Dysregulation of Cardiolipin Biosynthesis in Pediatric Heart Failure

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Background

Cardiolipin, mitochondrial function and heart failure. Cardiolipin (CL) is a major cardiac phospholipid found almost exclusively in the inner mitochondrial membrane where it is essential for the optimal function of key energy producing enzymes in the electron transport chain. For optimal cardiac mitochondrial function, evidence suggests that CL must be in the tetralinoleoyl form (4 linoleic acid side chains, or $(18:2)_4$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 $(18:2)_4$CL via a process where linoleoyl moieties are incorporated via tafazzin and monolysocardiolipin acyltransferase remodeling enzymes. Proper synthesis and remodeling of CL are 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 linoleoyl content of CL are dramatic in adult idiopathic dilated cardiomyopathy (IDC) and in a rat model of heart failure. Down regulation of enzymes in the CL biosynthesis pathway has been shown in cardiac tissue from adults with IDC. Additionally, in the Spontaneously Hypertensive HF rat model (SHHF), a well-established congenital model of IDC, a high linoleic acid diet can restore cardiac $(18:2)_4$CL levels and markedly increase survival. The aim of this work was to directly assess whether CL compositional abnormalities 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 tetralinoleoyl $(18:2)_4$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 ventricle tissue from pediatric patients with IDCM and non-failing controls: All samples were prepared from tissue obtained from the COMIRB-approved pediatric tissue bank at the University of Colorado. Subjects are male and female, ages 0.1 to 18 years, of all races and ethnic backgrounds who donated their heart at the time of transplantation. All IDC subjects had an ejection fraction <30%. Non-failing controls were obtained from subjects with normal ejection fraction unable to be donors for technical reasons. At time of explant, hearts are immediately cooled in ice cold oxygenated Tyrode's solution in the operating room. The LV is rapidly dissected ant flash frozen and stored at -80°.

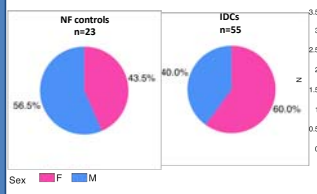
CL molecular species quantification: Lipid was extracted from LV tissue homogenates for quantification by electrospray ionizing mass spectrometry as described by Sparagna *et al*, J Lipid Res, 2005. Using 1,1',2,2'-tetramyristoyl CL as an internal standard EIS-MS was employed for quantification of total CL from the 6 most common molecular species present in human heart tissue (mass/charge 1422, 1446, 1448, 1450, 1470, 1472) measured individually. These species comprise >95% of CL present in human myocardium. CLs are expressed in nmol/mg protein.

Real-time PCR: RNA extracted from LV (Ambion mirVana isolation kit, manufacturers protocol) was reverse-transcribed to cDNA using the Qiagen miScript II RT kit (per manufactures protocol). The SYBR Green method was used to quantify enzyme expression using 10 ng cDNA per reaction using the AB 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. RT expression was measured using the delta delta CT method (values compared to non-failing controls).

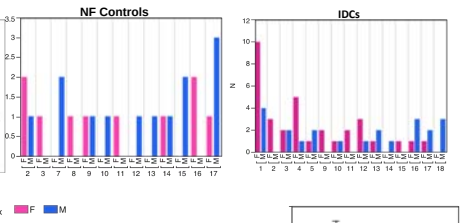
Statistical analysis: Data is expressed as means +/- SEM. The difference between two groups was evaluated by Students *t*-test. Comparisons were considered to be significant for p values < 0.05 unless otherwise noted.

