Ferroptosis Inhibition Combats Metabolic Derangements and Improves Cardiac Function in Pulmonary Artery Banded Pigs

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Abstract

Right heart failure (RHF) is a leading cause of mortality in multiple cardiovascular diseases and preclinical and human data suggest impaired metabolism is a significant contributor to right-sided cardiac dysfunction. Ferroptosis is a nonapopotic form of cell death driven by impaired metabolism. Rodent data suggests ferroptosis inhibition can restore mitochondrial electron transport chain function and enhance cardiac contractility in left heart failure models, but the effects of ferroptosis inhibition in translational large animal models of RHF are unknown. Here, we showed ferrostatin-1 mediated ferroptosis antagonism improve right heart structure and function in pulmonary artery banded pigs. Molecularly, ferrostatin-1 restored mitochondrial cristae structure and combatted downregulation of electron transport chain proteins. Metabolomics and lipidomics analyses revealed ferrostatin-1 improved fatty acid metabolism. Thus, these translational data suggest ferroptosis may be a therapeutic target for RHF.

***To the Editor**:* Right heart failure (RHF) is a leading cause of morbidity and mortality in multiple cardiovascular diseases, but we currently lack therapies that augment right heart function(1). Both rodent and human data suggest mitochondrial dysfunction, marked by impairments in fatty acid oxidation, drives RHF(1-3). While fatty acid oxidation (FAO) is a key source of ATP generation in the heart, disruptions in FAO can also be detrimental due via induction of ferroptosis, a lipid-dependent mode of cell death(4). Ferroptosis causes left ventricular dysfunction via suppression of electron transport chain (ETC) function as ferroptosis inhibition enhances ETC activity and cardiac function under multiple stressors(5). However, the role of ferroptosis in RHF is less well described. Moreover, the physiological and molecular effects of ferroptosis inhibition in a translational large animal model of RHF are unexplored.

Here, we addressed these knowledge gaps by evaluating the effects of ferroptosis inhibition in a porcine model of RHF induced by pulmonary artery banding (PAB)(6). To improve translatability, ferrostatin-1 (0.2 mg/kg diluted in 2% dimethyl sulfoxide, 50% polyethylene glycol, 5% Tween 80, and 43% double distilled water), a well validated small molecule ferroptosis inhibitor(4), treatment began three weeks after PAB to allow for the development of right heart dysfunction. The comparator group were PAB pigs treated with an equivalent volume of ferrostatin-1 vehicle (PAB-Placebo). End-point analyses were performed six weeks after PAB. Primary end-point was right ventricular ejection fraction as determined by cardiac magnetic resonance imaging (cMRI).

