Stimulated Acoustic Emission Detected by Transcranial Color Doppler Ultrasound

A Contrast-Specific Phenomenon Useful for the Detection of Cerebral Tissue Perfusion

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Background and Purpose—Experimental and clinical data suggest that insonation of echo-contrast agents with high acoustical power produces disintegration of microbubbles, resulting in a pseudo-Doppler phenomenon called stimulated acoustic emission (SAE). The purpose of this study was to investigate whether SAE might be detected by transcranial color Doppler imaging and whether these signals might be used for cerebral tissue perfusion measurements.

Methods—Nonmoving microbubbles (SHU 563 A) were insonated in vitro through the temporal parts of a human cadaver skull, and contrast signals were detected by velocity-coded color Doppler and power Doppler recordings. Transcranial color as well as power Doppler investigations were performed in 10 healthy volunteers with the echo-contrast agent Levovist (SHU 508 A).

Results—Color Doppler signals indicating SAE were observed in vitro and in transcranial human investigations. These signals were characterized by a mosaic of color Doppler pixels ranging over the full color scale. Apparent velocity information and spatial distribution of SAE signals changed from image frame to image frame. In the experimental model, the intensity of SAE signals decreased exponentially over time. With an increase of acoustic power, there was a significant increase of the maximum signal intensity ($P<0.01$) and a significantly shortened signal duration ($P<0.01$), consistent with stronger and more rapid disintegration. In humans, SAE signals were clearly detected in cerebral tissue regions. The intensity of SAE signals in those regions (eg, temporal cortex, $3.7 \pm 1.2$ dB) was approximately $8$ times lower than the signal enhancement in the major cerebral arteries (eg, in the MCA, $29.5 \pm 5.6$).

Conclusions—Echo-contrast specific color Doppler signals known as SAE are detectable by transcranial color and power Doppler sonography. Signals due to SAE might represent tissue perfusion, thereby providing a method for imaging flow with transcranial ultrasound. (Stroke. 2000;31:1661-1666.)

Key Words: stimulated acoustic emission ■ power Doppler imaging ■ transcranial ■ color Doppler ■ echo-contrast agents

There have been considerable efforts to develop ultrasound devices for visualization and quantification of tissue perfusion by use of echo-contrast agents. Great progress has been made by the development of echo-contrast agents optimized for arterial blood pool enhancement while introducing contrast-specific imaging modalities.1,2 A number of studies used echo-contrast agents for brain perfusion imaging.3–6 Although these studies provide some evidence that echo-contrast agents might be detected beyond the level of the cerebral macrocirculation, the understanding of signals related to cerebral tissue perfusion is still limited. In particular, interpretation of color Doppler studies3 remains difficult because blood flow in arterioles and capillaries produces Doppler shifts that are much below the common wall filter frequency of the ultrasound system and can therefore not be differentiated from tissue clutter.

The physical properties of microbubbles in an acoustic field are complex and depend on a number of factors, the most significant of which is acoustic power.7 At low emission power, the bubbles act as linear backscatters. With increasing power, they start emitting nonlinear frequencies that result from pressure-induced radial oscillations of their surface. At high power levels, they can be destroyed. In this process, the shell of the microbubbles is cracked, releasing a free gas bubble that rapidly grows and finally disappears. Because any change in a train of consecutive Doppler bursts results in a signal, it has been proposed that even stationary bubbles cause random pseudo-Doppler shifts due to their disintegra-
tion. These signals have been referred to as stimulated acoustic emission (SAE). They are considered to represent an echo-contrast–specific phenomenon useful for depiction of contrast agents of very low flow.

The purpose of this study was to investigate whether SAE signals can be detected by transcranial color Doppler imaging. Therefore, nonmoving microbubbles were insonated through a human skull in an experimental setup, and results were compared with echo-enhanced transcranial investigations in healthy volunteers.

