

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Journal of Pharmacological and Toxicological Methods xx (2006) xxx–xxx

**Journal of
Pharmacological
and
Toxicological
Methods**
www.elsevier.com/locate/jpharmtox

Original article

Safety and feasibility of percutaneous autologous skeletal myoblast transplantation in the coil-infarcted swine myocardium[☆]

Nabil Dib^{a,b,*}, Ann Campbell^a, Douglas B. Jacoby^c, Agatha Zawadzka^c, Judson Ratliff^c,
Brigitte M. Miedzybrocki^a, Amir Gahremanpour^a, Edward B. Diethrich^a, Shaun R. Opie^{a,b}

^a Arizona Heart Institute, Phoenix, AZ, USA^b Arizona State University, Tempe, AZ, USA^c GenVec, Inc., Charlestown, MA, USA

Received 11 December 2005; accepted 16 December 2005

Abstract

Introduction: Autologous skeletal myoblast transplantation (ASMT) for myocardial regeneration is a promising new treatment for patients with congestive heart failure secondary to myocardial infarction (MI). However, non-surgical delivery could broaden the utility of this approach. The present study was designed to evaluate the safety and feasibility of transplanting autologous skeletal myoblast (ASM) via endovascular delivery into the infarcted swine myocardium. **Methods:** Seven female Yorkshire swine successfully underwent induced left ventricular MI. ASM biopsies were obtained from the hind limb of each animal and myoblasts were expanded in vitro. In a pilot experiment, ASM were labeled with iridium and short-term retention and biodistribution was determined 2 h after ASM delivery via the MyoStar™ needle-injection catheter inserted through the femoral artery. At 30 days post-infarction, the remaining animals were divided into three groups containing 2 animals each for percutaneous catheter delivery into the infarcted zone: group 1 control animals were injected with media only, group 2 and 3 animals were injected with approximately 300×10^6 and 600×10^6 ASM, respectively. Sixty days post-transplantation, the swine hearts were harvested. **Results:** During the 60-day period between transplantation and harvest, no adverse events were recorded, and continuous rhythm monitoring revealed no arrhythmias. In the small sampling size, myocardial function assessments revealed a trend toward improvement in the treatment groups with respect to ejection fraction, viability, and cardiac index. However, histology of treated swine hearts identified no skeletal muscle cells. **Discussion:** Percutaneous ASMT into an infarcted swine myocardium is feasible and safe, and may contribute to overall improved heart function.

© 2006 Elsevier Inc. All rights reserved.

Keywords: Cell therapy; Electromechanical mapping; Endoventricular injection; Methods; Myoblast; NOGA; Porcine

1. Introduction

Myoblast transplantation is a promising new treatment for patients with congestive heart failure and/or MI. Successful autologous skeletal myoblast transplantation (ASMT) to the myocardium has been demonstrated in a variety of animal

models (Ghostine et al., 2002; Jain et al., 2001; Marelli, Desrosiers, el-Alfy, Kao & Chiu, 1992; Taylor et al., 1998). After injection into a damaged myocardium, surviving skeletal myoblasts can differentiate and develop into striated multinucleated myofibers which express skeletal muscle-specific markers and integrate into the scar (Jain et al., 2001; Ghostine et al., 2002). More importantly, transplantation of myoblasts is correlated with improved myocardial performance (Al Attar et al., 2003; Pouzet et al., 2001; Atkins et al., 1999; Jain et al., 2001). These improvements in cardiac function occur without formation of gap junctions (Reinecke et al., 2000), or transdifferentiation of skeletal to cardiac phenotype (Reinecke et al., 2002). However, there appears to be a propensity for

[☆] This study was supported by a research grant from Genvec, Inc. and Cordis Corporation.

* Corresponding author. Arizona Heart Institute, 2632 N. 20th Street, Phoenix, AZ 85006. Tel.: +1 602 266 2200; fax: +1 602 285 0867.

E-mail address: ndib@azheart.com (N. Dib).

formation of fatigue resistant slow twitch fibers (Murry et al., 1996; Pagani et al., 2003).

More recently, ASMT has been applied as an adjunct to coronary artery bypass grafting (CABG) and left ventricular assist device (LVAD) in humans with severe ischemic heart failure. Menasche et al. (2001) reported improvement in myocardial function following direct injection of autologous skeletal myoblasts (ASM) via the epicardial surface of the infarcted myocardium during a thoracotomy for bypass surgery. Cell survival was demonstrated up to 17 months after implantation (Hagege et al., 2003; Dib, McCarthy et al., 2005).

Since May 2000, Pagani et al. (2003), Dib, McCarthy et al. (2005), and Dib, Michler et al. (2005) have been using ASMT as an adjunct to CABG and LVAD in humans with severe ischemic heart failure. To date, these US studies have demonstrated safety, feasibility and engraftment of epicardial transplantation of ASM in 30 patients undergoing CABG or LVAD. In the LVAD arm of the study, engraftment of ASM in three out of four explanted hearts after an average 142 days post-transplantation was histologically documented (Pagani et al., 2003).

