Published in final edited form as:

Am J Physiol Heart Circ Physiol. 2005 June ; 288(6): H2995–H3000.

Catheter-based antegrade intracoronary viral gene delivery with coronary venous blockade

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Abstract

Hayase, Motoya, Federica del Monte, Yoshiaki Kawase, Brian D. MacNeill, Jennifer McGregor, Ryuichi Yoneyama, Kozo Hoshino, Tsuyoshi Tsuji, Alec M. De Grand, Judith K. Gwathmey, John V. Frangioni, and Roger J. Hajjar. Catheter-based antegrade intracoronary viral gene delivery with coronary venous blockade. *Am J Physiol Heart Circ Physiol* 288: H2995–H3000, 2005; doi:10.1152/ajpheart.00703.2004.—The purpose of this study is to evaluate the feasibility of percutaneous antegrade myocardial gene transfer (PAMGT). A consistent and safe technique for in vivo gene transfer is required for clinical application of myocardial gene therapy. PAMGT with concomitant coronary venous blockade was performed in 12 swine. The myocardium was preconditioned with 1 min of occlusion of the left anterior descending and left circumflex arteries. The anterior interventricular vein was occluded during left anterior descending artery delivery, and the great cardiac vein at the entrance of the middle cardiac vein was occluded during left circumflex artery delivery. With arterial and venous balloons inflated (3 min) and after adenosine (25 µg) injection, PAMGT was performed by antegrade injection of an adenoviral solution (1 ml of 10¹¹ plaque-forming units in each coronary artery) carrying β-galactosidase or saline through the center lumen of the angioplasty balloon. In one set of animals, PAMGT was performed with selective coronary vein blockade (*n* = 9); in another set of animals, PAMGT was performed without coronary vein blockade (*n* = 5). At 1 wk after gene delivery, the animals were killed. Quantitative β-galactosidase analysis was performed in the left and right ventricular walls. PAMGT was successfully performed in all animals with and without concomitant occlusion of the coronary veins. Quantitative β-galactosidase analysis showed that PAMGT with coronary blockade was superior to PAMGT without coronary blockade. β-Galactosidase activity increased significantly in the β-galactosidase group compared with the saline group: 1.34 ± 0.18 vs. 0.81 ± 0.1 ng (*P* ≤ 0.01) in the left ventricular wall and 0.91 ± 0.1 vs. 0.66 ± 0.07 ng (*P* ≤ 0.05) in the right ventricular wall. PAMGT with selective coronary venous blockade is feasible, reproducible, and safely achieved in a large-animal model.

Keywords

gene therapy; catheterization; balloon; occlusion

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CONGESTIVE HEART FAILURE is the most frequent cause of hospitalization, affecting nearly 5,000,000 patients in the United States alone (1). Even though novel treatments, including pharmacological and surgical techniques, have decreased mortality in patients with congestive heart failure, it continues to represent an enormous clinical problem (20,21). Alternative treatments that reverse or cure the underlying pathological state of the heart are being actively pursued (20,21). Cardiac gene transfer using viral vectors is one such novel modality. A number of approaches have been used to achieve cardiac gene transfer, such as intracoronary injection, direct myocardial injection, pericardial sac transduction, coronary venous retroperfusion (11), and selective intracoronary perfusion (6). An efficient and homogenous method for gene delivery during percutaneous coronary intervention would be very beneficial, especially if gene therapy for heart failure is to be applied to humans. A number of percutaneous gene delivery methods have been used in large animals. Antegrade coronary gene delivery with coronary artery and coronary sinus occlusion has been successfully performed in rabbits (7,14,23), and pressure-regulated retroinfusion of coronary veins during coronary artery occlusion has been accomplished in swine (3). The brief interruption of coronary flow and a high-pressure condition during viral delivery seem to be very important to increase diffuse and homogenous gene distribution. In addition, selective gene transfer to the atrioventricular node by intracoronary perfusion was recently shown to be effective in a porcine model (6).

In this study, our aim was to develop a percutaneous and clinically applicable catheter-based gene delivery system to allow selective antegrade myocardial gene transfer with or without concomitant specific coronary vein blockade to induce high gene expression in targeted myocardium.

METHODS

Construction of recombinant adenovirus.

