2nd THORAXCENTER EUROPEAN SYMPOSIUM ON

ULTRASOUND CONTRAST IMAGING

ABSTRACT BOOK

Folkert J. Ten Cate, MD
Nico de Jong, PhD

ICIN

January 23 - 24 1997
Rotterdam - The Netherlands

2nd THORAXCENTER EUROPEAN SYMPOSIUM ON ULTRASOUND CONTRAST IMAGING. 23 AND 24 JANUARY 1997, Rotterdam, The Netherlands.

WEDNESDAY 22 JANUARY 1997

18.00 - 20.00 **Registration and Welcome drink** **Atlanta Hotel**

THURSDAY 23 JANUARY 1997

08.00 - 09.00 **Registration**

Prof. dr. N. Bom — Opening address:

09.05 - 10.45 **CONTRAST AGENTS** **Chairperson: C.E. Angermann**

10.45 - 11.15 **Intermission**

11.15 - 12.30 **NEUROLOGIC APPLICATIONS** **Chairperson: F.J. Ten Cate**

12.30 - 13.30 **Lunch**

13.30 - 15.00 **TECHNOLOGY I** **Chairperson: K. Kristoffersen**

15.00 - 15.30 **Intermission**

15.30 - 16.30 **CONTRAST PEARLS** **Chairperson: R. Leischik**

16.30 - 17.00 **DRUGDELIVERY**

19.00 - 22.30 **Social Event "BUBBLES AND BALLS" (incl. Dinner)**

2nd THORAXCENTER EUROPEAN SYMPOSIUM ON ULTRASOUND CONTRAST IMAGING. 23 AND 24 JANUARY 1997, Rotterdam, The Netherlands.

FRIDAY 24 JANUARY 1997

08.00 - 08.30 **Registration**

08.30 - 10.00 **ORGANPERFUSION** **Chairperson: S. Feinstein, H. von Bibra**

10.00 - 10.30 **Intermission**

10.30 - 12.00 **TECHNOLOGY II** **Chairperson: P. Burns**

12.00 - 13.00 **Lunch**

13.00 - 14.00 **DRUGDELIVERY** **Chairperson: A. Man in 't Veld**

14.00 - 14.15 **DISCUSSION AND CONCLUSIONS**

14.15 - 15.00 **DISCUSSION** **Chairperson: P. Burns, E. Unger**

15.00 **Adjourn**

ASSESSMENT OF MYOCARDIAL BLOOD FLOW BY TRANSIENT HARMONIC POWER DOPPLER IMAGING USING SONOVUE ™

A. Broillet, J. Puginier, R. Ventrone, M. Schneider
Bracco Research SA, Switzerland

One of the main targets in contrast echocardiography is the assessment of myocardial perfusion. The combination of new technologies such as Transient Grey Scale Harmonic imaging (TGSH) with the use of transpulmonary contrast agents allows significant improvements in this field. With TGSH, the agent Sonovue (formely code named BR1) produced significant myocardial opacification in all our closed- chest mini-pig experiments.

When combining ECG-triggered imaging with second harmonic demodulation, power Doppler imaging also becomes a powerful tool in cardiac applications. This mode, called here Transient Power Doppler Harmonic imaging (TPDH), eliminates artefacts due to tissue motion, and thus allows fine assessment of myocardial perfusion.

The aim of this study was to compare TGSH and TPDH with Sonovue both *in vitro* and *in vivo*.

The instruments used was an ATL HDI-3000 with Harmonic software and a phased-array probe (P3-2). The experiments were carried out using a tissue-mimicking phantom with a flow channel (ATS Inc.) and 5 closed-chest mini pigs. They showed that TPDH was not more sensitive than TGSH, based on the same Sonovue dose ranging (0.01 to 1 ml/kg in bolus and 0.05 ml/kg/min in infusion).

However, TPDH has one specific advantage over TGSH, in that varying the Pulse Repetition Frequency (PRF) allows to discriminate blood flow velocities in the myocardium. Video sequences in non-pathological conditions will be shown to illustrate this potential application of Sonovue. This technology could become useful either for the detection of myocardial perfusion abnormalities or to differentiate large coronary arteries from the microcirculation.

CLINICAL EXPERIENCE WITH INTRAVENOUS BY 963

O. Kamp, P. Solleder, C.A. Visser
Free University Hospital Amsterdam, The Netherlands

BY 963 is a relatively new transpulmonary echocontrast agent for left ventricular opacification. In a phase II study we examined 14 patients with coronary artery disease in the catheterization laboratory. Four doses of BY 963 were randomized sequence. The patients were anaesthetized and invasive measurements of pulmonary resistance, cardiac output and cardiac index, first derivative of left ventricular pressure rise (dP/dt), area under intensity curves of the ventricles and duration of left ventricular opacification were analyzed. In addition, 9 other patients were studied comparing triggered fundamental with triggered second harmonic imaging to determine myocardial perfusion. Two different i.v. doses of BY 963 were administered in randomized order as well as the processing technique. Maximum changes of signal intensities from baseline for both signal techniques were determined in posterior, anterolateral and septal walls. It is concluded that there is no influence on pulmonary circulation and cardiac function using diagnostically relevant doses of BY 963. The intensity of left ventricular opacification correlates better with cardiac output and in lesser extend with pulmonary pressures. Using triggered second harmonic imaging, it is possible to determine myocardial perfusion with this agent.

LOC-IMAGING ("SONO-SCINTIGRAPHY") WITH SH U 563 A IN HUMANS - RESULTS OF A PHASE I CLINICAL TRIAL.

R. Schlief ,A. Bauer ,M. Mahler ,A, Urbank , M. Zomack ,T. Fritzsch
Clinical R&D Diagnostics, Schering AG Berlin, Germany

Basic mode of action:

Ultrasound diagnosis uses B-mode images to provide anatomical information and Doppler analysis to measure the velocity of blood flow. In the capillaries, however, the velocity of blood flow (0.03 - 0.3 cm/sec) is comparable to the speed of motion of the surrounding tissue. This capillary flow cannot be imaged by conventional Doppler techniques. A novel ultrasound agent, SH U 563 A, with special non-linear acoustic properties overcomes these limitations. When the new agent is exposed to ultrasound of sufficient amplitude, it produces a characteristic signal, which we call "stimulated acoustic emission"1 (SAE) which results into random velocity Color Doppler signals. Each Color-pixel signal thereby represents single "SAE-events" combined with the disappearance of microspheres which represents a sonographic analogous of scintigraphic procedures. The Doppler signals from quasi-static blood volumes can be explained as follows. Color Doppler flow mapping depends upon the correlation between successive pulses which is infringed by the non- linear response of SH U 563 A. We have described this new procedure as "LOC" imaging (Loss Of Correlation). The image provided by a conventional color Doppler ultrasound scanner after an injection of SHU 563 gives a map of the distribution of the contrast agent in the microvascular blood pool. The image gives an image of the microvascular compartment that contains anatomical and functional information on microvascular perfusion.

Material and Methods:

SH U 563 A is a novel transpulmonary agent with a cyanacrylate shell. The microspheres are filled with air and have an average diameter of about 2 µm. The microspheres are performed and presented as a dry powdery substance which is to be suspended by shaking in physiological saline for a few seconds before injection. In this first human study 5 volunteers were given three injections at varying doses, with an interval of at least one hour between doses to avoid any cumulative effect. A suspension of SH U 563 A microparticles was given as a bolus injection in ascending doses of 0.1, 1, 10 and 30 µl/kg body-weight. Safety assessment included heart rate, blood pressure, ECG, and laboratory analysis, (hematology, coagulation and clinical chemistry) and urinalysis. An conventional ATL Ultramark ultrasound scanner with a curved linear transducer array (C4-2) operating at 2 - 4 MHz was used for liver imaging. The

transducer was maintained in the same position manually throughout the imaging sequence.

Results:

SH U 563 A was well tolerated without any adverse events of clinical concern or limiting the usage. There were no hemodynamic changes and no clinically relevant changes in any of the safety parameters at any time during the trial. Color Doppler LOC-imaging provided a typical random-velocity localization map of microvascular blood volumes in the liver with dose dependent imaging durations. The principal feasibility of microvascular imaging was proven in all the volunteers. From 10 µl/kg BW the delineation of parenchymal perfusion was homogeneous and lasted for up to about 4 minutes. After higher doses the color Doppler delineation of perfused parenchyma was more persistent. It was possible to clearly distinguish perfused liver parenchyma from the surrounding structures.

Conclusion:

The polymer based microspheres of SH U 563 A represent an innovative step in the development of ultrasound contrast agents. The favorable acoustic properties of SH U 563 A enable reliable and long lasting stimulated acoustic emission response during ultrasound exposure. The LOC image shows all the blood vessels, including major arteries and veins. By its nature the technique is less suitable for distinguishing between the capillary, arterial and venous blood flow but the LOC image may distinguish perfused from non-perfused tissue quite clearly. Our results suggest that SH U 563 A could have a particularly valuable application in the assessment of organ and tissue perfusion. The extreme stability of SH U 563 A may in future bear a great potential for quantitative imaging and quantitative analysis of organ perfusion by ultrasound.

References:

1. Uhlendorf V, Scholle F O: Imaging of spatial distribution and flow of microbubbles using nonlinear acoustic properties. In: Tortoli P, Masotti L, Acoustical Imaging, Vol. 22, p. 233-238, Plenum Press, N.Y., 1996

LATEST DEVELOPMENTS OF THE ULTRASOUND CONTRAST AGENT BY 963

P. Solleder, C. Greis, K. Beller, R.Linder
Bracco-Byk Gulden GmbH, Max-Stromeyer-Str. 57, D-78467 Konstanz and Byk Gulden Pharmaceuticals, Byk-Gulden-Str. 2, D-78467 Konstanz, Germany

BY963, an ultrasound contrast medium (USCM) jointly developed by Byk Gulden and Bracco-Byk Gulden, is a blood pool marker USCM capable to pass the lung capillaries resulting in an opacification of right and left heart cavities, a perfusion dependent enhancement of myocardial and parenchymal tissue echogenicity as well as in an enhancement of Doppler signals obtained from central, cerebral and peripheral vessels.

BY963 is provided as a diagnostic kit. Each kit consists of the USCM and a special application set. The active ingredient of the pharmaceutical preparation is a chemically derived phospholipid isolated from soybeans: 3-SN-phosphatidyl-DL-glycero-distearoyl-Na (DSPG-Na). DSPG-Na belongs to the group of saturated, stearic acid containing phospholipids which are elementary parts of natural cell membranes. Further excipients, commonly used in pharmaceutical preparations, are added to obtain good physico-chemical properties. The reconstituted lyophile has an osmolality of 280 mosmol per kg H_2O, a viscosity of 1.86 mPa·s, and a pH value of 7–8. To prepare the minute bubbles (*spherosomes*) which elevate the echogenicity of the blood, a special application set was developed. The essential element is a cavitation chamber, integrated in a syringe which guarantees a homogeneous distribution of spherosomes of a defined diameter (3.8 µm with at least 95% < 8 µm).

Pharmacological studies on the safety and tolerability of BY963 were performed in conscious and anaesthetized dogs, anaesthetized and thoracotomized cats, rats as well as in sheep. BY963 proved to be well tolerated in all animal species studied. Possible effects of the spherosomes on microcirculation were investigated in the greater omentum of the rat and were compared to two commercially available USCM, an agitated non-ionic radiographic contrast agent and agitated saline. The study revealed no adverse effects of BY963 on microcirculation.

Single dose (in mouse and rat) as well as 4-week repeated dose (in rat, dog and monkey) toxicity studies were performed. There were no symptoms of a relevant local toxicity after i.m.-, i.v.-, p.v.-, and i.a.-administration of BY963. Additional studies performed did not reveal any sensitizing, mutagenic, or teratogenic properties.

Up to now, more than 500 healthy volunteers and patients with cardiac, vascular and cerebro-vascular diseases were studied with BY963. The studies showed a good efficacy, i.e. left heart cavities were homogeneously opacified and the Doppler signal intensity obtained from cerebral, central and peripheral vessels were amplified up to 40 dB. Usually, a intravenous bolus injections of 2.5–5.0 ml is sufficient to obtain a complete and homogeneous opacification of all 4 heart chambers and a pronounced enhancement of intracardiac and intravascular Doppler flow signals. Perfusion defects in the myocardium could be shown in patients with myocardial infarction. However, the correlation between perfusion defects and intensity changes derived from the myocardium is currently not proven. The tolerability of BY963 is excellent. Administration of single doses up to 30 ml was well tolerated. No serious adverse events occurred in all patient studies.

LATEST CLINICAL DEVELOPMENTS WITH QUANTISON ™

R. Johnson
Andaris Limited, Nottingham, United Kingdom

Recent trends in the development of echocontrast agents have focused on the use of low-soluble gases to enhance agent stability. This in turn, has led to improvements in bubble stability and enhance perfusion images. The downside of this has been the toxicological implications of the use of non-physiological gases.

Recent clinical trials developments with Quantison™ have shown for the first time that it is possible to achieve reproducible gray scale myocardial perfusion images with air filled microcapsules. This breakthrough is due to the improved understanding of the interaction of the impinging ultrasound with microcapsule wall and the associated air-liquid interface.

The phenomenon of Power Enhanced Scattering has now been described to explain the phenomenon whereby Quantison™ exhibits not only enhanced (non-linear) scattering but also well defined harmonics and sub-harmonics. In particular the application of harmonic imaging has not only improved the visualization of perfusion but also resulted in a significant reduction in the clinical dose (in comparison to fundamental imaging).

