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Intracoronary Adenovirus-Mediated Delivery and Overexpression of the \( \beta_2 \)-Adrenergic Receptor in the Heart

Prospects for Molecular Ventricular Assistance

Ashish S. Shah, MD; R. Eric Lilly, MD; Alan P. Kypson, MD; Oliver Tai, BS; Jonathan A. Hata, BA; Anne Pippen, BS; Scott C. Silvestry, MD; Robert J. Lefkowitz, MD; Donald D. Glower, MD; Walter J. Koch, PhD

Background—Genetic modulation of ventricular function may offer a novel therapeutic strategy for patients with congestive heart failure. Myocardial overexpression of \( \beta_2 \)-adrenergic receptors (\( \beta_2 \)-ARs) has been shown to enhance contractility in transgenic mice and reverse signaling abnormalities found in failing cardiomyocytes in culture. In this study, we sought to determine the feasibility and in vivo consequences of delivering an adenovirus containing the human \( \beta_2 \)-AR cDNA to ventricular myocardium via catheter-mediated subselective intracoronary delivery.

Methods and Results—Rabbits underwent percutaneous subselective catheterization of either the left or right coronary artery and infusion of adenoviral vectors containing either a marker transgene (Adeno-\( \beta \)Gal) or the \( \beta_2 \)-AR (Adeno-\( \beta_2 \)AR). Ventricular function was assessed before catheterization and 3 to 6 days after gene delivery. Both left circumflex– and right coronary artery–mediated delivery of Adeno-\( \beta_2 \)-AR resulted in \( \sim \)-10-fold overexpression in a chamber-specific manner. Delivery of Adeno-\( \beta \)Gal did not alter in vivo left ventricular (LV) systolic function, whereas overexpression of \( \beta_2 \)-ARs in the LV improved global LV contractility, as measured by \( \Delta P/\Delta t_{\max } \), at baseline and in response to isoproterenol at both 3 and 6 days after gene delivery.

Conclusions—Percutaneous adenovirus-mediated intracoronary delivery of a potentially therapeutic transgene is feasible, and acute global LV function can be enhanced by LV-specific overexpression of the \( \beta_2 \)-AR. Thus, genetic modulation to enhance the function of the heart may represent a novel therapeutic strategy for congestive heart failure and can be viewed as molecular ventricular assistance. (Circulation. 2000;101:408-414.)

Key Words: gene therapy ■ myocardium ■ receptors, adrenergic, \( \beta \) ■ ventricles ■ heart failure ■ signal transduction

Novel treatment strategies for ventricular dysfunction are of great importance, and the area of gene therapy is gaining increasing attention. Traditional approaches to the treatment and palliation of ventricular dysfunction and failure include medical management, myocardial revascularization, valve repair, and mechanical assist and transplantation. Although there have been advances in these therapies, congestive heart failure (CHF) remains a leading cause of death in the United States and worldwide. Thus, a molecular approach for treating the failing heart is attractive. Early work in cardiac gene therapy has concentrated on 2 distinct problems: reliable methods of delivery to working myocardium and identification of potential molecular targets. First, work by several groups demonstrated the feasibility of delivering transgenes via direct intramyocardial injection, ex vivo perfusion, and finally a transluminal intracoronary approach. Second, a spectrum of molecular targets has emerged through work done in genetically engineered mice.

