Cardiac Gene Delivery With Cardiopulmonary Bypass

Michael J. Davidson, MD; J. Mark Jones, AFRCS; Sitaram M. Emani, MD; Katrina H. Wilson, MS; James Jaggers, MD; Walter J. Koch, PhD; Carmelo A. Milano, MD

Background—Cardiac gene therapy offers the possibility of enhancing myocardial performance in the compromised heart. However, current gene delivery techniques have limited myocardial transgene expression and pose the risk of extraocular expression. Isolation of the coronary circulation during cardiac surgery may allow for more efficient and cardiac-selective gene delivery in a clinically relevant model.

Methods and Results—Neonatal piglets (3 kg) underwent a median sternotomy and cardiopulmonary bypass, followed by aortic cross-clamping with 30 minutes of cardioplegic arrest. Adenoviral vectors containing transgenes for either β-galactosidase (adeno-β-gal, n=11) or the human β2-adrenergic receptor (adeno-β2-AR, n=15) were administered through the cardioplegia cannula immediately after arrest and were allowed to dwell in the coronary circulation during the cross-clamp period. After 1 week, the animals were killed, and their heart, lungs, and liver were excised and examined for gene expression. Analysis of β-galactosidase staining revealed transmural myocardial gene expression among animals receiving adeno-β-gal. No marker gene expression was detected in liver or lung tissue. β-AR density in the left ventricle after adeno-β2-AR delivery was 396±85% of levels in control animals (P<0.01). Animals receiving adeno-β2-AR and control animals demonstrated similar β-AR density in both the liver (114±8% versus 100±9%, P=NS) and lung (114±7% versus 100±9%, P=NS). There was no evidence of cardiac inflammation.

Conclusions—By using cardiopulmonary bypass and cardioplegic arrest, intracoronary delivery of adenoviral vectors resulted in efficient myocardial uptake and expression. Undetectable transgene expression in liver or lung tissue suggests cardiac-selective expression. (Circulation. 2001;104:131-133.)

Key Words: gene therapy ■ cardiopulmonary bypass ■ signal transduction

Cardiac gene transfer of either the human β2-adrenergic receptor (β2-AR) or an inhibitor of β-adrenergic receptor kinase (βARKct) enhances cardiac performance.1,2 Use of such genetic strategies clinically will require a safe method of cardiac gene delivery. The technique that has been used in the laboratory setting involves intracoronary injection of an adenoviral vector with the heart beating. The principal disadvantage of this technique is that the viral vector is rapidly washed out to the systemic circulation and taken up in nontarget organs such as the liver and lung.1,3 A critical feature of any clinically relevant cardiac gene delivery technique, however, is limiting noncardiac delivery to prevent toxicity.

We hypothesized that cardiopulmonary bypass (CPB) may facilitate cardiac-selective gene transfer using recombinant replication-deficient adenovirus. CPB with aortic cross-clamping and cardioplegic arrest represent the fundamental components of many cardiac surgery procedures and uniquely isolate the coronary circulation. Administration of an adenoviral vector under these conditions maximizes contact time with the myocardium and may reduce systemic delivery, therefore limiting toxicity and offering a clinically relevant delivery system.

Methods

A replication-deficient, first-generation, type 5 adenovirus with deletions of the E1 and E3 genes was used to construct vectors for the human β2-AR (adeno-β2-AR) or β-galactosidase (adeno-β-gal) transgene.4 Large-scale preparations of these adenoviruses were purified from infected Epstein-Barr nuclear antigen-transfected 293 cells (Invitrogen Corp.).4