Phase II clinical studies are now underway or scheduled to start in 8 European clinical centres during 1997 to confirm the utility of Quantison™ in echocardiography and specific radiological applications. Phase II cardiac studies in the U.S. are scheduled.

Apart from the ability to achieve excellent, long-lasting myocardial perfusion images other less apparent features have been shown to be important in the clinical setting. These features include:

- Stability
- Robustness
- Ease of administration
- Flexibility of the formulation
- Superb safety profile
- Longevity of image

Whilst confirmation of the utility of Quantison™ in the cardiac setting will continue, studies are also being undertaken to utilize some of the unique features and scattering properties of the agent to obtain new diagnostic information during clinical studies.

WHICH CONDITIONS ARE NEEDED FOR A SUCCESSFUL LEFT VENTRICLE AND MYOCARDIAL PACIFICATION USING QUANTISON™

L. Varga , F. J. Ten Cate , P. Frinking
University Hospital Rotterdam, The Netherlands

Contrast echocardiography has benefited from the technological developments of the last decade. Physicians and manufacturers alike have made united effort to find ideal scatters for use within their imaging requirements.

This study was designed to assess the clinical utility of a recently developed contrast agent in a dose finding study in patients with stable coronary artery disease. The study population consisted of fourteen male patients with a mean age of 56 years, ranging from 40 to 70. Nine patients were administered two separate doses, given at least ten minutes apart. Two patients were given a single dose, one other patient was injected with a series of the drug, divided into sixteen equal parts with one minute's time being given between each dose of the series. One patient was administered Quantison™ through continuous intravenous infusion. The lowest concentration of Quantison™ used in the study was one million microparticles/kg for a single dose, and the highest was one hundred million microspheres/kg for a single dose. The maximum accumulative dose given did not exceed 150 million microparticles/kg. To analyze any enhancement apical views were monitored, with images recorded on video tape, and also stored on optical disc. All but one of the images had been performed using the HP Sonos 2500 prototype, and the ATL HDI 3000 ultrasound machines. These machines are able to use Transient Response Imaging in triggered mode.

Conclusion:
There were no side effects following any i.v. injection. In three cases, only the second injections could reach left ventricle opacification. The mean duration of the contrast effect in the left ventricle was 573 second, ranging between 120 end 1090. Complete left ventricle opacification had been obtained in 2 patients. In 4 patients a visual increase of myocardial intensity was noted after any of the Quantison injection. At present no definite dose is known for left ventricle and myocardial opacification for Quantison™ Further studies are needed.

IMPORTANCE OF ULTRASOUND CONTRAST IN CLINICAL NEUROLOGY

M.Kaps

Dpt. of Neurology, Medical University at Lübeck 23568 Lübeck, Germany

Ultrasound is the first choice to study the neurovascular status in patients with cerebrovascular disorders. Both extra- and intracranial arteries can be depicted with high diagnostic confidence. However, diagnostic problems due to ultrasound attenuation limit the clinical usefulness in a number of patients. Shadowing, unfavourable anatomic course and reduced Doppler signal amplitude due to high grade carotid stenosis are the main reasons for limited accessibility of the extracranial arteries („low flow, slow flow or no flow"). Transcranially, insufficient ultrasound penetration through the temporal scull hampers examination in up to 20 to 50% of patients, depending on age, sex and race.

Echocontrast agents (EA) are supposed to overcome these problems. There is increasing evidence, that ultrasound contrast imaging allows better depiction of high grade carotid artery stenosis, the vertebrobasilar system and intracranial cerebral artery occlusions. Acute stroke appears to be the most promising application.

Brain tumor vascularisation can be demonstrated with EA with a much higher sensitivity. Ongoing clinical trials aim to define the practical implications and the role of transcranial color duplex sonography as a tool for tumor diagnosis, classification and therapeutic monitoring.
Upcoming new technology will allow to investigate potentials of echocontrast enhancement in brain parenchyma.

ULTRASOUND CONTRAST - ENHANCEMENT IN THE CAROTID ARTERY TERRITORY

B. Griewing, C. Morgenstern, L. Motsch, C. Kessler
Department of Neurology, Ernst-Moritz-Arndt-University, Greifswald, Germany

Background:
Most clinical events of TIA or stroke occur in the area supplied by the carotid artery. Echo-enhancing agents improve signal intensity and the signal-to-noise ration, creating opportunities for diagnostic ultrasound of the extra- and intracranial carotid artery territory.

Patients and Methods:
Extra- and transcranial color Doppler (TCCD) imaging was performed using 10 MHz linear array and 2,25 MHz phased-array systems (Masters/Diasonics, Munich ,Germany). Levovist (Schering, Berlin, Germany), a galactose-based echo-enhancer was administered for extracranial imaging in a dose of 200 mg/ml and for transcranial duplexsonography in a dose of 400 mg/ml. 52 patients with the following vascular diseases in the carotid artery territory were examined prior to and following Levovist injection: Extracranial high-grade stenosis and occlusions (n=28), carotid artery aneurysms (n=4), intracranial stenosis of the middle cerebral artery (MCA: n=8), and arteriovenous malfomations (AVM: n=12). All patients underwent corroborative digital subtraction angiography (DSA).

Results:
In patients with carotid artery stenosis, aneurysms, or occlusions, Levovist either improved the quality of the color flow, permitted the initial diagnosis to be made with greater confidence, or corrected the initial diagnostic impression in 19/32 patients. Similar advantages for TCCD imaging were seen in 11/20 patients with MCA stenosis and AVM's. Echo-enhanced duplexsonography was unable to confirm DSA diagnoses in two cases of extracranial- and one of intracranial carotid artery disease.

Conclusions:
This study suggests that contrast-enhanced extra- and transcranial duplexsonography of the carotid artery territory facilitates the diagnosis and follow-up of associated vascular diseases.

TRANSCRANIAL COLOUR CODED DUPLEX SONOGRAPHY WITH AN ECHO CONTRAST AGENT (LEVOVIST®) IN ACUTE STROKE PATIENTS

M. Görtler

Department of Neurology, University of Magdeburg, Germany

Background:

There exists only a small time window for an effective treatment in acute stroke patients, ranging from up to 3 hours for an intravenous thrombolysis to 6 to 12 hours for decreasing an elevated body temperature. Diagnostic examinations in this situation needs to be fast, valid and of therapeutic relevance. The present study aims to evaluate prospectively the time period and intracranial vascular diagnostic ability of echo enhanced transcranial color coded duplex sonography (TCCS) in acute stroke patients. Results of the first 9 patients will be presented.

Patients and Method:

Nine patients were admitted to our hospital with symptoms suggesting an acute stroke. Six patients suffered a disabling, 2 patients a minor stroke, one had a prind. Age ranged from 36 to 89 (median 69) years. All patients underwent conventional and color-coded Doppler-/ duplex sonography of the extra- and intracranial arteries prior to the echo enhanced TCCS. Additionally all patients had a cerebral CT scan, a neurological examination, an ECG, an X-ray of the chest and a laboratory examination. The time period between onset of the first stroke symptoms and the sonographic examination ranged from 2 to 12 hours in 7 patients and was more then 12 hours in 2 patients. TCCS was performed with a 2.5 MHz sector probe through a temporal bone window. The echo contrast agent (Levovist®) was injected intravenously over 30s (4g, 300 mg/ml).

Results:

The echo enhanced TCCS lasted 4 to 6 minutes in 7 and 12 to 15 minutes in 2 patients (second examination). The whole sonographic examination ranged from 20 to 26 minutes. In 5/9 patients intracranial arteries could not be insonated and in 2/9 a definite diagnosis of the vascular pathology could not be given by conventional extra- and intracranial sonography. After the application of Levovist® 3 of these 5 patients sufficiently could be insonated and diagnosed definitively as well as the 2 patients with an insufficient diagnosis prior to the contrast agent.

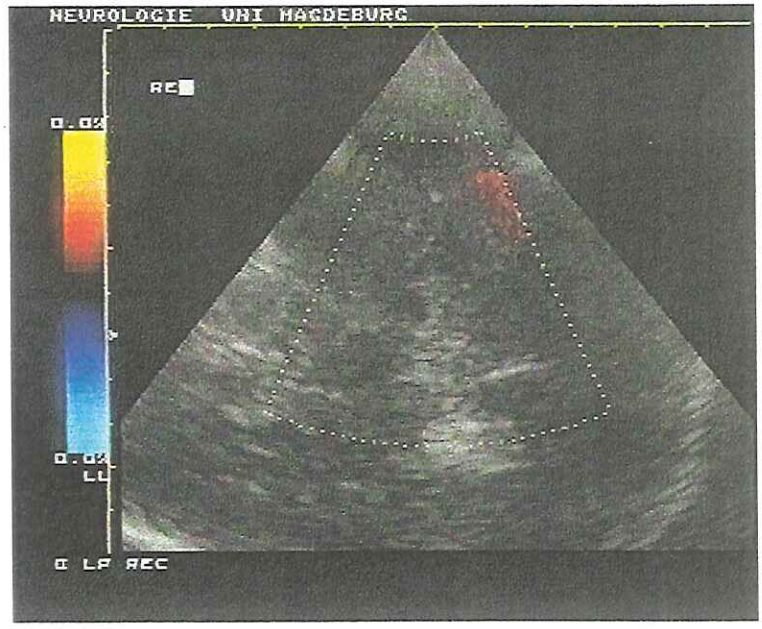
Conclusions:

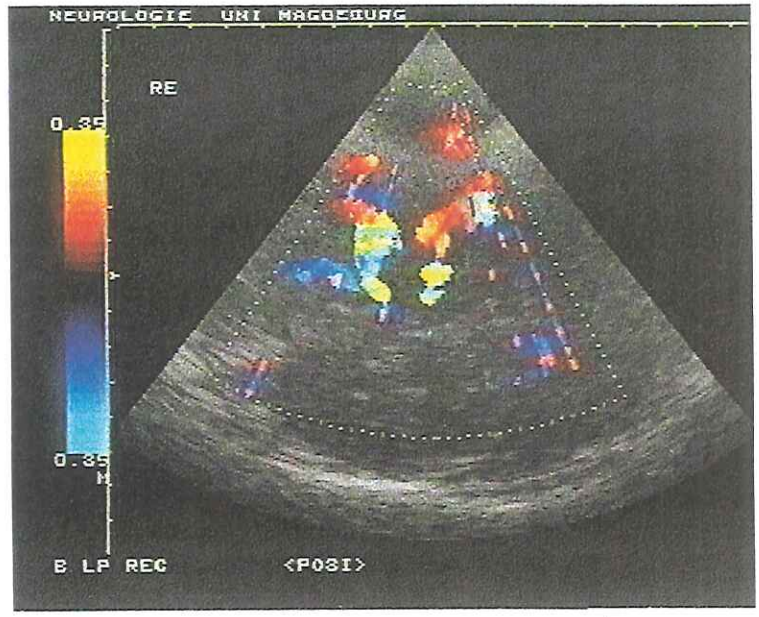
The application of echo contrast agent (Levovist®) seems to improve the diagnostic ability of transcranial Doppler-/ duplex examination in acute stroke patients without additionally time loss for therapeutic interventions.

Images: Patient with an left hemispheric stroke, acute onset 2:50 hours prior to the examination. No temporal bone window on the left side. Insonation through a right temporal bone window.

Image 1: TCCS without echo contrast agent. Arteries of the circle of Willis not detectable.

Image 2: TCCS after echo contrast agent. Occlusion of the main stem of the left middle cerebral artery.





ULTRASOUND CONTRAST IMAGING, THE HP APPROACH

A. D'Sa, P. Rafter
Hewlett Packard, Andover, USA

Hewlett-Packard's approach to contrast agent imaging can be summed up as follows: sensitive detection of contrast microbubbles, reliable and reproducible quantitation of the contrast effect, and joint partnership with pharmaceutical companies with the goal of succeeding in perfusion imaging using ultrasound technology. HP has made Harmonic Imaging available to the pharmaceutical companies for the animal and human phase trials of their agents. We have been working closely with many of these companies, trying to optimize the performance of our system and supporting them in the development of new agents. Acoustic Densitometry (AD) remains the only commercially available quantitation package based on acoustic or RF data. It's features include very flexible storage and triggering (a frame rate control and the ability of fire a frame every 1 - 99 cardiac cycles). However AD's benefits in providing acoustic intensity calculations need to be explored further.

The monitors used in current ultrasound systems have a display dynamic range of approximately 30dB (i.e,10 * log10 (Maximum Video Intensity) / Minimum Video Intensity) = 30 dB or a factor of 1000). The human visual system also has a dynamic range of somewhere around 30dB. Additionally, the human eye has difficulty distinguishing between grey levels which are close in intensity thus making it impractical for systems to display a large number of grey levels. The ultrasound transducers, and front- en system electronics, however, have the capability to far exceed 30dB. In fact ultrasound echo signals handled by scanners can range over 100dB, from the high amplitude signals reflected back from the pericardium / lung interface to the low-level scatter obtained from red blood cells. In order to display the entire range of echoes simultaneously on a 30dB range of monitor grey scales, a non-linear compression mapping (which is often logarithmic) is often implemented. In addition, post-processing curves or mappings are used to aesthetically enhance the images. Compression controls are available to allow the user to further optimize image quality by displaying a greater or smaller range of system signal levels. Often other post-processed non-linear filtering schemes are adapted. Many of these controls drastically change the displayed video intensity (i.e., the mapping of grey levels) without changing any signal levels within the system. Various manufacturers have different non-linear processing and sometimes even the same manufacturer will change their non-linear mappings between revisions of hardware / software. These reasons make the used of

video intensity programs to quantify concentration of contrast agents risky at best and cause reproducibility to become a serious concern between laboratories and even within the same lab.

