

Intracoronary Adenovirus Encoding Adenylyl Cyclase VI Increases Left Ventricular Function in Heart Failure

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Background—We tested the hypothesis that intracoronary delivery of an adenovirus encoding adenylyl cyclase type VI (Ad.AC_{VI}) would be associated with increased left ventricular (LV) function in pigs with congestive heart failure.

Methods and Results—Pigs (52±6 kg; n=16) underwent placement of pacemakers, LV pressure transducers, and left atrial and aortic catheters. Physiological and echocardiographic studies were obtained from conscious animals 13 days later, and pacing was initiated (220 bpm). Seven days later, measures of LV function were reduced, documenting severe LV dysfunction and dilation. Pigs then received intracoronary Ad.AC_{VI} (1.4×10¹² vp; n=7) or saline (PBS; n=9) (randomized, blinded), with concomitant infusion of nitroprusside (50 μg/min, 6.4 minutes) to increase gene transfer. Pacing was continued for 14 days, and final studies were obtained. The a priori key end point was change in LV dP/dt during isoproterenol infusion (pre-Ad.AC_{VI} value minus value after 21 days of pacing). Pigs receiving Ad.AC_{VI} showed a smaller decrease in both LV +dP/dt (P=0.0014) and LV -dP/dt (P=0.0008). Serial echocardiography showed that Ad.AC_{VI} treatment was associated with increased LV function and reduced LV dilation and that end-systolic wall stress was reduced. AC-stimulated cAMP production was increased 1.7-fold in LV samples from Ad.AC_{VI}-treated pigs (P=0.006), and B-type natriuretic peptide was reduced (0.035). Gene transfer was confirmed by polymerase chain reaction.

Conclusions—AC_{VI} gene transfer increases LV function and attenuates deleterious LV remodeling in congestive heart failure. (*Circulation*. 2004;110:330-336.)

Key Words: receptors, adrenergic, beta ■ genes ■ cAMP ■ nitroprusside

Adenylyl cyclase has long been recognized as a pivotal effector molecule in cardiac myocytes and other cells. In 1998, we showed that the amount of adenylyl cyclase type VI (AC_{VI}) sets a limit on the ability of cardiac myocytes to generate cAMP.¹ We then showed that cardiac-directed expression of AC_{VI} increases cardiac contractile function in transgenic mice.² When AC_{VI} is expressed in the background of G_q-associated cardiomyopathy, cardiac function and survival are improved.^{3,4} We then showed that global left ventricular (LV) function and responsiveness can be changed by gene transfer of AC_{VI} in a manner that can be applied clinically: intracoronary delivery.⁵

See p 242

Studies using transgenic mice have provided useful data but represent an early phase of the translation of basic science into useful clinical strategies. The models of heart failure available in genetically manipulated mice are rarely, if ever, found clinically, and the use of crossbreeding paradigms to test potentially therapeutic genes is limited. For example, successful studies using these paradigms to date have actually

prevented development of heart failure rather than treating heart failure per se.

In the present article, we address these 2 shortcomings. First, we use a large-animal model of heart failure that mimics aspects of clinical dilated heart failure and allows intracoronary delivery of an exogenous gene. Second, we treat subjects with active heart failure. We tested the hypothesis that intracoronary delivery of an adenovirus encoding AC_{VI} (Ad.AC_{VI}) would be associated with increased LV function when delivered to animals with congestive heart failure (CHF).

Methods

Animals and Surgical Procedure

The study was conducted in a randomized, saline-controlled, and blinded manner, and animals were treated in accordance with National Institutes of Health and institutional guidelines. Sixteen pigs (52±6 kg) completed 3 weeks of continuous LV pacing. Details of the surgical procedures have been reported previously.⁶ Catheters were placed in the LV, aorta, pulmonary artery, and left atrium, and a high-fidelity pressure transducer was placed in the LV chamber (Konigsberg). LV epicardial pacing (Prevail 8086, Medtronic Inc) was used as reported previously.⁶ Thirteen days later, measures of

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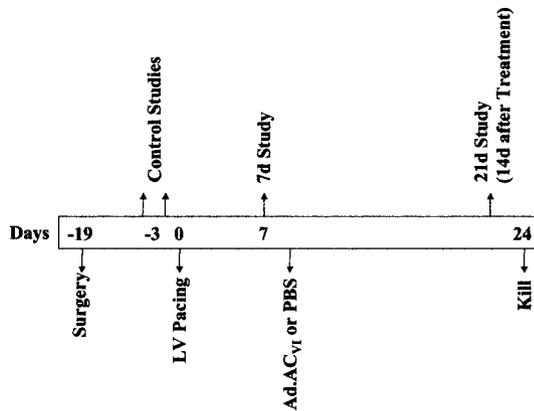