One-week-old piglets (3 kg) received humane care in compliance with the institutional committee on animal research and in accordance with the regulations adopted by the National Institutes of Health. Animals were given ketamine (20 mg/kg IM) just before inhaled isoflurane (1%) anesthesia.5 A median sternotomy was performed, and after systemic heparinization, CPB was established via an aortic cannula and a right atrial cannula. The CPB circuit consisted of a reservoir, a hollow fiber oxygenator/heat exchanger, and a roller pump. After stabilization, the aorta was cross-clamped and the heart arrested by infusion of cold (4°C), hyperkalemic cardioplegia solution (30 mL/kg) into the aortic root. Animals were randomized to receive either adeno-β2-AR or adeno-β-gal. Immediately after cardioplegic arrest, 1×1011 total viral particles, reconstituted in 8 mL of phosphate-buffered saline (PBS), were injected into the cross-clamp period. After 1 week, the animals were killed, and their heart, lungs, and liver were excised and examined for gene expression. Analysis of β-galactosidase staining revealed transmural myocardial gene expression among animals receiving adeno-β-gal. No marker gene expression was detected in liver or lung tissue. β-AR density in the left ventricle after adeno-β2-AR delivery was 396±85% of levels in control animals (P<0.01). Animals receiving adeno-β2-AR and control animals demonstrated similar β-AR density in both the liver (114±8% versus 100±9%, P=NS) and lung (114±7% versus 100±9%, P=NS). There was no evidence of cardiac inflammation.

Conclusions—By using cardiopulmonary bypass and cardioplegic arrest, intracoronary delivery of adenoviral vectors resulted in efficient myocardial uptake and expression. Undetectable transgene expression in liver or lung tissue suggests cardiac-selective expression. (Circulation. 2001;104:131-133.)

Key Words: gene therapy ■ cardiopulmonary bypass ■ signal transduction

Cardiac Gene Delivery With Cardiopulmonary Bypass

Michael J. Davidson, MD; J. Mark Jones, AFRCS; Sitaram M. Emani, MD; Katrina H. Wilson, MS; James Jaggers, MD; Walter J. Koch, PhD; Carmelo A. Milano, MD

Background—Cardiac gene therapy offers the possibility of enhancing myocardial performance in the compromised heart. However, current gene delivery techniques have limited myocardial transgene expression and pose the risk of extraocular expression. Isolation of the coronary circulation during cardiac surgery may allow for more efficient and cardiac-selective gene delivery in a clinically relevant model.

Methods and Results—Neonatal piglets (3 kg) underwent a median sternotomy and cardiopulmonary bypass, followed by aortic cross-clamping with 30 minutes of cardioplegic arrest. Adenoviral vectors containing transgenes for either β-galactosidase (adeno-β-gal, n=11) or the human β2-adrenergic receptor (adeno-β2-AR, n=15) were administered through the cardioplegia cannula immediately after arrest and were allowed to dwell in the coronary circulation during the cross-clamp period. After 1 week, the animals were killed, and their heart, lungs, and liver were excised and examined for gene expression. Analysis of β-galactosidase staining revealed transmural myocardial gene expression among animals receiving adeno-β-gal. No marker gene expression was detected in liver or lung tissue. β-AR density in the left ventricle after adeno-β2-AR delivery was 396±85% of levels in control animals (P<0.01). Animals receiving adeno-β2-AR and control animals demonstrated similar β-AR density in both the liver (114±8% versus 100±9%, P=NS) and lung (114±7% versus 100±9%, P=NS). There was no evidence of cardiac inflammation.

Conclusions—By using cardiopulmonary bypass and cardioplegic arrest, intracoronary delivery of adenoviral vectors resulted in efficient myocardial uptake and expression. Undetectable transgene expression in liver or lung tissue suggests cardiac-selective expression. (Circulation. 2001;104:131-133.)

Key Words: gene therapy ■ cardiopulmonary bypass ■ signal transduction

Cardiac gene transfer of either the human β2-adrenergic receptor (β2-AR) or an inhibitor of β-adrenergic receptor kinase (βARKct) enhances cardiac performance.1,2 Use of such genetic strategies clinically will require a safe method of cardiac gene delivery. The technique that has been used in the laboratory setting involves intracoronary injection of an adenoviral vector with the heart beating. The principal disadvantage of this technique is that the viral vector is rapidly washed out to the systemic circulation and taken up in nontarget organs such as the liver and lung.1,3 A critical feature of any clinically relevant cardiac gene delivery technique, however, is limiting noncardiac delivery to prevent toxicity.

We hypothesized that cardiopulmonary bypass (CPB) may facilitate cardiac-selective gene transfer using recombinant replication-deficient adenovirus. CPB with aortic cross-clamping and cardioplegic arrest represent the fundamental components of many cardiac surgery procedures and uniquely isolate the coronary circulation. Administration of an adenoviral vector under these conditions maximizes contact time with the myocardium and may reduce systemic delivery, therefore limiting toxicity and offering a clinically relevant delivery system.