Hewlett-Packard has recognized these problems early and has addressed them with the on-line AD analysis package, which has been cleared by the FDA. AD measurements are either based on the RF amplitude of the signal or on its Integrated Backscatter (IBS), independent of compression and post-processing controls. The AD program also documents and automatically stores all systems settings digitally along with the image. We believe the need for a quantitative tool is essential if ultrasound perfusion imaging is to replace its nuclear counterpart, which is inherently quantitative. Qualitative analysis (i.e., eyeballing) of video intensity has the same drawbacks discussed previously but also has additional problems - optical illusions, caused by the manner in which the human brain interprets grey levels. Intense microbubble signals from the left ventricular cavity are both distracting And can cause the eye to misinterpret the data.

AD currently offers "time-intensity" curves in dB (10 * log10 (RF Intensity)) or in RF-detected amplitude units. AD gives the user a standard which is recognized in many fields worldwide (dB) and also allows for the correction of a common problem. Baseline subtraction, although typically done on off-line packages in the video intensity domain, should only be done with RF Intensity if accuracy and reproducibility are desired. If done in the video intensity domain, or logarithmic domain, the answer will depend on the initial intensity. The following example illustrates this concept.

$$\mathbf{10^* \log10\,(It + Ib) - 10^*\log10\,(It) = 10 * \log10\,((It + Ib) / It)}$$

where:

It = Intensity from Tissue
Ib = Intensity from bubbles

It can be seen from the above equation that higher values of tissue intensity will "mask" the added intensity from the contrast agent. Due to the myocardial tissue anisotropy (i.e., acoustical drop-out due to fiber orientation) unequal it is not uncommon for tissue signals to vary over a range of 15 dB or more in an image scan. However, if proper baseline subtraction of RF Intensity is performed it would be possible to see signals much smaller than the tissue (i.e., {Ib + It} - It = Ib !!!!).

This method will undoubtedly be more sensitive to detection of smaller amounts of contrast.

References:

Eriksen, Morten (Nycomed), "Tissue Echo Intensity and Blood Attenuation Changes: The Pitfalls of Video Densitometry" (Abstract), The Leading Edge, May 7 1996; pg 31-35, Atlantic City, NJ, USA

Bos, L.J. et al, "Subtraction of Background Intensity from the Time Intensity Curves: A Pitfall in Flow Assessment Using Contrast Echocardiography" (Abstract), 1st European Symposium on Ultrasound Contrast Imaging, Jan 25-26 1996, Rotterdam, The Netherlands.

INSTRUMENT FACTORS AND STRATEGIES FOR LIMITING AND TAKING ADVANTAGE OF BUBBLE DESTRUCTION

D.J. Sahn, K.W. Walker, M. Williams, M. Crume, G.D. Giraud, S.E. Grauer, G. A. Pantely

Departments of Medicine and Pediatrics; Clinical Care Center for Congenital Heart Disease. Oregon Health Sciences University, Portland, OR, USA

The effect of time, temperature and acoustic power output on the stability of two echocontrast agents (*Aerosomes MRX115*, ImaRx Pharmaceuticals and *Imagent US*, Alliance Pharmaceuticals) was tested in vitro. Microbubbles were counted with an optical sizer over 60 minutes at water bath temperatures of 20 and 37 centigrade (°C), and after exposure to gain settings of 10, 20, 30, 40 and 50. The study used a Hewlett-Packard 2500 echocardiography machine and a 1.8 MHz transducer which imaged in standard and harmonic modes.

At 20 °C 81% of the microbubbles were still present after 60 minutes. Exposing the microbubbles to 37 °C resulted in an initial rapid fall in counts to 58% of baseline with a slower additional loss over the next 60 minutes to 45% of baseline.. A 3.5 second exposure of either microbubble to 1.8 MHz ultrasound produced no significant destruction at gain settings of less than 30, but over 80% of the microbubbles were destroyed at gain settings of 40 and 50 ($P<0.0001$). Both standard and harmonic modes demonstrated equivalent bubble destruction. A similar degree of bubble destruction occurred with a single triggered frame with *Aerosomes* and *Imagent*.

These two echocontrast agents are rapidly and thoroughly destroyed when acoustic power output exceeds a certain threshold, but they remain intact when power output remains below this threshold. These results have importance and the use of microbubbles as flow tracers or as delivery agents, and, although potentially disadvantages they could yield new strategies for quantitation.

STORY WITH CONTRAST AGENT MIMICKING CONTRAST AGENT

Y. Takeuchi
GE-Yokogwa Medical Systems, Ltd, Hino, 191 Japan

Instrumention engineer needs anyway some means to simulate the object to measured by one's newly made fantastic measuring instrument, including image or cookers or vehicles or any, before challenging to real object in real world. These means are called dummy load, test course, signal generator, or phantom, etc.. Eventually they are also necessary to calibrate or to test performance of the instrument. The story to be presented here is that of my case to find and use a real agent mimicking microballoon useful from basic research to system development, and possibly field calibration, of the fundamental and harmonics imagers that we concern.

My answer is to use PVC-AN (polyvinylidenchloride-acrylnitryl copolymer) micro-balloon, of avg. size about 4 micron, volume occupancy factor of gas (air) about 90% or slightly more, (as known), coated by undisclosed surfactant for hydrophilic behavior. The material in origins plastic filler to whiten or to light weight.

Almost every acoustical phenomenon seen with real agents can be simulated more or less with this material qualitatively, including harmonics and sub-harmonics emission or echogen, stimulated emission (it is actually not emission but sudden change of echo genicity on microballoon shell destruction) or, in my terminology, "scintillation".

With flow-through phantom, this material looks not so much different than known available blood cell mimicking solid particle, except it yields pseudo-Doppler signal, even when flow is stopping, with its scintillation mode.

Its 1/10,000 to 1/100,000 agar suspension looks in color (and power) mode a spectacular color mosaic pattern (in power mode, sunrise shininess) with its scintillation mode. In B-mode, crawling worm pattern is seen, which indicates sub-speckle echogenicity change by dying microballoon. In pulsed (and in some case CW also) Doppler mode the echogenicity scintillating by dying agent gives symmetrically double side-banded, very wide-band white pseudo-Doppler signal. When this is happening, range gated echo from the agar suspension has mild 2^{nd} harmonics and sub(1/2f)harmonics signals, together with unnegligible out of band leakage spectrum.

The color mosaic pattern dies by and by, the thinner agar suspension dying faster, in some second to even in an hour. After a total death of a scan plane its echogenicity is significantly lower (darker), which also can be visualized by the imager itself. The acoustic "wound" is also optically visible in major cases, as shown by someone in prior meeting, "records" beam and scan pattern. Postmortal specimen of the agar shows microscopic image of crushed shell of the balloon, obviously optically almost transparent. The record keeps months or more for the PVC shell doesn't melt or solve in water, until agar gets rotten.

Eventually by limited experiences with real agents like Echovist, Levovist, etc, author is confident that they show almost same phenomena as PVC microballoon here introduced show. Only big difference is in-water solubility/lifetime. However, shell thickness or strength seem relatively different, breakdown threshold seems much higher in case of my PVC microballoon than real agents.

In conclusion: this sort of microballoon seem known best mimicking material for the real agent, although with some major differences in shell thickness or strength. It eliminates the worry about availability, in-water lifetime, fragility, and expense. For engineer and engineering, anyway this sort of mimicking material is crucial, in order to proceed research and development activity in vitro. For application consideration, if scintillation mode is better sensitivity and specificity than 2nd harmonics mode in some application, the latter may be unnecessary or even unsuitable. However, it depends vastly on the natures of each microballoon agent.

CONTRAST IMAGING--THE ATL APPROACH

J. Powers, M. Averkiou, M. Bruce
Advanced Technology Laboratories, Bothell, WA, USA

The approach ATL is taking to the development of contrast agent imaging consists of many components. Some of the most important ones are the following:

- Work with contrast manufacturers and clinical researchers developing new technology.
- Study the physics of interaction of ultrasound with contrast agent microbubbles.
- Provide state of the art power control to prevent or cause microbubble destruction.
- Design the imaging system to be as sensitive as possible to detecting their presence.
- Develop quantification techniques for quantifying myocardial perfusion.

Contrast imaging requires a very strong alliance between the equipment manufacturer, contrast manufacturers, and clinical researchers. ATL has established ties with most contrast manufacturers and the physicians involved with their clinical trials. In addition, ATL is supporting more fundamental research in other labs directed toward better understanding of the underlying physics and development of quantification techniques.

The physics of microbubble interaction with ultrasound is complex. At low ultrasound intensities the bubbles act as linear scatterers. At intermediate intensities they produce harmonic components of the insonifying frequency. At the high end of FDA approved limits, the bubbles can be destroyed. The bubble destruction event also contains higher harmonic components. The acoustic pressure amplitude is variable over the incident sound beam so that a combination of the above mentioned effects may occur simultaneously. Even in the simplest case of a free gas bubble in water, bubble oscillation at pressures approaching the threshold of bubble destruction is quite challenging for the experts in the field. A number of other factors further complicate the situation in-vivo. The frequencies and power levels used by the ultrasound machine, the size, shell, and gas characteristics of the microbubbles, and the physical properties of blood and tissue are important factors in bubble oscillation and destruction. Better understanding of this complex situation will lead to more effective and sensitive detection of contrast agents in tissue.

Precise power control and display of incident acoustic energy is critical to contrast agent imaging. The Mechanical Index (MI) was recently developed to represent the likelihood

of ultrasound producing cavitation, assuming there are already cavitation sites present. It is defined as:

$$\mathbf{MI = P_- / SQRT\ (f_o)}$$

where:
P_- is the peak negative pressure of the incident acoustic field, and f_o is the center frequency. Through knowledge of this parameter, the user can estimate how likely it is that microbubbles are being destroyed. ATL was the first ultrasound company to implement the mechanical index display.

The broadband digital beamformer and signal processing in the HDI 3000 makes it the ideal platform for the development of new, more sensitive imaging techniques for contrast agents. ATL pioneered harmonic imaging in 1991 through our work with Dr. Peter Burns and Schering AG. Harmonic imaging selectively enhances microbubbles due to their nonlinear behavior and has become the de facto standard for cardiac contrast imaging. Harmonic B-mode on the HDI 3000, has been used in trials of many different contrast agents in more than 25 sites worldwide. Power Harmonics™, the newest contrast imaging technology from ATL, combines the harmonic signature of the contrast agent with the sensitivity of Doppler power imaging, leading to further increases in sensitivity. These two features are now available as a software upgrade to the HDI 3000.

Quantification of myocardial perfusion is the ultimate goal of both contrast and equipment manufacturers, as well as the many clinical researchers working in this field. While video densitometry, the only currently available tool, provides some level of quantification of contrast intensity, it suffers from a number of problems which need to be solved before it is likely to have significant clinical application. Cardiac motion, even with ECG gating, can cause misalignment from frame to frame requiring realignment of consecutive frames. Even with a rapid injection, the bolus becomes so spread out through the heart and lungs that the small changes in transit time due to stenosis will be extremely difficult to measure. These as well as other issues will need to be solved before video densitometry proves clinically applicable on a routine basis. ATL is investigating multiple avenues of contrast quantification with the ultimate goal of reliable myocardial perfusion measurement.

AN INVESTIGATION OF ULTRASONIC CONTRAST AGENTS BACKSCATTERING PROPERTIES WHEN INTRODUCED INTO SOLUTIONS WITH VARYING OXYGEN LEVELS AND DIFFERENT ULTRASONIC PRESSURES.

V. Sboros, C.M. Moran, I.C. Macleod., W.M. Geake, D.H. Glass, A.M. Millar, T. Anderson, G.R.. Sutherland**, W.N. McDicken.*

*Dept. Of Medical Physics and Medical Engeneering, *Dept. Of Chemical Engineering, ** Dept. Of Cardiology, University of Edinburgh, Edinburgh.*

An in-vitro system has been set-up to investigate the properties of ultrasonic contrast agents by measuring the integrated backscatter from different contrast agents under different oxygen saturation conditions, and in addition when subjected to different acoustic pressures. In an initial study the contrast agents were introduced into suspending media of different oxygen concentration levels, and their relative stability was assessed by measurements of change in integrated backscatter from the solutions. The partial pressures of oxygen in those solutions ranged between 1.5 and 26kPa. In a second study, the variation in integrated backscatter, from each of the solutions, was measured under seven different acoustic pressures. These pressures ranged from 0.27 Mpa to 1.27 Mpa (maximum peak negative pressure within the field). In all the experiments sterile water was used as the suspending medium. Three intravenous (Levovist, MRX-115, Quantison™), and one arterial (Quantison™ Depot) contrast agents were used in the first study,. Only MRX-115, Quantison™, and Quantison™Depot were used in the second study. The agents and solutions were put in a tank which was lined with an acoustic absorber. Image frames of digitized RF echo data were collected from an ATL Ultramark 9 Scanner (UM9) after being amplified and time gain compensated at present levels. Signal digitization was performed at 12MHz, to 16 bit precision. An image frame consisted of 128 lines, with 1024 samples in each. The images were transferred to a Sun workstation for statistical analysis. The integrated backscatter of a region of interest within the focal region was calculated. Over a period of one minute, during which the solutions were insonated for ½ sec at intervals of 30 sec, for an air-saturated solution (pO_2=26kPa), the decrease in integrated backscatter for MRX-115, Quantison™Depot, Quantison™, and Levovist was 0.26 ± 0.02 dB, 0.26 ± 0.02 dB, 0.12 ± 0.11 dB, and 2.12 ± 0.13 dB respectively. Over the same period of time and insonation conditions, for a degassed solution (pO_2=1,5kPa), the decrease in integrated backscatter for the above four agents was 0.31 ± 0.06 dB, 0.24 ± 0.03 dB, 0.04 ± 0.09 dB, and 10.60 ± 2.16 dB, respectively. From the agents that were tested, Levovist showed the highest sensitivity to oxygen concentration in the solution, while

the other three proved tolerant over the above range of oxygen concentration. Increasing the time of insonation to 2 sec per 30 sec, and over a period of 5 minutes in an air-saturated solution (pO_2=26kPa), the decrease in integrated backscatter for MRX-115, and Quantison™Depot was 5.80 ± 3.15 dB and 3.54 ± 1.16 dB for maximum ultrasonic pressure, and 1.91 ± 1.07 dB, and 1.96 ± 0.49 dB for the lowest ultrasonic pressure. Over the same period of time in a degassed solution (pO_2=1.5kPa), the decrease in integrated backscatter for the two above agents was and 4.07 ± 1.14 dB and 3.42 ± 0.22 dB for maximum ultrasonic pressure, and 1.08 ± 0.08 dB and 1.22 ± 0.07 dB for the lowest ultrasonic pressure. Quantison™, apart from the two lowest pressures, did not show good correlation with our model of exponential decay. The set-up used in this study showed that stability studies for contrast agents, and also other investigations of the properties of contrast, can be carried out reproducibly.