One molecular target identified is the overexpression of the \( \beta_2 \)-adrenergic receptors (\( \beta_2 \)-ARs). Transgenic mice with cardiac-specific overexpression of the human \( \beta_2 \)-AR at either >100-fold over endogenous \( \beta_2 \)-AR density or significantly lower overexpression have enhanced contractility without overt pathological conditions. These models were developed to replace receptors that are lost during the development of CHF. In the failing heart, there is a 50% reduction of myocardial \( \beta \)-adrenergic receptors (\( \beta \)-ARs), with remaining receptors being functionally uncoupled. In addition to the positive phenotype of the \( \beta_2 \)-AR-overexpressing mice, adenovirus-mediated overexpression of \( \beta_2 \)-ARs in failing rabbit ventricular cardiomyocytes in culture has resulted in the functional rescue of the signaling abnormalities present in failing heart cells. Thus, genetically replacing lost \( \beta \)-ARs in the failing heart represents a potentially novel therapeutic strategy to increase inotropy.
The primary hurdle to testing the feasibility of $\beta_2$AR gene transfer in vivo is the development of a clinically relevant gene delivery system. Recent work by our laboratory and others has shown that it is possible to deliver transgenes globally to the myocardium by adenoviral vectors. In these 2 studies, adenoviruses were delivered via a surgically invasive approach in which the transgenes are injected into the left ventricular (LV) cavity while the aorta is cross-clamped, directing the adenoviral solution to perfuse the coronary arteries. Using this method, we showed that overexpression of $2\Delta R$-ARs in the rabbit heart does enhance global in vivo LV function. Because this method of gene delivery has its limitations, we explored the feasibility of delivering the $2\Delta R$ AR transgene to the rabbit heart in vivo via percutaneous subselective coronary catheterization and injection. A previous report showed that transluminal intracoronary artery delivery of marker transgenes in the rabbit is possible; however, that report did not include a study of myocardial function. The purpose of our study was to develop a reproducible percutaneous subselective intracorony artery delivery method for efficient ventricle-targeted in vivo gene transfer of adenoviral transgenes to rabbit myocardium. Furthermore, we investigated whether ventricular overexpression of the $2\Delta R$-AR in the rabbit heart could alter biochemical and in vivo cardiac function.

### Methods

#### Adenoviral Constructs

Adenoviral constructs using a “first-generation” E1/E3-deleted replication-deficient adenovirus have been described previously. The $2\Delta R$-AR construct (Adeno-$\beta_2$AR) and the marker transgene $\beta_2$-galactosidase (Adeno-βGal) were driven by the CMV promoter. Large-scale preparations of these adenoviruses were purified from infected Epstein-Barr nuclear antigen–transfected 293 cells as described.

#### Percutaneous Intracoronary Adenovirus Delivery and Cardiac Functional Assessment

All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the NIH. Forty-seven adult male New Zealand White rabbits (3 kg) were used in the present study. At the time of the initial study, animals were sedated with ketamine (50 mg/kg) and acepromazine (0.25 mg/kg), and an incision was made over the right neck to expose the right carotid and jugular vessels. A 2.5F micromanometer (Millar Inc) was placed into the LV cavity via the carotid artery under fluoroscopic guidance, and a 22-gauge angiocatheter was placed into the jugular vein. The micromanometer was coupled to a PC-based data acquisition system (Physiological Systems Inc). The maximal rate of pressure rise ($dP/dt_{max}$) was computed from the digital pressure waveform as a running 5-point polyorthogonal transformation. All hemodynamic data were derived from the average of 20 steady-state cardiac cycles.

#### $\beta$-AR Density

$\beta$-AR binding was performed on myocardial sarcolemmal membrane preparations as we have previously described. Total myocardial $\beta$-AR density was determined by incubating 25 µg of sarcolemmal membranes with a saturating concentration of $\beta_2$-labeled cyanopindolol and 20 µmol/L alprenolol to define nonspecific binding. Assays were performed in triplicate, and $\beta$-AR density was normalized to milligrams of membrane protein.

#### $\beta$-Galactosidase Staining

After excision, transverse cross sections of myocardium at the midpapillary level were obtained for histological analysis and stained in 30% sucrose solution before paraffin embedding as described. Paraffin-embedded samples were mounted on a cryostat and sectioned into 5- to 10-µm sections, which were then transferred to a glass slide. $\beta$-Gal staining was performed by standard procedures as described.

#### $\beta_2$AR Immunohistochemistry

Frozen myocardial sections were cut at 10 µm for indirect immunofluorescence studies as we have described. Briefly, sections were rinsed in PBS and then in PBS with 0.05% Triton X-100 (Triton-PBS, blocked with serum diluent) before overnight incubation at 4°C with primary rabbit anti-human $\beta_2$AR antisera (1:500 dilution in serum diluent). The sections were then washed, incubated for 1 hour in FITC-conjugated goat anti-rabbit immunoglobulin G (1:50 dilution in serum diluent), rinsed in PBS, mounted with sodium iodide (25 g/L) in 1:1 PBS/glycerol solutions, and photographed.

