different brain areas and represents an ultrasonic tool related to brain perfusion. Absolute values of quantitative parameters show high variations caused by different temporal bone thicknesses and a complex relationship between echo contrast concentrations and measurements of optic intensities. Ratios between different ROIs help to compare contrast enhancement in different brain areas. Furthermore, because of the fact that attenuation of contrast enhancement in TRsHI depends strictly on the insonation depth, harmonic imaging studies of brain perfusion cannot be compared directly with other imaging techniques such as positron emission tomography. (Stroke. 1998;29:1901-1907.)

Key Words: echocardiography imaging, harmonic imaging, transient response perfusion, brain ultrasonography, Doppler, transcranial

The clinical utility of ultrasound contrast agents is limited by the poor performance of currently available B-mode scanners at discriminating echogenic microbubbles in the blood pool from the surrounding echogenic tissue. Harmonic imaging (HI) is a new contrast-specific imaging modality that uses the nonlinear properties of ultrasound contrast agents by transmitting at the fundamental frequency and receiving at multiples of this frequency.3,4 The first multiple of the fundamental frequency is generally the strongest of all possible harmonic frequencies (second harmonic imaging [sHI]).3,4 The major advantage of this technique is due to the difference in backscattering of the tissue and contrast agent at 2 frequencies. The magnitude of the backscattered contrast-enhanced signal at the harmonic frequency is greater than that of tissue, which leads to a significant increase of the signal to noise ratio. The nonlinearity of the ultrasound contrast agent allows capillary blood flow to be separated from tissue echoes.5 In this way sHI may clearly enhance the ability of B-mode scanners to differentiate bubbles in the tissue vascular space from the relatively echogenic surrounding avascular tissue. Three preliminary studies have demonstrated the potential clinical value of this technique in the assessment of myocardial perfusion in humans5,6 and liver perfusion7 in animal models. Furthermore, a HI study in echocardiography
has demonstrated that visually detectable myocardial perfusion is especially accentuated when the time interval between triggered frame rates is extended to once every 4 to 10 cardiac cycles (transient response [TR] imaging). This modification of the frame rate prevents the destruction of microbubbles and has been shown to significantly increase peak intensity (PI) of perfused tissues. The present study evaluates whether transient response second harmonic imaging (TRsHI) allows demonstration of cerebral contrast enhancement in individuals without cerebrovascular diseases.

Subjects and Methods

Subjects

This study included 18 patients (mean age, 39 years; range, 22 to 56 years; 8 men, 10 women) without history or physical signs of cerebrovascular disease. The exclusion criterion was galactosemia. Informed consent was obtained from all individuals. Patients had the following diagnoses: tension type headache (n=5), polynuropathy (n=5), radicular pain (n=2), trigeminal neuralgia (n=2), psychogenic headache (n=2), myasthenia (n=1), and myopathy (n=1). Extracranial and intracranial color-coded and spectral Doppler examinations were normal in all individuals. In 10 patients CT or MRI was performed and revealed no abnormalities.

Transcranial Sonography

For all ultrasound examinations, a Hewlett Packard SONOS 5500 duplex device capable of fundamental imaging and TRsHI in connection with a 2.5-MHz, 90-degree sector transducer was used. We first performed conventional transcranial gray-scale and color-coded real-time sonography using the transtemporal approach according to previously published studies. A low insolation depth (10 cm) was selected to improve the spatial resolution of parenchymal structures of the ipsilateral hemisphere. The butterfly-shaped mesencephalic brain stem with surrounding hyperechogenic basal cisterns was visualized in axial untilted sections. For depiction of the third ventricle and the thalamus, the ultrasound probe was tilted ∼10 degrees toward the parietal lobe. This plane of section was kept constant during the entire TRsHI examination. The second harmonic system operated at 1.8-MHz transmit and at the second harmonic frequency of 3.6 MHz. The instrument setting was not changed during the entire TRsHI examination. The second harmonic contrast application (cardiac cycles).