While surgical transplantation of myoblasts into injured myocardium appears promising, a less invasive means of delivering the myoblasts would be preferable. In a preliminary porcine study, we have shown that allogeneic skeletal myoblasts delivered percutaneously by endoventricular delivery into the infarcted myocardium was feasible (Dib et al., 2002). Others have also developed catheter approaches for the endocardial delivery of stem cells and myoblasts (Kraitchman et al., 2003; Kawamoto et al., 2003). However, there still remains a lack of knowledge about the short-term fate of cells after catheter-based endoventricular delivery, and the safety of delivering clinically relevant doses of ASM to the thin ventricular wall of ischemically damaged myocardium.

In this study, using the coil-infarcted swine model for MI, we have expanded on our earlier findings to quantify the short-term retention and biodistribution of ASM following endoventricular delivery. In addition, we have demonstrated the feasibility and safety of percutaneous transplantation of autologous skeletal myoblasts at clinically relevant doses.

2. Materials and methods

All experiments were conducted according to guidelines published in the *Guide for the Care and Use of Laboratory Animals* (DHHS publication number NIH 85-23, revised 1985) and Subchapter A of the Federal Animal Welfare Act written by the United States Department of Agriculture and in the spirit of FDA Good Lab Practices. The study protocol was approved by the Harrington Animal Care and Use Committee at Arizona State University, Phoenix, AZ, prior to the start of the study. A summary of the study design is shown in Table 1.

2.1. Animal preparation

Ten female Yorkshire swine between the ages of 3 and 6 months and weighing 41 ± 11 kg underwent baseline evaluations, echocardiography (ECG), cardiac output and index, and blood

Table 1
Study design

	Baseline		
	Day 0	Day 30	Day 90
Interventions			
MI	X		
Biopsy	X		
Transplant		X	
Harvest			X
Loop recorder implantation	X		
Left heart catheterization	X	X	X
Safety assessments			
Implanted loop recording		Continuous	
Observation — adverse events/death		Daily	
Blood lab evaluations	X	X	X
Myocardial functional assessments			
Electrocardiogram	X	X	X
Echocardiogram	X	X	X
Hemodynamics	X	X	X
Electromechanical mapping	X	X	X
LV gram, coronary angiography	X	X	X
Histology			X

values were assessed. Each animal was anesthetized with intramuscular Telazol (tiletamine hydrochloride and zolazepam hydrochloride; 500 mg), intubated, and mechanically ventilated with 2% isoflurane and 3-L/min oxygen. An 8-F arterial sheath was inserted into the right femoral artery using either percutaneous or cutdown technique, and selective left and right coronary angiography, left ventriculography and NOGA mapping were performed.

A skeletal muscle biopsy was taken from each of the seven study swine. Under sterile conditions, a 60 mm incision was made longitudinally along the right hind limb, and approximately 5–10 g of muscle from the thigh muscle was removed with a sharp dissection technique. The incision was closed in layers. The muscle biopsy was placed immediately in a biopsy transportation medium on ice and sent to a cell culturing facility for myoblast expansion.

Following the muscle biopsy, an implantable loop recorder (ILR) was inserted in each study swine. The ILR was the Medtronic Reveal® Plus 9526 (Medtronic, Minneapolis, MN), a single-use programmable device designed to continuously record a subcutaneous ECG during arrhythmic events. Using sterile technique, a single 20 mm incision was made along the left side of the spine just above the heart level. The wound was dissected to the fascia, and an approximate 40×20 mm subcutaneous pocket was formed over the muscle. The event monitor was placed subcutaneously, and ECG signal quality and amplitude were verified. Wound closure was performed in a conventional fashion.

2.2. Infarction model

The infarction model has been previously described (Dib et al., in press). Briefly, immediately following ILR implantation and left heart catheterization, an anterior infarction was induced in each of the seven study swine by coil embolization using either a 2×10 mm complex helical or a 3×23 mm diamond shape Vortex

coil (Boston Scientific/Target, Natick, MA) to the distal left anterior descending (LAD) artery. Coronary occlusion occurred in an average of 12 min after coil deployment, as demonstrated by coronary angiography and ECG showing ST elevation in V_1 – V_3 a few minutes after LAD occlusion. The femoral artery was closed with either an Angio-Seal vascular closure device or sutures, and the animals recovered per standard operating procedures. Significant ventricular arrhythmias were treated with a 50 mg intravenous lidocaine bolus and electrical cardioversion. Postprocedural discomfort was treated with intramuscular butorphanol tartrate (Dolorex, 1.0 mL). Three pigs died during or shortly after induction of the MI. One animal was used to evaluate short term retention and biodistribution of injected myoblasts, and six animals served as recipient animals for either ASM or transport medium only.

