Novel technique of aortic banding followed by gene transfer during hypertrophy and heart failure

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Novel technique of aortic banding followed by gene transfer during hypertrophy and heart failure. Physiol Genomics 9: 49–56, 2002; 10.1152/physiolgenomics.00035.2001.—Aortic banding in the rat has become a popular method to induce left ventricular (LV) hypertrophy and heart failure. However, because of often extensive intrathoracic adhesions and inflammatory cell infiltrates resulting from the traditional surgical approach, an uncomplicated second thoracic incision for genetic manipulation is impeded. In this study, we describe a novel surgical technique of aortic banding which avoids opening the sternum and thereby avoids adhesions and surgery-related inflammation. Placing a clip on the ascending aorta using a suprasternal approach in Sprague-Dawley rats created proximal aortic constriction. The present study was initiated to determine whether a replication-deficient adenovirus would enable efficient gene transfer to adult cardiac myocytes undergoing hypertrophy and transitioning to heart failure. Echocardiography performed at week 24 revealed significant concentric hypertrophy and increased fractional shortening followed by LV dilatation with decreased fractional shortening after 27 wk of banding. An adenoviral solution encoding for the reporter green fluorescent protein gene (GFP) was delivered to the heart. Fluorescent microscopy revealed global gene expression throughout hypertrophied and failing hearts. Our studies demonstrate that a novel suprasternal approach can be applied to create an LV hypertrophy model followed by heart failure which also allows investigators to perform genetic manipulations in vivo through gene transfer without the complication of adhesions and surgical trauma-induced inflammation. Furthermore, our approach to delivery of transgenes results in homogenous gene expression in both hypertrophied and failing hearts.

cardiac; gene therapy; rats

THE INCIDENCE OF HEART FAILURE continues to increase. Current methods of pharmacological therapy can only partly mitigate the disease progression, and the mortality in patients with advanced heart failure remains high.

Gene therapy is rapidly becoming an experimental treatment modality in heart failure (8), especially for patients where cardiac transplant is not an option. Animal models of heart failure are needed to enable investigators to evaluate the therapeutic effect and physiological function of genes delivered by gene transfer in vivo prior to studies in humans. Multiple animal models of left heart failure have been established in experimental research (1, 2, 7, 16). Aortic banding, coronary ligation, or administration of cardiotoxic substances such as adriamycin have frequently been used in rodents, whereas rapid pacing and emboli-induced infarction are more often used in large animal models (1, 2, 7, 16).

With aortic banding an increase in cardiac afterload is induced by placing a metal clip on the ascending aorta (14). Depending on the diameter of the clip, heart failure can develop in rats within 20–30 wk. The timing and progression to hypertrophy and heart failure is impacted by species, age at the time of banding, and the degree of stenosis applied (14). Nevertheless, this model, which closely mimics chronic hemodynamic stress or load seen in clinical heart failure, has proven useful in studying the progression to hypertrophy and transition to heart failure.

Commonly used surgical approaches in rats often result in extensive thoracic scaring, fibrous adhesions, and epicardial inflammatory cell infiltration. To investigate the efficiency of gene transfer in the setting of hypertrophy and heart failure as well as improve the utility of the aortic banded rat model in gene transfer experimentation, we have developed a new surgical approach to aortic banding to dissociate transgene-associated inflammatory cell infiltration and approach-induced scarring and adhesions which might impact global cardiac function and survival. This study was undertaken to determine whether a replication-deficient adenovirus can induce efficient gene transfer into adult cardiac myocytes undergoing hypertrophy and transition to heart failure in vivo. Here we demonstrate a novel surgical technique for aortic banding which enables homogenous adenoviral-mediated gene transfer in both hypertrophied and failing hearts.
METHODS