Figure 1. Experimental protocol.

hemodynamics, LV global and regional function (echocardiography), and contractile responses to graded doses of isoproterenol and dobutamine (assessed by pressure transducers implanted in the LV chamber) were made. Pacemakers were then activated (220 bpm, 5 V, 0.5 ms), and LV pacing was continued for the 21-day duration of the study (Figure 1). Heart rates were checked daily by ECG or auscultation. Seven days after initiation of pacing, pacemakers were inactivated and LV function was assessed by echocardiography and hemodynamic measurements. After data acquisition, animals received intracoronary delivery of a recombinant adenovirus encoding AC_{VI} (Ad.AC_{VI}) or PBS, and LV pacing was resumed for 14 additional days. Final studies were repeated (echocardiography, hemodynamics, and contractile responses to agonist infusion), and the animals were killed.

Hemodynamic Studies

Pulmonary artery, aortic, left atrial, and LV pressures were obtained after the pacemaker had been inactivated for at least 30 minutes and the animals were resting quietly. When necessary, diazepam (5 to 15 mg IV) was used to alleviate agitation. Studies were conducted before the initiation of pacing, after 1 week of pacing (just before intracoronary injection of saline or Ad.AC_{VI}), and after 3 weeks of pacing, 2 weeks after gene transfer (Figure 1).

Blood samples were obtained before initiation of pacing and weekly thereafter for measurement of plasma catecholamines by radioenzymatic assay⁷ and B-type natriuretic peptide (BNP) by radioimmunoassay (Peninsula Laboratories, Inc).

Two or 3 tests conducted on separate days were averaged before gene transfer and repeated 14 days after gene transfer. Basal LV pressure and heart rate were recorded before and after administration of glycopyrrolate (0.07 mg/kg) to remove vagal tone. LV pressure was recorded after intravenous bolus injection of (–)-isoproterenol (0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 μg/kg). Subsequently, LV pressure was recorded during intravenous administration of 3, 10, and 30 μg/kg of NKH477,⁸ a water-soluble forskolin derivative (Nippon Kayaku). Data from the high-fidelity pressure signal were differentiated to obtain LV +dP/dt, which was used to assess systolic function, and LV –dP/dt, which was used to assess LV relaxation. Data analyses were blinded.

Echocardiographic Assessment of Global and Regional Contractile Function

2D and M-mode images (Agilent Sonos 5500) were obtained as previously described.^{6,9} Images were recorded with animals in a basal state and during dobutamine infusion (20 μg · kg^{–1} · min^{–1}). These studies were performed 13 days after instrumentation, 1 or 2 days before gene transfer, and after 3 weeks of pacing. Meridional end-systolic wall stress¹⁰ was calculated for both lateral wall and interventricular septum (IVS) before the initiation of pacing and subsequently at weekly intervals (pacemakers inactivated).

Gene Transfer

An E1-deleted, replication-incompetent adenovirus encoding murine AC_{VI} driven by a cytomegalovirus promoter was generated by homologous recombination as previously described.^{1,5} Seven days after the initiation of LV pacing, animals underwent echocardiographic and hemodynamic studies and then received Ad.AC_{VI} or saline by intracoronary injection as previously described.⁵ A 5F multipurpose coronary catheter (A1, end hole only) was advanced 1 cm into the ostium in the proximal portion of each of the 3 coronary arteries: left anterior descending, left circumflex, and right coronary artery. Intracoronary sodium nitroprusside (Gensia Sicor Pharmaceuticals) was infused (50 μg/min) continuously beginning 3 minutes before and persisting 2.3 minutes after vector or saline delivery was completed via a separate portal in the manifold. Recombinant adenovirus (1.4 × 10¹² vp in 5 mL) was infused into each coronary artery at a rate of 1.5 mL/min. The total volume (5 mL) was delivered in proportion to the myocardial mass perfused by each major coronary artery (left anterior descending, 50%; left circumflex, 30%; and right coronary artery, 20%). A 50–50 mixture of contrast and saline (6.5 mL) was infused to reconfirm catheter placement. Vials containing saline or Ad.AC_{VI} were identical in appearance, and treatment assignment was random.

