Conclusions:
2-D TEE USD (12.5±7.9). 3-D TEE USD was marginally larger than fluoroscopic BSD.
Fluoroscopy (Fig. 1B) were measured. Results (mm, mean&SD) were: 3-D TEE USD was 4-6 mm larger than fluoroscopic BSD, the defects were irregular and non-uniform.
Fluoroscopic BSD was not significantly different from ICE BSD (p=0.16). Also, fluoroscopic BSD was significantly larger than ICE USD and 2-D TEE (p<0.001). Fluoroscopic BSD showed excellent concordance with the conventional methods. In patients with mitral valve disease, RT3DE provided an accurate information regarding prolapsed portion, calcification lesion and malcoaptation points. Images were significantly easier to interpret compared to the conventional methods. However, in patients with aortic valve diseases, it was not always possible to obtain adequate images for evaluation of its anatomical features. Conclusions: New RT3DE echocardiography provides accurate information regarding ventricular wall motion, and appears to be especially promising for evaluation of mitral valvular morphology.

Comparison of 2-D Intracardiac and Transesophageal Echocardiography and Fluoroscopy to 3-D Echocardiography in Sizing Atrial Septal Defect for Percutaneous Device Closure
Zheng Liu, Satish Surabhi, Bassam Roukoz, Tammana Nahar, Timothy Puri, Sheldon Thomas, The Cleveland Clinic Foundation, Cleveland, OH

Background: Sizing Atrial Septal Defects (ASD) is essential for the selection of optimal Amplatz occlusion device (AOD) during percutaneous closure. We compared the AOD diameter by transesophageal echo (TEE), intracardiac echo (ICE) and fluoroscopy to maximal 3-D Echo dimension of ASDs.

Methods: 16 patients with secundum ASD underwent AOD closure. 2-D TEE and maximal 3-D TEE (Fig. 1C) were measured. During the AOD closure, ICE USD and balloon stretched diameter (BSD) by both ICE (Fig. 1A) and fluoroscopy (Fig. 1B) were measured. Results (mm, mean&SD) were: 3-D TEE USD (21.4±7.2), fluoroscopic BSD (16.2±7.2), ICE BSD (17.7±6.3), ICE USD (13.2±6.5) and 2-D TEE USD (12.5±7.9). 3-D TEE USD was marginally larger than fluoroscopic BSD (p=0.042) and significantly larger than ICE BSD, ICE USD and 2-D TEE (p<0.001). Fluoroscopic BSD was not significantly different from ICE BSD (p=0.16). Also, fluoroscopic BSD correlated well with ICE USD (r=0.96) and 3-D TEE BSD (r=0.98) for both. In 2 cases where 3-D USD was 4-6 mm larger than fluoroscopic BSD, the defects were irregular and non-uniform (Fig. 2A-2C).

Conclusions: ICE and fluoroscopy are comparable in sizing asymmetrical ASDs. But fluoroscopy may not yield the largest diameter in non-uniform and complex ASDs. This is because balloon stretch deformation transforms an asymmetrical, ASD into a uniform shaped defect with a smaller maximal fluoroscopic BSD. Thus, 3DE TEE USD represents the best method to measure maximal diameter in asymmetrical ASDs.
flow, was mildly increased at 1 hr (2.0-2.3), then increased over the first week peaking at day 4 (5.5-6.6), then declined rapidly (0.9-0.7 at day 28). The MBG signal peak preceeded increases in normalized blood flow and blood volume, were not detected until day 14 (0.5x0.11 and 0.87x0.09, for blood flow and volume at 28 days). MBG signal peak also preceded an increase in ischemic muscle tissue PO2 (normalized values of 0.24x0.06 and 0.47x0.10, at 1 hr and 28 days, respectively).

We conclude that CEU with microbubbles targeted for endothelial α3 integrins can be used to non-invasively assess angiogenic responses in skeletal muscle. These results suggest that targeted CEU imaging of endothelial markers of angiogenesis may potentially be used for assessing intrinsic and therapeutic angiogenesis prior to changes in perfusion.

