Visualization of Brain Perfusion With Harmonic Gray Scale and Power Doppler Technology
An Animal Pilot Study

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Background and Purpose—It is unclear which harmonic imaging mode (power Doppler or gray-scale imaging) is superior and which measuring method is the most robust for the description of brain perfusion.

Methods—We performed an animal study on 6 beagles through the intact skull using a SONOS 5500 device and Optison injected intravenously in 3 different doses (0.15, 0.3, and 0.6 mL). Intensity versus heart-cycle plots for the brain parenchyma and the basal cerebral arteries were generated to evaluate the peak increase (PI) from baseline and the area under the curve (AUC).

Results—With harmonic gray-scale imaging, a homogeneous increase in echo contrast of the brain parenchyma was observed. The effect was dose dependent, resulting in a significant increase in PI as well as an insignificant increase of the AUC with 0.3 mL versus 0.15 mL contrast agent ($P<0.03$ and $P=0.65$, respectively; $n=5$). With harmonic power Doppler, injection of the 3 different doses resulted in a nonsignificant increase in PI and AUC ($P=0.17$, $n=6$ for both). After normalization of the brain signal to the peak arterial signal in individual dogs, a significant increase could be demonstrated ($P=0.03$ and $P=0.01$, respectively; $n=6$). The signal pattern of harmonic power Doppler was inhomogeneous, with stronger signal increases in the anterior part of the brain.

Conclusions—Gray-scale imaging leads to a more homogeneous increase in echo contrast of the brain tissue and may be more suitable for displaying brain perfusion. The PI of the signal intensity seems the most robust parameter for the description of cerebral perfusion with both imaging modes under investigation. (Stroke. 2000;31:1728-1734.)

Key Words: contrast media ■ perfluorocarbons ■ perfusion ■ ultrasonography ■ dogs

Several attempts have been made in the fields of neurosonology and echocardiography to measure capillary blood flow and tissue perfusion by means of contrast-enhanced ultrasound techniques. Harmonic imaging is a new ultrasound method that increases the signal-to-noise ratio in color-coded duplex sonography as well as gray-scale imaging. We have previously shown that an increased spatial resolution of the vertebrobasilar circulation can be achieved by using this novel technique in contrast-enhanced color-coded duplex sonography. In 2 studies in healthy human volunteers,$^{2,3}$ the use of second harmonic imaging in gray-scale ultrasound produced signal-enhancing effects in different regions of the brain. However, the extent of contrast enhancement in the parenchyma was highly variable and depth dependent, probably owing to the use of air-based ultrasound contrast agents.

The purpose of this animal study was to investigate the contrast-enhancing effects of a new perfluoropropane-based ultrasound contrast agent (Optison) and to compare harmonic gray-scale and harmonic power Doppler imaging with respect to their potential for the detection of contrast agent in the microcirculation of the brain. This study is of particular interest for the design of further investigations planning the visualization of ischemic defects in patients with acute stroke by means of ultrasound methods.

Materials and Methods

Animal Model
Six sedated male beagles (mean body weight, 20 kg) were used for the study. Sedation was achieved with 1 mL Rompun 2% (Bayer AG) and 10 mL Ketanest (Parke-Davis) in 14 mL NaCl 0.9% solution, administrated intravenously. The dogs were placed in a lying position and the contrast medium was administered through an indwelling 20-gauge catheter (Ventilon 2 [20G/32 mm], Fa. Boc Ohmeda AB) placed in a subcutaneous vein in the leg. Oxygen saturation (SPO$_2$) and heart rate were measured noninvasively (distal tail) before and after injection (Critikon Scolar 2); the ECG (SONOS...
The study complied with the animal protection legislation of the Federal Republic of Germany and was approved by an ethics committee.

**Ultrasound Contrast Agent**

Optison is a perfluoropropane-containing ultrasound contrast agent based on a 1% albumin solution. The contrast agent is commercially available and was originally developed for echocardiography (generic FSO69, Mallinckrodt Medical GmbH). The solution was prepared following the manufacturer’s instructions and injected in a dose relevant to humans (for left cardiac echocardiography, doses between 0.5 and 1 mL are used in humans).