Terminal Surgery

After 3 weeks of pacing, animals were anesthetized, hearts were removed and submerged in saline (4°C), the coronary arteries were rapidly perfused with saline (4°C), the right ventricle and LV (including the IVS) were weighed, and transmural samples were taken from regions of the heart and frozen (–70°C) for subsequent analysis. Ascites and pleural fluid were measured, and the liver was removed and weighed. Hematoxylin-eosin and Masson's trichrome stains were used to detect inflammatory cell infiltrates, cell necrosis, and fibrosis in samples of LV from animals of both groups (n=7 each). Histological analysis was blinded.

Assessment of cAMP Content

LV membranes were prepared as reported previously.⁵ Membrane protein (40 μg) was incubated with 10 μmol/L of NKH477 (30°C, 15 minutes). The sample was then boiled to stop the reaction, and the amount of cAMP measured with a cAMP enzyme immunoassay kit (Amersham). Net NKH477-stimulated cAMP values are reported (basal cAMP subtracted).

Presence of Transgene DNA

The presence of adenovirus encoding AC_{VI} was confirmed by polymerase chain reaction (PCR). Genomic DNA was prepared from LV samples using DNeasy Tissue Kit (Qiagen). The primers used included pE2B-1 (5'-TCGTTTCTCAGCAGCTGTTG-3') and pE2B-2 (5'-CATCTGAACTCAAAGCGTGG-3'). The annealing temperature was 58°C, the extension temperature was 72°C, and 40 cycles of PCR were used. The expected size of the PCR product was 841 bp.

Statistical Analysis

The a priori primary end point of this study was change in LV dP/dt during isoproterenol infusion after 3 weeks of continuous pacing. Secondary end points included LV chamber dimensions, LV wall stress, hemodynamic measurements, BNP, and LV cAMP generation. Data are expressed as mean ± SEM. To confirm procedural validity, the effects of LV pacing on the groups were evaluated with 2-way (group-by-time) ANOVAs with repeated measures on basal LV end-diastolic dimension, fractional shortening, mean left atrial pressure, and LV contractility (GraphPad Software). Hypothesis tests for treatment effects were made by assessing changes in hemodynamic measurements from the initiation of pacing versus 3 weeks after pacing using 2-way (treatment-by-dose) ANOVAs with repeated measures. Other comparisons between group means were conducted by use of Student's *t* test (2-tailed). The Fisher exact test was used to determine differences in histological assessment. The null hypothesis was rejected at a value of *P* < 0.05.

TABLE 1. Evidence for Heart Failure Before Randomization and Treatment

	Control	7 Days Pacing	<i>P</i>
EDD, cm	4.6±0.1	5.3±0.5	<0.0001
FS, %	42±1	27±2	<0.0001
LAP, mm Hg	10±1	21±2	<0.0001
LV +dP/dt, mm Hg/s	3019±123	2206±146	<0.0002

EDD indicates LV end-diastolic diameter; FS, fractional shortening; and LAP, mean left atrial pressure. *P* values are from Student *t* test (paired, 2-tailed). Data were obtained with pacemakers inactivated. Entries are mean values±SEM of 16 animals before randomization to Ad.AC_{VI} or PBS.

Results

Animals

Sixteen of the 34 (50.7±0.9-kg) pigs used in this study successfully completed 3 weeks of continuous LV pacing. Before randomization, 8 pigs were eliminated: 1 animal died during surgery, and 7 were withdrawn because of pacemaker or Konigsberg malfunction. Ten animals were randomized but did not complete the study. Two had pacemaker malfunction in week 3 (later identified as Ad.AC_{VI}-treated); the remaining 8 died or were electively killed (humane considerations with veterinarian consultation) before completion of the study: 4 had received Ad.AC_{VI} (1 died on day 21 of pacing, 1 died during the 21-day pharmacological study, and 2 were killed electively 11 and 18 days after treatment), and

4 had received PBS (3 were found dead on days 14, 18, and 21 of pacing, and 1 was killed 7 days after treatment).

Evidence for CHF Before Treatment

It was important to determine whether 7 days of continuous pacing resulted in signs of heart failure before animals randomly received Ad.AC_{VI} or PBS. LV end-diastolic dimension and mean left atrial pressure were increased, and fractional shortening and LV +dP/dt were decreased after 7 days of pacing for the 16 animals (Table 1). Group-by-time interaction tests were not significantly different (end-diastolic dimension, *P*=0.69; left atrial pressure, *P*=0.61; fractional shortening, *P*=0.48; +dP/dt, *P*=0.86), indicating that the degree of dysfunction present after 7 days of pacing was not different between groups.

