Myocardial Response to Milrinone in Single Right Ventricle Heart Disease

Stephanie J. Nakano, MD[1], Penny Nelson, MS[2], Carmen C. Sucharov, PhD[2], and Shelley D. Miyamoto, MD[1]

Objectives Empiric treatment with milrinone, a phosphodiesterase (PDE) 3 inhibitor, has become increasingly common in patients with single ventricle heart disease of right ventricular (RV) morphology (SRV); our objective was to characterize the myocardial response to PDE3 inhibition (PDE3i) in the pediatric population with SRV.

Study design Cyclic adenosine monophosphate levels, PDE activity, and phosphorylated phospholamban (PLN) were determined in explanted human ventricular myocardium from nonfailing pediatric donors (n = 10) and pediatric patients transplanted secondary to SRV. Subjects with SRV were further classified by PDE3i treatment (n = 13 with PDE3i and n = 12 without PDE3i).

Results In comparison with nonfailing RV myocardium (n = 8), cyclic adenosine monophosphate levels are lower in patients with SRV treated with PDE3i (n = 12, P = .021). Chronic PDE3i does not alter total PDE or PDE3 activity in SRV myocardium. Compared with nonfailing RV myocardium, SRV myocardium (both with and without PDE3i) demonstrates equivalent phosphorylated PLN at the protein kinase A phosphorylation site.

Conclusions As evidenced by preserved phosphorylated PLN, the molecular adaptation associated with SRV differs significantly from that demonstrated in pediatric heart failure because of dilated cardiomyopathy. These alterations support a pathophysiologically distinct mechanism of heart failure in pediatric patients with SRV, which has direct implications regarding the presumed response to PDE3i treatment in this population. *(J Pediatr 2016;174:199-203)*.

Single ventricle congenital heart disease is a subset of heart defects characterized by a univentricular circulation with persistent hypoxia, which is universally fatal without intervention.[1] Despite advances in surgical technique and perioperative care, the outcome for single ventricle heart disease remains poor,[2-5] with only one-third of children surviving, free from heart transplant, to age 14 years.[6]

Progressive heart failure is both a common cause of death and indication for heart transplantation in infants and children with single ventricle heart disease.[4,7] The dominant ventricle in single ventricle heart disease can be of right ventricular (RV), left ventricular (LV), or indeterminate morphology, and it is suggested that those with single ventricle heart disease of RV morphology (SRV) have worse outcomes.[8,9] The RV in particular is at risk for progressive failure secondary to the nonphysiological high afterload in SRV, as well as intrinsic embryologic and architectural differences between the RV and LV.[10,11] As a whole, the RV is not well-suited to pump against sustained pressure overload, and the mechanisms that help the RV adapt to high pressures ultimately lead to maladaptive remodeling, with RV dilation and eventual failure. However, the molecular mechanisms underlying RV failure in SRV are poorly understood, limiting the ability to identify effective therapies. Treatments for LV failure are aimed at preventing or reversing pathologic remodeling; these therapies have been applied to RV failure under the hypothesis that the same pathophysiological events occur in LV and RV failure. However, given the significant differences between the LV and RV, perhaps it is not surprising that the extrapolation of proven adult LV systolic heart failure medications to the pediatric population with SRV have demonstrated little benefit. Specifically, angiotensin-converting enzyme inhibitors and beta-blockers have been ineffective in preventing heart failure or even trended toward worsening heart failure in the setting of SRV, respectively.[12,13]

Management of SRV has included the empirical use of specific inhibitors targeting phosphodiesterase (PDE) enzymes. PDEs regulate the amplitude, duration, and compartmentalization of intracellular cyclic nucleotide signaling by

cAMP	Cyclic adenosine monophosphate
DCM	Dilated cardiomyopathy
LV	Left ventricular
PDE	Phosphodiesterase
PDE3i	PDE 3 inhibition
PKA	Protein kinase A
PLN	Phospholamban
RV	Right ventricular
SERCA	Sarcoplasmic reticulum calcium adenosine triphosphatase 2
SRV	Single ventricle heart disease of RV morphology
SRV+M	SRV treated with milrinone

From the [1]Division of Cardiology, Department of Pediatrics, University of Colorado School of Medicine, Children's Hospital Colorado; and [2]Division of Cardiology, Department of Medicine, University of Colorado Denver, Aurora, CO

Supported by the National Institutes of Health (NIH)/*Eunice Kennedy Shriver* National Institute of Child Health and Human Development (K12 HD068372 to [S.N.]), American Academy of Pediatrics Section on Cardiology and Cardiac Surgery (Research Fellowship Award [to S.N.]), NIH/National Center for Advancing Translational Sciences (UL1 TR000154 [to S.N. and S.M.]), NIH/ National Heart, Lung, and Blood Institute (R01 HL107715 [to B.S.], R01 HL126928 [to S.M.], R21 HL113846 [to S.M.]), Addison Scott Memorial Fund, Boedecker Foundation, and the Millisor Chair in Pediatric Heart Disease. C.S. is a scientific founder and shareholder at miRagen, Inc. The other authors declare no conflicts of interest.