Results

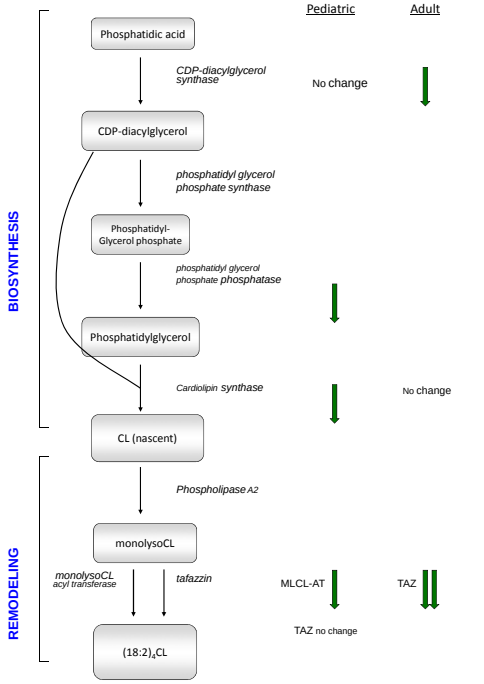
Patient Demographics

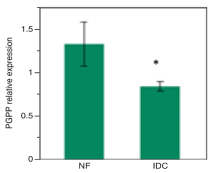


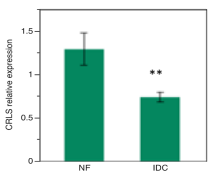
Age Distribution of Groups

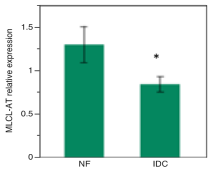


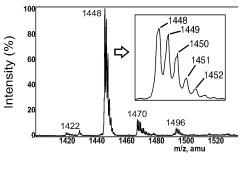
Cardiolipin Pathway Enzyme Expression





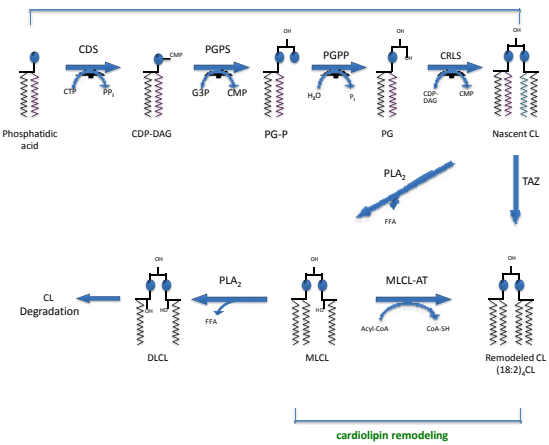






Example: CL MS profile

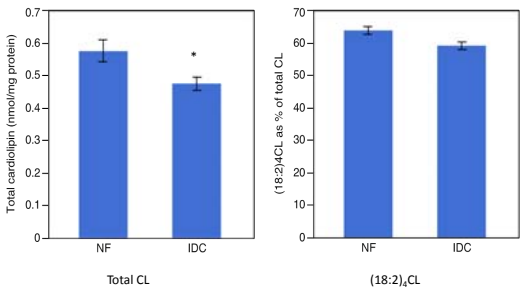
de novo cardiolipin biosynthesis



Legend:

CL- Cardiolipin
CDP- DAG- cytidinediphosphate-diacylglycerol
CDS- CDP-DAG synthase
CRLS- CL synthase
CMP- cytidinemonophosphate
CTP- cytidinetriphosphate
DLCL- Dilyso-CL
FFA- free fatty acid
G3P- glycerol-3-phosphate
MLCL- monolyso-CL
MLCL-AT- MLCL acyltransferase
PG- phosphatidylglycerol
PGPS- PG-P synthase
PGPP- PG-P phosphatase
P_i- inorganic phosphate
PLA_2- phospholipase A_2
TAZ-Tafazzin
*Figure adapted from Chicco and Sparagna, Am J Physiol, 2007

Cardiolipin EIS-Mass Spectrometry analysis



Conclusions

1. Total CL content is depleted in left ventricular myocardium from pediatric patients with IDC compared to non-failing controls, similar to what has been observed in adults with this diagnosis. The quantity and percentage of tetralinoleoyl $(18:2)_4$CL is similarly lower in pediatric 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 unlike that seen in adults. Specifically, PGPP and CRLS expression are significantly lower in children, while CDS and TAZ expression were shown to be lower in adults with IDC, with no difference in CRLS.
3. Alterations in expression of MLCL-AT, a CL remodeling enzyme are seen in pediatric IDC. There is lower expression in children, whereas higher MLCL-AT expression has been observed in adults with IDC.

Cardiolipin biosynthesis and remodeling is deranged in pediatric heart failure presenting as IDC which results in total myocyte CL depletion and lower levels of $(18:2)_4$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 secondary to a unique age-related mechanism.

1. Saini-Chohan, H. K. *et al.* 2009. Cardiolipin biosynthesis and remodeling enzymes are altered during the development of heart failure. *J. Lipid Res.* **50:** 1600–1608.
2. Chicco, A. J., and G. C. Sparagna. 2007. Role of cardiolipin alterations in mitochondrial dysfunction and disease. *Am. J. Physiol. Cell Physiol.* **292:** C33–C44.
3. Sparagna, G. C. *et al.* 2007. Loss of cardiac tetralinoleoyl cardiolipin in human and experimental heart failure. J. Lipid Res. 48: 1559–1570.
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Acknowledgments

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