Sequential Measures of Basal Cardiac Function

Over the course of 21 days of LV pacing, there were significant increases in arterial-mixed venous oxygen content difference, plasma norepinephrine concentration, heart rate, and mean pulmonary artery and left atrial pressures. Mean aortic pressure was reduced. However, no difference between groups was observed in any of these measures, although plasma concentrations of norepinephrine and epinephrine tended to be lower in animals receiving Ad.AC_{VI} (Table 2).

LV Contractile Function

Basal LV dP/dt was reduced after 7 days of continuous pacing (prepacing, 3019±123 mm Hg/s; 7 days of pacing,

TABLE 2. Sequential Measures of Basal Cardiac Function

Measures	Group	Days of Pacing				<i>P</i>
		0	7	14	21	
HR, bpm	AC _{VI}	117±7	118±3	117±9	132±4	T: 0.6048
	PBS	117±5	116±6	125±5	136±5	D: 0.0146
MAP, mm Hg	AC _{VI}	119±6	121±4	109±4	102±3	T: 0.6554
	PBS	121±3	111±4	108±4	105±5	D: 0.0015
PA, mm Hg	AC _{VI}	24±1	32±2	39±4	42±4	T: 0.4307
	PBS	23±1	30±2	37±3	40±2	D: 0.0001
LA, mm Hg	AC _{VI}	10±1	20±2	27±4	28±4	T: 0.1266
	PBS	9±1	16±2	24±2	28±1	D: 0.0001
LV +dP/dt, mm Hg/s	AC _{VI}	3433±288	2967±287	1706±147	1671±126	T: 0.6520
	PBS	3316±132	2581±253	1956±190	1655±181	D: 0.0001
LV -dP/dt, mm Hg/s	AC _{VI}	-2938±239	-2860±227	-2075±199	-1906±161	T: 0.7856
	PBS	-3130±140	-2455±163	-2243±149	-1815±144	D: 0.0001
AVO ₂ D, mL/dL	AC _{VI}	5.2±0.3	6.4±0.9	8.1±0.7	7.4±0.7	T: 0.4101
	PBS	5.7±0.4	5.4±0.4	6.8±0.5	7.8±0.5	D: 0.0003
Epi, pg/mL	AC _{VI}	108±34	85±19	133±26	89±21	T: 0.2808
	PBS	85±19	109±17	135±52	212±52	D: 0.4124
Norepi, pg/mL	AC _{VI}	220±44	211±55	433±170	491±98	T: 0.7488
	PBS	216±31	280±32	300±48	624±61	D: 0.0001

HR indicates heart rate; MAP, mean arterial pressure; PA, mean pulmonary artery pressure; LA, mean left atrial pressure; AVO₂D, arterial-venous oxygen content difference; Epi, epinephrine; Norepi, norepinephrine. Values are mean±SEM. Ad.AC_{VI}, n=7; PBS, n=9. Measurements were made with pacemakers inactivated; 0 days indicates before pacing and before treatment; 7 days, 7 days of pacing just before treatment; 14 days, 14 days of pacing, 7 days after treatment; 21 days, 21 days of pacing, 14 days after treatment. *P* values, from 2-way ANOVA, are for treatment effect (T) and duration effect (D).

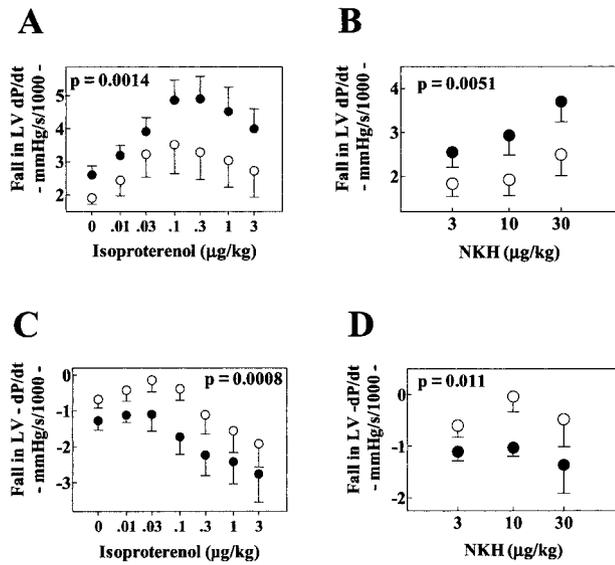


Figure 2. LV +dP/dt and -dP/dt. y axes of graphs represent mean values before pacing minus mean values obtained after 21 days of continuous pacing. PBS or Ad.AC_{VI} was given after 7 days of pacing. A and B, Pigs that received Ad.AC_{VI} (open circles) had less decrement in LV +dP/dt (a smaller decrease in contractile function) than pigs that received PBS (closed circles). This was observed through a wide range of infused isoproterenol concentrations (A) and also was evident when AC was directly stimulated with NKH477 (a water-soluble forskolin analogue) (B). C and D, Pigs that received Ad.AC_{VI} had less decrement in LV -dP/dt (increased relaxation) than pigs that received PBS. This was observed through a wide range of infused isoproterenol concentrations (C) and also was evident when AC was directly stimulated with NKH477 (a water-soluble forskolin analogue) (D). Symbols denote mean values, and error bars denote SEM. Probability value for treatment effect is from 2-way ANOVA. Ad.AC_{VI}, n=7; PBS, n=7.

