Cardiac Progenitor Cells Enhance Neonatal Right Ventricular Function After Pulmonary Artery Banding

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Background. C-kit$^+$ cardiac progenitor cells (CPCs) have been shown to be safe and effective in large-animal models and in an early-phase clinical trial for adult patients with ischemic heart disease. However, CPCs have not yet been evaluated in a preclinical model of right ventricular (RV) dysfunction, which is a salient feature of many forms of congenital heart disease.

Methods. Human c-kit$^+$ CPCs were generated from right atrial appendage biopsy specimens obtained during routine congenital cardiac operations. Immunosuppressed Yorkshire swine (6 to 9 kg) underwent pulmonary artery banding to induce RV dysfunction. Thirty minutes after banding, pigs received intramyocardial injection into the RV free wall with c-kit$^+$ CPCs (1 million cells, n = 5) or control (phosphate-buffered saline, n = 5). Pigs were euthanized at 30 days postbanding.

Results. Banding was calibrated to a consistent rise in the RV-to-systemic pressure ratio across both groups (postbanding: CPCs = 0.76 ± 0.06, control = 0.75 ± 0.03). At 30 days postbanding, the CPCs group demonstrated less RV dilatation and a significantly greater RV fractional area of change than the control group ($p = 0.002$). In addition, measures of RV myocardial strain, including global longitudinal strain and strain rate, were significantly greater in the CPCs group at 4 weeks relative to control ($p = 0.004$ and $p = 0.01$, respectively). The RV free wall in the CPCs group demonstrated increased arteriole formation ($p < 0.0001$) and less myocardial fibrosis compared with the control group ($p = 0.02$).

Conclusions. Intramyocardial injection of c-kit$^+$ CPCs results in enhanced RV performance relative to control at 30 days postbanding in neonatal pigs. This model is important for further evaluation of c-kit$^+$ CPCs, including long-term efficacy.

(Ann Thorac Surg 2017;104:2045–53)

Although extensively evaluated in adults with ischemic heart disease, cellular therapies for children with congenital heart disease (CHD) have only recently been investigated. A promising series of case reports and a recently published phase I trial in patients with hypoplastic left heart syndrome (HLHS) have demonstrated safety and early efficacy to boost ventricular function in children [1]. These findings, combined with preclinical evidence of robust regenerative mechanisms intrinsic to the very young, have garnered growing enthusiasm for the application of stem cell therapies in children with CHD and cardiomyopathy.

Among stem cell preparations approved for clinical use, c-kit$^+$ cardiac progenitor cells (CPCs) have yet to be explored in a pediatric population. C-kit$^+$ CPCs are a multipotent, clonogenic, and self-renewing population of cells characterized by a phenotype negative for hematopoietic and endothelial markers and positive for the tyrosine kinase marker, c-kit (cluster of differentiation [CD] 117). We, and others, have shown the regenerative potential of c-kit$^+$ CPCs isolated and expanded ex vivo for therapeutic use in the recovery of ischemic myocardium in a number of large-animal models [2]. In humans, a phase I clinical trial in patients with ischemic cardiomyopathy showed a mean improvement in ejection fraction of 12.3% and a reduction in infarct size of 30% at 1 year after delivery [3]. A comparable benefit to ventricular function in patients with CHD would represent a major therapeutic breakthrough. To date, however, c-kit$^+$ CPCs have had limited evaluation in preclinical models relevant to CHD.

We previously showed that a different stem cell type, bone marrow-derived mesenchymal stem cells (MSCs),

Accepted for publication April 26, 2017.

Presented at the Sixty-Third Annual Meeting of the Southern Thoracic Surgical Association, Naples, FL, Nov 9–12, 2016.

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The Videos and Supplemental Figure can be viewed in the online version of this article [http://dx.doi.org/10.1016/j.athoracsur.2017.04.058] on http://www.annalsthoracicsurgery.org.

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2017.04.058

Abbreviations and Acronyms

CD = cluster of differentiation
CHD = congenital heart disease
HLHS = hypoplastic left heart syndrome
CPCs = cardiac progenitor cells
MSCs = mesenchymal stem cells
LAA = left atrial appendage
PAB = pulmonary arterial banding
RV = right ventricular
TAPSE = tricuspid annular planar systolic excursion

preserved right ventricular (RV) size and function in a neonatal swine model of RV pressure overload at 4 weeks after intramyocardial injection [4]. At the time of this initial study, a contemporaneous cohort of animals underwent RV injection with c-kit$^+$ CPCs but had not yet been analyzed. The purpose of the present study was to therefore compare the results of the CPCs cohort with our recently published control group.