2.3. Expansion of myoblasts

The ASM were isolated by fine mincing of the muscle tissue followed by a three step enzymatic digestion containing a 0.5 mg/ml trypsin (Invitrogen, Carlsbad, CA) and 0.5 mg/ml collagenase (Crescent Chemical Co., Islandia, New York). Cells released in each step were washed and plated on gelatin coated dishes. The cells were expanded over two passages in a growth medium composed of SKBM (Cambrex, Walkersville, MD) supplemented with 15% (vol/vol) fetal bovine serum (Hyclone, Logan, UT), 10 ng/ml recombinant human epidermal growth factor (rhEGF) (Cambrex), 3.9 μ g/ml dexamethasone (American Reagent Laboratory, Shirley, NY), and 50 μ g/ml gentamicin (Invitrogen). The cells were maintained at less than 70% confluence to prevent spontaneous cell fusion, and were harvested by trypsin/EDTA digestion (Invitrogen) and cryopreserved. For the long-term survival study, approximately 10% of the culture was labeled with BrdU during the last 24 h of culture to aid histological identification of the transplanted cells.

In preparation for cell injection, frozen myoblasts were thawed and washed twice in growth medium and twice in transplantation medium. Finally, the cells were brought to the appropriate cell density, loaded into 1 cc syringes and shipped either on ice or cold packs.

To label cells with iridium, forty million cells from the animal were mixed with 13.4×10^{10} iridium particles (0.3 μ m diameter; supplied by BioPhysics Assay Laboratory, Worcester, MA) and incubated for 2.5 h at 37 °C to foster internalization of iridium by the myoblasts. Non-internalized particles were removed by washing the cells six times in growth medium. The remaining labeled cells were mixed with unlabelled myoblasts to formulate the final cell product in transplantation medium and loaded into 1 cc syringes. Aliquots of the final cell product were retained so that a standard curve could be generated (see below).

2.4. Characterization of cell population

Cells were analyzed for viability, sterility, purity, and potency. Viability was assessed using trypan blue, and sterility was measured using a membrane filtration method. The LAL Gel clot

assay was used to detect endotoxins. Cell purity was determined by FACS using a primary antibody against myoblast-specific $\alpha 7$ -integrin (H36 from Dr. Kaufman, University of Illinois). Myoblast potency was assessed using a fusion assay performed by switching confluent myoblast cultures to fusion media. Under these conditions, myoblasts fuse and form multinucleated myotubes. Contaminating fibroblasts do not have this property and remain as single cells.

At the time of final formulation in myoblast transplantation medium, the cell viabilities were between 60% and 96% (see Table 3). Upon receipt at the study site, the viabilities had decreased. The purity of the cell preparations ranged from 30% to 74%, with contaminating cells being fibroblasts. All transplanted cells passed sterility and endotoxin testing.

2.5. Autologous myoblast transplantation

Prior to initiating implantation studies using the MyoStar™ Intramyocardial Injection Device (Cordis, Diamond Bar, CA), preliminary biocompatibility studies were performed. Similar to a myogenic cell line (Oron et al., 2000), the data showed no significant alteration in cell number or cell viability after passing through the catheter at a range of cell concentrations from 10×10^6 to 100×10^6 cells/ml (data not shown). Approximately thirty days after infarction, ASM were transplanted into each of the treatment swine. Each animal was anesthetized as described earlier. An 8-F arterial sheath was inserted into the left femoral artery using a cutdown technique and myocardial assessments were repeated.

Percutaneous ASMT was performed using an 8-F arterial sheath to advance the MyoStar™ Intramyocardial Injection Device through either the right or left femoral artery. A 3-D unipolar voltage map (NOGA) determined the area of infarction and was used to guide the needle-injection catheter. An average of 137 points were used to map the left ventricle, and a mean unipolar voltage of 7.8 ± 1.5 mV (BP 2.3 ± 0.4 mV) was used to detect infarcted areas (Callans et al., 1999). The catheter used was either a B or C. The injection needle was measured in the straight and curved positions (90°) and adjusted to extend 3 to 5.5 mm into the infarcted region of the endocardium depending on the wall thickness measured by echocardiography. Penetration was verified by either fluoroscopy, ST elevations, or premature ventricular contractions during needle advancement.

Immediately prior to injection, each syringe was warmed to room temperature and inverted several times to ensure a homogenous cell suspension. The temperature was assessed by touch and homogeneity was assessed visually. The suspended cells were injected into the center and peripheral edges of the infarcted region of the myocardium. Group 2 animals received $\sim 300 \times 10^6$ cells, and Group 3 animals received $\sim 600 \times 10^6$ cells. Group 1 control animals were injected with myoblast transplantation medium using similar numbers of injections and injection volumes. Table 3 describes dosing characteristics in detail. After the injections were completed, the femoral artery was closed with either an Angio-Seal vascular closure device or sutures, and the animals were recovered.