2206±146; $P < 0.0002$; n=16) (Table 1). Significant reductions in basal mean LV +dP/dt and -dP/dt were also observed during the ensuing 14 days of pacing without group differences (Table 2).

TABLE 3. LV Dimension, Function, and Response to Dobutamine

	No Dobutamine						Dobutamine						<i>P</i>
	Ad.AC _{VI}			PBS			Ad.AC _{VI}			PBS			
	Pre	21 days	Abs Δ	Pre	21 days	Abs Δ	Pre	21 days	Abs Δ	Pre	21 days	Abs Δ	
EDD, mm	48±1	61±3	13±2	45±1	62±2	18±2	45±1	58±4	13±4	42±1	62±2	19±2	0.043; 0.073
ESD, mm	28±1	50±3	22±3	25±1	53±2	28±2	21±1	43±4	22±4	18±1	48±2	30±2	0.009; 0.71
Lat WTh, %	68±4	32±4	36±8	71±4	20±7	51±6	71±5	51±7	20±11	83±7	36±9	47±12	0.048; 0.34
IVS WTh, %	57±5	49±4	8±5	57±2	46±6	11±6	67±5	52±5	14±9	65±4	54±5	11±8	0.94; 0.66
FS, %	40±1	18±2	23±3	43±1	14±2	29±2	53±2	26±4	27±5	58±2	22±3	36±3	0.03; 0.13
Vcf, circ/s	1.9±0.1	1.1±0.1	0.7±0.1	2.1±0.1	1.0±0.1	1.1±0.1	3.6±0.2	1.9±0.2	1.7±0.4	4.1±0.1	1.5±0.1	2.5±0.2	0.008; <0.0001
LV +dP/dt, mm Hg/s/1000	2.8±0.2	1.9±0.2	0.8±0.3	3.2±0.2	1.3±0.1	1.8±0.2	7.0±0.9	3.6±0.4	3.4±1.2	7.9±0.6	2.4±0.3	5.5±0.8	0.039; 0.0002
LV -dP/dt, mm Hg/s/1000	-2.3±0.2	-2.2±0.2	0.1±0.4	-2.7±0.2	-1.6±0.1	1.1±0.2	-3.0±0.3	-2.8±0.4	0.2±0.5	-3.6±0.3	-2.1±0.1	1.5±0.4	0.005; 0.462
Lat wall stress, kdyn/cm ²	50±3	211±31	161±29	52±2	298±37	246±38	31±3	143±34	112±31	24±2	197±23	173±24	0.031; 0.069
IVS wall stress, kdyn/cm ²	52±3	78±5	27±6	50±4	148±9	98±10	32±3	96±14	65±12	24±2	110±9	86±9	<0.0001; 0.18

EDD indicates LV end-diastolic diameter; ESD, left ventricular end-systolic diameter; Lat. WTh, systolic thickening of lateral wall; IVS WTh, systolic thickening of interventricular septum; FS, fractional shortening; Vcf, velocity of circumferential fiber shortening; kdyn, kilodynes. Data entries (mean±SEM) represent values before pacing (Pre) and after 21 days of continuous pacing (21 days) and absolute difference between these two values (Abs Δ). Ad.AC_{VI}, n=6; PBS, n=9 except for the last 4 rows, where n=8 for the PBS group. Data were obtained with pacemakers inactivated. *P* values, from 2-way ANOVA, are for gene effect (first value) and dobutamine effect (second value).

To determine whether there were differences in LV contractile reserve, we performed studies to assess LV dP/dt responses to isoproterenol and also to NKH477, a water-soluble forskolin analogue⁸ that directly stimulates AC independently of β-adrenergic receptor and G_s. During adrenergic stimulation with isoproterenol, pigs that received AC_{VI} gene transfer exhibited improved LV +dP/dt through a wide range of isoproterenol doses ($P=0.0014$; Figure 2A). Stimulation with NKH477 was also associated with increased LV +dP/dt in AC_{VI}-treated pigs ($P=0.0051$; Figure 2B). LV -dP/dt, a measure of LV relaxation, was also favorably affected by AC_{VI} gene transfer compared with PBS treatment both during β-adrenergic receptor stimulation ($P=0.0008$; Figure 2C) and direct AC stimulation ($P=0.011$; Figure 2D).