**Subjects and Methods**

**Experimental Study**

**Model of Stationary Microbubbles**

Gelatin cylinders 8 cm long and 2 cm in diameter were prepared to study the effect of insonation on nonmoving microbubbles. Pork gelatin (50 g) (Sigma) was dissolved in water and heated before the cylinder-forming process to minimize incorporation of bubbles different from contrast agent within the gel. Approximately 40 000 spheres of the contrast agent SH-U 563 A (Schering AG) suspended in 9.6 mL of physiological saline were added to the gel. The suspension was homogenized until the gelatin was nearly solid and then filled into molds, avoiding the incorporation of bubbles different from the contrast agent within the gel. The molds were cooled down to 0°C to immediately congeal the gel. For adaptation of sound wave propagation and attenuation to a physiological level, the cylinders were immersed in castor oil at a depth of 6 cm by means of a special tripod. For each registration, a separate part of the gel cylinder at a minimum distance of 1 cm was insonated. Control investigations were performed in gelatin cylinders without contrast agents.

**Echo-Contrast Agent**

SH-U 563 A (Schering AG) is a transpulmonary stable echo-contrast agent under development. SH-U 563 A consists of air-filled microspheres (mean diameter, 2 μm) with a shell of a thin layer of a biodegradable cyanacrylate polymer.

**Ultrasound Investigations**

Registrations were performed by means of an HDI-5000 ultrasound machine (ATL-Ultrasound). The machine was equipped with an electronic phased-array transducer with an emission center frequency of 1.67 MHz. The insonation angle was 85° to minimize reverberation artifacts on the surface of the gel cylinder. Registrations were performed through the temporal bone of a human skull, which was obtained by autopsy. Imaging was done in a way comparable to the clinical situation, with the outer side of the bone placed 4 mm from the transducer. Castor oil was used for acoustic coupling. The investigation depth was set to 10 cm. The sector size of the color box was chosen to cover the entire gel cylinder. Pulse repetition frequency (PRF) was set at 1000 Hz. Color gain was adjusted to avoid system saturation and color noise. Frame rate was 1 Hz to visually observe effects of single ultrasound pulses.

**Experiments**

Experiments were performed to study SAE effects in velocity-coded color Doppler and in power Doppler at variable emission powers. The mechanical index (MI), displayed on the image screen of the HDI 5000, served as an estimate of the transducer output power. Ten color Doppler registrations were performed at an MI of 1.3, and 10 power Doppler registrations were performed at an MI of 0.7, 1.0, and 1.3.

Quantitative offline analyses of the power Doppler data were performed with a new calibrated software tool on a standard PC (HDI-Laboratory, ATL) that takes machine settings into account. A circular region of interest (ROI) (400 pixel/cm²) was placed to cover the entire cross-sectional area of the gel cylinder. Mean power Doppler values ± SD within this ROI were calculated in decibels. The digital acquisition allowed the removal of the compression curve for these measurements.

**Statistics**

The intensity of maximum signal enhancement at the first image frame and the number of image frames needed to reduce the signal intensity below 0.2 dB were compared for different mechanical indices by means of a Kruskal-Wallis multigroup analysis.

**Human Study**

**Subjects**

Subjects were 10 healthy volunteers (mean age, 33.1 ± 11.2 years; range, 24 to 65 years; 2 women, 8 men). All subjects had adequate temporal acoustic windows and normal findings in extracranial and transcranial color Doppler as well as spectral Doppler examinations of the anterior circulation. Exclusion criteria were galactosemia, current pregnancy or lactation, and history of cerebrovascular disease. Each volunteer provided informed consent before entering into the study. Recommendations guiding physicians in biomedical research involving human subjects had been followed (Declaration of Helsinki, 41st World Medical Assembly, 1990).

**Echo-Contrast Agent**

Leovist (SHU 508 A) is a galactose-based transpulmonary stable contrast medium (microbubble size <4 μm in 99%) that has been approved for use in humans in Germany since 1996. The agent was injected with an infusion pump (Perfusor Compact, Braun) via the right antecubital vein at a dose of 10 mL (400 mg/mL; injection speed, 1 mL/s). After the injection, the infusion line was cleared by a bolus of 5 mL of saline.

**Ultrasound Investigations**

Registrations were performed with the above-described ultrasound equipment. Axial intracranial sections of the right subtemporal region were investigated by color Doppler and power Doppler processing modes (MI = 1.3, PRF = 1000 Hz, focus = 6 cm). Doppler power and color information was achieved for the whole investigated area. Cerebral arteries were identified according to their anatomic location and flow characteristics; mesencephalic structures, including the pedunculi cerebri, were identified by anatomic B-mode information. Color gain was adjusted to avoid system saturation and color noise. To guarantee replenishment of the contrast agent in the investigated region after ultrasound-induced destruction of microbubbles, intermittent imaging was performed at a frequency of 1 image frame every third heart cycle. After injection of Leovist, power Doppler raw data were digitally stored and analyzed offline with the above-described software tools. Square ROIs (1 cm²) were placed within the distal middle cerebral artery (MCA) and the temporal cortex. Mean power Doppler signal intensity ± SD (decibels) within the ROIs was plotted versus the number of heart cycles.