Insonation per 30 sec (sec)	*pO_2 (kPa)*	*Percentage Output Power*	**Levovist***	**Quantison ™**	**Quantison ™ Depot**	**MRX-115**
0.5	1.5	2.24	10.60 ± 2.16	0.04 ± 0.09	0.24 ± 0.03	0.31 ± 0.06
0.5	26	2.24	2.12 ± 0.13	0.12 ± 0.11	0.26 ± 0.02	0.26 ± 0.02
2	1.5	100	-	r<<	3.42 ± 0.22	4.07 ± 1.14
2	1.5	2.24	-	0.18 ± 0.16	1.22 ± 0.07	1.08 ± 0.08
2	26	100	-	r<<	3.54 ± 1.16	5.80 ± 3.15
2	26	2.24	-	0.20 ± 0.02	1.96 ± 0.49	1.91 ± 1.07

(*Levovist was insonated ½ sec per 10 sec)

SECOND HARMONIC IMAGINGWITH LEVOVIST: INITIAL CLINICAL EXPERIENCE

H. Becher, K. Tiemann
Medische University Clinic Bonn, Germany

Harmonic imaging has been introduced to enhance the signals of contrast microbubbles. In a first clinical study 15 patients with ischemic heart disease were investigated using an HDI 3000 imager (ATL). Two dosages of intravenous Levovist were given (10ml 400 mg/ml and 10 ml 300 mg/ml). Four and two chamber views (4CV, 2CV) were obtained using fundamental B-scan (FB), harmonic B-scan (HB) and harmonic power Doppler (HPD). By HPD the presence of microbubbles in a cavity or a tissue is imaged by colored pixels overlaying a conventional B-scan. Left ventricular opacification as not homogeneous with continuous scanning in all three imaging modalities. Intermittent scanning (endsystole and enddiastole) was introduced to reduce dissolution of the microbubbles in the acoustic field. In FB and HB Levovist opacified the cavities, but reduced the differences in the grey levels between the cavities and the myocardium thus obscuring the borders of the myocardium. HPD provided intensive and homogeneous contrast effects in the LV cavity and good delineation of the myocardium in all patients. When HPD signals were detected in the myocardium, these signals appeared less homogeneous and more reticular than the opacification in the cavities. LV ejection fraction measured from HPD signals compared well with the results of the baseline B-scan (r=0.92).

Conclusion:
Harmonic imaging with Levovist is feasible. The power Doppler mode visualizes myocardial uptake of contrast and can be used for definition of endocardial borders.

ENHANCED DELIVERY ANTISENSE OLIGONUCLEOTIDES WHEN BOUND TO INTRAVENOUS PERFLUOROCARBON-FILLED MICROBUBBLES: EFFECT OF ULTRASOUND AND THERAPEUTIC IMPLICATION.

T. Porter, S. Li, K. Kilzer, J. Desjardins, P. Iversen.
University of Nebraska Medical Center, Omaha, Nebraska, USA

In addition to ultrasound contrast, perfluorocarbon-exposed sonicated dextrose albumin (PESD) microbubbles (MB) are capable of binding agents which modulate gene expression like antisense oligonucleotides (AON). Targeted AON delivery could selectively inhibit gene expression (exp). Since diagnostic ultrasound (DUS) is capable of destroying PESDA microbubbles, we tested the effect of DUS on the amount of vascular deposition of both a 15-mer, 20-mer, and 24-mer bound to intravenously injected PESDA in 14 dogs. The right carotid artery underwent external ultrasound with a 2.0 MHz diagnostic ultrasound probe, while the left carotid was not insonified. Secondly, we tested the ability of AON bound to PESDA to modulate gene expression in the liver by testing the changes in efficacy, potency, and specificity of AON's antisense to cytochrome P450 3A2 (CYP3A2) when given intravenously to adult male rats bound and unbound to PESDA in the absence of DUS.

Vascular uptake of 15-mer, 20-mer, and 24-mer AON in dogs were all significantly higher in both the right and left carotid artery when given intravenously bound to PESDA. There was a trend toward higher deposition in the insonified right carotid artery. In rats, it took 4mg/kg of AON to inhibit CYP3A2 expression in the absence of PESDA, while a dose of only 0.06 mg/kg of the same AON produced equivalent inhibition when given bound to PESDA microbubbles. This nearly 10-fold increase in antisense inhibition of CYP3A2 expression was also seen with other forms of antisense oligonucleotides, and was specific for inhibition of CYP3A2 expression. These studies demonstrate that PESDA microbubbles in the absence of ultrasound can significantly improve the biologic effectiveness of targeted gene therapy.

INCREASE OF THE MYOCARDIAL INTENSITY - IS IT SUFFICIENT FOR PERFUSION ANALYSIS? CLINICAL EXPERIENCE WITH SECOND HARMONIC AND TRIGGER-MODE AFTER INTRAVENOUS INJECTION OF TRANSPULMONARY CONTRAST AGENTS IN PATIENTS WITH CORONARY DISEASE

R. Leischik, R. Erbel
University Clinic Essen, Essen, Germany

Non-linear oscillations of the microbubbles and the use of second harmonic-technique create a real-time subtractions of the grayscale blood flow image in the tissue. This technique permits to detect the microbubbles with a high sensitivity and improves the visualization of the intracavitary contrast in comparison of the fundamental mode.

The great hope of the cardiologists is to detect perfusion defects after intravenous injection of transpulmonary contrast agents with this new imaging technique. The further hope is to quantify the perfusion by videodensitometry or raw data analysis using parameter of the wash-out curves.

At the moment it is enough, that a good left ventricular contrast can be achieved after intravenous injection of a contrast agent in all patients if second harmonic and triggering will be used. In young, healthy volunteers it is in most of the cases not necessary to use the second harmonic technique for good opacification of left ventricular cavity if the second generation of transpulmonary contrast agents will be used.

Second harmonic technique visualize an enhancement of myocardial intensity in a better was than conventional echocardiography but it is difficult to achieve reproducible and reliable measurements for perfusion analysis. The different scanning conditions, the poor transthoracic ultrasound window and not sufficient enhancement of the myocardial intensity makes it problematic to quantify the myocardial perfusion. In patients with two or three vessel coronary disease it will be more difficult to perform a reliable perfusion analysis caused by not predictable and not sufficient distribution of contrast agent in the myocardium.

We tested in 20 patients with coronary disease the use of second harmonic (ATL/HP) for visualization of perfusion defects or differences in enhancement of myocardial intensity after 4 x 5 ml and 1 x 10ml injections of BY 963. A reliable discrimination of differences in intensity distribution could not be seen. The different intensities of the

myocardium at the basic scanning conditions before administration of a contrast agent were a further possible cause for not reliable quantitative and qualitative analysis of perfusion defects.

Additionally there were differences in the myocardial opacification dependent of the use of ultrasound equipment (ATL HDI 3000 or HP 2500). Using ATL HDI 3000 we estimated the number of heart cycles with sufficient opacification of left ventricular cavity using conventional (TTE) and second harmonic technique (SH combined with trigger-Mode (TrM).

Results:

	TTE	TTE + TrM	SH	SH + TrM
Number of heart cycles	23 ± 7*	37 ± 10 #*	29 ± 6**	58 ± 11#*
Pulmonary transit time	6,7 ± 1	6,5 ± 2	6,1± 1	6,1± 2

*p=0,003; # p<0,001; ** p=0,001

A good systolic opacification of the apical region could be achieved in 4/10 patients by the conventional technique. If second harmonic technique and trigger was used a good opacification of left ventricular apex (even in the systole) in all patients (10 / 10) could be seen. The best contrast effect were estimated using second harmonic technique and triggering. The enhancement of myocardial contrast intensity was seen in all patients.

The myocardial intensity values increased in comparison to the basic conditions up to 10 - 20%. The increase of the intensity in the left ventricular septum was greater than in other myocardial segments (4-chamber-view / 2-chamber-view).

At the moment we have sufficient results for "myocardial intensity increase-assessment", but not sufficient for clinical useful and reliable quantitative or qualitative perfusion analysis.
Further studies with raw data analysis, power-mode-techniques and transesophageal approach should be performed for investigation of myocardial perfusion using transpulmonary contrast agents.

SECOND HARMONIC IMAGING OF QUANTISON

P.A. van der Wouw, C. Moran, A.C. Brauns, L. Fen, S.E. Bailey, R. Johnson
University of Amsterdam, TheNetherlands, University of Edinburgh and Andaris Limited, Nottingham, UK.

Quantison™ is a new ultrasound contrast agent, consisting of air-filled cross-linked albumin microcapsules (mc). It has been shown to give good LV opacification in volunteers, when using optimized ultrasound machine settings in fundamental mode. A study in healthy volunteers was undertaken to study the effect of second harmonic imaging on LV opacification and myocardial perfusion. Five healthy male volunteers received three doses of Quantison™ while triggered and continuous transthoracic imaging with two ultrasound machines equipped with second harmonic imaging took place. The ultrasound machines used where the Hewlett-Packard Sonos 2500 with 1.8-3.6 MHz transducer and the ATL HDI 3000 with P3-2 transducer and second harmonic software. Both machines where used simultaneously, one at the apical psition and the other at the parasternal position. As one machine was triggered in systole and the other in diastole, no interference was visible in the ultrasound image. The injected doses of Quantison™ ranged from 1-100 million mc/kg. In two subjects dipyridamol was given prior to the third injection, in order to improve myocardial perfusion.

In contrast with the earlier results with fundamental imaging, much lower doses gave good LV opacification using second harmonic imaging. Myocardial perfusion was clearly visible at doses of 10 mil mc/kg and more, while with a dose of 100 mil mc/kg shadowing caused decreased visibility of contrast farther away from the transducer.

LV opacification and myocardial perfusion:

dose (mil mc/kg)	total N subj	perfusion detected	adequate LV opacification
1	2	1	1
10	5	5	4
25	2	2	2
100	1	1	1

Second harmonic imaging improves both LV opacification and myocardial perfusion detection of Quantison™, in doses which are significantly lower compared to fundamental imaging. Dipyridamol improved myocardial perfusion detection.

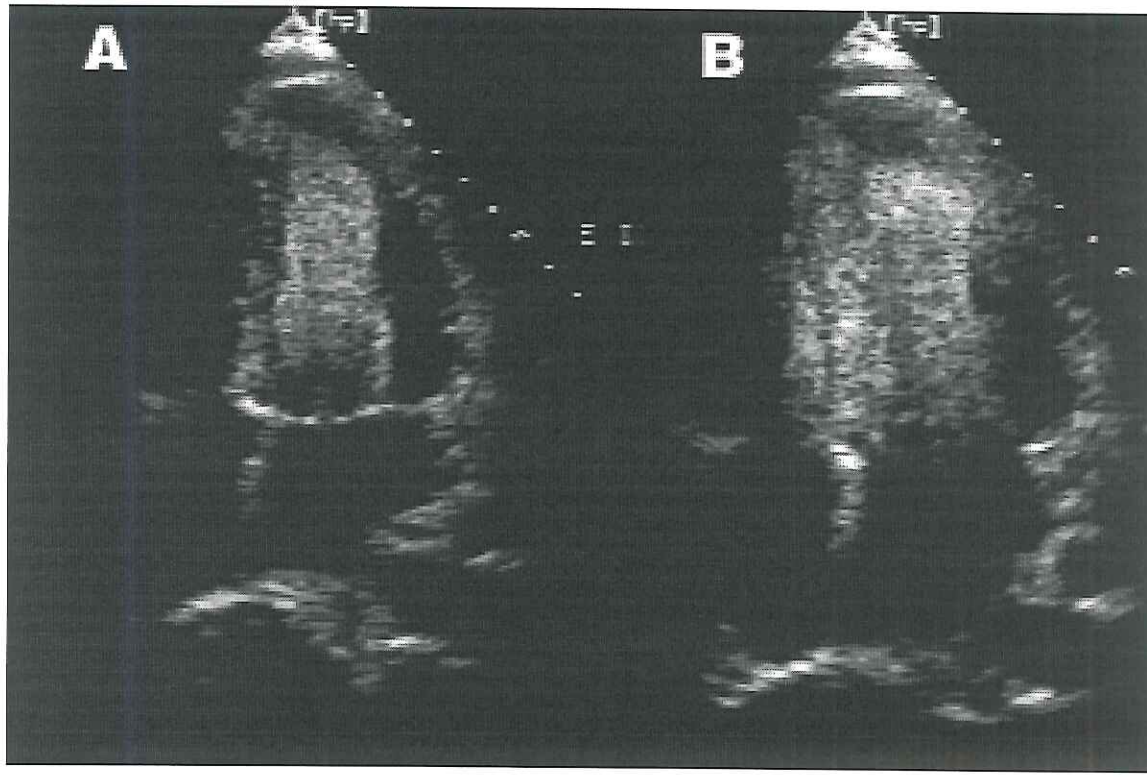


Figure 1: Second harmonic imaging of Quantison, with Second harmonic imaging of Quantison™, with (B) and without (A) dipyridamol. Note contrast enhancement of septum and lateral wall.