### Results

### Adenovirus-Mediated Myocardial Overexpression of Transgenes After Intracoronary Delivery

We delivered adenoviral transgenes via either the LCx or RCA in a subselective manner. Transgene expression in the rabbit myocardium was assessed 3 days after delivery to...
allow time for adequate protein translation and before adenoviral immunological processes were evident. As further protection toward the latter, we treated rabbits (including all control groups) for 2 days with corticosteroids.

To assess the volume of myocardium expressing transgene, we delivered Adeno-β Gal (5×10^11 TVP) via either the LCx or RCA. Figure 1 shows representative X-Gal–stained cardiac cross sections at low and high power of magnification after Adeno-β Gal delivery. Chamber-specific expression of the β-Gal gene that corresponded to staining in individual myocytes was evident (Figure 1). Thus, it appears that within the region served by the catheterized coronary artery, complete transmural expression is possible, especially in this adenoviral dose range.

We also studied the delivery and expression of the human β2 AR, which represents a potentially therapeutic transgene. As with Adeno-β Gal, we delivered 5×10^11 TVP Adeno-β2 AR via either the LCx or RCA and initially assessed β-AR density. As shown in Figure 2A, Adeno-β2 AR delivery in the LCx resulted in significant LV-specific overexpression, whereas catheterization and injection via the RCA resulted in RV-specific expression. β2 AR overexpression ranged from ~9- to 15-fold over endogenous myocardial β-AR density. Expression of the β2 AR transgene remained elevated at 6 days (n=4) after gene delivery (Figure 2B). Importantly, β2 AR overexpression after in vivo coronary delivery was found to be localized to the sarcolemmal membranes of individual ventricular myocytes as visualized by immunohistochemical staining with an antibody specific for the human β2 AR (Figure 3). As with the β-Gal transgene distribution, β2 AR overexpression found by immunohistochemistry was diffuse throughout the entire LV after LCx injection, and a representative section of an Adeno-β2 AR–treated LV is shown in Figure 3.
Functional Consequences of Subselective Intracoronary Adenovirus Delivery and Myocardial Overexpression of Adeno-βGal and Adeno-β₂AR Transgenes

The hemodynamic consequences of subselective intracoronary delivery of adenoviral transgenes were examined by use of dynamic intracavitary pressure measurements. The maximal first derivative of the LV intracavitary pressure (dP/dt max) was used as a measure of global LV contractile performance. As described in the Methods, the hemodynamics of each rabbit in the study was measured before and then 3 to 6 days after gene delivery. Thus, each animal served as its own control, increasing the power of the analysis. Furthermore, animals that underwent catheterization and injection of saline served as controls for the delivery technique itself. β AR density determined for all animals in the study confirmed β₂AR overexpression. Animals that received Adeno-βGal via the LCx did not show a significant change in systolic function compared with both precatheterization values and animals injected with saline (Figure 4A). In animals that received Adeno-β₂AR and subsequently showed β₂AR overexpression in the LV, a significant increase in baseline dP/dt max was seen compared with precatheterization values and Adeno-βGal–treated animals (Figure 4B). LV systolic functional responses to isoproterenol were also significantly greater in β₂AR-overexpressing animals compared with controls (Figure 4C).

Interestingly, in animals that received either Adeno-β₂AR or Adeno-βGal (5×10¹¹ TVP each), HRs and LV end-diastolic pressure (EDP) were significantly increased 3 days after gene delivery, whereas there was no change, with either treatment, in systolic blood pressure (Table 1). LV dP/dtₘᵡ in Adeno-βGal–treated rabbits was significantly reduced, whereas no reduction was evident in Adeno-β₂AR–treated rabbits (Table 1). Hemodynamic values were not altered 3 days after saline delivery including dP/dtₘᵡ (Figure 4A), dP/dtₘᵡ (before, -2387±148 mm Hg/s versus after, -2152±196 mm Hg/s, P=NS), and LV EDP (before, 0.7±0.15 mm Hg versus after, 0.9±0.4 mm Hg, P=NS).