Aortic banding. Male rats (Sprague-Dawley, 70–80 g) aged 4 wk, were obtained (Taconic, MA). These rats were free of antibody titers to a number of routinely tested rat viruses. In addition, the rats were free of all endo- and ectoparasites and mycoplasma species. The rats were maintained in a barrier room at 72 ± 2°F with a relative humidity at 50% ± 10% and fed a commercial laboratory diet and water ad libitum. Ventilation in the room was between 12–15 air changes per hour of 100% prefiltered outside air. The light cycle period was controlled at 12 h of light and 12 h of dark with no twilight transition. The Institutional Animal Care and Use Committee approved the study. Rats were anesthetized with intraperitoneal pentobarbital (60 mg/kg). A heating lamp maintained body temperature. The animal was intubated via the larynx by using a 16-gauge soft catheter (Angiocath; Becton-Dickson, Sandy, UT). The rats were ventilated with tidal volumes of 2 ml at 50 cycles/min with a P\textsubscript{ETCO\textsubscript{2}} of 0.21. The anterior chest of the animal was shaved, a 1-cm incision was made at the level of cricoid process, and dissection down to the clavicles was performed (Fig. 1). The right clavicle was cut, the thymus was exposed and retracted out of the field of dissection, and the aortic arch was exposed. A clip was placed around the ascending aorta. The clavicle was then re-adjointed, and the skin was closed. The animals were allowed to recover and then returned to their cages.

In vivo cardiac function. Animals were anesthetized with pentobarbital 60 mg/kg intraperitoneally, and the anterior chest was shaved. Transthoracic M-mode and two-dimensional echocardiography was performed with a Hewlett-Packard Sonos 5500 imaging system (Andover, MA) with a 15-MHz broadband transducer. A midpapillary level left ventricular (LV) short axis view was used to measure LV dimensions on the M-mode. The images were recorded and stored digitally. Measurements of LV posterior wall thickness, LV septum thickness, LV end-diastolic dimension (LVEDd), and LV end-systolic dimension (LVESd) were performed online and offline. Fractional shortening was then calculated as (LVEDd – LVESd)/LVEDd × 100. Gene transfer was performed in animals at the time of LV dilatation, which occurred at 27 wk postbanding. The same animals underwent echocardiography at 24 and 27 wk. Following delivery of the adenoviral vector (2–4 days), rats were euthanized with pentobarbital (200 mg/kg ip), and the heart weights, body weights, and tibial lengths were measured. Heart weight/tibial length and heart weight/body weight ratios were calculated as indicators of heart enlargement.

Construction of E1-deleted recombinant adenoviral vectors. A first generation type 5 recombinant adenovirus was used in these studies encoding for the green fluorescent protein (GFP) under the control of the cytomegalovirus promoter (CMV). Ad.GFP was constructed through homologous recombination in Escherichia coli bacteria by using pAdTRACK/Easy system (13). The recombinant virus was prepared as high-titer stocks by propagation in 293 cells as previously described (9–11, 13). The titer of stock used for these studies measured by plaque assays was as follows: 2 × 10\textsuperscript{10} pfu/ml for Ad.GFP. A second adenovirus, Ad.\textbeta-gal, was constructed in a similar fashion with \beta-galactosidase (\textbeta-gal) also under the control of CMV. A third virus with no insert or promoter was also constructed (Ad.\textemptyset).

Adenoviral delivery protocol. The chest was entered by a median sternotomy. A 22-gauge catheter containing 100 \mu l of adenoviral solution was advanced from the apex of the LV to the aortic root. The aorta and pulmonary arteries were clamped distal to the site of the catheter, and the solution was injected. The clamp was maintained for 30 s. This procedure allows the solution that contains the adenovirus to perfuse the coronary arteries of the heart. After removal of air and blood, the chest was closed. We have noted that the “blanching” of the heart during the injection and cross-clamping is a hallmark for effective gene transfer. We therefore ensured that during the surgical procedure and adenoviral gene delivery this blanching was observed.