Methods

A replication-deficient, first-generation, type 5 adenovirus with deletions of the E1 and E3 genes was used to construct vectors for the human β2-AR (adeno-β2-AR) or β-galactosidase (adeno-β-gal) transgene.4 Large-scale preparations of these adenoviruses were purified from infected Epstein-Barr nuclear antigen-transfected 293 cells (Invitrogen Corp.).4

One-week-old piglets (3 kg) received humane care in compliance with the institutional committee on animal research and in accordance with the regulations adopted by the National Institutes of Health. Animals were given ketamine (20 mg/kg IM) just before inhaled isoflurane (1%) anesthesia.5 A median sternotomy was performed, and after systemic heparinization, CPB was established via an aortic cannula and a right atrial cannula. The CPB circuit consisted of a reservoir, a hollow fiber oxygenator/heat exchanger, and a roller pump. After stabilization, the aorta was cross-clamped and the heart arrested by infusion of cold (4°C), hyperkalemic cardioplegia solution (30 mL/kg) into the aortic root. Animals were randomized to receive either adeno-β2-AR or adeno-β-gal. Immediately after cardioplegic arrest, 1×1011 total viral particles, reconstituted in 8 mL of phosphate-buffered saline (PBS), were injected into...
the aortic root and allowed to dwell in the myocardium. After 30 minutes of cardiac arrest, the cross-clamp was removed and the heart was reperfused. The animals were then weaned off CPB and allowed to recover.

Gene expression was assessed 1 week after delivery. A subset of animals (n=8) was studied at 4, 8, and 24 hours and 14 days after gene delivery to examine the time course of expression. Heart, liver, and lung tissues were either immediately stained with X-gal solution [2 mmol/L K₄Fe(CN)₆, 2 mmol/L K₃Fe(CN)₆, 2 mmol/L MgCl₂, and 0.5 mg/mL 5-bromo-4-chloro-3-indoyl-b-D-galactopyranoside] as whole-mount samples or frozen at −80°C, sectioned at 10 μm, and stained in X-gal as previously described. β-AR expression was quantified with radioligand binding assays to determine total β-AR density. Tissue samples were homogenized in lysis buffer (5 mmol/L Tris-HCl [pH 7.4] and 5 mmol/L EDTA), and membrane fractions were extracted. A radioligand binding assay was performed using 125I-cyanopindolol to determine total β-AR density, as previously described.

A subgroup of animals received only PBS during CPB (n=4). Standard hematoxylin and eosin histological sections of these hearts were made and compared with sections from hearts treated with adeno-β₂-AR to assess any inflammatory response. Data are expressed as mean±SEM and were assessed by Student’s t test. Significance was assumed at P<0.05.

**Results**

A total of 42 piglets underwent CPB-mediated gene delivery. Of these, 40 survived to the time of study (4 hours to 14 days). Twenty-six piglets were studied for myocardial transgene expression at 1 week. The piglets that received adeno-β₂-AR (n=15) demonstrated no background X-gal staining, whereas those that received adeno-β-gal (n=11) had transmural staining in all chambers (Figure, A and B). Micrographic sections of the myocardium of animals receiving β-galactosidase demonstrated staining of individual myocytes, consistent with transgene expression (Figure, C). There was no β-galactosidase expression in the liver or lung.

Animals treated with adeno-β₂-AR exhibited a left ventricular β-AR density ≈4-fold higher than those receiving marker transgene (P<0.01; Table). The right ventricular β-AR density was 1.6-fold higher than that of control animals, demonstrating lower but significant transgene expression in this chamber (P<0.01). β-AR density was not different in the liver and lung between adeno-β₂-AR and adeno-β-gal–treated animals (Table).

In addition, gene expression was studied at varying intervals from time of delivery (Figure, D). β-Galactosidase

**β-AR Density in Treated and Control Piglets**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Adeno-β₂-AR (n=15)</th>
<th>Adeno-β-gal (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricle</td>
<td>396±85%*</td>
<td>100±7% (94.4 fmol/mg)</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>164±19%*</td>
<td>100±9% (101.0 fmol/mg)</td>
</tr>
<tr>
<td>Liver</td>
<td>114±7%</td>
<td>100±9% (77.4 fmol/mg)</td>
</tr>
<tr>
<td>Lung</td>
<td>114±8%</td>
<td>100±11% (137.4 fmol/mg)</td>
</tr>
</tbody>
</table>

Values are mean±SEM and are expressed as percent of control. All studies were conducted 1 week after gene delivery.