LV dP/dtₘᵡ measurements can be confounded by changes in HR and afterload. Accordingly, a subset of animals was studied to determine the effect of HR on LV dP/dtₘᵡ by use of transvenous atrial pacing. As shown in Figure 5, the HR effect in the range of 200 to 300 bpm did not significantly increase LV dP/dtₘᵡ in a normal rabbit, demonstrating that increased HR is not accountable for the significant increase in basal and isoproterenol-stimulated LV dP/dtₘᵡ found in Adeno-β₂AR–treated rabbits.
The functional benefit of β2-AR overexpression in the LV was also studied 6 days (n=4) after gene delivery. Importantly, basal and isoproterenol-stimulated LV systolic performance remained elevated at 6 days compared with pre–gene delivery hemodynamic measurements (Table 2).

Finally, the effect of β2-AR overexpression in the RV after Adeno-β2-AR delivery via the RCA on LV function was also studied. No improvement in LV systolic performance was seen in animals that overexpressed the β2-AR in the RV (n=6) from precatheterization baseline values (before catheterization, 2725.8±211 versus after catheterization, 2858.7±48 mm Hg/s, P=NS).

**Discussion**

The present study reports the novel findings that in vivo molecular manipulation of ventricular function by a percutaneous intracoronary gene delivery method is feasible. Accordingly, we have demonstrated that chamber-specific delivery of an adenovirus containing the human β2-AR via subselective coronary catheterization and injection produces ventricular overexpression of the β2-AR at a level that increases contractile function in vivo, demonstrating a therapeutic strategy to effectively produce molecular ventricular assistance in CHF.

The β-AR signaling system is an appealing target for cardiac gene therapy, because specific molecular abnormalities have been well described in human CHF and in multiple animal models of failure and cardiac disease. A down-regulation of β-ARs, uncoupling from second messenger systems, and elevation of desensitizing G-protein receptor kinase activity are the fundamental alterations seen in heart failure. In myocytes isolated from hearts in CHF, these abnormalities can be reversed by β2-AR overexpression or G-protein–coupled receptor kinase inhibition by use of transgenes delivered via adenoviral vectors. These studies, as well as studies in transgenic mice, form the basis of our β2-AR gene therapy strategy.

The overexpression of β2-ARs in the LV free wall appears to improve global LV systolic performance. dP/dt max both at baseline and after isoproterenol administration was increased relative to the precatheterization values in each of the individual rabbits. This is consistent with our findings in a model of biventricular overexpression of β2-ARs. It is extremely encouraging that the ≈20% improvement seen in this study occurred after only LV free wall transfection and a relatively modest 10-fold increase in LV β2-AR density. Furthermore, this functional benefit was sustained at 6 days after gene delivery. Previous reports in transgenic mice demonstrated greater improvement in LV contractile function with β2-AR overexpression. However, these mice had considerably higher levels of β2-AR overexpression, which was global in nature and not limited to a single chamber, as is the case in the rabbits of this study. This is the first animal model to examine the in vivo effects of chamber-specific overexpression of the β2-AR in the intact circulation, where ventricular interactions limit the maximal improvement in performance. Rabbits treated with Adeno-β2Gal did not show a significant difference in systolic function compared with saline-injected animals. This demonstrates that adenovirus...
delivery, and transgene expression in general, does not depress baseline dP/dt\textsubscript{max}. Interestingly, β\textsubscript{2}AR overexpression directed to the RV did not enhance LV performance. Although this result is not surprising, RV delivery of β\textsubscript{2}ARs may offer benefit in conditions of isolated right-sided dysfunction such as pulmonary hypertension.