Experimental groups. In the first group of animals we used 60 rats aged 4 wk as described above. Thirty animals were randomized in the banding group, and thirty animals were randomized in the sham group. Immediately postoperatively and within 2 wk of surgery, six animals died in each group. Therefore, 24 animals in each group were followed by echocardiography. At the end of the study, 27-wk postbanding, 15 animals in each group were studied morphologically, and the rest of the tissues were used for protein analyses.

In a second group of animals, 10 rats aged 12 wk underwent either injection with either Ad.\textbeta-gal and the empty adenovirus (Ad.\textemptyset). The animals were studied 2–4 days following injection.

Histochemistry and fluorescence detection. A subset of the hearts which were infected with Ad.\textbeta-gal were examined by immunohistochemistry to evaluate the expression of \textbeta-gal. Hearts were fixed with a PBS containing 0.5% glutaraldehyde for 30 min and then in PBS with 30% sucrose for 30 min. The hearts were then permeabilized by incubation in solution containing sodium deoxycholate (0.01%) and Nonidet P-40 for 15 min. The hearts were then incubated overnight in a solution containing 5-bromo-4-chloro-3-indolyl-\textbeta-D-galactopyranoside (X-Gal), and 10-\mu m sections were then cut and examined under light microscopy. Lungs, livers, and

![Fig. 1. Banding of the ascending aorta using the supraclavicular approach.](http://physiolgenomics.physiology.org/)}
full-length aorta were also fixed and examined in a similar fashion.

The subset of animals which were infected with Ad.GFP were examined by fluorescent microscopy. The hearts were quickly removed and perfused in a retrograde fashion using PBS to remove blood products. The hearts were then cut in the midventricular area using 10-μm sections. These sections were first visualized with white light and then by fluorescence microscopy at 510 nm with single excitation peak at 490 nm of blue light.

Gross morphology. Hearts were harvested after four days posttransgene delivery and sectioned. The following morphological measurements were taken: heart weight, body weight, and tibial length. Heart weight/tibial length and heart weight/body weight ratios were calculated as indicators of myocardial enlargement.

Statistics. Data are presented as mean ± SD. A two-tailed unpaired Student’s t-test was performed to determine significance. A statistical significance was accepted at a the level of \( P < 0.05 \).

RESULTS

Aortic banding created LV hypertrophy. Banding of the ascending aorta resulted in LV hypertrophy 24 wk after surgery. Echocardiography showed concentric hypertrophy of the LV with significant increase in septal and posterior wall thickness (Table 1; Fig. 2; \( P < 0.0001 \)), as well as a decrease in LV end-diastolic and end-systolic diameter (Table 1; Fig. 2; \( P < 0.0001 \)). At 27 wk postbanding there was LV dilatation (Table 1; Fig. 3). LV end-diastolic diameter and end-systolic diameter were significantly increased, and fractional shortening was significantly reduced (Table 1).

Adenoviral gene transfer and morphological parameters. To examine the distribution of transgene expression, we used two adenoviruses carrying the reporter genes GFP and β-gal, Ad.GFP and Ad.βgal, respectively. Adenoviral gene transfer (Fig. 4) induced an expression pattern that was grossly global throughout
the ventricles in hypertrophied and failing hearts. In contrast, noninfected hearts had little background fluorescence. To address the potential impact of high-titer infection on adult ventricular myocytes and heart function in animals with heart failure, we compared pre- and postinfection on morphological parameters (Table 2). A significant increase in heart size was seen in the aortic banded rats at 27 wk postbanding. These data confirm echocardiographic results and demonstrate that there was no detrimental effect of the GFP transgene on the expected response to aortic banding at gross morphology.

Hearts were also examined with immunohistochemistry to evaluate the microscopic distribution of β-gal expression in vivo. As shown in Fig. 5, histochemical staining of a ventricular cross section from hearts infected with Ad.βgal revealed β-gal activity in myocytes. The distribution of β-gal was not uniform in all cross sections. Certain areas had diffuse staining while other sections had a more patchy distribution of expression. It is important to note that we used an adenovirus carrying a nuclear localized form of β-gal. Cytoplasmic β-gal activity is evident only in myocytes expressing the highest level of β-gal activity. There-
fore, a ventricular section that typically reveals a small minority of muscle nuclei may underestimate the uniformity and the level of nuclear β-gal expression. To evaluate whether other tissues are infected, we histologically examined sections of aorta, liver, and lung following infection with Ad.β-gal. There was no histological evidence of β-gal activity in the aorta; however, β-gal activity was present in the liver and lungs (data not shown).