2.6. Quantitation of distribution of iridium-labeled ASM

Two hours after the final injection, the animal injected with iridium labeled ASM was sacrificed and the heart, brain, kidneys, liver, lungs and spleen were weighed. The anterior, lateral, inferior and septal regions of the apex of the left ventricle were cut into eight equal segments (two vertical segments for each region, and 5–9 g of each organ was removed for analysis. All tissue samples and labeled cell standards were placed in vials and dried overnight at 70 °C.

The resulting dried samples were sent to BioPhysics Assay Laboratory for analysis involving two steps: activation and detection. During activation, the samples were exposed to high-energy neutrons allowing the iridium atoms in the cells to capture incident neutrons. The unstable radioactive products of the neutron flux were then allowed to decay for two days to reduce background interference. During the detection phase, the samples were placed in a high-resolution gamma-detection monitor that measured the energy level and the number of gamma particles emitted. A standard curve generated from samples containing known numbers of iridium-labeled cells was used to convert the gamma particle emission for each tissue sample to the number of retained iridium-labeled cells. To calculate the total number of labeled cells within each whole organ (other than the heart), the value for each tissue sample was multiplied by the weight of the organ divided by the sample tissue weight.

2.7. Safety assessments

Safety was evaluated by animal survival, well-being, heart rhythm, blood tests and adverse events. Well-being and survival were continuously monitored and recorded during the 90-day study period. Heart rhythm was monitored using a standard 12-lead ECG, obtained in a resting, supine position at selected time-points, and by an implantable loop recorder (ILR). The ILR was activated during, and 24 h following, MI and transplant. Additional interrogations were performed 3 times/week for 2 weeks after each procedure and weekly between transplant and harvest. Each device

Table 2
Retention of myoblasts in tissues

Tissue sample	Number of cells	% Total cells*
Total left ventricle	4,514,540	4.0
–Anterior wall 1	2,068,321	1.9
–Anterior wall 2	3333	<0.1
–Lateral wall 1	49,800	<0.1
–Lateral wall 2	2065	<0.1
–Inferior wall 1	4093	<0.1
–Inferior wall 2	4605	<0.1
–Septal wall 1	95,414	0.1
–Septal wall 2	2,286,909	2.1
Lung	5,616,687	5.1
Spleen	87,196	<0.1
Brain	0	0
Kidney 1	0	0
Kidney 2	0	0
Liver	0	0

*Percent of 110,600,000 cells injected that were retained in the tissue.

Table 3

Skeletal myoblast cell and dosing characteristics

Group	Cell viability (%)						
	# Injected cells	At shipment	Prior to transplant	% Purity*	Number of inj.	Vol/ inj. (ml)	Total vol inj. (ml)
Control	Medium	N/A	N/A	N/A	20	0.15	3.0
Control	Medium	N/A	N/A	N/A	28	0.10	2.8
1	233 × 10 ⁶	77	54	ND	19	0.10	1.9
1	302 × 10 ⁶	96	79	30	19	0.10	1.9
2	595 × 10 ⁶	89	60	35	39	0.15	5.9
2	756 × 10 ⁶	81	73	62	31	0.15	4.7

*Percent $\alpha 7$ integrin immunostaining by FACS.

N/A—not applicable ND—not done.

was programmed as follows: Storage mode –13 auto-activated events for 42 min to detect bradycardia <30 bpm, tachycardia >230 bpm, asystole >3 s for 16 consecutive beats. All ILR devices were set for a maximum gain of 8 (+/–0.2 mV) and sensitivity adjusted between 10 and 13 to archive optimal sensing.

Hematology and chemistry specimens were drawn at each intervention after the animals were fasted overnight. Blood was collected from the femoral access under anesthesia.

2.8. Myocardial function assessment

Functional assessment of the hearts was performed at selected time points to compare the effects of cell transplantation from baseline and to compare the treated animals with controls. Ejection fraction (EF) and ventricular wall thickness were assessed using a standard resting echocardiogram (ECHO). The ECHO was 2-dimensional and performed in the parasternal long and short axis views, four, two and long axis apical and subcostal four and short axis views. ECHO results were interpreted in a blinded fashion by an experienced cardiologist. Additional EF assessments were made by ventriculography (LV gram). Coronary arteries were visualized for patency through coronary angiography during left heart catheterization using the right and left anterior oblique projections, and were interpreted by the investigator. Cardiac index was assessed by noninvasive impedance cardiography (ICG) using a BioZ device (Cardio Dynamics International Corporation, San Diego, CA). Four ICG sensors were attached to each animal (one on both sides of the neck and torso), and a correction factor of 1.48 was used to adjust the values for pig chest anatomy (Broomhead, et al., 1997).