To construct the adenovirus containing β -galactosidase cDNA, we used the method described by He et al. (13), whereby the backbone vector, containing most of the adenoviral genome (pAd.EASY1), is used and the recombination is performed in *Escherichia coli*. The shuttle vector also has a concomitant green fluorescent protein under the control of a separate cytomegalovirus promoter. The adenoviruses were propagated in 293 cells. The titers of stocks used for these studies measured by plaque assays were 3×10^{11} plaque-forming units/ml. The recombinant adenoviruses were tested for the absence of wild-type virus by PCR of the early transcriptional unit E1.

Experimental study.

The study was carried out according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and was approved by the Massachusetts General Hospital Subcommittee on Research Animal Care. A total of 22 swine (40–50 kg) were anesthetized first with atropine (0.04 mg/kg) and acepromazine (0.1 mg/kg) and then with Telazol (tiletamine and zolazepam, 4.4 mg/kg) and xylazine (2 mg/kg) and subsequently intubated and ventilated with oxygen and 2% isoflurane. After sterile skin preparation, a 7-Fr sheath was placed in the right femoral artery and an 8-Fr sheath was placed in the right femoral vein using a standard Seldinger technique. Before catheterization, heparin (5,000 U) was injected to maintain an activated clotting time of 250–300 s.

Percutaneous coronary venous access.

Via a right femoral approach, a 50-cm 8-Fr modified Left Amplatz 1 catheter (Cordis, Miami, FL) was advanced to the coronary sinus. A 0.025-inch guide wire (Terumo, Tokyo, Japan) was advanced via the great cardiac vein (GCV) to the anterior interventricular vein (AIV). A 110-cm 5-Fr wedge balloon (Allow International, Reading, PA) was advanced to the GCV or AIV

over the guide wire, and the guide wire was withdrawn. A pressure manometer was connected to the center lumen of the balloon catheter to measure coronary venous pressure. The wedge balloon was inflated until coronary venous occlusion was confirmed by angiography and a rise in the coronary venous pressure.

Percutaneous coronary arterial access.

A 7-Fr hockey-stick guiding catheter (Cordis) was placed in the left coronary artery. After a 100- μ g nitroglycerin injection, coronary angiography was performed. A 0.014-inch guide wire (Guidant, Temecula, CA) was advanced into the left anterior descending coronary artery (LAD). A 9-mm-long, 3.5-mm Maverik over-the-wire balloon (Boston Scientific Scimed, Natick, MA) was advanced over the wire and positioned after the first diagonal, and the guide wire was withdrawn. The coronary balloon was inflated incrementally until complete occlusion was confirmed by angiography.

Similarly, a standard coronary wire was inserted into the distal circumflex artery. Over the wire, an angioplasty balloon was placed proximal to the bifurcation of the second obtuse marginal artery. The coronary balloon was inflated incrementally until complete occlusion was confirmed by angiography.

Gene delivery.

For the LAD and left circumflex artery (LCX) territories, the myocardium was preconditioned with a 1-min arterial balloon occlusion. The AIV was occluded during LAD delivery, and the GCV at the entrance of the middle cardiac vein was occluded during LCX delivery (Fig. 1). With the arterial and venous balloons inflated (total 3 min) and after an intracoronary adenosine (25 μ g) injection to increase cellular permeability (23), percutaneous ante-grade myocardial gene transfer (PAMGT) was performed by ante-grade injection of an adenoviral solution (1 ml of 10^{11} plaque-forming units in each coronary) carrying β -galactosidase with ($n = 9$) or without coronary vein occlusion ($n = 5$) or saline ($n = 6$) through the center lumen of the angioplasty balloon. Arterial blood pressure was continuously monitored, and antibiotics were administered (cefazolin, 1 g as a single bolus intravenous injection). At the end of the first experiment, all catheters and introducer sheaths were removed, and the animals were allowed to recover.

Euthanasia.

After 1 wk, the animals were again anesthetized and intubated, and catheter introducer sheaths were placed as described above. The patency of the LAD and LCX was confirmed by coronary angiography. Thereafter, the animals were euthanized with pentobarbital sodium (100 mg/kg iv), and the heart was excised for histology.

Detection of cardiac gene expression.

