New Efficient Catheter-Based System for Myocardial Gene Delivery
Ronen Beeri, MD; J. Luis Guerrero, BS; Gregory Supple, BS; Suzanne Sullivan, BS; Robert A. Levine, MD; Roger J. Hajjar, MD

Background—Manipulating gene expression in the failing heart has therapeutic promise, but until now efficient and homogeneous cardiac gene delivery has required an open-chest approach. This study examines the hypothesis that vector delivery promoted by echo contrast microbubbles will be maximized by injection of the vectors into the aortic root with brief balloon occlusion above the sinuses, while at the same time prolonging diastole and vasodilating with acetylcholine (ACh) to maximize coronary exposure.

Methods and Results—After incubation with albumin-coated perfluorocarbon microbubbles, an adenovirus encoding a reporter gene was infused into the aortic root of rats. To maximize delivery, the aortic root was transiently occluded with a balloon catheter during a brief ACh-induced asystole. Ultrasound was used to image the delivery and disrupt the microbubbles. Aortic occlusion with concomitant ACh increased myocardial gene expression for virus + microbubbles by >2.5-fold, from 925 ± 165 to 2358 ± 376 relative units (RU; P < 0.01). This delivery system also produced substantial expression with vector alone (1473 ± 549 RU). All uptakes were significant compared with 433 ± 332 RU without virus.

Conclusions—An adenoviral delivery system combining echo contrast with a catheter-based technique to maximize coronary perfusion increases gene delivery compared with echo contrast alone. This novel method permits efficient percutaneous gene delivery in closed-chest animals. (Circulation. 2002;106:1756-1759.)

Key Words: gene therapy • echocardiography • catheterization

Improvements in vector technology and our understanding of the pathogenesis of myocardial dysfunction have prompted consideration of gene therapy for heart failure. Efficient global cardiac gene delivery, however, has until now required open-chest methods. Recently, effective in vivo myocardial gene delivery has been achieved by directly injecting vectors into the myocardium or by perfusing the heart with them under cardiopulmonary bypass; it has also been achieved by trans-apical injection, but this has required an open-chest approach with cross-clamping to block outflow from the cardiac chambers. Our next challenge is to determine whether we can achieve comparable efficiencies in closed-chest animals.

Exposure time and high coronary flow are major determinants of efficient intracoronary gene delivery. As maximal coronary flow is diastolic, prolonging this phase would maximize myocardial exposure to the vector. In addition, a major obstacle to efficient transcoronary gene delivery is the endothelial barrier, which hinders the passage of macromolecules and cells to the myocardial interstitium. Echocardiographic contrast agents disrupt this microvascular barrier, permitting passage of colloidal particles and red blood cells from the lumen to the interstitium. Specifically, albumin-shelled microbubbles enhance gene delivery to cardiomyocytes and are effective when delivered intravenously and caused to burst by ultrasound energy when they reach the myocardial vasculature.

We therefore sought to test the hypothesis that combining a catheter-based adenoviral vector delivery system that increases coronary exposure with echocardiographic contrast microbubbles could enhance the efficiency of semi-invasive gene delivery.

Methods
A recombinant adenovirus carrying the β-galactosidase (βGal) gene under the control of the cytomegalovirus (CMV) promoter (Ad.CMV.Gal) was constructed as described previously. The stock solution titer was $1.3 \times 10^{12}$ particles/mL, and the particle-to-plaque forming units (pfu) ratio was 1:40. A 200-µL aliquot per animal was mixed in 0.25 mL of an albumin-coated perfluorocarbon-containing microbubble suspension (Optison, Mallinckrodt), incubated for 30 minutes, and shaken before injection.

Delivery Protocol
Male Lewis rats (Charles River Laboratories, Wilmington, Mass) were anesthetized with intraperitoneal pentobarbital (60 mg/kg), intubated, and ventilated (Harvard Apparatus). A linear phased-array 10-MHz echocardiographic probe (AcuNav, Acuson) was inserted...
into the esophagus and connected to an echocardiographic scanner (Sequoia, Acuson). The catheter was oriented to produce a long-axis view of the left ventricle (Figure 1), and the heart continuously imaged at a low mechanical index (0.5) to preserve microbubbles. A coronary balloon angioplasty catheter was inserted through the right carotid artery, and its tip was placed just above the aortic valve with the use of echo guidance. The balloon was inflated with saline to occlude the aorta, and 1.5 µg acetylcholine in normal saline was immediately injected through the catheter, followed by 0.25 mL of the virus-microbubbles suspension and then a 1-mL saline flush. When asystole occurred and the myocardium filled with contrast, the balloon was deflated. Echo mechanical index was raised again whenever myocardial microbubble replenishment was noted. The maximum asystolic time was 5 seconds. The carotid artery was repaired using 7-0 sutures, the skin closed, and the animals allowed to recover. They were euthanized 5 days later using high-dose pentobarbital, and the heart, liver (positive control), lung, and rectus abdominis muscle (negative control) were harvested and snap frozen. This study was approved by our institutional Animal Research Committee and conformed to American Heart Association principles.

### Experimental Groups

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<th>Group</th>
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<th>Echo Contrast</th>
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+ indicates present; −, not present.

### Histochemistry and β-Galactosidase Activity

The extent of gene transduction in the heart muscle was evaluated by a qualitative histochemical staining and quantitative colorimetric assay for β-galactosidase activity. Ventricular tissues were fixed in 0.5% glutaraldehyde for 30 minutes, and then in 30% sucrose for 30 minutes. After permeabilization, tissues were incubated overnight in Xgal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), sectioned every 10 µm, and viewed under a light microscope with the images digitally stored.