We used bolus injections of 0.15, 0.3, and 0.6 mL Optison, which is comparable to the doses used for parenchymal imaging in other animal studies.4

The mean diameter of the microbubbles is 2.0 to 4.5 μm, and the concentration is 5 to 8 × 10^6/mL suspension. The ultrasound contrast agent bolus was immediately followed by a second bolus of 3 mL 0.9% NaCl solution to flush the injection line.

**Transcranial Sonography**

Harmonic gray-scale and power Doppler imaging was performed with a SONOS 5500 ultrasound system (Agilent Technologies) in connection with a 1.8/3.6-MHz sector transducer (S4 probe, Agilent Technologies) in an investigation depth of 8 cm (focus on 6 cm). For gray-scale imaging we used the integrated backscatter (IBS) mode and the study type T-INT (mechanical index 1.0 to 1.1). In harmonic power Doppler mode, the cranial thermal index was 1.9 and the pulse repetition frequency 700 Hz.

After each contrast agent injection, 62 digitized gray-scale or 150 power Doppler images of the brain triggered by ECG were stored in continuous loop review memory and then recorded on an optical disc for later offline analysis. We used the transient response imaging mode with a frame rate of 1 image every 4 cardiac cycles. Gain and transmit power setting were optimized for each dog at the beginning of each investigation and were not changed throughout the procedure. The entire investigation was also recorded on videotape.

**Harmonic Gray-Scale Imaging**

For the analysis of harmonic gray-scale data, the IBS of the brain tissue was measured offline using the acoustic densitometry unit (AD) of the HP SONOS 5500. This unit assists in the quantification of ultrasound images by measuring the scattered energy received by the transducer. Because AD measurement is made upstream in the imaging chain, it is less influenced by postprocessing functions of the imaging chain. The IBS is a relative measure of the total ultrasound energy scattered by a small volume of the interrogated tissue. The IBS data measurements were displayed on a logarithmic scale in decibels.6,7 We specified the regions of interest (ROIs) to the ipsilateral brain parenchyma and the supplying artery of this brain region. The identification of the anatomic regions of interest (ROIs) to the ipsilateral brain parenchyma and the contralateral skull. Note the homogeneous increase in brightness of the brain parenchyma without enhancement in the muscle.

**Data Analysis**

Noninvasive determination of blood flow based on the dye dilution theory has been described for contrast echocardiography, digital intra-arterial angiography, ultrafast CT, MRI, and SPECT.9–13 The combined washout and appearance phase of the intravascular indicator is quantitatively described by the area under the time-intensity curve (AUC) and the peak intensity (PI).

The AUC is directly related to the cerebral blood volume.14 The ratio between the AUC of a parenchymal region and the supplying artery is related to the blood distribution volume. The PI from baseline after contrast agent injection is related to the maximum amount of agent bubbles in the sample volume. Because ultrasound contrast agents are markers for the moving blood, they are ideal intravascular indicators, according to the dye dilution theory. For increasing doses of an intravenously injected agent, increasing values of the AUC and the PI should be detected.

In this study we compared for both imaging modes the AUC and the PI in the brain parenchyma for increasing doses of Optison with a nonparametric test for related samples (Friedman ANOVA test, SSPS Inc). Baseline for each ROI was the mean value from the first 2 AD values after the injection.

For harmonic power Doppler imaging, we performed a normalization of the parenchymal signals on the maximal arterial signal of the basal cerebral arteries to take into account the depth-dependent attenuation of the power Doppler signals.15

Additionally, we calculated the AUC for the 2 ROIs (basal cerebral arteries and brain parenchyma) and compared the ratio between the AUC (which is related to the blood distribution volume) of the brain parenchyma and the basal cerebral arteries with a Friedman ANOVA test. The normalization of the data could not be performed for gray-scale imaging because a clear differentiation of the basal cerebral arteries was not possible in all animals under investigation.

**Harmonic Power Doppler Imaging**

Harmonic power Doppler images were analyzed offline with special software for videodensitometry (QuantiCon, 3D-EchoTech) on a separate workstation. This software counted the pixels in different ROIs with various sizes, using the formula

\[ \sum P_{\text{color}} / \sum P_{\text{all}} = (p_{\text{all}} = \text{color pixels} / p_{\text{all}} = \text{all pixels}) \]

The mean washout curves of the 6 dogs were displayed graphically.