INTRACORONARY SITE SPECIFIC DRUG ADMINISTRATION

E.Camenzind
Thoraxcenter, Rotterdam, The Netherlands

Site specific drug administration has been advocated as an adjunctive therapeutic modality to deal more efficaciously with critical issues of interventional cardiology such as thrombolysis, vessel wall passivation and, in particular, restenosis. Attempts to reduce the incidence of restenosis using systemic administration of many different drug classes during and/or following PTCA have all failed to be effective. One exception has been with the use of platelet glycoprotein IIb/IIIa receptor antibodies, but these findings await confirmation. The lack of efficacy of systemic drug administration to prevent restenosis has been attributed to insufficient compound concentration at the site of angioplasty. Site specific delivery has been proposed to result in higher concentrations of chemical compounds at the site of angioplasty.

The used modalities to deliver drugs endovascularly are of 2 types: (1) catheter based delivery and (2) stent based delivery.

Historically, the first local drug delivery device used for endovascular drug administration was the porous balloon and consisted of an angioplasty balloon with laser-made perforations around its circumference. Since, a few different catheters have been developed to avoid the jet-stream lesions to the vessel wall induced by the high local infusion pressure.
'Eluting' stents are stents allowing the release of the drug from the stent surface and are object of important research efforts. In contrast, the 'coated' stents, already in clinical use, are not designed to release pharmacological agents.

As far different pharmacological agents have been used in man, which may be classified into 2 main categories either 'non-genetic'/conventional or genetic - in the broad sense of the term - including the use of antisense oligonucleotides. The site specific drug delivery modality has open the way to endovascular gene therapy, as therapeutic option.

The future perspective of local drug delivery will depend on the ability to demonstrate and to quantify site specific infused drugs in man and thus allow to establish a link between acute delivery efficiency and long-term clinical outcome.

MYOCARDIAL PERFUSION USING SECOND GENERATION CONTRAST AGENTS

M. J. Monaghan
Consultant Clinical Scientist & Care Group Director for Cardiology.
King's College Hospital, London, UK

Approximately 12 ultrasound contrast agents are currently undergoing phase I or phase II trials at present. In most of these trials, demonstrating myocardial perfusion is a major end point. However, it is also evident that many agents, when coupled with appropriate imaging technology will also be able to demonstrate kidney and liver perfusion following intravenous injection.

Many studies have indicated that in order to effectively demonstrate myocardial perfusion, changes in imaging technology will be necessary in order to provide maximum sensitivity. These changes include the utilization of second harmonic imaging or other methods based upon analyzing the backscatter frequency characteristics of contrast agents. In addition, reducing the ultrasound exposure of contrast microspheres within the tissues has been shown to greatly enhance evaluation of perfusion by limiting micro-bubble destruction. This difference appears to be more pronounced with second harmonic imaging rather than fundamental imaging, where only one two dimensional frame is fired every single or every other cardiac cycle, as opposed to conventional continuous imaging, where approximately 30 frames are fired per second are methodologies which reduce contrast agent destruction.

However, there are challenges which still remain in order for echocardiography to be able to provide clinically useful evaluation of myocardial perfusion following intravenous contrast injection. These include the effect of attenuation by the contrast agent within both the right and left ventricular cavities. This effect can substantially mask visualization of some myocardial segments. Unfortunately, by the time that the contrast agent concentration within the cavity has reduced to a level where attenuation no longer exists, the concentration of the contrast agent within the tissues is often insufficient to permit detection.

Finally, techniques also have to be established to facilitate evaluation of reduced perfusion rather than just normal and absent perfusion. Myocardial segments with reduced perfusion may become as saturated with contrast microspheres as those segments with normal perfusion and this can make it difficult to differentiate between the two. Methodologies which utilize destruction of the contrast agent by the ultrasound field, may prove useful,

since well perfused segments will replenish destroyed microspheres quicker than those with poor perfusion, possibly allowing differentiation between the two.

Whilst this field remains very rapidly moving and exciting with clear potential clinical applications, there still remains some methodological hurdles to overcome before it can be routinely applied.

QUANTIFICATION OF MICROBUBBLE ENHANCEMENT USING DOPPLER TECHNIQUES; APPLICATIONS TO PARENCHYMAL IMAGING.

M. Blomley
Hammersmith Hospital, London W12 OHS, UK

There are several reasons why we might wish to quantify the changes produced by microbubble enhancement, but the most important would be to try to calculate physiological indices in order to improve the sensitivity and specificity of ultrasound. This could be useful in both tumor imaging and also in the study of diffuse parenchymal disease, for example, in the kidney. Although B-mode enhancement is likely to be more important in the rapidly-moving myocardium, Doppler based techniques are more sensitive. In this paper, in vitro work on the formal quantification of color Power Doppler imaging will be presented, and a comparison made of the use of harmonic and fundamental modes, different machines, different quantification algorithms and wider and narrow color dynamic ranges.

Current evidence suggest that the most accurate measurements can be made using dedicated software supported by the manufacturer, and that wide color dynamic ranges are appropriate. The clinical relevance to tumor enhancement quantitation will be discussed.

MICROVASCULAR IMAGING WITH SH U 563 A IN A CAPILLARY PHANTOM

A. Bauer, M. Zomack, W. Wendler, A. Urbank, R. Schlief
Clinical R&D Diagnostics, Schering AG Berlin, Germany

Introduction:
With SH U 563 A stimulated acoustic emission due to exposure to high power color Doppler generates a specific response: During a sequence of color Doppler pulses the echo-signal intensity varies substantially.

The color Doppler system interprets this varying intensity from pulse to pulse as random Doppler shifts. Because of this stimulated acoustic emission, the signal returned by one pulse no longer bears any correlation to the pulse sent immediately before it. The resulting loss of correlation pattern gives a map of the distribution of the contrast agent - the localization of the responding microspheres, and we describe this novel technique as LOC-imaging. The accuracy of detecting the contrast agent's position as well as the spatial resolution in LOC-imaging was tested in an in vitro capillary phantom.

Material and Methods:
A microvascular flow phantom was built consisting of 200 capillaries with an average diameter of 200 µm. The flow phantom in a water tank can be designed into varying spatial arrangements. Two bundles of capillaries were separated with by a varying distance to mimics analysis of non perfused vs. perfused areas in organs. The accuracy of delineation of the capillary bundles was imaged by introduction of position markers, and the distance between bundles was compared to the distance seen in the ultrasound image. In all experiments, SH U 563 A was used as a test substance for LOC imaging in varying on concentrations. An 128 ultrasound system, capable of harmonic imaging, was used for all experiments.

Results:
Imaging of the microvascular phantom perfused with SH U 563 A by application of high amplitude Color Doppler Ultrasound (LOC imaging) was feasible independent on geometry. Color Doppler and Power Doppler (color Doppler energy) resulted in a clear delineation of the capillary phantom, and with both even single capillaries could be imaged. The delineation of the phantom was even possible under non-flow conditions for a certain time. A spacing of less than 2 mm between bundles could clearly be separated. The delineation of the phantom was also possible in B-Mode, intermittent

harmonic B-Mode clearly improved the detection limit and the delineation of the capillaries.

Conclusion:

LOC imaging provide an accurate map of microparticle distribution representing microvascular blood volume as proven in a capillary phantom. With the appropriate equipment settings, any of today's color Doppler ultrasound scanners is suitable for LOC imaging, provided a sufficiently high ultrasound amplitude is transmitted. The use of adapted modes, like intermittent harmonic scanning, significantly improves sensitivity.

FACTORS INFLUENCING ULTRASOUND CONTRAST QUALITY

Christiane E. Angermann, Danielle S. Noll, Thorsten Lück, Wolfgang Freiberger, Marie C. Herregods, Bart Bijnens*, Jan D'hooge*, Bruno DeMan*, Sorin Pislaru*, Christina Pislaru*, Carmel Moran+, George R. Sutherland+*

for the European Tissue Characterisation Group, Universities of Munich, Germany, Leuven, Belgium and Edinburgh+, Scotland*

Introduction:

The European Tissue Characterisation (ETC) Group performs joint experiments whose aims are the quantitative assessment of myocardial tissue structure and function. Within the framework of this programme, systematic studies on ultrasonic contrast imaging of the ventricular myocardium and cavities have also been performed.

Methods:

BY 963 (Byk Gulden) and Quantison™ (Andaris) were used as transpulmonary contrast agents. Ultrasonic imaging was performed using a Toshiba SSH 160A, a Hewlett Packard SONOS 2500 equipped with prototype second harmonic facilities or an ATL HDI 3000 CV prototype scanner. 8 anaesthetised closed chest dogs were investigated. During pervenous contrast injections, the following factors were varied in a systematic fashion: Transducer frequency (range 1.8 to 5.0 MHz center frequency), imaging modality (continuous vs. intermittent, fundamental vs. second harmonic (1.8/3.6 MHz (Hewlett-Packard), 1.6/3.2MHz and 2.3/4.6MHz (ATL)), conventional grey scale vs. Power Doppler imaging), transmission power, focal depth.To date, visual evaluation of the studies has been performed.

Results obtained with BY 963:

With conventional grey scale continuous imaging of the left ventricular short axis after a 5 ml pervenous injection, ultrasound contrast quality within the cavity was optimal with the 5MHz transducer with full and lasting homogenous contrast. Contrast quality decreased with a decrease in transducer frequency; using a 1.8 MHz transducer, left ventricular cavity contrast was hardly detectable. Second harmonic imaging with a 1.8/3.6 MHz transducer

did not improve cavity contrast quality, however, intermittent second harmonic imaging with end-diastolic triggering improved contrast quality significantly. Due to a rather fast decrease in cavity contrast intensity after a bolus injection of BY 963, the effects of differing focal depths were not studied in this agent. Irrespective of the transducer frequency used, maximum transmission power reduced contrast quality, whereas a decrease of transmission power accompanied by an increase in gain improved contrast quality. From pervenous injections, no visible myocardial contrast was observed with grey scale imaging. Power Doppler imaging with a 3.2/6.4 MHz transducer further increased left ventricular contrast brightness and homogeneity. Optimal contrast quality was achieved with Power Doppler second harmonic intermittent imaging with end-diastolic or end-systolic triggering. Using this imaging modality, myocardial opacification appeared to be visible afer a 5 ml pervenous injection of BY 963. However, due the complex effects of acoustic shadowing, this was not observed in all segments of the myocardial circumference, thus the clinical diagnostic usefulness of this finding may be limited.

Results obtained with Quantison^{TM}:

With conventional grey scale continuous imaging of the left ventricular short axis after 0.5 to 5ml pervenous injections, ultrasound contrast quality within the cavity was optimal with low transducer frequencies and tended to decrease with higher transducer frequencies. Using a 1.8 MHz transducer, a very prolonged and almost constant left ventricular cavity contrast could be observed. Second harmonic imaging with a 1.8/3.6 or a 2.0/4.0 MHz transducer seemed to improve cavity contrast quality to some extent, however, only intermittent second harmonic imaging with end-diastolic triggering improved contrast quality significantly. A marked effect of the focus position was noted with reduced or absent contrast below the focus with immediate return of visual contrast in these areas after more distant positioning of the focus. Maximum transmission power was found to increase contrast quality, suggesting a non-linear effect. Power Doppler imaging with a 2.0/4.0 MHz transducer further increased left ventricular contrast brightness and homogeneity. Very satisfacory contrast quality was again achieved with second harmonic intermittent imaging with end-diastolic or end-systolic triggering. With this imaging modality, myocardial contrast was also observed.

Conclusion:

These results in 2 different contrast agents seem to further emphasise the necessity of precise characterisation of the optimal imaging conditions for each individual contrast agent in vivo to achieve the best possible ultrasound contrast effects for clinical application. All parameters investigated in this study might be useful tools during the process of modeling of ultrasound contrast agents.

CONTRAST ENHANCED STRESS ECHOCARDIOGRAPHY

P. Nihoyannopoulos
Hammersmith Hospital, London, UK.

Stress echocardiography has become widely available for assessing patients with known or suspected coronary artery disease. In a number of patients however the endocardial definition of one or more myocardial regions cannot be readily identified thus hampering the assessment of regional myocardial thickening. Although regional myocardial perfusion is the ultimate goal of contrast echocardiography, it is possible at present to provide comprehensive left heart cavities opacification and improved endocardial border detection.

In this study we tested the hypothesis that intravenous injection of BR1, will improve endocardial border detection at rest and during dobutamine stress echocardiography. We prospectively studied 12 patients with previous myocardial infarction and known coronary anatomy. The average age was 60±11 years (range 33 to 74). BR1 was injected through a 20 or 18G cannula at randomized dose of 0.5 to 4 mls. The apical 4- and 2-chamber views as well as the parasternal long- and short-axis views were employed at rest and at peak arbutamine infusion, first without contrast immediately followed by a single BR1 injection at rest and another at peak stress. The left ventricle (LV) was divided in 16 regions (ASE criteria). Endocardial border definition was assessed from each of the classic 4 views and for each region separately (total of 22 regions) and graded from 0 (not seen) to 2 (best) before and after BR1 administration. The total score was then added and compared. Regional myocardial thickening was scored as normal=1, hypokinetic=2, akinetic=3, dyskinetic=4. The total ischaemic score and score index was then calculated before and after BR1 administration.

