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Cardiac Adenylyl Cyclase and Phosphodiesterase Expression Profiles Vary by Age, Disease, and Chronic Phosphodiesterase Inhibitor Treatment

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ABSTRACT

Background: Pediatric heart failure (HF) patients have a suboptimal response to traditional HF medications, although phosphodiesterase-3 inhibition (PDE3i) has been used with greater success than in the adult HF population. We hypothesized that molecular alterations specific to children with HF and HF etiology may affect response to treatment.

Methods and Results: Adenylyl cyclase (AC) and phosphodiesterase (PDE) isoforms were quantified by means of quantitative real-time polymerase chain reaction in explanted myocardium from adults with dilated cardiomyopathy (DCM), children with DCM, and children with single-ventricle congenital heart disease of right ventricular morphology (SRV). AC and PDE expression profiles were uniquely regulated in each subject group and demonstratde distinct changes in response to chronic PDE3i. There was unique up-regulation of AC5 in adult DCM with PDE3i (fold change 2.415; $P = .043$), AC2 in pediatric DCM (fold change 2.396; $P = .0067$), and PDE1C in pediatric SRV (fold change 1.836; $P = .032$). Remarkably, PDE5A expression was consistently increased across all age and disease groups.

Conclusions: Unique regulation of AC and PDE isoforms supports a differential molecular adaptation to HF in children compared with adults, and may help identify mechanisms specific to the pathogenesis of pediatric HF. Greater understanding of these differences will help optimize medical therapies based on age and disease process. (*J Cardiac Fail 2016;*■■*:*■■–■■)

Key Words: Adenylyl cyclase, phosphodiesterase, single ventricle congenital heart disease, dilated cardiomyopathy.

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Despite clear improvement in adult heart failure (HF) outcomes over the past several decades, pediatric HF outcomes remain comparatively poor. Treatments for children with HF, largely regardless of etiology, are based on clinical trials performed in adults with systolic HF, but a growing body of clinical evidence suggests that established adult HF medications, such as β-adrenergic receptor blockers and angiotensin-converting enzyme inhibitors, may not provide the same benefit to children with HF.[1–4] Additionally, there is differential adaptation of β-adrenergic receptors and adrenergic signaling pathways in children with HF secondary to dilated cardiomyopathy (DCM) as well as single right ventricle heart disease (SRV), suggesting that both age and disease etiology may influence response to therapy.[5,6] Certainly, the treatment of pediatric HF patients comprises unique challenges, including (1) diverse etiologies of HF, (2) age-related changes, (3) treatment of a vulnerable population, and (4) a relatively low incidence of HF. Therefore, investigations into the differences between a spectrum of pediatric and adult HF populations may further our understanding of disparate mechanisms of disease as well as the variable therapeutic potential of certain medications.

The second messenger cyclic adenosine monophosphate (cAMP) is highly regulated, in terms of both concentration and location. A decrease in global myocardial cAMP level is a common feature across multiple HF populations and phenotypes, specifically adults and children with DCM[7] and children with SRV,[8] suggesting that alterations in cAMP generation via adenylyl cyclases (ACs) or cAMP hydrolysis via phosphodiesterases (PDEs) may be involved in HF. Both ACs and PDEs have numerous isoforms with differing degrees of expression and activity in the myocardium. Activation of ACs via β-adrenergic receptor stimulation leads to increased production of cAMP, which provides short-term improvement in cardiac contractility. Nevertheless, prolonged and excessive adrenergic stimulation, as in the setting of HF, leads to adverse cardiac remodeling as well as induction of cardiac myocyte apoptosis.[9,10] Inhibition of PDEs can also contribute to improved inotropy and lusitropy through decreasing cAMP and/or cyclic guanosine monophosphate (cGMP) hydrolysis in the appropriate subcellular compartments. Therefore, stimulation or inhibition of AC isoforms and inhibition of select PDEs have been investigated as potential HF therapies, with varying success and utility. Most notably, PDE3 inhibition (PDE3i) is commonly used on a chronic basis as a bridge to transplant or recovery in children with HF,[11,12] with a low incidence of sudden death, whereas a clinical trial of PDE3i in adults with severe HF demonstrated a 34% increase in cardiovascular mortality,[13] tempering its long-term use in adult HF. Specifically, our previous work demonstrated that only pediatric DCM patients treated with PDE3i benefit from augmentation of myocardial cAMP and phospholamban phosphorylation, which may contribute to sustained hemodynamic benefits; these changes were not demonstrated in myocardium from adults with DCM[7] or children with SRV.[8] The differential response to PDE3i, both clinically and molecularly, is suggestive of fundamental differences in molecular adaptation to HF secondary to age and disease etiology. AC and PDE isoforms can uniquely target cAMP or cGMP and be present in different compartments. The physiologic consequences of activation of these isoforms varies from increased contractility to relaxation (which is addressed in the Discussion section). Although multiple AC and PDE isoforms are expressed in human heart tissue, little is known about the isoform levels in response to HF or PDE3i. Therefore, our objective was to identify changes in isoform expression of myocardial AC and PDE to provide insight into which subcellular compartments may be altered by disease, age, and PDE3i treatment. These expression profiles can be used in conjunction with additional studies to ultimately (1) characterize molecular alterations that may contribute to HF pathogenesis, particularly in the pediatric population, (2) identify targets unique to pediatric DCM or SRV that have therapeutic potential, and (3) further elucidate the mechanism of the beneficial effects of PDE3i in the pediatric population and, conversely, the mechanism underlying adverse outcomes with PDE3i in adults.

Methods

Human Samples

All subjects gave informed consents and donated their hearts to the Institutional Review Board–approved Pediatric or Adult Cardiac Transplant Tissue Bank at the University of Colorado, Denver. Nonfailing tissues were from adult or pediatric organ donors without previous heart disease; nonfailing hearts were deemed to be suitable for transplantation but could not be placed owing to technical reasons (size or blood type mismatch). All adult patients with DCM had nonischemic cardiomyopathy without any definitive contributing comorbidity. Pediatric hearts from patients transplanted for SRV morphology were included in this study; patients with single left ventricle heart disease or indeterminate morphology were excluded. Subjects with HF (DCM or SRV) were divided into 2 groups based on whether they were on PDE3i at the time of explantation. At the time of cardiac transplantation or donation, the heart tissue is rapidly dissected, flash frozen and stored at −80°C until further use.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from homogenized cardiac myocardium with the use of the mirVana kit (Ambion, Austin, Texas) and reverse transcribed into complementary DNA with the use of the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, California). Quantitative real-time polymerase chain reaction (qRT-PCR) was then performed with Power Sybr Green PCR Master Mix (Applied Biosystems/Life Technologies, Carlsbad, California) with the use of the ABI StepOnePlus system. Primer sequences are listed in Supplemental Table 1. Melt curve analysis was performed on each primer pair to confirm target specificity.