http://dx.doi.org/10.1016/j.jpeds.2016.04.009

hydrolyzing cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate. Pharmacologic inhibition of select PDE enzymes has become an increasingly common therapy for SRV and is often used in pediatric heart failure as a bridge to transplant[14]; specifically, PDE 3 inhibition (PDE3i) with milrinone is thought to augment contractility through increasing intracellular cAMP. Classically, milrinone-mediated improvements in contractility are attributed to the downstream effects of increased cAMP through phosphorylation of proteins that affect excitation/contraction coupling, including the sarcoplasmic reticulum calcium adenosine triphosphatase 2 (SERCA)-regulatory protein phospholamban (PLN). By de-inhibiting SERCA, increased protein kinase A (PKA)-mediated phosphorylated PLN accelerates calcium re-uptake into the sarcoplasmic reticulum and increases sarcoplasmic reticulum calcium content, contributing to both lusitropic and inotropic effects, respectively.[15] We have previously demonstrated that PKA-mediated phosphorylated PLN is increased in children with dilated cardiomyopathy (DCM) chronically treated with PDE3i, and may contribute to increased contractility resulting in the sustained clinical benefit of PDE3i treatment in that population.[16] However, it is unlikely that the molecular perturbations in SRV are akin to those in pediatric DCM; thus, given the widespread clinical use our objective was to characterize the molecular response to PDE3i in this unique population.

Methods

All subjects gave informed consent and donated their hearts to the Institutional Review Board-approved Pediatric Cardiac Transplant Tissue Bank at the University of Colorado Denver. Nonfailing tissues were from pediatric (<18 years of age) organ donors with normal heart function, whose hearts could not be placed for technical reasons (size or blood type mismatch). Hearts from patients transplanted with SRV were included in this study (single ventricle disease of LV or indeterminate morphology were excluded). Two groups of patients with SRV were included: (1) patients with SRV listed for transplant secondary to surgical palliation failure; and (2) patients with SRV listed for primary transplant that met predefined criteria. Surgical palliation failure included those patients suffering from RV failure, protein losing enteropathy, and/or plastic bronchitis. Patients with SRV listed for primary transplant were included only if they were >4 months of age at the time of transplant and had signs and symptoms of heart failure, evidence of ventricular dilation, hypertrophy, or decreased cardiac function. Patients that were transplanted primarily for SRV lacking these defined clinical characteristics of heart failure were excluded. Patients with SRV were then categorized based on PDE3i treatment at the time of transplant and all patients with SRV were compared with nonfailing RV controls. At the time of cardiac transplantation (SRV group) or donation (nonfailing group), the heart tissue was rapidly dissected in the operating room, flash frozen, and stored at −80°C until further use.

cAMP levels were measured by enzyme-linked immunosorbent assay in the core facility at Children's Hospital Colorado, Aurora, Colorado using the R&D Parameter immunoassay kit (R&D Systems, Minneapolis, Minnesota) according to manufacturer's recommendations.

Approximately 150 mg of myocardium was homogenized and separated into nuclear, cytosolic, and sarcoplasmic reticulum-enriched microsomal fractions by differential sedimentation, as previously described.[16]

cAMP-hydrolytic activity was quantified at 30°C by the 2-step snake-venom method with (^{3}H) cAMP (1 μmol/L) as substrate (previously described[16]). Western blots were performed as described previously.[16] Serine 16 residue phosphorylated PLN (A010-12; Badrilla, Leeds, United Kingdom) and total PLN (05-205; Millipore, Darmstadt, Germany) were quantified on separate blots and normalized to glyceraldehyde 3-phosphate dehydrogenase (Santa Cruz Biotechnology, Dallas, Texas). Blots were quantified using ImageJ v 1.46r (U.S. National Institutes of Health, Bethesda, Maryland).

Statistical Analyses

Statistical analyses were performed using GraphPad Prism v 6.0c (GraphPad Software, La Jolla, California). Statistical significance was set a priori at P value of <.05. Normality of data was confirmed and when appropriate, comparison of 3 normally distributed groups was conducted using 1-way ANOVA; if the overall comparison reached significance, Tukey post hoc tests were performed. Nonnormally distributed data were analyzed using nonparametric testing (Mann-Whitney or Kruskal-Wallis with Dunn multiple comparisons test). Comparisons of nonfailing LV and RV were conducted using unpaired t test (cAMP), paired t tests (PDE activity in RV and LV from the same patient), or Wilcoxon matched-pairs signed rank test (phosphorylated PLN). Linear regression was performed to evaluate for association between milrinone treatment, duration of heart failure, and dose of milrinone and cAMP levels, PDE activity, and PLN expression.

Results

Subject characteristics and medications are listed in the **Table** (available at www.jpeds.com). Median age at tissue collection for pediatric nonfailing subjects (n = 13) was 8.0 years with an IQR of 8.9 years; for subjects with SRV (n = 12), 0.67 years with an IQR of 5.5 years; and, for subjects with SRV treated with milrinone (SRV+M) (n = 13), 2.9 years with an IQR of 4.1 years. The nonfailing group was significantly older than the SRV group (P = .005), however, the ages of the SRV and SRV+M groups were similar. Mean duration of milrinone therapy was 139 days, with a median of 100 days (range 19-355 days). Duration of heart