There was no change with regard to the positivity of the test following BR1 injection. There was an overall improvement in endocardial border detection from after BR1 at rest and peak stress. This improved border detection was particularly evident for the anterior basal, inferior basal and lateral regions.

It is concluded that BR1 injection significantly improves endocardial border detection at rest and during peak dobutamine infusion thus facilitating wall motion assessment during arbutamine stress echocardiography.

FUNCTIONAL IMAGING USING CONTRAST ENHANCED-ULTRASONOGRAPHY: THE IMPACT OF RENAL ARTERY STENOSIS ON CORTICAL B-MODE ENHANCEMENT IN DOGS.

J.M. Correas [1], M. Lafortune [2], P.N. Burns[3]

[1]. Department of Radiology, Necker Hospital, Paris, France, [2]. Department of Radiology, St. Luc Hospital, Montreal, Canada, [3]. Imaging Research Sunnybrook Health Science Center and Department of Medical Biophysics University of Toronto, Toronto, Canada.

Purpose:

To determine the relationship between B-mode enhancement of the renal cortex and backscatter enhancement of renal artery blood flow and to correlate this enhancement to the grade of renal artery (RA) stenosis.

Materials and Methods:

An activated emulsion of dodecafluoropentane (EchoGen®, Sonus Pharmaceutical , Bothell, WA) was injected intravenously in 10 dogs at 0.1 ml/kg. The renal cortex was imaged using B-mode at 7.5 MHz (Acuson, Mountain View, CA), S-VHS frames were digitized to a Power PC and mean pixel intensity from the anterior cortex was calculated from 45 end-expiration frames. A vascular occluder was located on the RA or its first segmental branch. The stenosis was evaluated by arteriography. The RA flow signal was recorded using a 10 MHz pulsed Doppler transducer cuff proximal to the occluder. The signals were digitized and analyzed for instantaneous Doppler power. Measurements of Doppler power and B-mode enhancement were plotted after injection for all cases.

Results:

Cortical and medullar enhancements were detected after EchoGen® administration. Grayscale cortical enhancement showed a sigmoid relationship to Doppler power enhancement. There was a slight linear increase under 18 dB and a sharp increase above 18 dB. The signal enhancement in the RA required to achieve cortical enhancement above 10% ranged from 15 to 18 dB. Peak enhancement was 25.3 ± 3.6 dB. With significant RA stenosis (≥ 50% in diameter), the exponential decay of intensity with time disappeared. With major stenosis (> 95%), peak enhancement was delayed and the time-intensity curves exhibited a plateau. When stenosis was located in a RA branch, redistribution of the contrast agent toward the non stenotic cortex was seen.

Conclusion:

A visible cortical enhancement on B-mode imaging was deleted when the integrated backscatter power of blood in the RA increased by at least 18 dB after EchoGen® administration. If the renal cortex can be reliably enhanced on B-mode and individual variations in enhancement controlled, ultrasound contrast agents can be used as bolus indicators of occlusive diseases.

OPTIMAL CHARACTERISTICS OF ULTRASOUND CONTRAST IMAGING

V. Uhlendorf
Research Laboratories of Schering AG, 13342 Berlin, Germany

Currently, almost all ultrasound contrast agents contain microbubbles. Their interaction with diagnostic ultrasound has been studied in recent years, as it forms the basis for improved procedures in ultrasound contrast imaging. Diagnostic efficacy is influenced by equipment parameters as well as by characteristics of the contrast agent. Main areas for optimization are: 1. manufacturing and handling of contrast agents, 2. imaging modes, 3. instrument settings, and 4. potential applications. Since these areas are not independent from each other, a simultaneous development of contrast agents and optimized detection methods is necessary. As a first step Harmonic Imaging was jointly developed by ultrasound contrast manufacturers, equipment companies and clinicians. Clinical studies are starting now and will lead to further improvements in the near future. It may be helpful at this point to summarize some basic characteristics of ultrasound contrast imaging.

It was found to be most important, that in general the response of a single bubble depends on absolute values of the ultrasound peak pressure in situ (transmit amplitude reduced by attenuation), on transmit frequency (transducer), and on pulse duration and repetition rate (imaging mode). In most agents the bubbles are coated with stabilizing films or shells. The core consists either of a gas which is nearly insoluble in water, or it is formed by air. The shell materials of various agents differ considerably, as do their sizes. This leads to different mechanical properties and therefore influences the stability and life time of bubbles under static as well as acoustic pressure, and their acoustic response. The overall response depends on amplitude and signature of the single particle response, on concentration (and flow rate in some cases), but also on the background signal from tissue. Some agents are more sensitive to high amplitude diagnostic ultrasound than others, but both cases can have an advantage in different situations. Sufficient stability under static pressure in the physiological range is required for handling of a contrast agent and for good enhancement on the arterial side.

Although up to now all agents were developed mainly for conventional imaging modes, some may be more useful in special contrast imaging modes. These are Harmonic Imaging and more recently Stimulated Acoustic Emission which uses transient signals during bubble disruption. Both techniques may be combined and each can help to overcome basic physical limitations of conventional contrast imaging, particularly in

tissues with a high scattering coefficient like liver or myocardium. The large variety of possible parameter combinations calls for optimization to find those imaging modes which are most suitable for implementation in prototype scanners. Clinical research will hopefully make use of these characteristics and lead to new applications which may extend the use of diagnostic ultrasound.

Proper choice of instrument settings can be very important for optimal results in ultrasound contrast imaging, both with conventional and with new techniques. The general rules found by investigators progressively will be taken into account in scanner software as "contrast set-ups". Except for a few cases where contrast agents will not improve an insufficient image quality (e.g. in the shadow from bone), the given dose and the instrument settings together with anatomy determine the possible enhancement. Different instrument settings which all result in adequate image quality before contrast injection may significantly differ in image quality during passage of a contrast bolus. In some cases (e.g. in cardiology) there is not a unique optimum unless one imposes additional restrictions. Examples for the influence of the above mentioned parameters will be given.

PRELIMINARY STUDIES IN ULTRASONIC SPECTRAL CONTRAST IMAGING

M. Arditi, T. Brenies, M. Schneider
Bracco Research SA, Switzerland

All microbubble-based contrast agents known have backscattering properties which differ greatly from those of soft tissues. The agent Sonovue™ (formally code named BR1) naturally displays such properties. In addition to the non-linear scattering properties of these agents in response to incident acoustic energy (causing harmonic components in reflected energy), an important difference is observed in the frequency dependence of their backscatter coefficient, when compared to that of tissues. In this situation, any such differential behavior can be exploited by imaging instrumentation to enhance the detection of agent-containing vessels against background tissue, in possibly dramatic proportions. The object of this presentation is to report on imaging studies performed with Sonovue™, aimed at demonstrating the contrast - enhancing potential of spectral rf-signal processing.

The techniques used, termed here "Ultrasonic Spectral Contrast Imaging", is applicable in real-time B-mode imaging. It is based on an on-line subtraction approach, using multi-channel signal processing to implement differential filtering and demodulation. In this preliminary work, USCI was implemented off-line, on rf-echo signals digitized from commercial B-mode scanners. Data acquisition, USCI processing, as well as complete B-mode image reconstruction, was programmed on a personal computer.

The images presented were produced from test phantoms as well as animal phased-array scanning. The phantom included a flow channel, background scattering material, fixed echogenic targets, and echo-free regions. Animal scanning included rabbit liver and mini-pig carotid and kidney. Because of the slow transfer rate to the personal computer, care had to be taken to reduce motion artefacts during animal experiments. Therefore, individual rf-vectors were acquired in synchronization with breathing.

The results obtained from USCI show interesting contrast-enhancing properties. The regions containing no contrast agent are quasi-completely "erased" from the image, leaving only the regions perfused by the contrast agent. This property was favorable to experimenting with image-overlay presentations, superimposing color-coded USCI imaging with standard log-compressed grayscale B-mode, in a way analogous to duplex-

imaging combining color-Doppler and B-mode. Further work in USCI will focus on the optimization of filter characteristics, weighting factors and process algorithms.

GENERATION AND DETECTION OF NEGATIVE CONTRAST BOLUSES FOR USE IN BLOOD FLOW STUDIES

J.B.Fowkles ,D.W. Sirkin, R. Rhee, J.M. Rubin, P.L. Carson
University of Michigan Medical Center, Department of Radiology, Ann Arbor, MI

Our laboratory is currently conducting research on the production of temporally short contrast boluses in arterial blood flow using ultrasound to interrupt microbubble contrast agent. Under normal IV injection of agents, the bolus produced in the arterial blood supply is temporally very long. One possible solution is to use an acoustic beam targeted in an upstream vessel to eliminate contrast flow momentarily. The duration and extent of contrast elimination will determine the ultimate resolution of the flow measurements made in the tissues supplied by this vessel. During preliminary in vivo experiments, ultrasound contrast agent (MRX-115 ImaRx Pharmaceutical Corp.,Tucson, AZ) was administered IV in a drip infusion through the ear vein of a rabbit. A single, sinusoidal tone bursts (1.8 MHz with duration's of 0.25 to 1s) were applied transcutaneously to the femoral artery of rabbits and spectral Doppler data from a position downstream in the arteries measured the duration and degree of signal reduction.

The stored Doppler spectra were processed on a Digital Equipment Corporation Alpha workstation and then tabulated on an Apple Macintosh computer. Each spectrum was integrated over frequencies using AVS software (Advanced Visual Systems, Waltham, Massachusetts) to give a time record of power Doppler. Since the pixel intensities were on a logarithmic scale ("logarithmically compressed" data) in order to enhance the ability of a user to discriminate intensity levels when looking at the screen, these values had to be linearized prior to integration.

The data processing of the power data was one additional point of interest which may have some broader applications to bolus studies. Using the software package Matlab (The MathWorks, Inc., Natick, Massachusetts), a cumulative power Doppler curve (integral over time of the power Doppler record) was generated and then fitted with three connected line segments. This is based on the initial assumption that the interruptions will occur abruptly to a previously constant signal and continue for a specified duration at a lower signal level and then the signal will be restored resulting in a notch or negative pulse. The integral of this waveform would then be a series of three line segments. The program was provided with a first approximation of the function consisting of three connected line segments. The "fmins" function of Matlab was then

applied to get a least squares local best fit of the three-line function to the data. Fmins uses the Nelder-Mead simplex algorithm to find a local minimum. The computer program was provided with the beginning and end points of the data to fit (the entire 4- or 8-second saved data set or else a smaller region around the apparent interruption of contrast was fitted), and starting values of 5 parameters: the slopes of the 3 line segments, m1, m2, m3, and the x-coordinates of the two end points of the middle line segment, p and q. These 5 parameters were adjusted by the computer to get a local least squares fit. The parameters m1 and m3, which represent the average Doppler power before and after the interruption respectively, were allowed to take values that were different from each other. This allowed for better fits in some instances in which the average enhancement from the contrast agent changed somewhat over the recording time segment. The left-most line segment was anchored to the curve at the left end of the region to be fit, obviating the need for a y-intercept as a 6th fitting parameter.

The following quantities were defined by the five fitting parameters m1, m2, m3, p, and q defined above: The fractional reduction in the Doppler power signal is the difference between m2 and the average of m1 and m3, expressed as a fraction of the average of m1 and m3. The interruption delay is the time between onset of the high intensity ultrasound pulse and p. The interruption duration is the time from p to q. The total input is the product of the amplifier pulse duration and voltage, and the total effect is the product of the fractional reduction and the interruption duration.

In all 5 rabbits, transient decreases in contrast enhancement were easily discernible in the Doppler spectra and particularly easy to detect using the data analysis described above. Our quantitative analysis yielded fractional reductions in Doppler power up to 90% and interruption and acoustic burst duration's were approximately equal under specific amplitude conditions. Fractional reductions and duration's generally increased with increasing burst duration and amplitude. The minimum ISPPA required to produce an average fractional reduction of at least 50% for an ultrasound pulse of 0.25 s duration was approximately 2 W/cm^2. Current experiments are being conducted to increase the pressure amplitude in shorter burst in order to reduce the average intensity.

TECHNIQUES OF PERFUSION IMAGING OF THE LIVER USING MICROBUBBLE CONTRAST AGENTS

F. Moriyasu
Department of Gastroenterology and Hepatology, Kyoto University School of Medicine,54 Sakyo-ku, Kyoto, 606-01, Japan

Key words: microbubble, intermittent transmission, harmonic imaging, perfusion imaging, passive cavitation, non-linear emission

1. INTRODUCTION

Why is perfusion imaging necessary for liver diseases? Ultrasound is one of screening examinations in clinical procedures dealing with patients with high risk for liver malignancy; hepatocellular carcinoma in patients with hepatitis virus infection and metastasis in patients with cancers in other organs. In addition to gray scale ultrasound and color Doppler ultrasound, power Doppler ultrasound has been utilized recently to evaluate vascular abnormalities seen in malignant tumors of the liver; that is, arterial hypervascularity, deficit in portal supply, velocity abnormalities in tumor blood flow, portal and hepatic venous thrombosis and etc. Although the criteria of diagnosis of malignant tumors are dependent on abnormalities of blood flow in the tumor tissue, these modalities are not always able to visualize these flow abnormalities. Power Doppler ultrasound in a fully digitized echo equipment, the limitation of minimum detectable velocity would be more than several millimeters per second. Blood flow velocity which perfuses the liver tissue is assessed to be less than 40 micrometers per second at the sinusoidal level. In order to assess abnormalities of perfusion condition in malignant tumors, it is necessary to evaluate and quantify the perfusion rate which is different between normal and pathologically abnormal tissues.

