ORAL PRESENTATIONS

New applications of contrast echocardiography

Thursday, 8 December 2005, 14:00–15:30

Location: Giotto

411 Adenosine contrast echocardiography has a very high long-term (up to 6 years) negative prognostic value of in patients with suspected or known coronary artery disease

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Background: We have previously shown that continuous infusion of PESDA at rest and after a bolus injection of adenosine (ACE) accurately elicit a further contrast enhancement in the normal region making this observation the principal goal of this protocol. However, no information exists regarding the negative prognostic value (NPV) of ACE. So we sought to investigate the long-term (up to 66 months) NPV of ACE in pts known or at high-risk for coronary artery disease (CAD).

Methods: We examined the outcomes of 306 pts (66.6±11.1 years, 194 men) who underwent ACE with continuous infusion of PESDA associated with triggered (fixed 1:1) 2nd harmonic imaging technology, at rest and after a bolus injection of adenosine. A reversible defect was considered positive test while normal perfusion or isolated fixed defect were considered negative tests. The end-points analyzed were cardiac death and non-fatal myocardial infarction (MI).

Results: Pts were followed up for 33.6± 16.1 months. Negative tests were detected in 212 pts with only 2 events (1 non-fatal MI and cardiac death) while positive tests were detected in 94 pts with 20 events (6 non-fatal MI and 14 cardiac deaths). [chi-square = 71.83, 25, p<0.001 with OR = 49.615 (11.365 to 216.603 - 95% CI)]. The presence of fixed defects did not affect this distribution (p = 0.081 - Cox proportional model). The cumulative event free survival (figure) for hard events was 98.4% in pts with negative and 62% in pts with positive ACE (Log Rank = 47.46, p < 0.001).

Conclusion: ACE has a very high NPV for late cardiac events in pts with known or at high-risk for CAD and could be used in the clinical scenario.

412 Formation of reactive oxygen species in the presence of ultrasound-exposed microbubbles is related to transient permeabilization of cell membranes

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It has been shown that microbubbles and ultrasound increase transfection efficiency of transgenes, however, the exact mechanism(s) underlying increased uptake of genes are still unknown.

We assessed the hypothesis that reactive oxygen species (ROS) are involved in transient permeabilization of cell membranes in vitro after ultrasound exposure at low diagnostic power, in the presence of stable oscillating microbubbles, by measuring intracellular accumulation of ROS and calcium influx.

Ultrasound, in the absence or presence of Sonovue microbubbles, was applied to H9c2 cardiomyoblast cells, with a HP 5500 ultrasound system (Philips) at 1.8 MHz with a mechanical index (MI) of 0.1 or 0.5 during ten seconds. This was repeated every minute for five times. Intracellular ROS accumulation was measured with CM-H2DCFDA and cell membrane permeability was assessed by measuring real-time changes in intracellular calcium with Fluo-4 using live-cell fluorescence microscopy. Cell viability was assessed by detecting externalized phosphatidylserine using flow cytometry; DNA degradation by TUNEL and overall cell morphology.

In the presence of microbubbles, however, there was a significant increase in intracellular ROS accumulation at MI 0.1 of 50% (SEM 5.0, 566 cells) and at MI 0.5 of 110% (SEM 11.8, 217 cells) compared to control (p<0.001). Furthermore, we detected a variety of calcium responses reflecting increases in intracellular calcium (up to 100%) at both MI 0.1 and 0.5 in the presence of microbubbles, which was not detected in the absence of extracellular calcium. In addition, in the presence of the extracellular ROS scavenger, catalase, less cells showed an increase in intracellular calcium. Cell viability was not significantly affected, even 24h later.

In conclusion, present study showed increased intracellular ROS accumulation after ultrasound exposure at low diagnostic intensities in the presence of stable oscillating microbubbles. Increased membrane permeability, as reflected by calcium influxes, can be reduced by extracellular scavenging ROS, implicating a role for ROS in transient permeabilization of cell membranes.

413 Microvascular and myocardial correlates of persistent st-segment elevation after PCI: results from the acute myocardial infarction contrast imaging (A.M.I.C.I) multicenter study

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Background: Microvascular and myocardial correlates of persistent ST segment elevation after PCI are still undefined. We tested the hypothesis that persistent ST segment elevation after PCI is associated with sustained microvascular obstruction and, consequently, with unresolved myocardial dysfunction and LV remodeling.