All patients had adequate acoustic bone windows, enabling visualization of the third ventricle and the thalamus in transcranial real-time images. When the second harmonic mode was used the structural components of the B-mode image were preserved, albeit at a lower intensity. Thirty-six intravenous injections of galactose-based microbubbles were given; no side effects could be observed. Three characteristic phases of contrast enhancement could be visually observed in all subjects. After a baseline period of ≈10 cardiac cycles (phase 1), gray-scale intensity (particularly in ROIc and ROIc) increased to a maximum within a few cycles (phase 2), whereas anterior and posterior parts of the thalamus exhibited only a minor intensity increase. In the third phase, contrast enhancement slowly disappeared. This tidying effect was best observable in those regions with most prominent contrast enhancement during phase 2. An example for these characteristic phases is given in Figure 2.

Results

General

All patients had adequate acoustic bone windows, enabling visualization of the third ventricle and the thalamus in transcranial real-time images. When the second harmonic mode was used the structural components of the B-mode image were preserved, albeit at a lower intensity. Thirty-six intravenous injections of galactose-based microbubbles were given; no side effects could be observed. Three characteristic phases of contrast enhancement could be visually observed in all subjects. After a baseline period of ≈10 cardiac cycles (phase 1), gray-scale intensity (particularly in ROIc and ROIc) increased to a maximum within a few cycles (phase 2), whereas anterior and posterior parts of the thalamus exhibited only a minor intensity increase. In the third phase, contrast enhancement slowly disappeared. This tidying effect was best observable in those regions with most prominent contrast enhancement during phase 2. An example for these characteristic phases is given in Figure 2.
Quantitative Analysis of Time-Intensity Curves

Data of all quantitative parameters are summarized according to different ROIs in Tables 1 to 4. Figure 3 demonstrates typical time-intensity curves in the ROIs. Characteristic time-intensity contrast curves that allowed quantitative analysis were evident in 32 of 36 examinations of ROIc and ROId on the left side and in all examinations of ROIc and ROId on the right side. Four curves in ROIa (3 on the left, 1 on the right side) and 5 curves (4 on the left, 1 on the right side) in ROIb could not be assessed because of insufficient contrast enhancement. Peak enhancement in ROIa was observed 24 to 88 cardiac cycles after the injection of the contrast agent. For the other ROIs the time intervals varied between 24 and 60 (ROIb), 20 and 76 (ROIc), and 20 and 80 (ROId) cardiac cycles, respectively. In ROIc and ROId, high AUCs (all in decibels $\times$ cardiac cycles) (404 to 2334 in ROId; 145 to 5160 in ROIc) could be observed. Highest AUCs in ROIa and ROId were 1249 and 1264, respectively. PI ranged between 0.4 and 7.6 dB for ROIa, 0.8 and 17.6 dB for ROIb, 3 and 54.6 dB for ROIc, and 2.7 and 32.6 dB for ROId. AUCs in ROIc and ROId were significantly elevated compared with ROIa and ROIb (each $P<0.0001$).

Comparison Between Both Examination Sides

Side differences (>2-fold increase of PI or AUC in all ROIs on 1 side compared with the contralateral side) could be found in 3 individuals (patients 8, 9, and 11). In all those examinations the right-sided ROIs were elevated compared with the left side. In the remaining 15 subjects, 46 of 49 ROIs that could be evaluated had a <2-fold side difference with respect to the AUC. In 1 subject ROIa and ROIc demonstrated a >2-fold AUC side difference; in another individual ROIa and ROIb demonstrated a >2-fold AUC side difference.

AUC Ratios of ROIs

Detailed data about all AUC ratios are summarized in Table 5. AUCc/a, AUCc/b, and AUC d/b were $>2$ in all examinations; AUCd/a was $>2$ in 28 of 30 examinations. In 13 (c/a), 13 (d/a), 15 (c/b), and 13 (d/b) investigations, AUC ratios were $>5$. In a comparison of AUC ratios of the left and right sides, none of the subjects, including those (patients 8, 9, and 11) with marked side differences in absolute AUC values, showed a >2-fold difference when any ratio was considered. There was no significant difference between right and left examination side in AUC ratios in any ROI.