**Results**

Harmonic Gray-Scale Imaging

Figure 1 shows the contrast-enhancing effects of an intravenous bolus injection of Optison in 1 dog. We observed a strong and homogeneous increase in brightness of the brain parenchyma, without enhancing effects in the masticatory muscles or signal attenuation in the region of the contralateral skull. This indicates a selective enhancement of the brain tissue, with little or no attenuation by the temporal muscles.

When the washout curves after 0.15 and 0.3 mL Optison were analyzed in the 6 dogs under investigation, a wide variation of the increase in IBS measurements of the brain...
parenchyma (Table 1) was noted. There was a dose-dependent increase in the contrast-enhancing capacity of the agent when the 2 different doses (0.15 and 0.3 mL) were compared (Figure 2, Table 1). Although the difference in PI from baseline PI proved significant ($P = 0.03$, $n = 5$), no significant difference was detected with respect to the area under the intensity versus heart-cycle curve for the 2 different dosages ($P = 0.65$, $n = 5$). This was probably owing to the wide variation of the enhancing effect with the different doses used.

**Harmonic Power Doppler Imaging**

Optison bolus injection led to a dose-dependent increase in power Doppler signals in the brain parenchyma, which resulted in an inhomogeneous signal pattern, as displayed in Figure 3. A strong signal enhancement was frequently found in the anterior part of the brain (Figures 3B, 3D, and 3F, left side); however, in the posterior part of the brain few signals could be differentiated (Figures 3B, 3D, and 3F, right side). In accordance with the results of gray-scale imaging, we saw no contrast-enhancing effects in the masticatory muscles, even with the high dose (0.6 mL) of Optison.

The pixel versus heart-cycle plots (Figure 4) for the brain parenchyma and the basal cerebral arteries indicated a strong difference between the intensities obtained with 0.15 mL and the 2 higher doses (0.3 and 0.6 mL) of contrast agent. In the arteries we found a typical 1-phase washout curve (Figure 4 B). In the brain parenchyma, both the slope of the curves during the washout phase and the enhancing effect achieved were lower than those of the arterial signal.

The statistical workup of the data revealed a significant dose-dependent increase of the AUC in the basal cerebral artery ($P = 0.04$, $n = 6$) but not in the brain parenchyma ($P = 0.17$, $n = 6$). Furthermore, the PI in the brain showed no dose-dependent increment ($P = 0.17$, $n = 6$).

The mean ratio of the AUCs of the brain parenchyma and the artery ranged between 0.17 and 0.19, and the variation coefficients ranged from 35.3% to 57.7%, which indicated a large fluctuation of the data (Table 2). There was no dose dependence ($P = 0.052$) of this proportion, which is related to the blood distribution volume.

To take the interindividual ultrasound attenuation of the tissue into account, we performed a normalization of the brain signals on the peak arterial signal (Figure 4 C). In contrast to the analysis of the unadjusted signals, the normalized approach led to a dose-dependent increment of the PI and the AUC ($P = 0.03$ and $P = 0.01$, $n = 6$, respectively).

**Discussion**

This study describes the echo-enhancing potentials of the new octafluoropropane-filled, albumin microsphere–based ultrasound contrast agent Optison, which is currently used for myocardial imaging as well as for the visualization of other parenchymatous organs, such as the liver.

Through the use of harmonic imaging technology and gray-scale as well as power Doppler ultrasound modes, we

### Table 1. Harmonic Gray-Scale Imaging: Mean Values, SD, and Range of AUC and PI for Brain Parenchyma, by Optison Dose

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
<th>$P$ (n=5)</th>
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</thead>
<tbody>
<tr>
<td>AUC</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0.15 mL</td>
<td>144.4</td>
<td>208.9</td>
<td>0.9–513.6</td>
<td>0.65</td>
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<tr>
<td>0.30 mL</td>
<td>244.6</td>
<td>220.4</td>
<td>66.4–493.6</td>
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<tr>
<td>PI</td>
<td></td>
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</tr>
<tr>
<td>0.15 mL</td>
<td>3.6</td>
<td>3.5</td>
<td>0.9–9.6</td>
<td>0.03</td>
</tr>
<tr>
<td>0.30 mL</td>
<td>7.0</td>
<td>3.9</td>
<td>3.4–11.9</td>
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</tbody>
</table>

**Figure 2.** Harmonic gray-scale imaging. Mean IBS intensity versus heart-cycle plot of the 5 dogs with intravenous injection of 0.15 and 0.3 mL Optison in the brain parenchyma (see Figure 1, area 3).