The short-axis cross section of the left ventricle (LV) was divided into four sections, the anterior, lateral, inferior, and septal walls, and a fifth sample was taken from the right ventricular (RV) wall. Histology of gene expression and quantitative analysis were performed in each section. Each section was examined by immunohistochemistry to evaluate the expression of β -galactosidase. Each section was fixed with a phosphate-buffered solution containing 0.5% glutaraldehyde for 30 min and then in phosphate-buffered solution with 30% sucrose for 30 min. The sections were permeabilized by incubation in solution containing sodium deoxycholate (0.01%) and NP-40 for 15 min. The sections were then incubated overnight in a solution containing 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal), and 10- μ m sections were cut and examined under light microscopy.

Detection of viral distribution through fluorescent microspheres.

To examine the distribution of the fluorescent spheres, we performed two additional studies, where we injected fluorescent beads, instead of the virus. The large-animal intraoperative fluorescence imaging system used in this study has been described in detail previously (4). Briefly, the system simultaneously displays color video and fluorescence images of the surgical field in real time. The near-infrared (NIR) fluorophore indocyanine green (Akorn, Decatur, IL) was resuspended at 2 mg/ml in saline and mixed 1:1 with 10- μ m-diameter red fluorescent beads (catalog no. F-8834, Molecular Probes). Five milliliters of this solution were injected intravenously into the coronary artery under investigation, and NIR fluorescent and red fluorescent images of the heart were acquired using optimized filter sets at exposure times of 67 and 250 ms, respectively.

Quantitative β -galactosidase analysis.

Quantitative β -galactosidase analysis was performed using a Galacto-Light Plus kit (Applied Biosystems) on tissue lysate. The activity was evaluated using a luminometer (Microumat). At 7 days after gene delivery, the heart of each animal was removed and dissected into cross sections that are distal to the balloon occlusion sites and represent the different walls of the ventricles. Each sample from each section was shock frozen (-70°C) until assessment of β -galactosidase activity. For the β -galactosidase assay, 1 g of tissue was sampled from the entire wall, which included epi-, mid-, and endocardium. After the tissue was minced for homogenization in lysis buffer (Galacto-Light Plus kit) with protease inhibitor (Complete, Roche), an aliquot was taken for protein assay. Homogenates were centrifuged at 14,000 g for 10 min, and the resulting supernatants were further clarified by means of a second centrifugation at 14,000 g for 10 min. Duplicate enzymatic assays for β -galactosidase activity were performed with 5- μ l samples of cleared supernatant using a transilluminator. A 35- μ l aliquot of assay reagent (reaction buffer, dilute Galacto-Light Plus substrate, 1:100, reaction buffer diluent, Applied Biosystems) was added to each 5- μ l sample, and light production was measured over a 10-s period. Samples were measured and light units were determined. β -Galactosidase activity (light units/ml) was then converted to nanograms per milliliter of protein.

Statistics.

Values are means $\pm$ SE. Data from the β -galactosidase and saline PAMGT groups were compared using the unpaired *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

PAMGT was successfully performed in 20 animals without complication. Antegrade adenoviral injection with or without selective AIV and GCV occlusion resulted in a significant gene expression in the inferior walls and the RV wall. There were no acute procedural complications, nor were there any deaths, in follow-up. From a hemodynamic standpoint, the pigs tolerated the procedure with or without coronary vein occlusion (Table 1).

Gene expression histology.

Different sections were obtained from the five different walls within the heart as described above. X-Gal staining for β -galactosidase expression, identified by the blue discoloration of the sections, was observed in all sections within the heart (Fig. 2). The most intense X-Gal activity was observed in the inferior wall of the heart. Infected hearts demonstrated an inflammatory response; however, there was no evidence of disruption of normal myocardial architecture or collagen deposition. Serum antibodies to adenoviral capsid proteins were measured on the day of gene transfer and 7 days later by ELISA. Between *days 0* and 7, pigs

infused with Ad. β gal by PAMGT with or without coronary vein obstruction developed significant titers ($\geq 1:100$) of antibodies to adenoviral capsid proteins

Quantitative β -galactosidase analysis.

PAMGT was successfully performed in all animals with and without concomitant occlusion of the coronary veins. Quantitative β -galactosidase analysis showed that PAMGT with coronary blockade was superior to PAMGT without coronary blockade. A significant increase in β -galactosidase activity was observed in the β -galactosidase group compared with the saline group: 1.34 ± 0.18 ($n = 9$) vs. 0.81 ± 0.1 ($n = 5$) ng ($P \leq 0.01$) in the LV wall and 0.91 ± 0.1 ($n = 9$) vs. 0.66 ± 0.07 ($n = 5$) ng ($P \leq 0.05$) in the RV wall (Fig. 3).