Echocardiographic Assessment of Global and Regional Contractile Function

Table 3 shows changes in cardiac dimensions and function after 21 days of pacing, with and without stress conferred by infused dobutamine, a β₁-adrenergic receptor agonist. There was a favorable effect on LV end-diastolic ($P=0.043$) and end-systolic ($P=0.009$) dimension associated with AC_{VI} treatment. Regionally, the lateral wall showed less systolic dysfunction (measured by percent systolic wall thickening) in animals that received Ad.AC_{VI} ($P=0.048$; Table 3). The IVS was not affected as much by pacing as reported previously⁶ and was invariant between groups. Global LV function was also improved in animals receiving Ad.AC_{VI}. For example, there was improved fractional shortening ($P=0.03$) and velocity of circumferential fiber shortening ($P=0.008$; Table 3).

The end-systolic meridional wall stress increased over the course of the study. The increase in wall stress, however, was significantly reduced in animals that had received AC_{VI} gene transfer, an improvement that was evident in both the IVS and lateral walls (Table 3).

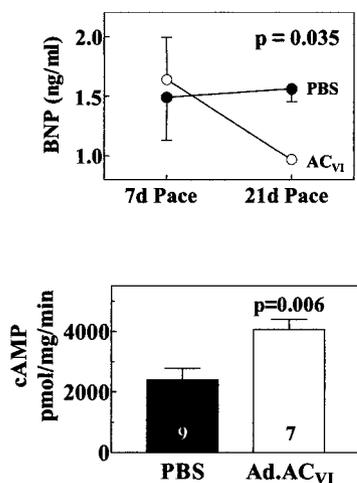


Figure 3. Top, Plasma concentration of BNP was reduced in animals that received intracoronary Ad.AC_{vI} (n=7) compared with animals receiving PBS (n=9). Symbols represent mean values; error bars denote SEM. Bottom, LV cAMP generation. Samples of LV from animals that had received Ad.AC_{vI} showed increased cAMP generation in response to AC stimulation with NKH477 (10 μ mol/L). Bars represent mean values (net stimulation, ie, basal subtracted); error bars denote SEM; numbers on bars denote number of animal per group.

LV cAMP-Generating Capacity and BNP

Intracoronary delivery of Ad.AC_{vI} was associated with a 1.7-fold increase in net NKH447-stimulated cAMP generating capacity 2 weeks after gene transfer (PBS, 2399 \pm 376 pmol \cdot mg⁻¹ \cdot min⁻¹, n=9; Ad.AC_{vI}, 4061 \pm 328 pmol \cdot mg⁻¹ \cdot min⁻¹, n=7; $P=0.006$; Figure 3). LV membranes obtained 14 days after AC_{vI} gene transfer also showed an increase in basal cAMP (PBS, 65 \pm 9 pmol \cdot mg⁻¹ \cdot min⁻¹, n=9; Ad.AC_{vI}, 90 \pm 7 pmol \cdot mg⁻¹ \cdot min⁻¹, n=7; $P=0.055$). Plasma concentrations of BNP were lower in animals that had received AC_{vI} gene transfer (Figure 3).

Evidence for Gene Transfer

The presence of Ad.AC_{vI} in LV after coronary gene transfer was confirmed by PCR. A 841-bp PCR product was found in LV samples from all pigs that had received intracoronary injection of Ad.AC_{vI} but not in samples from pigs that received PBS (Figure 4), confirming AC_{vI} gene transfer in the LV.

Necropsy and Histopathology

Although there were no differences in body, liver, and heart mass between the 2 groups, the PBS-treated group had 6 times more ascites than the AC_{vI}-treated group (Table 4). Pleural fluid was present in 2 of the 9 PBS pigs, whereas none was found in the AC animals. Scattered perivascular chronic inflammation was detected in 1 pig from each group. One pig

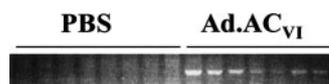


Figure 4. PCR conducted on LV samples from study animals confirmed presence of Ad.AC_{vI} transgene in LV samples from animals that had received intracoronary Ad.AC_{vI}, but samples from animals that had received PBS showed absence of transgene. See Methods for details.