**Figure 3.** Harmonic power Doppler scans before (panels A, C, and E) and at the peak of the enhancing effect after 44 to 52 heart cycles in 1 dog with intravenous injection of 0.15 (B), 0.3 (D), and 0.6 (F) mL Optison. Area 1 is the masticatory muscle; 2, ipsilateral skull; 3, brain parenchyma; and 4, contralateral skull. Note the inhomogeneous power Doppler signal mainly in the anterior (left) region of the brain.

**Figure 4.** Pixel versus heart-cycle plots for the brain parenchyma and the basal cerebral arteries (A and B).
evaluated the potentials of Optison for the enhancement of the brain parenchyma. With gray-scale imaging, a homogeneous, dose-dependent increase in echo enhancement of the brain parenchyma was detected. However, the effect showed a high interindividual variation, with increases between 0.9 and 11.9 dB, which could be explained by the various insonation conditions of the dogs. We found no shadowing effect (ie, an attenuation of signals emerging distally from the probe) that could be detected with other contrast agents. In our opinion, the most robust measuring method of the enhancing effects is the PI from baseline, which showed a significant dose-dependent increase.

For the qualitative visualization of brain perfusion, gray-scale imaging seems an appropriate imaging mode because of the homogeneous echo pattern of the brain obtained after the injection of contrast agent. Comparing our data with human studies that used gray-scale harmonic imaging for the analysis of brain perfusion, Optison seems to have a contrast-enhancing effect of at least comparable strength with less depth-dependent decrease of echo enhancement.

Power Doppler with fundamental technology has been used in several studies for the quantification of brain perfusion. The authors correlated the area under the pixel-intensity curves with the cerebral blood flow and found good correlations. We observed a dose-dependent increase of the AUC in the basal cerebral arteries but not in the brain parenchyma. One parameter of the blood distribution volume is the ratio of AUCbrain and AUCartery. The mean value of this proportion showed no dose dependence (0.17 to 0.19) but showed a high variation coefficient (between 35.3% and 57.9%). The high variation of the quantitative parameters and the inhomogeneity of the power Doppler images with stronger signal increases in the anterior part of the dog brain compared with the posterior part (Figure 3) indicates that harmonic gray-scale imaging is probably superior to power Doppler imaging for the qualitative analysis of cerebral perfusion. This observation might be explained by the localization of the major intracranial vessels, which are predominantly in the rostral part of the dog brain. For the high-dose range (0.3 mL and 0.6 mL), PI in the artery was not different (Figure 4). This might be a measurement artifact produced by the limited dynamic range of the ultrasound system to detect high-contrast concentrations in the vessel.

The most robust measuring method of the enhancing effect using power Doppler were the normalized values of the PI and the AUC, which showed a significant dose-dependent increase. This normalization was first introduced by Rubin for the analysis of the fractional moving blood volume, to take into account the ultrasound attenuation in different depths of the investigated tissue.

One possible explanation for the more homogeneous enhancement of harmonic gray-scale imaging compared with power Doppler imaging is that the latter requires either movement or destruction of contrast agent bubble to generate signals. That means that contrast agent bubbles could be detected in the larger vessels because of the movement between ultrasound pulses. However, in the microcirculation the blood flow is very slow, and bubble destruction is required for the system to detect changes between pulses. Harmonic gray-scale imaging will display bubbles if they have a significant harmonic response, not just if they are destroyed. Bubble destruction requires application of a higher ultrasound power compared with the harmonic response. It is well known that ultrasound going through the skull attenuates by >90%, and therefore bubbles are not destroyed as easily as in other parenchymal organs such as the heart or the liver, in which harmonic power Doppler imaging is fairly homogeneous.
In conclusion, harmonic imaging is a technique for visualization of brain perfusion. Harmonic gray-scale imaging showed a more homogeneous enhancement effect in the parenchyma compared with power Doppler imaging. A quantitative analysis of perfusion seems difficult because of the high fluctuation of the data and methodical problems such as the attenuation of ultrasound passing through the skull and the depth dependence of ultrasound imaging. The most robust parameters are the PIs from baseline for harmonic gray-scale imaging and the normalized peak values, as well as the AUC of the normalized power Doppler curve.