**Gross morphology.** As demonstrated in Fig. 6, compared with the traditional approach (Fig. 6A), which often resulted in adhesions and myocardial scarring, there were no adhesions in rats that underwent the supraclavicular surgical approach (Fig. 6B). Survival was 100% for the supraclavicular approach. No deterioration was noted in heart function in virus-infected hearts (data not shown). Table 2 confirms the expected increase in heart size as a result aortic banding. The delivery of high-titer infection did not appear to result in loss or inhibition of the expected hypertrophic response. Thus under the conditions tested, adenovirus infection by itself had no obvious detrimental effect on animal survival rate, cardiac size, or global cardiac function.

**Inflammatory response.** To evaluate the inflammatory response, we used two different adenoviruses, Ad.β-gal and the empty adenovirus (Ad.Ø), to rule out effects due to the transgene. Four days postinjection, rat hearts demonstrated a mild inflammatory response within myocardium (Fig. 7). Magnification of these areas identified the infiltrating cells as being primarily mononuclear, with only rare polymorphonuclear leukocytes present. Interestingly, most of the infiltration occurred around arterioles. However, there were no significant differences between Ad.β-gal and the empty adenovirus (Ad.Ø) in terms of the immune response.

**DISCUSSION**

Accurate knowledge of the cellular processes involved in the contractile performance of hypertrophied myocardium while transitioning to overt failure is critical to our understanding of cardiac muscle disease and the development of innovative strategies for its treatment. To be able to study carefully phenotyped animal models at selected time points under controlled conditions of therapeutic interventions would be ideal. Furthermore, gene transfer may facilitate tests to determine the impact of putative regulatory proteins involved in the signaling pathways of hypertrophy and the transition to heart failure and would help us understand some of these mechanisms.

Although molecular techniques have made giant steps forward, the methods for inducing reproducible and highly consistent surgical models in small rodents has lagged behind the improvements in molecular bi-
ology techniques. However, with the development of rodent models, there arise several clearly recognized needs: 1) a need to fully explore the phenotype at the whole organ and in vivo animal level and 2) the need to evaluate them at the molecular or genetic level (2, 4, 5). Microsurgical interventions used to study the interrelationship of genes and pathophysiology in vivo is a powerful approach. Surgical models may resemble the pathophysiological response to chronic hemodynamic stress or load, thereby resembling components of clinical heart failure seen in humans. Although techniques for exploring aspects of cardiac function are well developed for larger species, their modification for the small size of rodents with rapid heart rates and small surgical planes has proven a formidable challenge.

Using this approach, we obtained a model of hypertrophy and heart failure that allows a less challenging second surgical intervention for the in vivo transduction of adenoviral genes.

**In vivo transduction by adenoviral gene transfer.** We have previously reported that the catheter-based adenoviral technique induces global gene transfer in adult rat hearts with an expression pattern that is grossly global throughout the ventricle (3, 11). In single myocytes isolated from the infected hearts, we have also previously reported that ~50–70% of cardiomyocytes were expressing the reporter transgene (3, 11). Even though not every single cardiomyocyte was infected, we have shown previously that this degree of myocyte transduction was sufficient to induce morpho-

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**Fig. 6.** A: scarring associated with traditional approach to aortic banding in rats. Notice the severe adhesions with the standard aortic banding approach. B: there are no adhesions or cellular infiltrates with the supraclavicular approach.