TABLE 4. Data From Necropsy

	PBS (n=9)	Ad.AC _{vI} (n=7)	<i>P</i>
Body weight, kg	64 \pm 4	60 \pm 2	0.427
Liver weight, g	1958 \pm 102	2179 \pm 102	0.154
Liver/body wt, g/kg	31 \pm 2	37 \pm 2	0.055
LV, g	180 \pm 9	179 \pm 7	0.935
LV/body wt, g/kg	2.8 \pm 0.1	3.0 \pm 0.1	0.161
RV, g	83 \pm 4	87 \pm 6	0.575
RV/body wt, g/kg	1.3 \pm 0.1	1.5 \pm 0.1	0.244
Ascites, mL	1812 \pm 736	300 \pm 141	0.097
Pleural fluid, mL	33 \pm 24	0	NS

RV indicates right ventricular.

receiving PBS showed mild LV fibrosis; no pigs receiving Ad.AC_{vI} showed fibrosis. Three pigs in the AC group and 1 in the PBS group showed mild scattered interstitial inflammation. There were no statistically significant differences between the 2 groups (Fisher's exact test, $P=0.28$).

Discussion

The most important finding of this study is that gene transfer of AC_{vI} to failing hearts was associated with favorable effects on LV function and remodeling. These changes included reductions in LV end-diastolic and end-systolic dimensions and wall stress and increases in LV fractional shortening and velocity of circumferential fiber shortening, an echocardiographic correlate of contractile function. Stimulation with isoproterenol and NKH477 revealed increases in LV +dP/dt and -dP/dt, reflecting improved β -adrenergic receptor signaling and increased AC function and direct physiological impact on LV systolic function and relaxation. LV samples showed increased cAMP generation capacity, and plasma concentrations of BNP, a marker of LV dysfunction, were reduced. Gene transfer was documented by PCR. The study was conducted in a randomized and blinded manner, and treatment was given after evidence of heart failure was present.

We chose to use the pacing model for CHF in pigs because this model of heart failure is easy to generate and is associated with uniform degrees of LV dysfunction, thereby facilitating comparisons between treated and untreated animals. Pigs were used because of the relative ease of coronary catheterization for delivery of vector or PBS. Key features of this model: LV chamber dilation and severe depression of global LV function, decreased LV +dP/dt in response to β -adrenergic receptor stimulation, marked depression of cAMP-generating capacity, and increased plasma norepinephrine, mimic key features of clinical dilated heart failure. We thought that this model would provide a suitable means to determine whether intracoronary delivery of adenovirus encoding AC_{vI} would have beneficial or deleterious effects.

An important element in the present study was initiation of treatment only after we had firm evidence of heart failure. This was achieved by initiating pacing 7 days before treatment and documenting that severe LV dysfunction was present before intracoronary delivery of Ad.AC_{vI} or PBS was

performed. In this manner, we truly tested the effects of AC_{VI} gene transfer when performed in the presence of heart failure.

The pacing model of heart failure mimics aspects of clinical dilated CHF but also differs in important ways. For example, clinical heart failure, in general, develops slowly over a long period of time (months to years), but the pacing model is associated with the rapid onset of heart failure (days to weeks). Second, the cause of heart failure as a result of rapid sustained pacing is not entirely clear, and, although it may involve relative myocardial ischemia, it certainly is not associated with obstructive coronary artery disease, as is so often the case in clinical dilated CHF. Finally, the mortality rate from pacing-induced CHF is very high. In the present study, we witnessed a 33% mortality resulting from 21 days of continuous LV pacing at 220 bpm. We designed this study to focus on functional and biochemical indications of improved cardiac function, recognizing that a mortality end point would require the study of many more animals.

The pacing model, unlike other models of CHF, is associated with sustained toxicity (pacing). Thus, a therapeutic entity must not only overcome baseline LV dysfunction but also the accumulated dysfunction brought on by sustained pacing. The proposed therapeutic intervention must overcome these cumulative impediments to be efficacious. Because of these features, we envisioned that pacing-induced CHF would be a useful means to detect toxic effects, but we were skeptical that a gene transfer strategy would be sufficiently effective to overcome the cumulative and inexorable severity of LV dysfunction associated with this model. In this regard, it is noteworthy that LV dysfunction was still present in animals that received Ad.AC_{VI}, albeit to a lesser degree than that seen in animals that received PBS.