Method: A total of 109 patients with first successfully reperfused STEMI were enrolled in the acute myocardial infarction contrast imaging (A.M.I.C.I) multicenter study. After PCI, ST-segment resolution was considered a reduction $> 50\%$ of ST-segment elevation. At day 1 and 3 months follow-up, the endocardial length of contrast defect (CD) was evaluated by myocardial contrast echocardiography (MCE) using continuous infusion of Sonovue (Bracco) in real-time imaging. Regional wall motion scores (RWMs), LV end-diastolic and end-systolic volumes (EDV, ESV) were calculated by echocardiography at the same time intervals.

Results: Patients with and without ST-segment resolution had similar symptoms-to-PCI time, peak CK, RWMSI and CD at day 1. At 3 months follow-up, in patients with ST segment resolution, RWMSI and CD significantly improved (1.9±0.8 vs 2.7±0.6 p<0.001 and 12.3±16.5 vs 22.6±20.3, p<0.01, respectively) and EDV
technique is expected to have excellent time and cost performance for assessing the reconstructed short axis view of apical, papillary muscle and basal level of the ventricle in 12 patients with coronary heart disease. MCE was performed using 1:1 consistent ultrasound contrast effect. Aim of the present study was to test the feasibility of newly developed 3D imaging application to depict myocardial perfusion. Methods: We obtained 3D myocardial perfusion image utilizing 1.5RSI by a single scan to assess all the 16 segments of the entire left ventricle (16 segments in total) after scanning apical 4-chamber view. Results: Reconstructed myocardial perfusion images were analyzable in 30 segments (68%) of apical short axis view. 52 segments (79%) of short axis view of mid-papillary muscle level and 44 segments (70%) of basal short axis view. Several artifacts were recognized in apical and basal short axis images while there were fewer artifacts in the short axis image of mid-papillary muscle level. Conclusions: Subtracted 3D-MCE made it possible to depict the short axis perfusion images at multiple levels from a single scan of apical 4-chamber view. Therefore, 3D technique is expected to have excellent time and cost performance for assessing contrast myocardial perfusion.

415 The potential of a new stable ultrasound contrast agent for site-specific targeting

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Background: Microparticle-based ultrasound contrast agents can be used for specific site targeting, but demonstrate time-limited opacification. We have previously demonstrated the potential of gold-bound microtubules to provide a stable and persistent ultrasound contrast effect. Aim of the present study was to test the feasibility of gold-bound microtubules to specifically bind to human thrombi in vitro. Methods: Microtubule samples were prepared by an in vitro self-assembly process of tubulin and gold colloids were immobilized on microtubes walls. Human clots were incubated with biotinylated monoclonal antifibrin antibodies. Control and antibody treated clots were incubated with avidin followed by biotinylated gold-bound microtubules emulsion and conjugated to Cy3-anti-f-tubulin antibody. Foils were filled with buffer containing control and antibody treated clots and were ultrasonographically imaged in harmonic mode (3.2 MHz). After ultrasound imaging clots were fixed and scanned by fluorescence microscopy. Results: The feasibility of gold-bound microtubules conjugation to antibody treated but not to control thrombi was confirmed using immune fluorescence analysis (figure 1A-B). Antibodies immobilized on gold-bound microtubules were stable and persistently immobilized in thrombi. Conclusions: Gold-bound microtubules can specifically bind to human thrombi providing a significant contrast effect, which remains stable in the ultrasound field. This may be a promising approach for site-specific targeting.

416 Optimal timing of microvascular damage assessment in the prediction of post-infarct LV dilatation. Insight into post-ischemic perfusion-contraction mismatch and in the definition of viability

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Background: To evaluate the optimal timing of assessment and cut-off values of myocardial damage in the prediction of LV dilatation after acute myocardial infarction (AMI).

Methods and Results: Forty-one patients with first AMI and successful PCI were studied by myocardial contrast echocardiography (MCE) at 24 hours and 1 week. Real time MCE was performed by contrast pulse sequencing (CPS) (Siemens) and intravenous Sonovue® and regional contrast score index (CSI) (abstract =3, patchy=2, normal=1) was calculated within dysfunctional myocardium. At 3 months, 35% of patients showed LV dilatation (>20%) without any change in perfusion and function. Patients without LV dilatation showed a significant improvement in microvascular perfusion (p<0.0001) from 24 hours to 1 week after PCI, but limited change in function. ROC analysis demonstrated that microvascular damage has a better efficiency in predicting LV dilatation, with a cut off of 1.7 MCE score and of 3 non perfused segments, as compared to wall motion analysis (p<0.05) with improvement from 24 hours to 1 week (p<0.05).

Conclusions: The extent of microvascular damage at 1 week identifies patients evolving in LV dilatation better than wall motion abnormality. Microvascular damage at 1 week gives more information than that at 24 hours in predicting LV dilatation. Segments with perfusion-contraction mismatch are crucial in preventing LV remodeling and they have to be defined "viable".