9:30 a.m.

802-2

Increased Suppression of Intracoronary C-myc Protein Synthesis Within the Stent or Balloon Injury Site Using an Intravenous Microbubble Delivery System Containing Antisense to C-myc: Comparison With Direct Intracoronary Injection

Thomas R. Porter, Derek Knapp, Lucia Venneri, Joseph Oberdorfer, John Lof, Patrick Iverson, Feng Xie, University of Nebraska Medical Center, Omaha, NE, AVI BioPharma, Inc., Owahtina, MN

BACKGROUND: Although perfluorocarbon containing albumin microbubbles (PESDA) can bind large quantities of antisense (AS) to the c-myc protooncogene (anti-c-myc), which promotes minimal hyperperia, it is unknown how much c-myc suppression within the intracoronary (IC) stent or balloon injury is actually suppressed by this intravenous (IV) targeting technique in the early period following vascular injury. To examine this, we performed high phase liquid chromatography of AS to c-myc uptake and Western Blot studies of c-myc protein synthesis in coronary arteries from eight pigs 50 minutes following IC stent and balloon injury (two vessels per pig). Pigs were treated with either direct IC anti-c-myc (4 milligrams), or the same dose of anti-c-myc IV bound or unbound to PESDA. IV PESDA containing anti-c-myc was given in the presence or absence of transthoracic 1 megahertz ultrasound (TTU) (pulsed wave at 0.6 W/cm2). RESULTS: C-myc protein synthesis in the injured coronary arteries (normalized for control vessels) was significantly lower when pigs were given IV anti-c-myc bound to PESDA irrespective of whether TTU was concomitantly delivered (TABLE). Suppression of c-myc synthesis was comparable to direct IC injection. CONCLUSION: These data confirm that simply binding anti-c-myc to IV PESDA is a non-invasive method of targeting therapeutic genes to selective sites of IC balloon or stent injury and suppressing the formation of the c-myc protooncogene which mediates intimal hyperplasia and restenosis.

* p<0.05 compared to other groups (ANOVA).

Direct IC AS IV AS/PESDA IV AS/PESDA IV AS + TTU

c-myc protein ratio 0.94±0.26 0.88±0.11 0.89±0.28 2.11±0.28

anti-c-myc Uptake (nanograms) 13±18 24±3 31±6 29±38

9:45 a.m.

802-3

Improvement in Epicardial Recanalization Rates With Transcranial Therapeutic Ultrasound and Intravenous Microbubbles Containing Ligands Which Attach to the Glycoprotein IIb/IIIa Receptor on Activated Platelets

Thomas R. Porter, Joseph Oberdorfer, Lucia Venneri, John Lof, Feng Xie, University of Nebraska Medical Center, Omaha, NE

BACKGROUND: Intravenous (IV) perfluorocarbon containing microbubbles (PCMB) and transthoracic ultrasound (TTU) (pulsed wave at 0.6 W/cm2) were found to be promising methods for non-invasive gene therapy and can be extended to plasmid vectors. Recombinant adenoviruses or plasmids containing expression constructs of beta-galactosidase and luciferase were incorporated into albumin-coated perfluoropropane-filled microbubbles during their preparation. These bubbles were infused into the internal jugular vein of rats and destroyed with ultrasound while passing through the target organ. Reporter gene activity in organs targeted with adenovirus was 104 times higher than in control organs. We now show that this technique markedly enhances delivery of plasmid DNA into specific tissues. However, liver activity was even higher. Histological examination revealed transgene-derived beta-galactosidase activity in subsets of brain neurons and pancreatic islets. Luciferase transfection with plasmids showed highly specific gene expression in the heart, 10-fold lower than with adenovirus, but with negligible activity in liver (figure). We conclude that ultrasound targeted microbubble destruction can markedly improve the range and specificity of gene delivery.

10:15 a.m.