*P<0.05 vs control.
expression was first detected 8 hours after gene delivery. Expression at 8 hours was transmural and comparable to that seen at 24 hours and at 1 week. At 2 weeks, an additional 4 animals treated with adeno-β2-AR had increased left ventricular (275±126 fmol/mg) and right ventricular (181±31 fmol/mg) β-AR density.

Hematoxylin and eosin micrographs of hearts 1 week after delivery of adeno-β2-AR or PBS (n=4) are shown in the Figure (panels E and F, respectively). There was no evidence of an inflammatory response in either group.

Discussion
This study demonstrates the feasibility of myocardial gene delivery during CPB and cold hyperkalemic cardioplegic arrest. This protocol simulates conventional cardiac surgery and tests the effectiveness and potential advantages of gene transfer during cardiac surgery. Unlike prior attempts at intracoronary gene transfer, CPB-mediated gene therapy seems to limit extracardiac gene expression. Our laboratory has previously found high levels of gene expression in the liver and lung after non-CPB intracoronary delivery.1 When delivered to the beating heart, the intracoronary vector is rapidly washed out of the heart and delivered systemically.

Because the coronary circulation is uniquely isolated during CPB, gene delivery to the myocardium may be improved relative to injection into the coronary circulation with the heart beating. By using CPB and cardioplegic arrest, the virus is allowed to dwell in the coronary circulation for 30 minutes. At the end of this time, in contrast to beating-heart delivery, a higher percentage of viral particles may be taken up by myocytes or be inactivated. Furthermore, any remaining viable virus is ultimately washed out of the coronary circulation via the coronary sinus and returned to the CPB apparatus. Because the CPB circuit has a high surface area for potential virus-binding, particularly at the membrane oxygenator, the remaining viable virus may become bound. Indeed, Marshall et al8 demonstrated that the replication-deficient adenoviral vectors commonly used for gene delivery are rapidly inactivated on exposure to nonbiological surfaces such as polycarbonate, cardiac catheters, and syringes.

This approach may have multiple applications to clinical cardiac surgery. Such genetic treatments might support end-stage heart failure patients in a manner similar to left ventricular assist devices, as a bridge of support until heart transplantation. It may also provide support for high-risk patients with severely reduced ventricular function undergoing revascularization or valve replacement procedures. Indeed, impairment of the myocardial β-AR system during cardiac surgery has been documented, including receptor desensitization with reduced adenyl cyclase response, possibly due to increased βARK1 activity.9,10 This method of gene therapy would achieve transgene expression during the first postoperative day and continue for ~2 to 3 weeks. This time course would correlate with the early postoperative period during which inotropic support is most important. These studies also raise interest in the possibility of gene therapy with retrograde cardioplegia or with percutaneous methods of CPB, such as Heartport.

This study represents the first use of CPB for global myocardial gene delivery. Moreover, it demonstrates the feasibility of intracoronary gene delivery in the pig, whose heart is similar to humans. The study is limited insofar as the subjects were healthy neonatal piglets. Further work is needed to characterize the effectiveness of this technique in adult animals and those with ventricular dysfunction. In addition, current efforts are directed at demonstrating the biochemical and hemodynamic consequences of gene delivery using functional transgenes.

Acknowledgments
This work was supported in part by National Institute of Health grants HL61690 (to W.J.K.) and HL56205 (to W.J.K.) and by National Research Service Award 5 F32 HL10179 (to M.J.D.). The authors thank George Quick, Ronnie Johnson, and Kurt Campbell for their invaluable assistance in the animal setup and use of CPB. We also thank Robert J. Lefkowitz, who was instrumental in initiating gene therapy efforts with β2-AR and who provided much of the adenoviral vector for these studies.

References
Cardiac Gene Delivery With Cardiopulmonary Bypass
Michael J. Davidson, J. Mark Jones, Sitaram M. Emani, Katrina H. Wilson, James Jaggers, Walter J. Koch
and Carmelo A. Milano

Circulation. 2001;104:131-133
doi: 10.1161/01.CIR.104.2.131

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/2/131

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/