Discussion

Ultrasound contrast agents producing enhancement of the backscattered ultrasound signal are of increasing interest in neurosonology.$^{11-13}$ Recently published studies have shown their diagnostic potential in cerebrovascular disease.$^{14,15}$ How-

Figure 2. Contrast enhancement at different time points in axial diencephalic gray-scale images. A, Before echo contrast application: visualization of the third ventricle (arrow) and the adjacent ipsilateral thalamus (T). B, Visible contrast appearing first in projection to the lentiform nucleus (1) after 24 cardiac cycles. C, Maximal contrast causing an increase in optic intensity in all ROIs; contrast enhancement is most accentuated in projection to the lentiform nucleus (1) and the white matter (2), with only moderate increase of optic intensity in the region of the thalamus (3). D, Disappearing contrast enhancement after 244 cardiac cycles; compared with panel A, optic intensity in gray-scale images is still increased in the region of the lentiform nucleus and the thalamus.
ever, all transcranial echo contrast studies concentrated on the assessment of intracranial vessels of the circle of Willis in conventional color-coded images. In contrast, harmonic ultrasound is an approach that exploits the nonlinear resonance of microbubbles when exposed to an acoustic field, allowing enhanced detection of contrast-containing parenchymal and vascular structures while suppressing the reception of echoes from noncontrast-containing structures.16 In the field of neu-
rosonology, a preliminary color-coded HI study on the assessment of vessels of the vertebrobasilar system has been published. \(^{17}\) The authors found that the diagnostically useful period could be prolonged by HI. Fundamental frequency and echo contrast concentration identical to those in our study were used. Furthermore, spatial resolution was improved compared with conventional color-coded images, enabling visualization of more arterial and venous vascular structures.

Preliminary echocardiographic studies demonstrated the potential of HI for the noninvasive detection of myocardial perfusion in humans in a variety of clinical settings. \(^{6,18}\) Additional use of TR imaging has been shown to prevent the destruction of microbubbles and is regarded as a helpful supplementary tool for HI. \(^{6}\) In the present study we succeeded for the first time in visualizing parenchymal cerebral contrast with ultrasound. Visually evident contrast could be assessed quantitatively in different brain areas. In areas with marked contrast enhancement caused by the ultrasound contrast agent, a mean gain of 15.3 dB (range, 3 to 54 dB) was observed. This mean signal increase is comparable to that in liver (20.5 dB) \(^{7}\) and myocardial (17 to 37 dB) \(^{5}\) examinations. High peak values in our study (54 dB) are mainly attributed to the high echo contrast concentrations compared with liver and myocardial investigations. For physical reasons TRsHI results of brain parenchyma show a different pattern of

![Figure 3. Time-intensity curves in different ROIs: A, posterior part of thalamus; b, anterior part of thalamus; C, lentiform nucleus; and D, white matter. All curves show a characteristic baseline phase before contrast enhancement (phase 1), a sudden increase of contrast enhancement causing the peak intensity (phase 2), and a slow washout phase (phase 3). Note the higher PIs and AUCs (different values on the y axis) for the lentiform nucleus and the white matter compared with the thalamus.](image)