β-galactosidase activity was measured in the microscopic sections by a colorimetric assay (Sigma) and expressed as relative Xgal units (RU). In each sample, 10 contiguous fields from the anterior wall (most evident visible staining) were quantitatively analyzed and the results were averaged.

### Experimental Groups

The rats were divided into treatment groups of 5 animals each (Table), which were adenovirus + microbubbles, with and without delivery system (balloon catheter + ACh); adenovirus + delivery system without microbubbles; and controls without virus (with microbubbles + delivery system).

### Statistical Analysis

Colorimetric data were compared among groups using ANOVA, with 2-way comparisons by Student-Neumann-Keuls test (StatView 5, SAS Institute Inc; significance at P<0.05).

### Results

#### Localization

All hearts in groups 1 and 2 (adenovirus + microbubbles with and without delivery system) macroscopically demonstrated blue Xgal staining that was confined to the anterior and posterior walls, which were insonated by the linear echo probe, as opposed to the septal and lateral walls which were not (Figure 1). This is consistent with the importance of ultrasound-induced microbubble disruption to this process. This was corroborated by the qualitative microscopic examination. None of the hearts from group 4 rats (controls) showed any staining. No myocardial damage was noted in the treated areas.

#### Gene Expression by Group

By colorimetric assay, group 1 (adenovirus + microbubbles + delivery system) demonstrated significantly increased transgene expression compared with the other groups (Figure 2A). Aortic occlusion with concomitant ACh increased myocardial gene expression for the virus + microbubbles group by >2.5-fold, from 925±165 to 2358±376 RU (P<0.01). This delivery system also produced substantial expression with virus alone (1473±549 RU), although less than with delivery system + virus + microbubbles (P<0.05), suggesting the additional importance of the microbubbles.
uptakes were significant compared with 433±332 RU without virus (*P<0.01).

Extracardiac Tissue Gene Expression
Extracardiac gene expression was noted in the liver (840±151 RU, n=4) and the lungs (604±126 RU, n=4), though it was relatively low in both. β-galactosidase activity in skeletal muscle did not exceed baseline (294±778 RU, n=4) and had no evident staining, suggesting high myocardial selectivity of this delivery method.

Discussion
This study demonstrates that the combination of echo contrast microbubbles and a catheter-based viral vector delivery system provides superior gene transduction to any one of those mechanisms alone, as well as to simply injecting the virus into the aortic root. This system was designed to overcome the major obstacles to efficient percutaneous myocardial gene delivery, including endothelial barrier crossing, exposure time, and specific delivery to cardiomyocytes (Figure 2B).

Endothelial Crossing
Increasing coronary permeability using agents such as serotonin enhances myocardial gene delivery, as does ultrasound contrast infused along with the viral vector. These agents are microbubbles that resonate when ultrasound energy is applied. When that energy exceeds a threshold, the bubbles are destroyed, thereby disrupting the endothelium and allowing passage of cells and macromolecules into the myocardial interstitium. Microbubbles may also “carry” viral vectors on their shell.

Coronary Delivery
Exposure time critically influences gene delivery. Donahue et al demonstrated that coronary vasodilation with a low-calcium perfusate increases transduction efficiency. We chose acetylcholine because it both dilates normal arteries and induces a short-lived asystole, prolonging diastole to maximize coronary flow. It also has an extremely short half-life.

Transport
Another factor to consider in maximizing gene delivery efficiency is the fraction of virus that is actually delivered to the heart. This has been addressed by direct virus injection into the heart, into a coronary artery, retrogradely into a coronary vein, or via a cardiopulmonary bypass cardioplegia cannula. We reported efficient myocardial gene expres-
sion using isovolumic contraction against a closed aorta with direct aortic root virus injection.\textsuperscript{2,16,17} The delivery system reported here is also based on aortic occlusion using a balloon catheter, thus directing the bulk flow of viral suspension into the coronary arteries. The first contractions after the short asystole actually occur against an inflated balloon, further enhancing coronary delivery. The inflated balloon limits viral transport to the rest of the body, while localized microbubble destruction further promotes selective delivery. In principle, cardiac-specific promoters such as those for myosin light chain, cardiac troponin T, and brain natriuretic peptide could be used to target the myocardium; however, local delivery would still be important because they have low activity compared with the CMV promoter.

As it was beyond the scope of this initial study, we did not assess any functional gene beyond a reporter to determine whether efficient delivery will translate into long-term function change. Our previous studies confirm that SERCA2a gene transduction using this vector significantly affects protein expression, cellular and cardiac function, and eventually, survival.\textsuperscript{16,17} The reported delivery system is designed to increase the efficiency of such transduction. More widespread uptake could be promoted by scanning the ultrasound beam across the heart during infusion, or by using a broader beam. We elected to use a transesophageal probe to maintain a sterile field on the chest and neck of the animals. This narrow beam allowed comparison of insonated to non-insonated areas, thus demonstrating the importance of ultrasound.

All animals did well except for 2 early postoperative deaths, 1 each in groups 2 and 4 (no delivery system/no-vector controls), both related to upper airway obstruction. Mild momentary conjunctival hemorrhage with no sequelae was associated with rapid aortic balloon deflation (pressure upsurge) in the relevant groups.

This new system therefore permits efficient percutaneous gene delivery in closed-chest animals by maximizing access of adenoviral vectors to the coronary circulation and myocardial uptake. We can envision that this minimally invasive procedure could potentially increase the future accessibility of gene therapy, particularly as approaches are developed to benefit heart failure patients who would be at higher risk for open-chest procedures.

Acknowledgments
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References
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