Ad.AC_{VI} was associated with reductions in LV end-diastolic and end-systolic dimensions and increases in LV fractional shortening and velocity of circumferential fiber shortening. LV +dP/dt and -dP/dt responses to β -adrenergic receptor and direct AC stimulation were both improved. However, we saw no changes in arterial-mixed venous oxygen content difference or mean left atrial or pulmonary artery pressures. This may reflect the sustained toxicity of this model of CHF, which requires continuous rapid LV pacing. Basal measures of cardiac function in heart failure are not as predictive of overall functional capacity as are measures of cardiac function during stress. For example, LV ejection fraction, a measurement obtained at rest, does not predict functional capacity measured by maximal oxygen consumption or treadmill time.¹¹ In the present study, we found differences in the 2 groups in terms of inotropic reserve, estimated by infused isoproterenol and NKH447, and in echocardiographic assessment of LV function during dobutamine stress. In addition, there were also changes in static measures of LV geometry and reduced plasma concentrations of BNP, indicating improved global LV function.

We have no data regarding the effects of treatment on the liver in the present study. However, in a previous study conducted in normal pigs with the same adenovirus vector and doses up to 3.5-fold higher, we found no abnormal findings in liver samples from the study animals.⁵ During hemodynamic and agonist infusion studies, no arrhythmias

were observed in the present study. In a previous study using transgenic mice with cardiomyopathy, we found that coexpression of AC_{VI} in the heart prolonged life by reducing the incidence of sudden death.⁴

Two previous studies have used cardiac gene transfer to treat failing hearts.^{12,13} Both used adeno-associated virus vectors in rodents: 5- to 6-week-old hamsters with genetic cardiomyopathy¹² or 7- to 8-week-old rats.¹³ Gene transfer was facilitated by hypothermia and occlusion of the aorta and pulmonary artery. A strength of the present study is that we have shown the feasibility of a method that could be applied safely in clinical settings. These methods do not require interventions such as surgery, hypothermia, or occlusion of major vessels, any 1 of which would be associated with morbid complications in patients with severe heart failure.

One wonders whether the strategy presented here, which uses adenovirus vectors, would provide a long-term benefit. A previous study from our laboratory, using the same vector and delivery methods in normal pigs, showed that the effects of AC_{VI} gene transfer on LV cAMP generation and cardiac function persisted without recrudescence for at least 18 weeks after gene transfer.⁵ The present study was designed so that all animals were killed at the completion of the protocol, which occurred 3 weeks after pacing was initiated, which was 2 weeks after treatment. This model is not suited for studies of longer duration because of its progressively high mortality when pacing is continued for longer than 3 to 4 weeks. One option would be to decrease the pacing rate, but then there would be recovery of LV function, which would confound data analysis. We have shown in transgenic mice that long-term expression of AC_{VI} is associated with improved cardiac function and increased survival in cardiomyopathy.⁴

We did not include a contemporaneous instrumented but unpaced control group in the present study to determine the degree to which LV cAMP generation was reduced by pacing-induced heart failure. However, previous studies from our laboratory found a 41% reduction in forskolin-stimulated LV cAMP production¹⁴ in this model of heart failure. By inference, the increase in LV cAMP generation associated with Ad.AC_{VI} treatment that we report in the present study (1.7-fold) would represent an increase to near normal levels.

Numerous statistical tests were conducted, and therefore, the issue of type I errors must be addressed. Many of these tests were conducted to provide confirmation of the effectiveness of the model (Tables 1 and 2) or describe outcomes (Table 4). In addition, the conclusions do not appear to be at risk because of the strength of the findings. For example, the central results reported in Figure 2 are all significant far beyond the stipulated $P < 0.05$ level. Likewise, in Table 3, 9 of the 10 tests reported showed statistical significance. Therefore, we do not believe that the type I error risk is excessive.

Determining the molecular pathways that are involved in these salutary effects of AC_{VI} gene transfer in the failing heart was not the goal of the present work but is the focus of ongoing studies in our laboratory. The concept that the favorable effects of AC_{VI} gene transfer are a manifestation of increased cAMP generation is, however, overly simplistic. More recent unpublished data from our laboratory indicate

that AC_{VI} alters the expression of genes that have favorable effects on contractile function. For example, phospholamban expression is markedly reduced by AC_{VI} gene transfer, and this is associated with favorable alterations in myocardial calcium handling. These events may be important in understanding why protein kinase A seems to have deleterious effects on the heart,¹⁵ whereas AC does not.

In conclusion, we have shown that intracoronary delivery of an adenovirus encoding AC_{VI} is associated with increased LV function when delivered to animals with CHF. LV dysfunction was present before gene transfer, underscoring the potential therapeutic relevance of this study.

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Intracoronary Adenovirus Encoding Adenylyl Cyclase VI Increases Left Ventricular Function in Heart Failure

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