Three-dimensional electromechanical mapping was performed using the NOGA Biosense Navigational System (Biosense-Webster, Diamond Bar, CA,) via a 7-F NOGA-STAR™ catheter advanced through the 8-F sheath into the left ventricle. The mapping was used to identify areas of normal tissue, ischemia and infarction of the ventricle, as described previously (Kornowski et al., 1998). These maps included unipolar and bipolar voltage maps which were used to calculate left ventricular unipolar voltage (LVUPV), apical unipolar voltage (APUPV). A number and color scale to indicate the voltage in each area of the myocardium were assigned by the computer.

Table 4
Cardiac functional parameters at the time of ASMT (baseline) and 60 days later (sacrifice)

	Ejection fraction (%)				
	ECHO	LV gram	Cardiac index	LVUVP	APUPV
Control: group 1 (n=2)					
Baseline	49	43	0.85	7.8	7.5
Sacrifice	44	38	0.65	9.3	7.2
% Change	-10	-12	-24	19	-4
Treated: group 2 and 3 (n=4)					
Baseline	46	42	0.95	7.9	7.7
Sacrifice	53	41	0.83	9.3	9.5
% Change	15	-2	-13	18	23

LVUVP: Global left ventricular unipolar voltage; APUPV: Apical unipolar voltage.

2.9. Histology

Histological analysis was performed on all hearts from the treatment and control group animals following harvest at day 90 of the study. The hearts were weighed and preserved in 10% neutral-buffered formalin. The infarcted portion of each heart was embedded in paraffin, sectioned, mounted on slides, and stained to identify presence of cellular engraftment and inflammatory reaction to the procedure. Histological stains included Hematoxylin and Eosin, and Trichrome. Immunohistochemical

stains included skeletal muscle-specific myosin heavy chain (MY32, Sigma, St. Louis, MO), and immunoreactivity to BrdU (Zymed, South San Francisco, CA).

3. Results

3.1. Retention and biodistribution

The retention of myoblasts in the selected tissues 2 h following catheter-based injection into the myocardium is listed in Table 2. No iridium-labeled ASM were detected in the brain, kidney or liver. Very low numbers of cells were detected in the spleen and in areas of the left ventricle not targeted for cell injection (<0.1% of the injected cells). Two adjacent myocardial regions, which were the targets of the injections, contained the majority of the cells retained in the heart. In total, 4.0% of the injected cells were detected in the apical region of the heart that contained the scar tissue. The primary site of outside of the heart where cells were detected was the lung which contained 5.1% of the injected cells.

3.2. Safety

Injections of control media or cells for determining safety and effects on myocardial function were performed as summarized in Table 3. In all groups, there were no complications or deaths related to the catheter-based delivery of ASM. No significant