**Statistics**

Time-intensity curves obtained in the MCA and the temporal cortex were compared for differences in the mean appearance time (enhancement > 1 dB) and mean time to peak echo enhancement by means of a Wilcoxon test.

**Results**

**Experimental Study**

Neither color Doppler nor power Doppler signals were detected in gel cylinders without SH-U 563 A, whereas the round shape of the gel could be clearly identified in B-mode images. In gel cylinders with the echo-contrast agent, color Doppler as well as power Doppler signals appeared. In the color Doppler mode, these signals were distributed randomly within the gel and presented a mosaic of color pixels ranging over the full variance scale (Figure 1a). Both the apparent
velocity information and the spatial distribution of color signals changed from image frame to image frame.

In power Doppler, the first frame of each imaging sequence showed an almost homogeneous distribution of the power signals. In consecutive image frames, a decrease of the signal intensity was observed, and the distribution of the signals started to have a random nature (Figure 1b). At the end of each imaging sequence, only a few widely distributed power signals appeared. Color as well as power signals were transient in nature, disappearing over a period of 5 to 60 frames.

Quantitative analysis of power Doppler signals revealed an exponential decay of signal intensity for all registrations, reflecting continuous destruction of stationary microbubbles (Figure 2). When registrations at different mechanical indices were compared, a significant increase of the maximum signal intensity at the first image frame ($P<0.01$) and a significantly shortened signal duration ($P<0.01$) were observed with an increase of acoustic power (Table 1).

**Human Study**

In all subjects, the major branches of the basal circle of Willis could be identified by unenhanced color and power Doppler, whereas the brain tissue revealed no color signal. In color Doppler recordings, the arrival of the echo-contrast agent resulted in a significant blooming of the major cerebral arteries (Figure 1c). The blooming was characterized by color Doppler signals coherent with the signals in the overestimated arteries (eg, the MCA) in terms of direction and velocity information. However, distinct from the blooming, color signals appeared in the color window covering parts of the tissue of the temporal lobe up to an investigation depth of 6 cm, which was the level of focus placement. These color signals are depicted by velocity-coded color Doppler (1a) and by power Doppler imaging (1b). Lower row shows a transcranial section of the basal circle of Willis insonated through the temporal bone window as depicted by velocity-coded color Doppler (1c) and power Doppler (1d) at maximum echo enhancement. TC indicates temporal cortex.

**Figure 1.** Upper row demonstrates SAE signals in a gel cylinder as depicted by velocity-coded color Doppler (1a) and by power Doppler imaging (1b). Lower row shows a transcranial section of the basal circle of Willis insonated through the temporal bone window as depicted by velocity-coded color Doppler (1c) and power Doppler (1d) at maximum echo enhancement. TC indicates temporal cortex.

**Figure 2.** Decay of Doppler intensity (power Doppler mode) of SAE events in the gel cylinders during continuous scanning reflecting continuous destruction of stationary microbubbles. The mean ± SD of the power Doppler values for MIs 1.3, 1.0, and 0.7 are plotted versus time (10 measurements for each curve).
signals had a random spatial distribution and were characterized by a mosaic of color pixels ranging over the full variance scale. Both the apparent velocity information and the spatial distribution of color signals changed from image frame to image frame.

Also, in power Doppler recordings, the arrival of the echo-contrast agent produced a significant blooming of the major cerebral arteries (Figure 1d), and contrast signals appeared in cerebral tissue regions covering the entire near field of the color window. Beyond an investigation depth of 6 cm, there was a considerable interindividual variability of echo enhancement, ranging from almost no signals, eg, in the cerebral peduncles, to echo enhancement in parts of the contralateral hemisphere (see Figure 1d). The intensity of the power signals in cerebral tissue regions decreased rapidly, and the distribution of the signals started to have a random nature, which changed from image frame to image frame.