We did observe a degree of myocardial injury with this model. In Adeno-β\textsubscript{2}Gal controls and β\textsubscript{2}AR-treated rabbits, EDP was elevated and Adeno-β\textsubscript{2}Gal–treated rabbits had depressed dP/dt\textsubscript{max} values. Saline-injected controls did not show alterations of EDP or dP/dt\textsubscript{max}. Overall, these data suggest that bolus delivery of adenovirus caused an increase in LV stiffness. Interestingly, animals that overexpressed the β\textsubscript{2}AR had less depression of dP/dt\textsubscript{max} at 3 and 6 days, suggesting a superimposed lusitropic effect. Nevertheless, this apparent negative effect of intracoronary adenovirus injection needs to be examined further.

The present study highlights a number of challenges to in vivo cardiac gene therapy. First, myocardial delivery of the adenoviral transgene has been achieved in this percutaneous model, but whether the methods used here will be applicable to other viral vectors is not certain. The kinetics of adenovirus-mediated transfer in the intact coronary vasculature favor high coronary perfusion flow and pressure as well as enhanced permeability, most of which can induce myocardial injury. We use a relatively large injection volume for individual coronary arteries, which we have found leads to significant improvement in gene transfer and transgene expression, consistent with the ex vivo kinetic studies of intracoronary adenoviral gene delivery. Furthermore, the present study uses higher concentrations of virus than previously reported; however, transmural myocardial expression is also greater than in previous reports. Accordingly, a significant percentage of ventricular myocytes must be transfected to achieve a global functional impact.

The duration of transgene expression was not examined in this study. Work in our laboratory has documented that the first-generation vectors used yield expression that lasts for 1 to 2 weeks in vivo in the myocardium. However, we have clearly shown that adenovirus-mediated transgene delivery can alter the in vivo function of the heart for >1 week, and the use of this method can only improve in the future as vector technology advances to allow for less inflammatory vectors that also support longer-term expression. For example, long-term myocardial expression has recently been reported with adeno-associated virus. It will be of particular future interest to determine whether our delivery method will effectively deliver adeno-associated virus transgenes.

There are several implications of our experimental findings. The present study is among the first to demonstrate the feasibility of cardiac gene transfer by clinically relevant and available methods. Previous studies have used open thoracotomy and aortic cross-clamping. However, we have also demonstrated the ability to enhance baseline myocardial function in the otherwise normal LV. This improvement of baseline systolic function is consistent with previous findings in β\textsubscript{2}AR-overexpressing transgenic mice, including transgenic mice that had myocardial-targeted β\textsubscript{2}AR overexpression in the range reached by our adenovirus-mediated gene transfer. Thus, the possibility of genetic manipulation via the coronary circulation offers potential therapy to patients not amenable to traditional approaches to cardiomyopathy. Recent work has documented polymorphisms of the human β\textsubscript{2}AR gene, which result in dysfunctional G-protein coupling and predict poor prognosis in affected patients with CHF.

Therefore, gene delivery of a normal β\textsubscript{2}AR gene might be particularly critical in this patient population.

The recent clinical evidence that failing cardiomyocytes have the capacity to reverse their remodeling and regain normal contractile function supports the hypothesis that molecular ventricular assistance, as shown here with β\textsubscript{2}AR overexpression, could serve as a novel form of therapy. This hypothesis is further strengthened by the finding that overexpression of β\textsubscript{2}ARs and an inhibitor of β\textsubscript{2}AR desensitization reverse abnormal signal transduction in cultured cardiomyocytes isolated from rabbits in CHF.

A growing body of evidence supports the use of β-blockade in the treatment of heart failure. Although our strategy would seem contradictory to this clinical experience, it may in fact be supported. Recent experimental work has shown that β-antagonist therapy with carvedilol is associated with a restoration of desensitized β-AR signaling that may be the mechanism of benefit of this agent in CHF. Therefore, overexpression of functional β\textsubscript{2}ARs serves as an alternative method to restore β-AR signaling in failing myocardium.

In summary, the present study has demonstrated that subselective adenovirus-mediated delivery of a functional transgene is possible via the intact coronary circulation. Future studies will examine the consequences of acquired β\textsubscript{2}AR overexpression in models of CHF, and the present approach lends itself to that task. Furthermore, subselective gene delivery may allow the examination of new strategies for the management of hypertrophy, RV failure, and genetic cardiac maladaptation.

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