Detection of viral distribution through fluorescent microspheres.

To examine distribution of the fluorescent spheres, we injected fluorescent beads, instead of the virus, using PAMGT with coronary vein occlusion. Immediately after injection of the NIR fluorophore indocyanine green and red fluorescent beads, we acquired NIR fluorescent and red fluorescent images of the heart (Fig. 4). Figure 4A shows the anterior surface of the heart after removal. The fluorescent beads were visualized in Fig. 4B and on cross section in Fig. 4C. At a lower cross section, the indocyanine green staining shows areas of perfusion (Fig. 4D).

DISCUSSION

This study demonstrates that percutaneous coronary gene delivery can be safely and effectively performed in a large-animal model. Clinical application of cardiac gene transfer necessitates an efficient, safe, and targeted technique for gene delivery. The present study demonstrates modification of standard interventional techniques to provide a minimally invasive gene transfer.

Method of delivery.

A number of approaches have been reported to achieve cardiac gene transfer. Antegrade adenoviral gene injection from the aortic root during aortic and pulmonary artery cross clamping (5,12,17), direct injection into the ventricular wall (8,10,22), and gene transfer into the pericardial sac (9) have demonstrated efficient gene transfection. However, the invasive nature, relatively high morbidity, and associated risks of these techniques preclude widespread clinical application.

A percutaneous catheter-based delivery would be particularly attractive as a minimally invasive strategy. A number of catheter-based methods for PAMGT have been reported. Barr and Leiden (2) reported that percutaneous intracoronary gene delivery caused transfection of ~30% of the regional myocytes served by the targeted artery. Subsequent studies demonstrated reduced efficiency of the intracoronary adenovirus delivery, presumably related to rapid washout of the virus before sufficient myocyte transfection (14,16,18). Thus this technique can be limited by high systemic delivery and low target organ transfection. To solve this problem, antegrade coronary gene delivery was combined with coronary artery or coronary vein occlusion to create brief interruption of coronary blood flow and prolong exposure to the vector (7,14,16,18). This delivery technique resulted in significant gene expression compared with the procedures without coronary sinus occlusion (14). More recently, Donahue et al. (6) showed that selective intracoronary gene transfer to the atrioventricular node can modulate electrical conduction in pigs. The present study expands on this method by substituting global coronary venous occlusion with a focal or targeted approach. The technique of selective occlusion in the AIV was effective for the LV and RV walls. Unexpectedly, our study showed high levels of transfection in the RV, even though the right coronary artery was not targeted. Potential causes

of this finding include drainage from the LAD into the RV through the septal branches or AIV shunts that drain into the RV wall (19). This finding raises the potential of clinical application not only for LV failure but also for RV failure. Our study also showed that PAMGT with coronary vein occlusion is superior to PAMGT without coronary vein occlusion. Therefore, if this method is to be used in humans, especially in cases of heart failure, the added benefits of coronary vein occlusion must be taken into account. Other studies have also evaluated percutaneous gene delivery through selective pressure-regulated retroperfusion into the coronary veins. Although this has shown promise in adenoviral gene delivery to the regional myocardium in pigs, a prolonged arterial occlusion (3) is required to induce any reasonable gene expression. Our PAMGT with a short occlusion time, performed during standard percutaneous balloon angioplasty, offers a better strategy for patients who also need treatment for narrowed coronary vessels.

Limitation of the study.

Substantial variability exists in the venous drainage, making complete venous blockade complex (15). However, we demonstrated significant gene expression in the targeted myocardium.

Summary.

Our study has demonstrated efficient gene expression with the percutaneous antegrade myocardial gene transfer using coronary venous blockade. This PAMGT is relatively easy and requires only conventional techniques of percutaneous transluminal angioplasty with commercially available catheters. Our previous studies confirm that sarco(endo)plasmic reticulum Ca^{2+} -ATPase type 2a gene transduction using this vector significantly affects protein expression, cellular and cardiac function, and, eventually, survival. Although a study is still needed with a large-animal heart failure model, we have shown that PAMGT permits efficient percutaneous gene delivery by maximizing access of viral vectors to the coronary circulation and permitting myocardial uptake. The PAMGT technique holds promise in bridging the gap between investigative studies and clinical gene therapy, particularly as approaches are developed to benefit high-risk patients with heart failure. PAMGT with coronary venous blockade is feasible and reproducible and may be safely achieved in human heart failure.