802-4

Site-Specific Imaging of Tumor Angiogenesis Using Contrast-Enhanced Ultrasound Imaging With Microbubbles Targeted to Alpha-V Beta-3

Howard Liang-Pai, Gamma Vetelina, Juan Casper, Alexander L. Nikonov, Yiu Donung, Sanjir Kaul, Mark E. Shaffrey, Jonathan R. Lindner, University of Virginia, Charlottesville, VA

BACKGROUND: Identification of tumor angiogenesis could potentially be used for early diagnosis of neoplasms and for prognosis. We hypothesized that contrast-enhanced ultrasound (CEU) with microbubbles targeted to endothelial αβ3 integrins expressed in neovessels could be used to assess tumor angiogenesis.

METHODS: We created a brain tumor model where 105 U87MG cells derived from a human glioblastoma tumor cell line were embedded in gel foam and injected intracerebrally in athymic rats. Tumors were assessed after either 14 or 26 days of growth (n=4 for each). Control rats (n=4) were injected with gel foam alone. Targeted CEU imaging was performed 15 min following I.V. injection of control microbubbles (MB) or αβ3 targeted microbubbles (MB3) bearing the disintegrin echistatin on their surface. Cerebral perfusion was assessed by CEU during continuous infusion of non-targeted microbubbles. A corresponding brain slice was processed for immunohistochemistry.

RESULTS: Tumors were ~4-fold larger at day 28 compared to day 14 (p<0.05). Perfusion was reduced within all tumors and was characterized by low microvascular blood velocity. On histology, 15-50 µm neovessels were abundant within the tumor and stained positive for endothelial PECAM-1. Dense staining for αβ3 on the endothelium was found within tumors, especially at the outer margin, whereas minimal staining was seen in control regions. On CEU, MB3 signal intensity in gel foam injection sites was low and similar to that in the contralateral hemisphere. At both 14 and 26 days, MB3 signal in tumors was significantly (p<0.01) greater that for MB in normal control regions, or for MB within the tumor. MB3 signal in tumors was greater at 28 versus 14 days (p<0.05), and was greatest at the peripheral margins of the tumors. MB3 intensity within tumors correlated well with microvascular blood volume derived from CEU perfusion imaging (r=0.83, p<0.01).

CONCLUSIONS: CEU with microbubbles targeted for endothelial αβ3 can be used to assess tumor angiogenesis. These results have important implications for developing methods for early detection of primary or metastatic disease, or for developing novel anti-angiogenic therapies with microbubble delivery systems.

10:15 a.m.

802-5

Ultrasonic Targeted Microbubble Destruction Can Direct Adenoviral or Plasmid Gene Expression to the Heart, Pancreas, and Brain

Raffi H. Bekeredjian, Shuyuan Chen, Peter A. Frenkel, Paul A. Grayburn, Ralph V. Snoner, University of Texas Southwestern Medical Center at Dallas, Dallas, TX

We have previously shown that ultrasonic targeted microbubble destruction (UTMD) can augment expression of an adenoviral reporter in the heart. We now show that this method can selectively deliver transgenes to two organs that are particularly desirable for non-invasive strategies (pancreas and brain) and can be extended to plasmid vectors. Recombinant adenoviruses or plasmids containing expression constructs of beta-galactosidase and luciferase were incorporated into albumin-coated perfluoropropane-filled microbubbles during their preparation. These bubbles were infused into the internal jugular vein of rats and destroyed with ultrasound while passing through the target organ. Organ were harvested after 4 days and analyzed for reporter gene activity. Luciferase activity in organs targeted with adenovirus was 104 times higher than in control organs. However, liver activity was even higher. Histological examination revealed transgene-derived beta-galactosidase activity in subsets of brain neurons and pancreatic islets. Luciferase transfection with plasmids showed highly specific gene expression in the heart, 10-fold lower than with adenovirus, but with negligible activity in liver (figure). We conclude that ultrasound targeted microbubble destruction can markedly improve the range and specificity of gene delivery. This technique heralds a new class of strategies for non-invasive gene therapy.