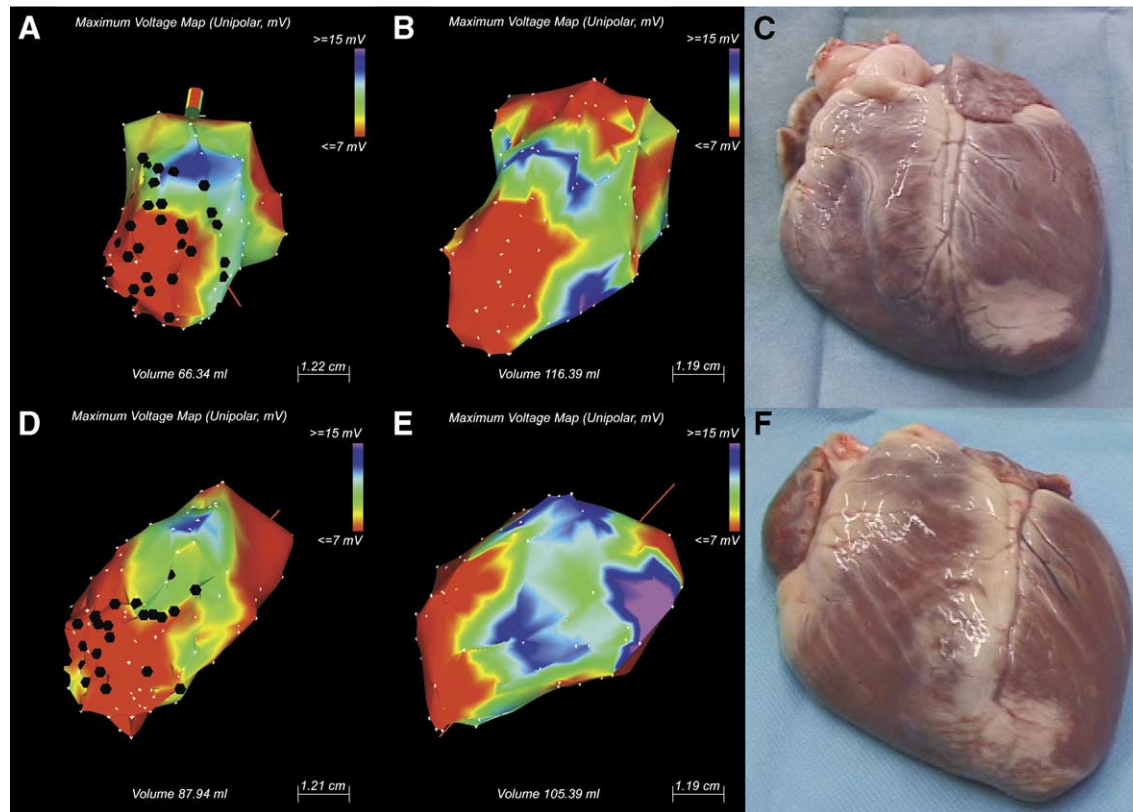


Fig. 1. Results of 3-dimensional NOGA unipolar endocardial voltage mapping at transplantation/injection (A, D), sacrifice (D, E), and gross pathology of hearts at harvest (C, F). A representative control animal is shown in the top row and an animal injected with 600 million cells in the bottom row. Black dots in A and D indicate the sites of injection within the left ventricle and septal wall of heart. A color scale is shown in the upper right corner of each NOGA map with an upper and lower limit of 15 and 7 mV, respectively.

differences in hematology and blood chemistry were seen between the two treatment groups and controls at any selected time point (data not shown). In addition, no premature ventricular contractions, sinus tachycardias, or arrhythmias were recorded by ILR in any group during the 60-day period following ASMT. One episode of non-sustained ventricular tachycardia and 2 episodes of sinus tachycardia were recorded, all three prior to transplantation.

3.3. Myocardial function

Functional assessments of the hearts were performed to detect and compare changes in viability and function that may have occurred in the treatment groups compared to controls. At the time of transplant (baseline), no significant differences in EF by ECHO, EF by LV gram, cardiac index by Bio-Z, left ventricular unipolar voltage (LVUPV) by NOGA, and apical unipolar voltage (APUPV) by NOGA were found between the treatment and control groups.

At sacrifice, a consistent trend toward improved cardiac function was seen in the treatment groups relative to controls (Table 4). Given that there were no obvious differences between improvements in cardiac function between animals which were injected with 300 million versus 600 million ASM, the data from both treatment groups were pooled for analysis. By blinded echocardiographic assessment, the treated animals exhibited a 15% improvement in EF by ECHO versus a –10% deterioration in control animals, and a 2% decrease in EF by LV gram versus a 12% deterioration in control animals. Finally, the mean APUPV improved by 23% in treated animals but declined 4% in control animals. Representative examples of the 3-dimensional NOGA unipolar voltage maps at baseline and at the completion of the study are shown in Fig. 1.

3.4. Histology

Histological analysis of sections taken through the anterior left ventricular wall of each treatment pig showed lack of cell survival 60 days after implantation. No injected myoblasts or more mature multinucleated myotubes were detected using H and E and trichrome stains, or myoblast specific myosin heavy-chain immunostaining (MY-32). Also, immunostaining for nuclear BrdU was negative on all animals. Lesions in graft-recipient pigs were not more severe or qualitatively different than those in the control animals.

4. Discussion

Experimentally, myoblasts have been delivered into the injured heart using a number of methods, including intravascular infusion into the coronary circulation (Taylor et al., 1997), transvenous delivery (Brasselet et al., 2005), direct epicardial injection into the injured myocardium (Dib, McCarthy, et al., 2005; Dib, Michler et al., 2005; Jain et al., 2001; Ghostine et al., 2002; Taylor et al., 1998), and, most recently, by catheter-based endoventricular delivery (Dib et al., 2002; Chazaud et al., 2003). Catheter-based delivery is more challenging than direct injection

since the myocardial wall of the infarcted zone is thinner than in healthy tissue. Thus, we have examined the accuracy, retention, biodistribution and safety of using a needle injection catheter to deliver the cells into a thin wall and assess the risk of perforation and cell leakage. The safety data indicate that percutaneous, catheter-based transplantation of ASM does not have a deleterious effect on the general well-being of the recipient animals or the infarcted swine heart muscle. In addition, the trend toward improved myocardial function seen in the two treatment groups compared to controls not only supports the safety findings, but also indicates that catheter-based delivery is feasible and results in greater overall heart function.

Using percutaneous catheter delivery of iridium labeled myoblasts, the cells were accurately targeted to the infarct zone in the anterior apex and septal apex of the pig heart. Within 2 h, 4.0% of the cells were retained in the site of implantation and 5.1% were localized in the lung. Biodistribution to other areas of the heart and the spleen was very low, and no cells were detected in the other analyzed tissues: brain, kidney and liver. In total, only approximately 9% of the cells were detected in the tissues examined, indicating that the remaining cells were distributed in other fluids and tissues. Other short-term retention studies using catheter-based delivery methods which have reported 43% retention of microspheres immediately after injection (Grossman et al., 2002), and 11% retention of myoblasts 2 h after injection (Brasselet et al., 2005).