Quantitative analysis of power Doppler recordings revealed that there was a transient increase of signal intensity with the typical shape of a bolus first-pass effect in both the MCA ROI and the ROI of the temporal cortex (Figure 3). In the MCA, this first pass of the contrast agent was followed by a smaller recirculation effect. In the temporal cortex, signal intensity again approached the baseline range (mean signal intensity of 0.5 to 1 dB) after a maximum period of ≈30 heart cycles. In this area, recirculation of the agent did not produce sufficient signal enhancement to result in a detectable second increase of signal intensity in the time-intensity curve. Signal enhancement in the MCA was 8 times stronger than in the temporal cortex (Table 2). Moreover, there was a significant time delay between the appearance time (P<0.01) and the time to peak concentration (P<0.01) in the MCA and the temporal cortex.

Table 1. Model of Stationary Microbubbles

<table>
<thead>
<tr>
<th>MI</th>
<th>Maximum Signal Intensity, dB</th>
<th>Duration, frames</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>0.25 (0.03)</td>
<td>3.0 (1.58)</td>
</tr>
<tr>
<td>1.0</td>
<td>2.24 (0.14)</td>
<td>35.8 (3.4)</td>
</tr>
<tr>
<td>1.3</td>
<td>4.34 (0.29)</td>
<td>58.4 (6.5)</td>
</tr>
</tbody>
</table>

Mean duration of SAE signal enhancement (>0.2 dB) and intensity of maximum signal enhancement (dB)±SD for the mechanical indices 0.7, 1.0, and 1.3. Indicated P values refer to the Kruskall-Wallis analysis of multigroup comparisons.

Discussion

In this feasibility study related to ultrasonic measurement of cerebral tissue perfusion, we demonstrate that echo-contrast-specified color Doppler signals known as SAE, which have previously been described in echocardiography and abdominal ultrasound,8–10 are detectable by transcranial color Doppler sonography. Insonation of stationary microbubbles (SH-U 563 A, Schering AG) through a human skull produced a mosaic of color Doppler as well as power Doppler signals that were distributed randomly within the investigated area. Both the apparent velocity information and the spatial distribution of the signals changed from image frame to image frame. SAE signals were transient in nature, suggesting that they mainly reflect effects due to disintegration of microbubbles. A mosaic of color Doppler signals that closely resembled SAE in vitro was detectable in echo-contrast–enhanced transcranial investigations of healthy volunteers. Those signals briefly enhanced cerebral tissue regions, showing them to be clearly different from the echo-contrast enhancement in the blooming major cerebral arteries with regard to color appearance. In power Doppler registrations, echo enhancement of cerebral tissue regions was even more extended than in color Doppler because of the better signal-to-noise ratio of this imaging modality. Unlike color Doppler, power Doppler signals in cerebral tissue regions could not be differentiated from blooming of the major cerebral arteries in terms of their visual appearance. However, a substantial delay between the time to appearance and time to peak intensity of power Doppler enhancement in representative regions of the microcirculation and the microcirculation could be observed. These findings suggest that the observed tissue signals derive primarily from contrast agents in the periphery of the vascular tree comprising various vessel compartments such as arterioles, capillaries, and venules.

There is still little experimental knowledge about the generation of SAE. It has been suggested that SAE derives from rapid changes of the size of the microbubbles and their disintegration during insonation.8–10 These changes during a train of color Doppler bursts result in a high-frequency Doppler signal. We refer to this signal as a pseudo–Doppler signal, because the signal can originate from nonmoving microbubbles. Except for system noise, all other Doppler signals detected in the body are strictly due to movement relative to the scan head. Wei et al7 demonstrated that the applied acoustic power is of critical importance for the disintegration of echo-contrast agents. In transcranial investigations, the effective acoustic power for bubble destruction severely decreases because of ultrasound attenuation at the temporal...
area of the skull. Under these circumstances, a high-emission power appears to be a prerequisite for generation of SAE signals. Most likely as a consequence of the individual thickness of the temporal bone, we could observe considerable interindividual differences with respect to the extension and intensity of tissue echo enhancement in healthy volunteers. Further reflecting acoustic power as a critical variable, it has to be considered that the thickness of the slice in which bubbles possibly could be altered is a function of the emission power as well. Therefore, the observed increase of SAE signal intensity with increasing emission power may result from recruitment of bubbles of different acoustic properties or widening of the active sound field. In this line, SAE signals have been shown to cluster at the level of the focal zone of the ultrasound beam and are more likely to be detected at peak negative sound pressure in the near field of the transducer. Finally, we suggest that the variability of echo-contrast preparations in terms of microbubble size and shell will have a considerable impact on the generation of SAE signals. Because SAE signals result primarily from disintegration of microbubbles, they will more likely occur with soft-shell agents. Moreover, the intensity of SAE signals will be increased if microbubbles are insonated with emission frequencies corresponding to their size-dependent resonance frequency.

