Cardiac Gene Therapy: Optimization of Gene Delivery Techniques \textit{In vivo}

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**Vector-mediated Cardiac Gene Therapy**

Holds tremendous promise as a translatable platform technology for treating many cardiovascular diseases

*However*, in spite of development of adeno-associated viral (AAV) vector serotypes with significant tropism for the heart and in spite of the extremely promising findings in murine species (Gregorevic et al., 2004; Wang et al., 2005; Inagaki et al, 2006), these results *have not* yet been confirmed using a transvascular delivery route in large animals in vivo and *in situ*. 
Vector-mediated Cardiac Gene Therapy

Furthermore, on the basis of previous murine studies (Gregorevic et al., 2004; Wang et al., 2005; Inagaki et al., 2006), the doses of vector required to transduce the human heart by intravenous injection would likely be prohibitive or certainly impractical.

The quest for more efficient, cardiac gene delivery methods is currently a critically important, rate-limiting challenge in clinical cardiac gene therapy.
Requirements for successful heart failure gene therapy

1. Transgene with therapeutic potential in heart failure

2. A vector with cardiac muscle tropism resulting in long-term gene expression

3. A method for global myocyte gene delivery
Prerequisite for Effective Cardiac Gene Therapy

- Reliable
- Safe
- Clinically Relevant

Successful Cardiac Gene Therapy
Ideal Technique for Cardiac Gene Therapy

- Efficient and Practical
- Global Gene Expression
- Minimal Collateral Expression

Successful Cardiac Gene Therapy
Current Cardiac Gene Delivery Techniques

Cardiac Circulation
- Beating Heart
- Ex Vivo Perfusion Pre-Transplantation
- Cardiopulmonary Bypass

Site of Injection

Interventional Approach
- Percutaneous Catheter Based
- Thoracotomy
- Sternotomy
- Subdiaphragmatic

LOCAL:
- Intramyocardial
- Endocardial
- Epicardial

SYSTEMIC:
- Peripheral Veins
- Coronary Veins and Coronary Sinus
- Coronary Arteries
Local Cardiac Gene Delivery -
Epi/Intra-myocardial Gene Delivery with Syringe

• Simple and safe.
• Direct *in vivo* gene transfer into ventricular cardiomyocytes is possible using both naked DNA and viral vectors.

• Gene expression not limited exclusively to cardiac muscle.
• Upper limit to delivery secondary to spillage of vector into systemic circuit—leading to collateral gene expression.
• Inhomogeneous expression
Local Cardiac Gene Delivery-
Catheter-mediated Percutaneous Endomyocardial Gene Delivery

• Experimental models demonstrate significantly higher microsphere retention than open-chest, epicardial/intramyocardial injection.

• Still has limitations of saturation kinetics, collateral gene expression, inhomogeneous distribution, and immunogenicity.
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<th>Technique</th>
<th>Characteristics</th>
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| Local Cardiac Gene Delivery | • Allows for local gene delivery but transgene expression is limited to injection site—making it impossible to genetically alter the whole organ or system. *French et al. (1994), Magovern et al. (1996)*  
• Does not preclude systemic vector spread, *Fromes et al. (1999)*  
• Most readily applicable to therapeutic angiogenesis, *Koransky et al. (2002)* or focal arrhythmia therapy, *Edelberg et al. (2001)*  
• Feasibility and safety has been established in phase 1 clinical trials; *Isner et al. (2001), Lathi et al. (2001), Vale et al. (2001), Lorsordo et al. (1998), Rosengart et al. (1999)*  
• Results in a pattern of mild or moderate but multi-focal transgene expression, *Acsadi et al. (1991)*  
• In spite of inhomogeneity may be sufficient to affect global phenotypic changes such as improved ventricular contractility and alteration of ECG intervals, *Tomiyasu et al. (2000)*  
• Local delivery provides for short-term gene expression; *Zhang et al. (1999), Muhlausser et al. (1996)*  
• Allows for targeted delivery and high local concentration of agent can be achieved, *Gwon et al. (2000)*  
• Simple and reproducible, *Guzman et al. (1993)* |
Systemic Cardiac Gene Delivery - Catheter-mediated Percutaneous Antegrade Intracoronary Gene Delivery

- Believed to be most clinically relevant method because of the possibility of delivering vectors to the whole myocardium and because of the extensive clinical experience in coronary catheterization procedures, Shah et al. (2000), Logeart et al. (2001), Hayase et al. (2005)

- Low transfection efficiency (1-<10% of myocytes)
- Single pass delivery
- Increased collateral expression
Systemic Cardiac Gene Delivery - Antegrade Intracoronary Gene Delivery via Left Ventricle with Short-term Aortic Cross-clamping

• Allows for perfusion of coronary bed under pressure—increasing efficiency of gene transfer.

• Single pass delivery
• Clinical applicability highly questionable
Systemic Cardiac Gene Delivery - Catheter-mediated Percutaneous Gene Delivery through the Coronary Venous System in the Beating Heart

- Selective pressure-regulated retroinfusion of the coronary veins prolongs adhesion time of the vector with the cardiac endothelium and increases endothelial permeability, Boekstegers et al. (2000).
- Increased adenoviral gene transfer to the targeted myocardium.

- Single pass
- Collateral gene expression
Systemic Cardiac Gene Delivery - Catheter-mediated Percutaneous Retrograde Gene Delivery through the Coronary Venous System with Concomitant Myocardial Ischemia in the Beating Heart

- Gene expression distributed more homogenously.
- Overall gene expression in the targeted left anterior descending artery region after adenoviral gene transfer is superior to that achieved after intramyocardial injection, Boekstegers et al. (2004), Raake et al. (2004).

