Dysregulation of Cardiolipin Biosynthesis in Pediatric Heart Failure

Background
Cardiolipin, mitochondrial function and heart failure. Cardiolipin (CL) is a major cardiac phospholipid found almost exclusively in the inner mitochondrial membrane and is essential for the function of key energy-producing enzymes in the electron transport chain. For optimal cardiac mitochondrial function, evidence suggests that CL must be in the tetrolipid form [4 hexose acid side chains, or 18:2(6Z)/CL]. Nascent CL is biosynthesized de novo by a pathway that assembles fatty acid side chains into a double-glycerol-phosphate backbone. CLs are then remodeled into 18:2(6Z)/CL via a process where hexose moieties are incorporated via tation- and monofosfodilipasin acyltransferase remodeling enzymes. Proper synthesis and remodeling of CL is essential to maintain the function of the mitochondria, preserving the ATP content, concentrations of which are reduced in severe heart failure.

Previous work has shown that decreases in the (6Z)-content of CL are dramatic in adult idiopathic dilated cardiomyopathy (IDC) and in a rat model of heart failure. Oxygen regulation of enzymes in the CL biosynthesis pathway has been shown in cardiac tissue from adults with IDC. Additionally, in the Spontaneously Hypertensive Rat model (SHR), a well-established congenital model of IDC, a high hexose acid diet can restore cardiac 18:2(6Z)/CL levels and markedly increase survival. The aim of this work was to directly assess whether CL compositional alterations contribute to development of heart failure in pediatric IDC. Identification of changes in CL composition in pediatric IDC may lead to a better understanding of the pathophysiology of this disease, distinct from that observed in adults, and ultimately lead to the design of agents that can specifically alter the cardiac CL profile, target mitochondrial function, and improve cardiac function.

Hypothesis
We hypothesize that changes in cardiolipin quantity and composition play a significant role in the progression of idiopathic dilated cardiomyopathy in children.

1. We predict that total and tetrolipidated (18:2(6Z))/CL will be depleted in left ventricular myocardium from children with idiopathic IDC compared to non-failing (NF) controls.
2. We expect dysregulation of enzymes in the CL biosynthetic or the remodeling pathway will be associated with these changes in mitochondrial phospholipid composition.

Methods
Left ventricular tissue from pediatric patients with IDC and non-failing controls. All specimens were prepared from tissue obtained from the CORRAB (spontaneous) pediatric tissue bank at the University of Colorado. Subjects are male and female, ages 0 to 12 years. All tissues were obtained by donating their heart at the time of transplantation. All IDC subjects had an ejection fraction < 40%. Non-failing controls were obtained from subjects with normal ejection fraction unable to be donors for technical reasons. At time of implant, hearts are immediately cooled in ice-cold oxygenated Tyrode’s solution in the operating room. The heart is rapidly dissected and flash frozen and stored at −80°C.

Cardiolipin mass quantification: Cardiolipins extracted from LV tissue homogenates for quantification by LC-MS were obtained as described by Sparagna et al. [1]. Lipid extracts were obtained using 1,1,2,2’-tetrachloroethane (TCE) as an internal standard. Cardiolipin was quantified for total CL from the 4 common molecular species present in human heart tissue (mass/charge 1222, 1246, 1248, 1250, 1270) measured individually. These species comprise ~80% of CL present in human myocardium. CLs are expressed in n mole/mg (wet weight) of tissue.

Real-time PCR: RNA extracted from LV (kambio mirVana isolation kit, manufacturer protocol) was reverse-transcribed to cDNA using the iScript cDNA Synthesis Kit (Iv) and per manufacturer protocol. The 384-well method was used to run quantitative expression using 50 ng total RNA and using the ABI StepOne Rapid RT-PCR protocol. All reactions were performed in duplicate with melting curves to ensure specificity of PCR product and normalized to 18S expression. ET expression was measured using the delta delta CT method values corrected to non-failing controls.

Acknowledgments
Data is expressed as mean +/- SEM. The differences between two groups was evaluated by Student’s t-test. CL comparisons were considered to be significant for p values < 0.05 unless otherwise noted.

Conclusions
1. Total CL content is depleted in left ventricular myocardium from pediatric patients with IDC compared to non-failing controls, similar to what is observed in adults with this diagnosis. The quantity and percentage of tetrolipidated (18:2(6Z))/CL is similarly lower in patients with IDC.
2. Significant differences in expression of enzymes in the CL biosynthesis pathway are observed in pediatric IDC compared to non-failing controls. The pattern of biosynthetic enzyme down-regulation is similar to that seen in adults. Specifically, PGSS and CTS expression is significantly lower in children, while CDT and TAZ expression appear to be lower in children with IDC, with no difference in CTS.
3. Alterations in expression of NCL-AT, C1 remodeling enzymes are seen in pediatric IDC. There is lower expression in children, whereas higher NCL-AT expression has been observed in adults with IDC.

Cardiolipin biosynthesis and remodeling is abnormal in pediatric heart failure presenting as IDC with both reduced CL content and lower levels of (18:2(6Z))/CL which is necessary for normal mitochondrial function. The effect of heart failure on CL levels is similar to that seen in adults, but is likely unique to a single age-related mechanism.

References

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