This study indicates that it is possible to visualize changes of ultrasound intensities in perfused areas of the brain through the intact skull. This observation is encouraging for further studies evaluating brain perfusion in humans with the use of harmonic imaging technology. This method could be of particular value for the investigation of patients with acute brain infarctions.

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References

Transcranial color duplex sonography with or without intravenous administration of echocontrast agents (ECAs) has been shown to be a reliable tool for the assessment of abnormal hemodynamics in the basal cerebral arteries of patients with acute ischemic stroke.1-2 The prognostic and therapeutic impact of different occlusion and collateral patterns in the basal cerebral arteries of such patients is not well understood, because these vessels were not examined in the large placebo-controlled, randomized trials investigating the therapeutic effect of intravenously administered fibrinolytic3 or neuroprotective4 drugs. Nevertheless, the assessment also of the cerebral perfusion deficit and, especially, the ischemic penumbra is important, because it may allow prediction of clinical outcome and identification of the patients with the potential to respond to acute stroke therapy, and it may help to monitor the therapeutic effect of new drugs.

The magnitude of the backscattered contrast-enhanced signal is greater at twice the fundamental (harmonic) frequency than that of biological tissue.5 The resulting increase in signal-to-noise ratio is exploited by contrast harmonic imaging (cHI). Subsequent studies have shown that the intermittent transmission of ultrasound signals, the so-called transient response imaging (TRI), reduced the insonation-related destruction rate of ECAs and increased the intensity of the backscattered signal.6 Recently, gray scale TRI-cHI was used to estimate brain perfusion by measuring time-intensity curves to assess the passage of intravenously injected ECAs through cerebral regions of interest in normal subjects7–9 and patients with hemispheric stroke.10,11

In the accompanying article, Seidel and coworkers present animal data that compare the time-intensity curves obtained in intracranial regions of interest by B-mode and power Doppler TRI-cHI with use of different dosages of intravenously infused ECA. The authors did not compare their measurements with a gold standard such as the radiolabeled microsphere technique,12 and it would have been ideal if they could have performed their experiments in this manner. For gray scale imaging they found a dose-dependent increase of signal intensity that was significant for the peak increase from baseline with large interindividual variations. The poorer results of power Doppler imaging suggest that this technique is not appropriate for the assessment of cerebral perfusion. This and previous studies7-11 show that TRI-cHI has several drawbacks that prevent the reliable quantification of global and regional cerebral blood flow and its use in clinical routine: the relation between measured signal intensity and actual microbubble concentration is nonlinear at the useful dosage13; the attenuation of the ultrasound signal depends on the depth of insonation; transcranial B-mode imaging gives no precise anatomic localization, has a limited spatial resolution, and intrindividual and interindividual differences of bone thickness of the temporal ultrasonic window may cause misleading intensity values; and transcranial ultrasonic investigation is restricted to one insonation plane. Furthermore, the sonicated albumin microbubbles used in the present study behave like red blood cells only when the endothelium is functionally normal. When the endothelium is damaged (eg, after ischemia and reperfusion), they bind to activated leukocytes located at endothelial surface of venules, which prolongs the mean transit time.14 In conclusion, transcranial TRI-cHI has been investigated only in a few animals and patients, and a final judgment of this promising technique is therefore premature. Improvements of ultrasound machines, ECAs, software, and new techniques such as ultrasound-induced destruction of microbubbles12 may allow quantitative or even quantitative assessment of cerebral perfusion. The future will show whether transcranial color duplex sonography enables the investigation of brain perfusion in addition to cerebral hemodynamics in acute ischemic stroke.

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