Patients and Methods

This study was approved by the University of Maryland School of Medicine Institutional Review Board, and parental consent was provided for use of all cardiac tissue biopsy specimens. Tissue specimens were obtained from the right atrial appendage of infant pediatric patients during routine congenital cardiac operations. Animal protocols were reviewed and approved by our Institutional Animal Care and Use Committee and followed the 1996 *Guide for the Care and Use of Laboratory Animals* [5].

Study Design

Immunosuppressed Yorkshire swine (6 to 9 kg, 14 to 21 days of life) underwent pulmonary artery (PA) banding (PAB), followed by injection of c-kit$^+$ CPCs (n = 5) or control (phosphate-buffered saline) at 30 minutes postbanding. The c-kit$^+$ CPCs were isolated from right atrial appendage samples, expanded in c-kit growth media, and characterized as previously described [6]. Results from the CPCs group were compared with values from a contemporaneous control group that underwent the identical protocol described in the present study [4]. Conventional and speckle-tracking echocardiography was performed to assess structural and functional changes at baseline (before banding), at day 1 postbanding (24 to 48 hours from banding), and at 30 days postbanding (day 30).

Induction of RV Pressure Overload

Sedation and anesthesia of the swine was performed as previously described [4]. Briefly, a left anterior thoracotomy was performed in the fifth intercostal space to expose the RV, main PA, and aorta. A 5-mm-wide Gore-Tex band (W. L. Gore & Associates, Flagstaff, AZ) was placed around the PA 10 mm from the pulmonary valve. The band was progressively tightened around the PA using medium-sized hemoclips to induce an RV-to-systemic systolic pressure ratio of approximately 75%. In the event this ratio exceeded 75% or the animal showed signs of hemodynamic compromise, 1 or more hemoclips were removed. When the animal was hemodynamically stable at the desired pressure ratio, the band was sutured to the PA with a 5-0 polypropylene suture to prevent migration of the band. The animal was then monitored for 30 minutes before injection of CPCs. All animals were then kept alive 30 days.

Our protocol for immune suppression, intramyocardial stem cell injection, echocardiography, and myocardial strain analysis was previously described [4]. Briefly, the control and experimental animals were treated with cyclosporine and methylprednisone to attenuate the immune response to the injected human cell xenografts [7]. Cyclosporine doses were titrated to target a serum level of 125 to 225 ng/mL [8]. Findings from our previous study showed this regimen produced a serum cyclosporine level that was on average within the targeted goal (144.8 ± 47.0 ng/mL) [4].

On the morning of CPC delivery, c-kit$^+$ CPCs (passage 3) were harvested with TrypLE Express (Gibco, Life Technologies, Grand Island, NY) and resuspended in sterile Plasma-Lyte solution (Baxter Healthcare, Deerfield, IL). Animals underwent stem cell (n = 5, 1×10^6 CPCs) or control (n = 5, phosphate-buffered saline) injection to the RV myocardium by 6 separate 200 μL aliquots (total volume, 1.2 mL) using a 29-gauge needle at a 30-degree angle to the epicardium. We administered 1×10^6 CPCs, which corresponds to a weight-based dose of approximately 125,000 CPCs/kg. This dose was chosen based on demonstrated safety in preclinical large-animal models as well as safety in a phase I trial in adult humans [3, 8].

Transthoracic echocardiograms were performed using a General Electric Vivid Q Ultrasound and a 3S probe (General Electric, New York, NY) as previously described [4]. Variables for assessment of RV function and size were selected from current recommendations [9] for RV assessment in the biventricular heart, which included RV fractional area change (FAC), defined as ([end-diastolic area – end-systolic area]/end-diastolic area) × 100. Speckle-tracking measures of deformation from the apical 4-chamber view included peak global longitudinal strain and strain rate and were calculated from the combined deformation of the myocardial segments in each imaging plane. All echocardiographic images were reviewed and analyzed by a single, blinded investigator.

At necropsy, multiple biopsy specimens were obtained from the RV free wall. Primary antibodies used were c-kit (AbD Serotec, Raleigh, NC), smooth muscle actin (#F3777; Sigma-Aldrich, St. Louis, MO), α-sarcomeric actinin (Sigma-Aldrich), and human anti-mitochondrial antibody (EMD Millipore, Billerica, MA). Myocardial fibrosis was assessed using Masson trichrome staining to detect the percentage of collagen. Random fields were selected while viewing the slides, and the mean number of