Double blood supplying channels of the liver.

The liver has double channels of blood supply, portal vein and hepatic artery. This matter makes diagnosis and treatment of liver diseases complicated and interested such as arterial embolization therapy. In terms of contrast enhancement with microbubbles, this is important not only because double peaks are observed but also because stickiness of microbubbles against the vascular epithelium is different from that seen in other organs in which artery is the sole blood supplier.

Which kinds of characteristics are necessary for contrast agents in perfusion imaging of the liver ?

The time intensity curve obtained from the liver parenchyma shows a low peak and a slow washout decrease even when the contrast agents are administered by bolus injection into the peripheral vein. This is explained by the fact that the contrast agents in the portal vein have to come through the capillary beds two times, pulmonary and splanchnic ones. Therefore, the ultrasound contrast agents applicable to liver diagnosis should have characteristics described as follows. 1) Enough amount of dose can be administered to reach the liver. 2) They are tolerate to high pressure such as arterial pressure while re-circulating in the body for a certain period.

2. HARMONIC IMAGING OF THE LIVER WITH MICROBUBBLE CONTRAST AGENTS

Correlation between emitted ultrasound and characteristics of the shells and gas contents of microbubbles. Non linear properties of ultrasound backscattered from microbubbles are different between kinds of microbubbles. The ratio of amplitude between fundamental and second harmonic signals is mainly dependent on the shell parameters. At the same time, crushing or destruction of microbubbles provoked by transmitted ultrasound emits non linear components of RF signals in vivo. Many kinds of microbubbles which are under clinical or pre-clinical studies are fragile by ultrasound transmission at the diagnostic level. This means that harmonic imaging system can visualize the microbubbles while destroying microbubbles. Contribution of harmonic imaging to diagnosis of liver malignancy. It has been discussed which harmonic modality is most suitable for imaging of the liver, especially malignant lesions. There have been considered harmonic color Doppler, harmonic power Doppler, and harmonic gray scale imaging. Spatial and temporal resolutions are important in diagnosis of malignant tumors in the liver rather than information of blood flow velocity. Therefore harmonic gray scale imaging is more feasible for diagnosis of neoplastic diseases of the liver. Using harmonic gray scale imaging with intravenous injection of ultrasound contrast agents, it is possible to observe behavior of the microbubbles flowing in the tumor in arterial phase. Contrast enhancement is observed in the viable tumor tissue as subtracted images from the fundamental signals. Non-tumor tissue is fulfilled with bubble signals in the venous phase and it lasts longer. Quantification of blood perfusion of the tissue is performed by videodensitometry obtained from VTR data. A software for intensitometry with large sized image memory should be installed in an ultrasound equipment applicable to contrast enhancement in future.

3. FLASH ECHO IMAGING OF THE LIVER USING INTERMITTENT TRANSMISSION.

RF signals from destruction of microbubbles in the liver by ultrasound transmission.

Microbubbles which have been developed for general radiology and myocardial perfusion have a tendency to be able to recircuit in blood circulation. However many of the microbubbles have been known to be easily destroyed by ultrasound transmission. Therefore intermittent transmission is now most practical method to observe enough amount of microbubbles flowing in the parenchyma. There might be a threshold of acoustic power for destruction and it is different between kinds of contrast agents. The difference between resonance and passive cavitation of microbubbles in terms of acoustic power is not identified. From our studies using frequency analysis of RF signals received from passive cavitation, non linear properties of signals are similar between them. Both harmonic and color Doppler imaging methods can be used for depiction of this flash echo signals. Spatial and temporal resolution is superior in harmonic gray scale imaging and sensitivity detecting flash echo signals is better in color Doppler ultrasound.

Flash echo imaging of the liver tumors.

Both factors of suspension time and amplitude of acoustic power are important for flash echo imaging. If all of the microbubbles existing in the scanned plane are crushed, the amount of signals depicted on the next first frame is linearly dependent on blood perfusion rate of the tissue. There is a possibility to differentiate pathologically abnormal tissue from the normal tissue by changing the suspension time since there may be a difference in perfusion rates between them. In hepatocellular carcinoma, flash echo imaging with a shorter suspension time depict the tumor more strongly than the normal liver tissue.This suggest that re-filling time of the HCC tissue is shorter than that of normal tissue because blood supply of the HCC tissue is dependent only on hepatic artery. Fragility of microbubbles against ultrasound is different between kinds of contrast agents. If the microbubbles are more resistive against ultrasound transmission, the suspension time can be reduced and frame rate can be increased.. On the other hand, a higher acoustic power is necessary to provoke resonance and passive cavitation for stronger microbubbles.

CONCLUSIONS

In summary, harmonic gray scale imaging and flash echo imaging are useful methods to evaluate perfusion of the liver.

TECHNOLOGICAL DEVELOPMENTS IN ULTRASOUND CONTRAST IMAGING

P.N. Burns PhD, Professor of Medical Biophysics,
University of Toronto Sunnybrook Health Science Centre ,2075 Bayview Avenue S660,
Toronto, Ontario, Canada M4N 3M5

From the point of view of the imaging process, one of the major objectives in ultrasound contrast agent development is to identify an acoustic signature of the agent by which means its echo may be distinguished from that of solid tissue and flowing blood. As more is understood about the physical behavior of microbubble agents in an acoustic field, so better methods are being developed to create a mode of imaging which is sensitive specifically to their presence in the circulation.

The problem is a substantial one: if we are to realize our ultimate aim of imaging blood flow in the capillary circulation of, say, the moving myocardium, we must be capable of detecting the scatter's diluted in a volume of blood that is less than one hundredth that of the ultrasound resolution cell, whose flow velocity might be one hundred times slower than the velocity of the solid tissue that contains it. At first sight, Doppler, which relies on motion to detect a target, would seem an unlikely choice to image the agent in such a setting, as the echo signal would be expected to be swamped by that of the solid tissue clutter.

However, by exploiting some nonlinear properties of microbubble agents, we can show that this interference can effectively be eliminated, leaving us with some of the recognized advantages of Doppler imaging, namely its better use of the available dynamic range of the signal and its real time segmentation of the flow component. These nonlinear properties of the agents are the result of the asymmetry of the radial oscillation between the negative and positive portions of the acoustic cycle. At relatively low incident pressures (about 100-1000 kPa) these result in the generation of even harmonics in the echo signal. These can be detected by transmitting at one frequency and receiving preferentially at double the frequency, the technique known as *harmonic* imaging. At higher incident pressures, irreversible changes in the bubble structure occur, at which a very brief, high intensity, highly nonlinear signal is emitted. Detecting this signal (often known as *transient* imaging) results in a further improvement in sensitivity to the agent. By combining the ability of the harmonic method to suppress the clutter from moving solid tissue in Doppler mode and enhancement provided by

transient behavior of the bubbles, Doppler imaging of extremely low vascular volumes is possible, even in the moving tissue of the myocardium.

Future refinements of these methods is closely linked to the need for a better understanding of bubble behavior, especially in the first few cycles after initial insonation.

ULTRASOUND DIRECTED DRUGDELIVERY (UDDD)

F. J. Ten Cate, A. Bouakaz
Thoraxcenter University Hospital and Erasmus University Rotterdam, The Netherlands

Local delivery of drugs has been accomplished by conjugating a drug to biocompatible or biodegradable macromolecules.

Several methods in the past have been described to prepare drugs containing gelatin particles or solid serum albumin spherules. Also aqueous suspensions of drugs containing spherules of phospholipids have been described. Recently the biophysical properties of ultrasound contrast agents have been used to attach to a drug to be used as drugdelivery compounds.

In this way drugdelivery systems can be developed containing an ultrasound reflector, and a drug. Using the physical properties of ultrasound the drug may be released in a site specific and time adjusted manner at a given dosage which has been determined before. Since ultrasound agents nowadays can be administered to the mammalian body by parental, oral or other methods, these agents may be visualized after introduction into the body.

Release of the drug may be accomplished using specific ultrasound properties such as increase of the mechanical index, frequency shifts or other ultrasound mechanical vibrations.

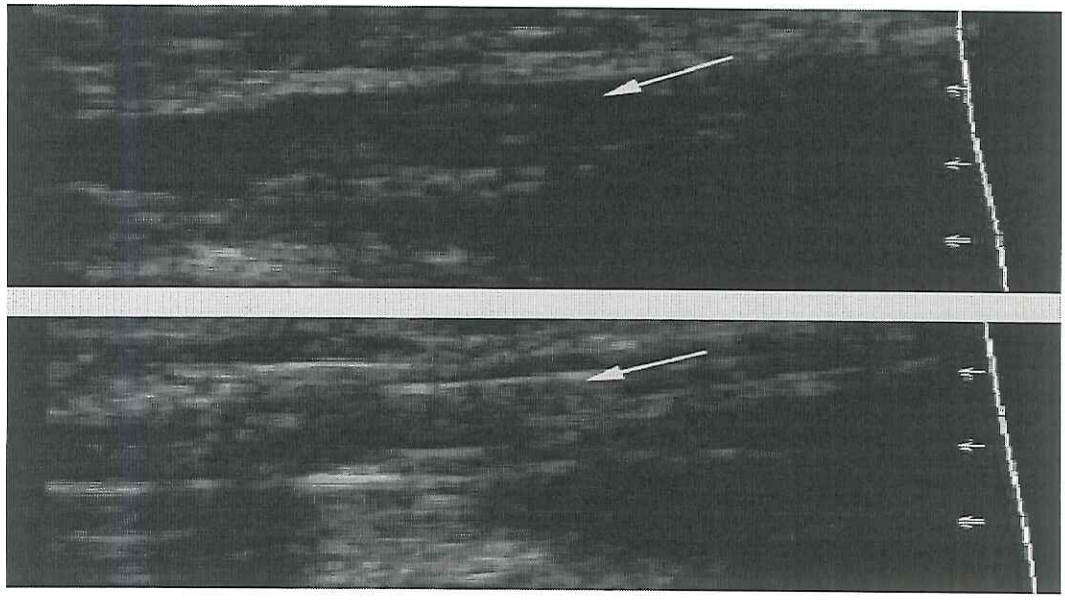
DRUG DELIVERY APPLICATIONS OF ULTRASOUND CONTRAST AGENTS

E.C. Unger,
President and CEO, ImaRxPharmaceutical Corp. Professor of Radiology, University of Arizona

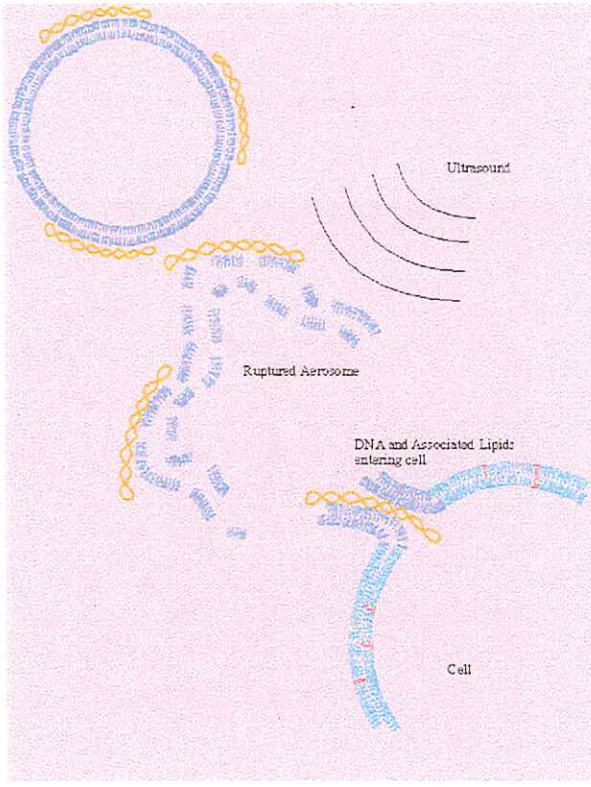
Gas-filled microspheres/ coated microbubbles pose unique opportunities for drug delivery. We have developed lipid coated microbubbles (gas-filled microspheres with a lipid coating known as Aerosomes®) and have used this as a platform for developing drug delivery technologies.

Microbubbles are known to decrease the cavitation thresh-hold of ultrasound energy. It is well known that microbubbles can be destroyed by ultrasound. A number of years ago we conducted experiments showing how ultrasound can be used to augment hyperthermia. Microbubbles can be used to increase the deposition of local heat during ultrasonic hyperthermia. Drugs and pro-drugs can be incorporated into gas-filled microspheres and released locally by ultrasound energy. Simultaneous ultrasound imaging and therapy can be performed.

We have developed targeted Aerosomes® bearing peptides which bind to the GPIIBIIIA receptor of activated platelets. We have performed in vitro and in vivo tests showing enhancement of thrombus. Ultrasound energy may be applied to clot in association with targeted microbubbles to increase the rate of clot dissolution during sonolysis. Site specific sonolysis with targeted microbubbles may represent an effective new means of treating vascular thrombosis. Other thrombolytic drugs may be delivered with the microbubbles or systematically.



Cationic lipids have been used to formulate Aerosomes® which bind DNA. Ultrasound energy may be applied to the Aerosomes® to release the DNA as well as to potentially aid in gene delivery.



Pro-drugs have been incorporated into the wall-forming materials of Aerosomes® to create a drug delivery system for ultrasound. Ultrasound imaging can be used to monitor the target tissue and energy applied as the drug bearing contrast agent circulates through the region of interest. Vast potential exists to use diagnostic ultrasound imaging and drug carrying microbubbles for therapy.