In our safety and feasibility study of 6 animals, there were no complications related to the transplant procedure. Interrogation of a surgically implanted loop recorder revealed that no significant arrhythmias occurred following endocardial catheter injection of up to 756 million cells in a total volume of 5.9 cc. There was also no elevation of cardiac enzymes at 2 months which might indicate inflammatory or tumorigenicity processes. However, we did observe complications that occurred after MI and prior to the cell transplant procedure; three pigs did not survive the MI, one pig had sustained VT and two had sinus tachycardia leading to sudden death.

Albeit with a small sampling size, we observed a trend toward improvement in heart function by ECHO, LV gram and conductive output, despite negative histochemical staining with MY-32. This paradoxical finding suggests that the improvement in the treated arm might be due to transient myoblast cell survival, recruitment of other cell types to the area of MI, nascent angiogenesis or prevention of further ischemic damage. Yet, we have no data to support these mechanisms and cannot rule out the possibility that the observed improvements are not significant or reproducible. A larger animal study would be necessary to confirm the reported cardiac changes in this study. It is known from a large number of animal and clinical studies in species other than pigs including rats (Jain et al., 2001), rabbits (Taylor et al., 1998), sheep (Ghostine et al., 2002), humans (Pagani et al., 2003; Hagege et al., 2003) that myoblasts transplanted by epicardial delivery survive and form myotubes and myofibrils, suggesting that grafted myoblasts are able to survive in a foreign environment. We currently speculate that porcine myoblasts have a unique property which does not allow them to survive long-term in the normal or infarcted myocardium. This

conclusion is based on unpublished findings in other studies from our lab using epicardially injected porcine myoblasts which did not show ASM survival beyond a few days after implantation (data not shown). In the literature, there are references to short term myoblasts transplant studies in the porcine heart (Chazaud et al., 2003; Brasselet et al., 2005), but no long-term studies describing ASM survival.

In summary, our data indicate that delivery of autologous skeletal myoblasts via a percutaneous endoventricular technique into a coil-infarcted swine myocardium may be performed safely, without adverse events related to the procedure or toxicity of the cells. Secondly, our findings suggest that implantation of ASM via percutaneous catheter may improve cardiac function.

Acknowledgments

We would like to thank K. Wetzel for her histological assistance. *Conflict of Interest Disclosure* DBJ, AZ and JR are employees of GenVec, Inc.