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**Fig. 7.** Low-magnification image of cross section from rat hearts 4 days following gene transfer with either Ad.βgal (A) or Ad.Ø (B). Note the small infiltrations around the coronary vessels. High-magnification image around arteriolar vessels from rat hearts 4 days following gene transfer with either Ad.βgal (C) or Ad.Ø (D).
logical and physiological changes. Together our data using reporter constructs demonstrates highly effective gene transfer to adult rat hearts with hypertrophy and heart failure in vivo.

It has been reported that coronary reserve is decreased in the setting of heart failure (15). This might be expected to impact our delivery method and efficiency of gene transfer. However, we obtained similar levels of efficiency for both control aged matched rats as seen with hypertrophied hearts and failing hearts. These data indicate that in the presence of differences in coronary reserve and/or flow, our method of gene delivery can still result in significant transgene expression levels.

Recombinant adenoviral vectors offer the opportunity to investigate the role of local signal transduction pathways in the pathogenesis of heart failure both in vitro and in vivo. Recombinant adenoviral vectors offer several significant advantages: the viruses can 1) be prepared at extremely high titer, 2) successfully infect non-replicating cells, and 3) confer high-efficiency and high-level transduction of cardiocytes. Transgenic approaches targeting specific proteins in the signaling pathway are also possible. Furthermore, adenoviral transduction offers several advantages over transgenic approaches. First, developmental issues can be removed from consideration by working in adult animals and in vivo systems. For example, transgenic mice have been generated by overexpressing SERCA2a or by knocking out phospholamban. However, these animals develop changes in other calcium cycling proteins that dilute the direct effects of overexpression of the cDNA of interest (12). The differences in the phenotype seen in transgenic mice may be related to differences in the timing and/or level of transgene expression or differences in genetic backgrounds. Second, the high efficiency of adenoviral transduction makes feasible expression of multiple exogenous cDNAs. In this way, one can potentially deduce whether effects involve overlapping or distinct mechanistic pathways. We previously demonstrated that coinfection of neonatal rat myocytes with two viruses did not significantly alter the expression level of either transgene, compared with myocytes infected with one virus alone (10). Third, adenoviral vectors can be used in vitro and in vivo, as well as in multiple species, thus providing optimal flexibility in selecting a model (9–11). Fourth, with the use of adenoviral vectors that simultaneously encode for GFP, investigators can rapidly identify the distribution of the transgene delivery and expression as well as identify individual cells that are expressing the transgene of interest and study these single cells functionally. The major disadvantages to adenoviral gene transfer have been its transient expression and the host immune/inflammatory response to the vectors themselves (6). Gene transfer to adult myocardium in vivo has generally been found to mediate a high level of expression for ~1 wk. By 2–4 wk after infection, transgene expression has usually either declined dramatically or been extinguished completely. Furthermore, it has been suggested that late inflammation can conceivably be exacerbated by the mechanical trauma of gene delivery approaches (6). By reducing myocardial trauma using our approach to gene delivery, one should be able to avoid exacerbation of inflammatory responses.

Adenoviral gene transfer to rodent hearts has been largely performed by direct injection into the myocardium. This has usually resulted in focal areas of overexpression of the gene carried by the adenovirus and inflammatory responses. Some of the disadvantages of this method are 1) direct injection results in damage to muscle tissue at the site of injection, and 2) the overexpression is focal rather than homogeneous and therefore is unlikely to modulate global cardiac function. Therefore, a catheter-based technique was previously developed to deliver adenoviruses to the heart in a global fashion (11). This technique offers the distinct advantage over established methods of gene delivery to the heart in that the overexpression is global in distribution and the coronary arteries do not have to be manipulated, a procedure that carries a very high mortality in rodents. Using this combined approach, we have achieved global cardiac transgene expression in a model of hypertrophied and heart failure.

Our novel approach will enable investigators to deliver transgenes without extensive cardiac adhesions and increased scarring due to surgical manipulation, thereby enabling an easier second surgical manipulation and a clearer picture of the effects of transgene delivery on cardiac function as opposed to a complicated picture that results from the surgical intervention.

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