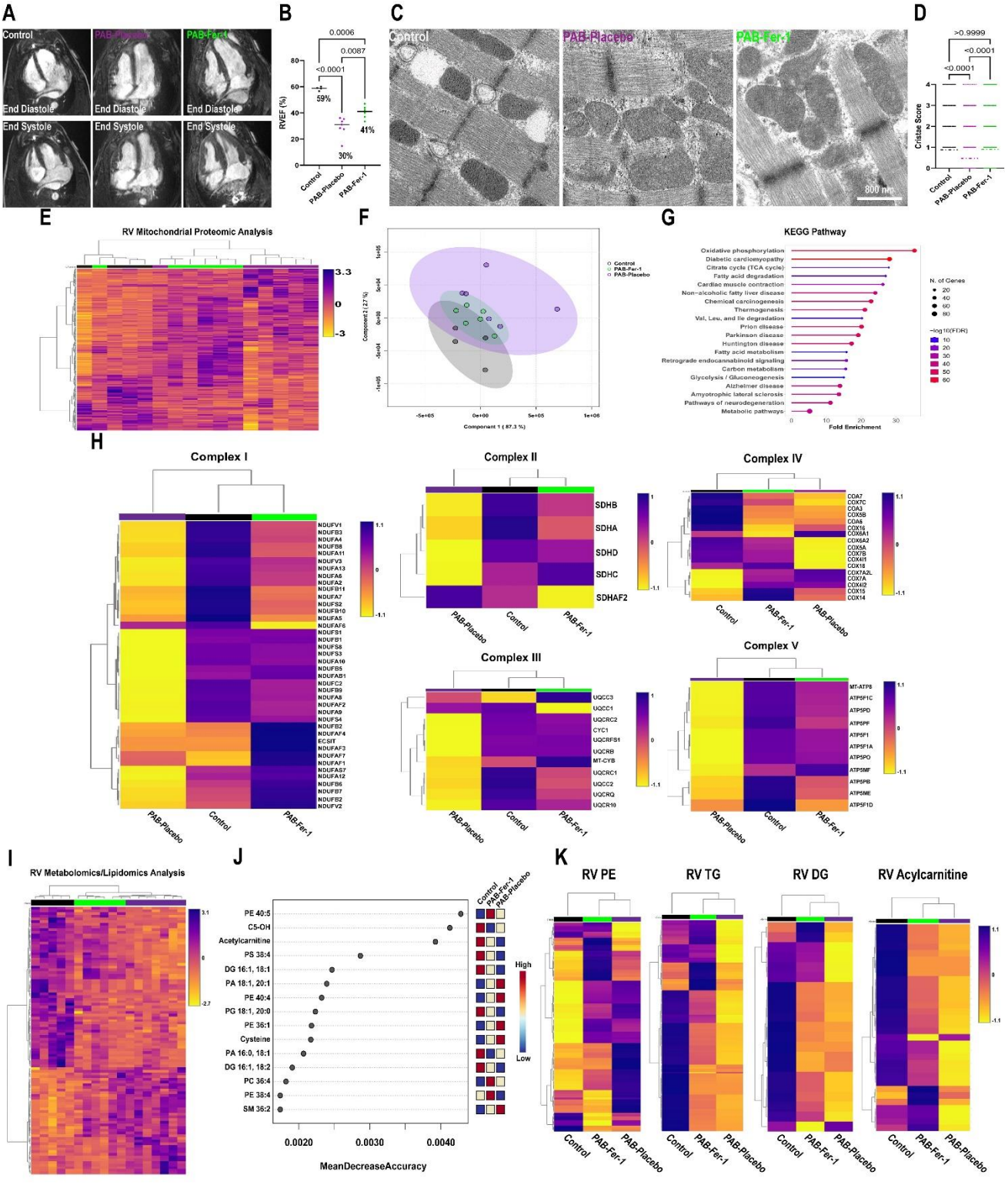
Ferroptosis inhibition via ferrostatin-1 exerted significant therapeutic effects as cMRI revealed ferrostatin-1 increased right ventricular ejection fraction by 11% (Control:59±2%, PAB-Vehicle: 30±3%, PAB-Fer-1: 41±2%). (Figure A and B). In addition, ferrostatin-1 reduced pathological RV dilation and tricuspid regurgitation severity (Supplemental Figure 1). At the cellular level, ferrostatin-1 normalized cardiomyocyte size despite no significant differences in anatomical or hemodynamic measures of RV afterload (Supplemental Figure 1). The ferrostatin-1 mediated corrections in RV remodeling and function also counteracted PAB-induced right atrial dilation and dysfunction (Supplemental Figure 1).

Because ferroptosis modulates mitochondrial homeostasis(5), we evaluated mitochondrial morphology, protein regulation, and function using a combination of electron microscopy, proteomics, and metabolomics/lipidomics analyses. Electron microscopy revealed mitochondrial cristae structure was deranged in PAB-Placebo animals, but corrected by ferrostatin-1 (Figure C and D). To evaluate the functional consequences of the observed mitochondrial morphological deficits, we profiled RV mitochondrial enrichments using proteomics and found ferroptosis inhibition shifted the overall molecular signature towards control as demonstrated by hierarchical cluster analysis and partial least squares discriminate analysis (Figure E and F). KEGG pathway analysis of the 250 proteins most important for differentiating the three experimental groups identified oxidation phosphorylation as the most enriched pathway (Figure G). Therefore, we profiled all five components of the ETC and found ferrostatin-1 prevented the downregulation of nearly all subunits of complex I, II, III, and V (Figure H). Then, we probed RV metabolism using metabolomics/lipidomics profiling of RV specimens. The global RV metabolomics/lipidomic signature was clearly changed when PAB-Placebo animals were compared to control. However, PAB-Fer-1 animals were an intermediate between control and PAB-Placebo (Figure I). Random forest classification identified the 15 metabolites/lipids that were divergent comparing the three groups, and changes in phophatidylethanolamines and metabolites of fatty acid metabolism [diacylglycerides and acylcarnitines] were among the most important variables that differentiated the three groups (Figure J). Hierarchical cluster analysis of phosphatidylethanolamines, the lipid species that can promote ferroptosis(4), demonstrated ferroptosis inhibition blunted the increase of a subpopulation of phosphatidylethanolamines. Moreover, ferrostatin-1 likely modulated fatty acid metabolism as the reduction in triacylglycerides, diacylglycerides, and acylcarnitines in PAB-Placebo RVs was partially mitigated in PAB-Fer-1 RVs (Figure K).