References

- Al Attar, N., Carrion, C., Ghostine, S., Garcin, I., Vilquin, J. T., Hagege, A. A., et al. (2003). Long-term (1 year) functional and histological results of autologous skeletal muscle cells transplantation in rat. *Cardiovascular Research*, *58*, 142–148.
- Atkins, B. Z., Huelman, M. T., Meuchel, J., Hutcheson, K. A., Glower, D. D., & Taylor, D. A. (1999). Cellular cardiomyoplasty improves diastolic properties of injured heart. *Journal of Surgical Research*, *85*, 234–242.
- Brasselet, C., Morichetti, M. C., Messas, E., Carrion, C., Bissery, A., Bruneval, P., et al. (2005). Skeletal myoblast transplantation through a catheter-based coronary sinus approach: An effective means of improving function of infarcted myocardium. *European Heart Journal*, *26*, 1551–1556.
- Broomhead, C. J., Wright, S. J., Kiff, K. M., & Withington, P. S. (1997). Validation of thoracic electrical bioimpedance as a porcine research tool. *British Journal of Anaesthesia*, *78*, 323–325.
- Callans, D. J., Ren, J. F., Michele, J., Marchlinski, F. E., & Dillon, S. M. (1999). Electroanatomic left ventricular mapping in the porcine model of healed anterior myocardial infarction. Correlation with intracardiac echocardiography and pathological analysis. *Circulation*, *100*, 1744–1750.
- Chazaud, B., Hittinger, L., Sonnet, C., Champagne, S., Le Corvoisier, P., Benhaïem-Sigaux, N., et al. (2003). Endoventricular porcine autologous myoblast transplantation can be successfully achieved with minor mechanical cell damage. *Cardiovascular Research*, *58*, 444–450.
- Dib, N., Diethrich, E., Campbell, A., Gahremanpour, A., Miedzybrocki, B., McGarry, M., et al., (in press). A percutaneous swine model of myocardial infarction. *Journal of Pharmacological and Toxicological Methods*.
- Dib, N., Diethrich, E. B., Campbell, A., Goodwin, N., Robinson, B., Gilbert, J., et al. (2002). Endoventricular transplantation of allogenic skeletal myoblasts in a porcine model of myocardial infarction. *J Endovasc Ther*, *9*, 313–319.
- Dib, N., McCarthy, P., Campbell, A., Yeager, M., Pagani, F. D., Wright, S., et al. (2005). Feasibility and safety of autologous myoblast transplantation in patients with ischemic cardiomyopathy. *Cell Transplantation*, *14*, 11–19.
- Dib, N., Michler, R. E., Pagani, F. D., Wright, S., Kereiakes, D. J., Lengerich, R., et al. (2005). Safety and feasibility of autologous myoblast transplantation in patients with ischemic cardiomyopathy: Four-year follow-up. *Circulation*, *112*, 1748–1755.
- Ghostine, S., Carrion, C., Souza, L. C., Richard, P., Bruneval, P., Vilquin, J. T., et al. (2002). Long-term efficacy of myoblast transplantation on regional structure and function after myocardial infarction. *Circulation*, *106*, 1131–1136.
- Grossman, P. M., Han, Z., Palasis, M., Barry, J. J., & Lederman, R. J. (2002). Incomplete retention after direct myocardial injection. *Catheterization and Cardiovascular Interventions*, *55*, 392–397.
- Hagege, A. A., Carrion, C., Menasche, P., Vilquin, J. T., Duboc, D., Marolleau, J. P., et al. (2003). Viability and differentiation of autologous skeletal myoblast grafts in ischaemic cardiomyopathy. *Lancet*, *361*, 491–492.
- Jain, M., DerSimonian, H., Brenner, D. A., Ngoy, S., Teller, P., Edge, A., et al. (2001). Cell therapy attenuates deleterious ventricular remodeling and improves cardiac performance after myocardial infarction. *Circulation*, *103*, 1920–1927.
- Kawamoto, A., Tkebuchava, T., Yamaguchi, J., Nishimura, H., Yoon, Y. S., Milliken, C., et al. (2003). Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. *Circulation*, *107*, 461–468.
- Kornowski, R., Hong, M. K., Gepstein, L., Goldstein, S., Ellahham, S., Ben-Haim, S. A., et al. (1998). Preliminary animal and clinical experiences using an electromechanical endocardial mapping procedure to distinguish infarcted from healthy myocardium. *Circulation*, *98*, 1116–1124.
- Kraitichman, D. L., Heldman, A. W., Atalar, E., Amado, L. C., Martin, B. J., Pittenger, M. F., et al. (2003). In vivo magnetic resonance imaging of mesenchymal stem cells in myocardial infarction. *Circulation*, *107*, 2290–2293.
- Marelli, D., Desrosiers, C., el-Alfy, M., Kao, R. L., & Chiu, R. C. (1992). Cell transplantation for myocardial repair: An experimental approach. *Cell Transplantation*, *1*, 383–390.
- Menasche, P., Hagege, A. A., Scorsin, M., Pouzet, B., Desnos, M., Duboc, D., et al. (2001). Myoblast transplantation for heart failure. *Lancet*, *357*, 279–280.
- Murry, C. E., Wiseman, R. W., Schwartz, S. M., & Hauschka, S. D. (1996). Skeletal myoblast transplantation for repair of myocardial necrosis. *Journal of Clinical Investigation*, *98*, 2512–2523.
- Oron, U., Halevy, O., Yaakobi, T., Hayam, G., Gepstein, L., Wolf, T., et al. (2000). Technical delivery of myogenic cells through an endocardial injection catheter for myocardial cell implantation. *International Journal of Cardiovascular Interventions*, *3*, 227–230.
- Pagani, F. D., DerSimonian, H., Zawadzka, A., Wetzel, K., Edge, A. S., Jacoby, D. B., et al. (2003). Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. Histological analysis of cell survival and differentiation. *Journal of the American College of Cardiology*, *41*, 879–888.
- Pouzet, B., Vilquin, J. T., Hagege, A. A., Scorsin, M., Messas, E., Fiszman, M., et al. (2001). Factors affecting functional outcome after autologous skeletal myoblast transplantation. *Annals of Thoracic Surgery*, *71*, 844–850 (discussion 850-1).
- Reinecke, H., MacDonald, G. H., Hauschka, S. D., & Murry, C. E. (2000). Electromechanical coupling between skeletal and cardiac muscle. Implications for infarct repair. *Journal of Cell Biology*, *149*, 731–740.
- Reinecke, H., Poppa, V., & Murry, C. E. (2002). Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. *Journal of Molecular and Cellular Cardiology*, *34*, 241–249.
- Taylor, D. A., Atkins, B. Z., Hungspreugs, P., Jones, T. R., Reedy, M. C., Hutcheson, K. A., et al. (1998). Regenerating functional myocardium: Improved performance after skeletal myoblast transplantation. *Natural Medicines*, *4*, 929–933.
- Taylor, D. A., Silvestry, S. C., Bishop, S. P., Annex, B. H., Lilly, R. E., Glower, D. D., et al. (1997). Delivery of primary autologous skeletal myoblasts into rabbit heart by coronary infusion: A potential approach to myocardial repair. *Proceedings of the Association of American Physicians*, *109*, 245–253.