References:

Sutherland GR, Grauer SE, Moran C, Ishii M, Sahn DJ: Aerosomes ™ MRX-115 echo contrast agent demonstrates myocardial opacification after intravenous injection in humans, without significant side effects, in phase I clinical trial. Circulation (Suppl) 9:8, Oct 15, 1995, p. 463.

Grauer SE, Xu J, Pantely GA, Ge S, Gong Z, Shiota T, Zhou X, Sahn DJ, MRX-115, an Echocardiographic Contrast Agent, Produces Myocardial Opacification in Primates: Studies Before and After Occlusion of Left Anterior Descending Coronary Artery. Academic Radiology, Vol 3: S405-406, 1996.

Unger E, Fritz T. Phase I/II Clinical Trials of MRX-115 - A New Ultrasound Contrast Agent. Presented at RSNA, Chicago, IL, Dec 1-6. 1996.

US patent: 5,149,319 Methods for Providing Localized Therapeutic Heat to Biological Tissues and Fluids, Unber: Filed Sep. 11, 1990, Issued Sept. 22, 1992.

US patent: 5,209,720 Methods for Providing Localized Therapeutic Heat to Biological Tissues and Fluids Using Gas Filled Liposomes, Unber; Filed Jun. 18, 1991, Issued May 11, 1993

US patent: 5,558,092 Methods and Apparatus for Performing Diagnostic and Therapeutic Ultrasound Simultaneously, Unger, Yellowhair: Filed Jun. 6, 1995, Issued Sept. 24, 1996

US patent: 5, 542, 935 Therapeutic Delivery Systems Related Applications, Unger, Fritz, Matsunaga, Ramaswami, Yellowhair, Wu: Filed Nov. 30, 1993, Issued Aug 6, 1996

M^{c}Creery TP, Sweitzer RH, Wu G, Shen D, Yellowhair D, Unger EC. Targeted Ultrasound Contrast Agents for the Detection of Thrombosis. Presented at AHA, New Orleans, LA, Nov 9 -13, 1996

RELEASE OF ENCAPSULATED X-RAY CONTRAST AGENT BY ULTRASOUND IRRADIATION

P. Frinking[1], N. de Jong[1,2], S. Keating[3]

[1] Department of Cardiology and Experimental Echocardiography, Thoraxcenter, Erasmus University Rotterdam, The Netherlands.

[2] Interuniversity Cardiologic Institute Netherlands (ICIN), Utrecht, The Netherlands.

[3] Andaris Ltd., 1 Mere Way, Ruddington, Nottingham, UK

Ultrasound contrast agents are biocompatible and biodegradable gasfilled microspheres in the range of 1 to 8 mm and used to enhance the backscattered acoustical signal. These particle sizes make them excellent drug carriers in a drug delivery system which can be administered intravenously. By encapsulation highly soluble drugs can be prevented from rapidly dissolving in the blood. Their ability to enhance the backscattered acoustical signal makes them good tracers in the human body. The combination of loading the microspheres with a drug and the ultrasound *interrogation* gives the opportunity to locate the loaded microspheres in specific regions in the body and to force them to accelerate the release their contents at specific times and locations.

For drug releasing induced by ultrasound, the following constraints for the microspheres have to be fulfilled:

- The release rate must be low when the microspheres are resuspended
- The microspheres have to be echogenic
- An increase in release rate should occur for high acoustic pressures

A unique process has been used to produce microspheres, based on a biocompatible and biodegradable polymer, which encapsulates an X-ray contrast agent. The size ranges from 1 to 5 mm with a mean size of 2 mm. The microspheres show good echogenic properties when they are put under a standard cardiographic echomachine.

The release rate of the X-ray contrast agent in a control volume has been measured with a UV/VIS spectrophotometer at 243 nm. Compared to this control volume a significant increase in release rate was measured when the microspheres were insonified with ultrasound. Experimental data will show of the influence of the acoustic pressure amplitude on the release rate.

INCREASE OF THE MYOCARDIAL INTENSITY - IS IT SUFFICIENT FOR TARGETING AND CONTROLLED DRUG DELIVERY WITH ULTRASOUND CONTRAST AGENTS

V.Uhlendorf, T.Fritzsch
Research Laboratories of Schering AG, 13342 Berlin, Germany

Some ultrasound contrast agents of the latest generation not only use backscatter for signal enhancement, but also "Acoustic Emission" during or shortly after acoustically induced rupture of individual hollow particles. In this novel imaging mode, the sensitivity is sufficiently high to detect extremely low concentrations of hollow particles in tissue or blood, if the transient response of the particles is detected. Both effects - acoustically controlled rupture of particles and their sensitive detection - are easily demonstrated. Potentially, they give rise to several new applications of gas-filled microcapsules in ultrasound diagnostics, and in therapy as well. The most advanced part of the proposed methods is currently given by the new generation of all-digital ultrasound scanners, which are flexible enough to implement additional functions for controlled drug release and recording of trace amounts of specific contrast agents.

When microcapsules are partially filled with a drug, it is possible to release this drug at a well-defined point in the body. This is done just by directing a focused beam of ultrasound to the region of interest (like positioning the gate in Spectral Doppler mode) and then increasing the diagnostic output level sufficiently. The drug may be either a therapeutic agent or it could be used as an indicator for diagnostic purposes (functional diagnostics). The estimated maximum amount of drug that can be encapsulated looks rather promising, but sometimes technological limits will be imposed by the process of particle preparation. However, the concentration of microcapsules may also be larger than normal diagnostic levels, or their acoustic efficacy may be reduced in order to carry more of the drug. In other cases, e.g. with segments of DNA as the encapsulated active drug, the loading factor can be less important.

Drug release and (transient) contrast imaging may be made even more specific, if the particle surface is coated with suitable molecules which act as homing devices or help to modify the rate of uptake by the MPS. A number of different strategies for targeting and drug delivery seem to be possible, and necessary to overcome biochemical and pharmacokinetic restrictions.

Further research will show how many of these ambitious goals can be reached within the near future. The prospect of combining diagnosis and therapy seems worth to invest into this field.

ULTRASOUND CONTRAST IMAGING

N. de Jong and P. Frinking

Department of Cardiology and Experimental Echocardiography, Thoraxcenter, Erasmus University Rotterdam and the Interuniversity Cardiologic Institute Netherlands (ICIN), Utrecht, The Netherlands

The contrast agents currently under development are safe, passes the lung circulation, recirculate in the human body after intravenous administration, do have a long persistence and scattered the ultrasound field. The agents are good enough for the time being. It is now up to the equipment to detect the presence of the agents in order to determine perfusion, flow and other parameters.

The scattering of gas (encapsulated) bubbles depends on the applied acoustic pressure. Depending on the agent three acoustic pressure regions can be distinguished:

Linear scattering	0	-	50	KPascal
Harmonic scattering	50	-	200	KPascal
Transient Power scattering	0.2	-	2	MPascal

Current 2D echomachine generate acoustic pressure amplitudes in the above mentioned regions.

Linear and harmonic (stationary) are well described. Transient Power Scattering occurs above a certain threshold which is dependent on the contrast agent. This transient power scattering results in an increased scattering cross-section. Other phenomenon's occurs as well which is shown by measurements on Quantsion™ (Andaris, Nottingham,UK). A 1 MHz single element transducer with a focus at 7.5 cm is mounted in a waterbath and is used as a transmitter. Perpendicular to the acoustic beam of this transducer a 10 MHz broadband transducer is mounted and is used as a receiver. The sending transducer generates a burst of 10 periods with an acoustic amplitude of 0.6 MPascal. The waterbath contains the Quantison™ contrast agent.

Three measurements are carried out;

a) Quantison™ (5 µl in 200 ml Isoton) acoustic power of 0.6 MPascal,
b) Same concentration of Quantison™ and an acoustic power of 0.3 MPascal,
c) Carborundum (Silicium carbide particles of ~ 5 µm) and an acoustic pressure of 0.6 MPascal.

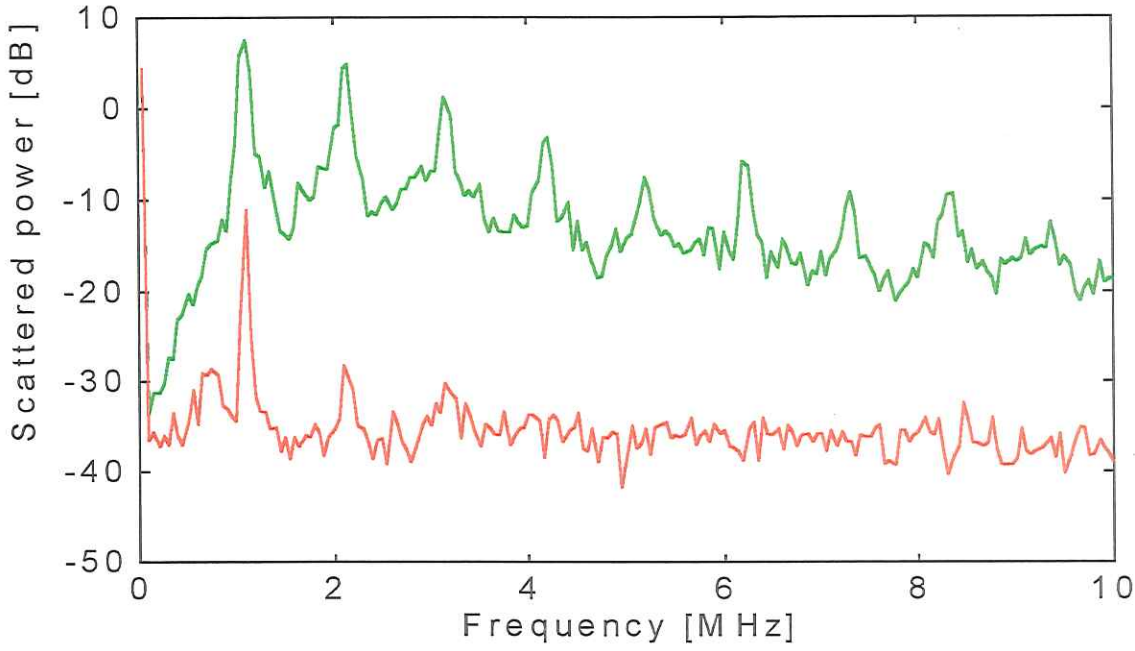


Figure 2: Scattered power as function of the frequency. Transmitting frequency 1 MHz, broadband receiving. -- acoustic amplitude 0.6 MPascal, -- acoustic amplitude 0.3 MPascal

The results of the first two measurements are given in figure. The average is calculated over the FFT of 10 time traces as received with the 10 MHz transducer with a repetition rate of 1 Hz. It can be concluded that for the highest acoustic amplitude, higher harmonics appear up to 10 MHz. For a 50 % drop in the amplitude, the decrease in the 1 MHz component is about 20 dB. The higher harmonics decrease even more. The measurements with the Carborundum, not given in the figure, reveal that there are no artifacts.

The development of the 2D echomachine for ultrasound contrast imaging which started a few years ago has to be seen as only the beginning of a longer process. Employing all the properties of the contrast agents is still a long way to go. For the transient power scattering modalities like real-time differentiation between contrast agent and surrounding tissue is obvious and easy to perform. Also performing simultaneously imaging at low acoustic pressure for tissue imaging together with (triggered) imaging at high acoustic pressure for ultrasound contrast is useful. Optimalisation of transmit frequency and bandwidth of the transmitted signal will improve image quality, together with optimalisation of focussing and amplitude. Further development of the phased array probe is also needed. In order to minimize the cross talk between the first harmonic and higher harmonics the transducer has to be split into a part which is sensitive for the first harmonic and a part for the second harmonic. Current machinery uses frequencies above 2 MHz and mechanical index (MI) below 1.9. For optimal contrast imaging these limits have to be reconsidered.

The 2nd THORAXCENTER EUROPEAN SYMPOSIUM ON ULTRASOUND CONTRAST IMAGING. 23 AND 24 JANUARY 1997, Rotterdam, The Netherlands.

is sponsored by:

Andaris Limited
1 Mere Way Ruddington
Nottingham NG11 6JS
UK

A.T.L.
22100 Bothell Everett Highway
Bothell, Washington 98021 - 8491
USA

Bracco Byk Gulden
Max-Strameyer Strasse 57
78467 Konstanz
Germany

Bracco Research S.A.
7, Route de Drize
CH-1227 Carouge - Geneve
Switzerland

Hewlett Packard GmbH
Schickardstrasse 4
71034 Böblingen
Germany

ICIN
Gebouw Trindeborgh
Hoog Catharijne
3511 GD Utracht
The Netherlands

ImarX Pharmaceutical Corp.
1635 E 18th Street
Tucson, AZ 85719
USA

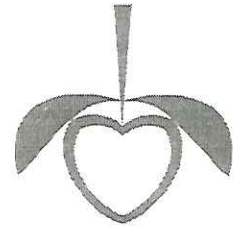
Nycomed AS
Nycoveien 1-2
N-0401 Oslo
Norway

Schering AG
Müllerstrasse 178
Berlin D 13342
Germany

FIRST ANNOUNCEMENT :

3RD ULTRASOUND CONTRAST SYMPOSIUM

21, 22, AND 23 JANUARY 1998



GENERAL INFORMATION:
Mrs. H. F. Gerritsen
University Hospital Rotterdam
Thoraxcenter, room Ba 350
Dr. Molewaterplein 40
3015 GD Rotterdam
The Netherlands
tel: 31 10 4635356
fax: 31 10 4362995
Email: Gerritsen@card.azr.nl

NYCOMED

Schering