- Demonstrates relevance of retrograde perfusion
- May not be clinically applicable—particularly in the setting of pre-existing atherosclerotic disease
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<td>• Short-lived contact between vectors and cardiomyocytes, <em>Griscelli et al.</em> (2003)</td>
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<td>• The ability to attain more homogeneous though often limited gene expression in the heart, <em>Griscelli et al.</em> (2003)</td>
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<td>• Efficient transgene transfer possible using intracoronary delivery, using surgical manipulations poorly compatible with clinical setting (i.e. aortic and pulmonary artery, cross-clamping), <em>Shah et al.</em> (2000)</td>
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<tr>
<td></td>
<td>• Possible to deliver vector to whole myocardium, <em>Logeart et al.</em> (2001)</td>
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<td>• Extensive clinical experience in coronary catheterization procedures</td>
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<td>• Minimally invasive: intracoronary delivery may not be effective in coronary artery disease (70% of heart failure patients in the U.S.)</td>
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<td>• Single-pass intracoronary delivery leads to rapid virus washout</td>
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<td>• Potential difficulties with injection and distribution in atherosclerotic vessels</td>
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<td>• Inability to delivery genes to individual, selected target cells, <em>Baker et al.</em> (2002)</td>
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<td>• Selective intracoronary gene transfer to the atrioventricular node can modulate electrical conduction, <em>Donahue et al.</em> (2000)</td>
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CPB-mediated Cardiac Gene Delivery
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<td></td>
<td>• Extended vector residence time in the coronary circulation, <em>Bridges et al. (2005), Logeart et al. (2001), Emani et al. (2003)</em></td>
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<td>• Increased myocardial transcapillary gradient using physical methods like increasing perfusion pressure and flow rate and decreasing the resistance to filtration by increasing endothelial permeability within coronary circulation with pharmacological agents like VEGF or histamine that can enhance transendothelial transport of viral particles from the vasculature into the interstitium, <em>Donahue et al. (1997), Donahue et al. (1998), Wright et al (2001), Logeart et al. (2001), Bridges et al. (2005)</em></td>
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<td></td>
<td>• Isolation of the cardiac circulation from the systemic circulation to allow for maximization of coronary vector concentration and washout of vector after gene delivery to minimize collateral gene expression with interruption of coronary flow during vector transfer, <em>Bridges et al. (2005).</em></td>
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<td>• Removal of blood components, <em>Donahue et al. (1998), Griscelli et al. (2003)</em></td>
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<td>• Closed loop allows for multiple pass cardiac recirculation, increased contact time with the coronary vasculature, control of temperature and ionic composition of the perfusate and removal of blood cells, <em>Bridges et al. (2005), Ly et al. (2007), Bryne et al. (2008), Bridges et al (2009)</em></td>
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<td>• Absence of significant influence of cold temperatures on transgene expression, <em>Jones et al. (2002), Ikeda et al. (2002), Griscelli et al. (2003)</em></td>
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<td>• Minimal collateral expression, <em>Goto et al. (1998), Griscelli et al. (2002)</em></td>
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<td>• Attendant morbidity and intrusiveness associated with CPB</td>
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• What is the Rationale for Retrograde: Venous to Arterial Perfusion for Gene Transfer?
Stable restoration of the sarcoglycan complex in dystrophic muscle perfused with histamine and a recombinant adeno-associated viral vector


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Limb-girdle muscular dystrophies 2C–F represent a family of autosomal recessive diseases caused by defects in sarcoglycan genes. The cardiomyopathic hamster is a naturally occurring model for limb-girdle muscular dystrophy caused by a primary deficiency in sarcoglycan1,2. We show here that acute sarcolemmal disruption occurs in this animal model during forceful muscle contraction. A recombinant adeno-associated virus vector encoding human sarcoglycan conferred efficient and stable genetic reconstitution in the adult cardiomyopathic hamster when injected directly into muscle. A quantitative assay demonstrated that vector-transduced muscle fibers are stably protected from sarcolemmal disruption; there was no associated inflammation or immunologic response to the vector-encoded protein. Efficient gene transduction with rescue of the sarcoglycan complex in muscle fibers of the distal hindlimb was also obtained after infusion of recombinant adeno-associated virus into the femoral artery in conjunction with histamine-induced endothelial permeabilization. This study provides a strong rationale for the development of gene therapy for limb-girdle muscular dystrophy.
Uniform Scale-Independent Gene Transfer to Striated Muscle After Transvenular Extravasation of Vector


Circulation 2005;112;1780-1788; originally published online Sep 12, 2005;
Myocardial Capillary Network
Pathway of Gene Delivery via Coronary Sinus
Positioning of Coronary Sinus Catheter for Retrograde Cardiac Gene Delivery

Snare Placement Middle Cardiac Vein

Coronary Sinus Occlusion

Placement of Retrograde Catheter in Coronary Sinus

Posterior Left Ventricle Vein
Retrograde Infusion without CPB vs. Molecular Cardiac Surgery with Re-circulating Delivery

Efficient myocyte gene delivery with complete cardiac surgical isolation in situ


LacZ encoding β-galactosidase
Clinically Translatable Technique for Global Cardiac Myocyte Transgene Delivery Must Ideally Incorporate...

1. Retrograde transvenous delivery through the coronary sinus or coronary veins
2. Extended vector residence time in the coronary circulation
3. Increased myocardial transcapillary gradient using physical methods such as increasing perfusion pressure and the flow rate and decreasing the resistance to filtration by increasing endothelial permeability within the coronary circulation with pharmacological agents that can enhance transendothelial transport of viral particles from the vasculature into the interstitium.
4. Isolation of the cardiac circulation from the systemic circulation to allow for maximization of coronary vector concentration and washout of vector after gene delivery to minimize collateral gene expression with interruption of coronary flow during vector transfer.
5. Removal of blood components.
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