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contrast distribution compared with other imaging techniques. First, it is important to note that ultrasound attenuation is dependent on frequency and image depth. Therefore, backscattered ultrasound signals at the second harmonic frequency will be attenuated approximately twice as much as signals of the fundamental frequency. Furthermore, the deeper the structure is, the greater the attenuation will be. This effect will occur twice as fast at the second harmonic than at the fundamental frequency.19,20 Second, the relationship between concentrations of microbubbles and measurements of optic intensity is complicated because of the acoustic properties of echo contrast agents and signal processing of ultrasound scanners. This refers not only to TRsHI studies but to quantitative measurements of echo contrast concentrations in general. At low microbubble concentrations, a linear relationship between microbubble concentration and optic intensity can be observed. For higher concentrations (>30,000 bubbles per cubic centimeter), backscatter intensity saturates, and an additional increase in tracer concentration cannot be registered as an increase in optic intensity.10,21 This fact explains why AUCs and PI may appear equal despite different flow rates and why AUC and PI of time-intensity curves cannot be related directly to cerebral perfusion unless these parameters are not calibrated. Echocardiographic studies have shown that the correlation between blood flow and echo contrast agent improved when a polynomial function was applied to the data.22 For this reason a standardization of the relationship between tracer concentration and system response is needed to derive quantitative data directly from time-intensity curves. Depth-dependent attenuation of TRsHI examinations and nonlinear signal processing explain that this ultrasound technique cannot be compared with other imaging techniques assessing brain perfusion, such as positron emission tomography (PET).

However, our results show that contrast enhancement may be placed in some relation to cerebral perfusion if the physical properties of TRsHI are taken into account. Corresponding to the functional activity, regional blood flow values show distinct differences in PET studies. Perfusion of the white matter (20 mL/100 g per minute) is found to be lower than that of the basal ganglia (60 to 70 mL/100 g per minute).23,24 In our study gray-scale contrast intensities after echo contrast application were comparable for the white matter and the lentiform nucleus; the thalamus exhibited a lower signal intensity. Because of the different insonation depths of these areas, ultrasound reflexion of the lentiform nucleus is more attenuated than the white matter, thus simulating a similar perfusion in these regions. Furthermore, thalamic contrast enhancement exhibits the highest attenuation of the ultrasound waves in comparison to the other ROIs. In additional studies including more patients, depth-adjusted amplification factors for each region may be developed that may allow direct comparison of blood perfusion with PET studies irrespective of insonation depth. The side difference of AUC ratios did not exceed factor 2 in any patient. The comparison of left- and right-sided AUC ratios can partially compensate for the great interindividual differences of TRsHI values.

Apart from nonlinear signal processing, high interindividual variations of absolute values and side differences in the quantitative analysis of TRsHI examinations in our study may be explained by temporal bone thickness and the attenuation of the backscattered second harmonic frequency. Small variations of bone thickness in the same individual may potentiate asymmetric attenuation of the second harmonic frequency (3.6 MHz); marked differences of bone thickness in different subjects may cause misleading absolute values.

A further limitation of TRsHI is due to the limited spatial resolution of transcranial real-time sonography. Since the lentiform nucleus and the white matter appear isoechogenic to surrounding brain parenchyma9 in transcranial real-time images, precise anatomic localization of contrast enhancement in these areas is difficult. It cannot be excluded that parts of the internal capsule and the lentiform nucleus are included in ROIb; in ROIa parts of the internal capsule may be incorporated. For this reason experienced ultrasound examiners are necessary to accurately visualize the third ventricle and the thalamus as landmarks for orientation. Although extended time intervals between frame rates produce prolonged contrast, it may be impractical in some patients to hold the transducer in 1 position for a period of ≈4 minutes. Another limitation with triggering frame rates is that patient and transducer motions can scarcely be corrected during the examination. Modified frame rates (eg, once every cardiac cycle) can shorten the examination period and improve anatomic orientation.

In conclusion, the present study demonstrates that qualitative and to a minor extent quantitative TRsHI is applicable to neurosonology and provides characteristic phases of contrast enhancement and fading contrast effect in different brain areas. Our study indicates that gray-scale TRsHI provides detection of ultrasound contrast in brain parenchyma by improving the signal to noise ratio. It is a promising and noninvasive new method for visualization of focal cerebral contrast enhancement, allowing real-time “digital subtraction” imaging of capillary blood flow in the brain with ultrasound. Because TRsHI can be rapidly performed as a bedside examination, this technique may be a cost-effective method of detecting abnormalities of echo contrast enhancement, particularly in cerebrovascular diseases.

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References


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