SAE has a number of clinical implications on contrast-enhanced color Doppler applications. First, like harmonic imaging, SAE signals are contrast-specific even at the fundamental frequencies. This might be of considerable value in transcranial ultrasound, because attenuation due to the temporal area of the skull severely impairs the detection of higher harmonics. Second, the usual wall filtering does not allow the detection of flow velocities <1 to 5 cm/s. However, the majority of the blood vessels within the cerebral microcirculation are capillaries with blood flow velocities <1 mm/s. Therefore, SAE is a prerequisite for the detection of low-contrast flow beyond the cutoff frequency of the tissue-clutter wall filter by means of Doppler devices.

To date, only 1 human study tried to estimate cerebral tissue perfusion by use of transcranial ultrasound. In that study, the indicator dilution principle was applied to echo enhancement of harmonic B-mode registrations, demonstrating relative flow differences within the thalamus, the cortical gray matter, and the white matter. However, even in the harmonic mode, the evaluation of echo-enhanced B-mode images suffers from the presence of tissue signals before contrast injection. To truly evaluate the contrast effect, these signals have to be subtracted offline in a time-consuming and fault-prone procedure. By contrast, power Doppler imaging allows the online assessment of contrast enhancement, because no Doppler signals are present in low-flow areas at unenhanced baseline registrations. Moreover, Doppler techniques are known to have a markedly higher contrast sensitivity than B-mode registrations. In line with these considerations, it has been shown in animal studies that contrast-induced time-intensity curves obtained by power Doppler correlate better with scintigraphic measurements of cerebral blood flow than B-mode registrations. Therefore, we suggest that power Doppler is more suitable than B-mode imaging for detection and quantification of cerebral tissue perfusion.

However, valid ultrasonic methods for quantification of cerebral tissue perfusion have not yet been developed. The basis of perfusion measurements using the indicator dilution principle is the determination of contrast concentrations by the intensity of the contrast-enhanced ultrasound signal. However, the relation between contrast concentrations and the resulting ultrasound signal might be influenced by imaging conditions. As observed in this study, a major problem for quantitative analysis in transcranial investigations results from the heterogeneity of temporal bone structure and thickness, which may falsify absolute perfusion values. Therefore, algorithms for quantitative perfusion analysis will have to consider differences in individual imaging conditions as well as changes in the geometry of the ultrasound beam passing the temporal skull. Moreover, placement and size of the ROI analyzed will have a considerable impact on the quantitative analysis of tissue perfusion. Previous studies have shown that small ROIs may result in a large variability of perfusion measurements, whereas larger ROIs generate less noisy data, resulting in more accurate perfusion values. Conversely, larger ROIs might be affected by signals due to blooming arteries. Therefore, placement and size of representative tissue ROIs have to be considered carefully in the analysis of echo-enhanced images for tissue perfusion. In addition, time-intensity curves in different sectors of the scan plane must be compared with caution, because the intensity of ultrasound signals decreases with investigation depth and is subject to focal attenuation. As a consequence, cerebral perfusion measurements are currently limited to near-field structures, such as the temporal cortex.

Taken together, for ultrasonic measurement of cerebral tissue perfusion, it will be of critical importance to further optimize ultrasound systems for transcranial contrast investigations and to develop suitable algorithms for quantitative analysis of the images obtained. In this feasibility study, we could demonstrate that Doppler-based imaging modalities might also be valuable.
for further development, because they detect signals due to SAE, which represent a specific echo-contrast phenomenon useful for the detection of contrast agents at very low flow velocities. We suggest that these ultrasound techniques might become a promising tool for rapid, noninvasive, bedside investigations of cerebral tissue perfusion.

References
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