GRANTS

This work was supported in part by National Heart, Lung, and Blood Institute Grants HL-069842 (to F. del Monte), HL-057623 and HL-071763 (to R. J. Hajjar), and R43 HL-075934 (to J. K. Gwathmey). Other support was provided by grants from the Center for Integration of Medicine and Innovative Technology (R. J. Hajjar and J. V. Frangioni), National Institutes of Health Grants R33-CA-88256, R21-CA-88870, and R33-EB-00673 (J. V. Frangioni) and R01-HL-078691 (R. J. Hajjar and J. V. Frangioni), and Department of Energy (Office of Biological and Environmental Research) Grant DE-FG02-01ER63188 (J. V. Frangioni). R. J. Hajjar is a Paul Beeson Scholar of the American Federation of Aging Research. R. J. Hajjar and J. V. Frangioni are Clinical Scientist Development Award recipients of the Doris Duke Charitable Foundation.

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Fig. 1.

Angiography showing gene delivery technique. *A*: left anterior oblique 30° projection.

Coronary balloon (black arrow) was inflated in left anterior descending coronary artery, and contrast medium was injected through the over-the-wire balloon. Wedge balloon (white arrow) was inflated in the anterior interventricular vein. *B*: right anterior oblique 90° projection.

Coronary balloon (black arrow) was inflated in the left circumflex artery, and contrast medium was injected through the over-the-wire balloon. Wedge balloon (white arrow) was inflated in the proximal great cardiac vein at the entrance of the middle cardiac vein.

Fig. 2.

Expression of β -galactosidase in left ventricular (LV) sections 7 days after infection with Ad. β gal. Photomicrographs show LV sections (anterior, lateral, inferior, and septal walls), along with the right ventricular (RV) wall, stained for X-galactosidase (X-Gal) activity. Sections show variability of β -galactosidase expression within the same heart with our catheter-based method of gene delivery.

Fig. 3.

Comparison of quantitative β -galactosidase analysis between the percutaneous antegrade myocardial gene transfer (PAMGT) groups with and without coronary vein occlusion. Values are means $\pm$ SE; $n = 5$ animals without coronary vein occlusion, $n = 9$ with coronary vein occlusion, and $n = 6$ in saline group. $*P \leq 0.01$ vs. saline. $^{\#}P \leq 0.01$ vs. PAMGT without coronary vein occlusion.

Fig. 4.

Fluorescence imaging of cardiac perfusion and microsphere distribution. *A*: color video image of the intact heart. Dashed white lines define the 1-cm tissue slices shown in *C* and *D*. *B*: red fluorescence image of the heart from *A* showing the distribution of microspheres in the anterior wall. *C*: 1-cm tissue slice from the heart shown in *A* viewed for red fluorescence. Indocyanine green (ICG) near-infrared (NIR) fluorescence is pseudocolored in green and overlaid with the color video image to precisely define agent distribution.

Table 1

Effect of PAMGT with and without coronary vein occlusion on blood pressure and heart rate

	Baseline	During PAMGT	Immediately After PAMGT	1 wk After PAMGT
		<i>Saline (n = 6)</i>		
BP, mmHg	92 ± 4	88 ± 3	87 ± 4	91 ± 5
HR, beats/min	101 ± 10	98 ± 8	103 ± 6	104 ± 9
		<i>With coronary vein blockade (n = 9)</i>		
BP, mmHg	89 ± 6	94 ± 8	87 ± 6	90 ± 6
HR, beats/min	104 ± 8	109 ± 11	103 ± 5	106 ± 7
		<i>Without coronary vein blockade (n = 5)</i>		
BP, mmHg	88 ± 7	90 ± 4	86 ± 5	92 ± 4
HR, beats/min	100 ± 9	106 ± 10	101 ± 8	113 ± 7

Values are means ± SE; *n*, number of animals. PAMGT, percutaneous antegrade myocardial gene transfer; BP, blood pressure; HR, heart rate.