In conclusion, our translational porcine study demonstrates ferroptosis inhibition improves mitochondrial cristae structure and combats ETC protein downregulation. Our metabolomics/lipidomics data suggest ferrostatin-1 counteracts disrupted fatty acid metabolism. These molecular changes are paired with significant improvements in right heart structure and function. Thus, our data provide translational evidence that

ferroptosis inhibition may be an effective therapeutic strategy for RHF, a deadly and currently untreatable form of heart failure.

Figure: Ferrostatin-1 Treatment Improves Right Ventricular Function and Mitochondrial Metabolism in Pulmonary Artery Banded Pigs. (A) Representative four-chamber cardiac magnetic resonance images in end-systole and end-diastole of control PAB-Placebo, and PAB-Fer-1 animals. (B) Blinded quantification of right ventricular ejection fractions, *p*-values determined by one-way *ANOVA* with Tukey's multiple comparison test (C). Representative electron micrographs depicting mitochondrial cristae structure. (D) Blinded quantification of cristae structure, *p*-values as determined by Kruskal-Wallis test with Dunn's multiple comparisons test. (E) Hiearchical cluster analysis of mitochondrial enrichments. (F) Partial least squares discriminate analysis of mitochondrial proteomics. (G) KEGG pathway analysis of the top 250 proteins that differentiated the three groups. (H) Hierarchical cluster analysis of ETC protein subunit abundances of complexes I-V as determined by proteomics analysis. (I) Hierarchical cluster analysis of metabolites/lipids in RV specimens. (J) Random forest classification of metabolites/lipids that differentiated the three experimental groups. (K) Hierarchical cluster analysis of phosphatidylethanolamines (PE), triacylglycerides (TG), diacylglycerides (DG), and acylcarnitines. Ferroptosis inhibition partially prevented the accumulation of some PE and the reduction of TG, DG, and acylcarntines in RV samples.


A
Control
PAB-Placebo
PAB-Fer-1
End Diastole
End Systole
B
RVEF (%)
0.0006
0.0087
<0.0001
59%
30%
41%
Control
PAB-Placebo
PAB-Fer-1
C
Control
PAB-Placebo
PAB-Fer-1
800 nm
D
Cristae Score
>0.9999
<0.0001
<0.0001
E
RV Mitochondrial Proteomic Analysis
F
Control
PAB-Fer-1
PAB-Placebo
Component 1 (87.3 %)
Component 2 (27 %)
G
KEGG Pathway
Oxidative phosphorylation
Diabetic cardiomyopathy
Citrate cycle (TCA cycle)
Fatty acid degradation
Cardiac muscle contraction
Non-alcoholic fatty liver disease
Chemical carcinogenesis
Thermogenesis
Val, Leu, and Ile degradation
Prion disease
Parkinson disease
Huntington disease
Fatty acid metabolism
Retrograde endocannabinoid signaling
Carbon metabolism
Glycolysis / Gluconeogenesis
Alzheimer disease
Amyotrophic lateral sclerosis
Pathways of neurodegeneration
Metabolic pathways
N. of Genes
-log10(FDR)
Fold Enrichment
H
Complex I
Complex II
Complex III
Complex IV
Complex V
SDHB
SDHA
SDHD
SDHC
SDHAF2
PAB-Placebo
Control
PAB-Fer-1
I
RV Metabolomics/Lipidomics Analysis
J
PE 40:5
C5-OH
Acetylcarnitine
PS 38:4
DG 16:1, 18:1
PA 18:1, 20:1
PE 40:4
PG 18:1, 20:0
PE 36:1
Cysteine
PA 16:0, 18:1
DG 16:1, 18:2
PC 36:4
PE 38:4
SM 36:2
MeanDecreaseAccuracy
High
Low
K
RV PE
RV TG
RV DG
RV Acylcarnitine
Control
PAB-Fer-1
PAB-Placebo

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