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Adult and neonatal models of chemogenetic heart failure caused by oxidative stress

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With preterm infants often surviving into adulthood, prematurity has been identified as a risk factor for heart failure, leading to the recognition of a new disease entity: "heart failure of prematurity" (1). Preterm infants are susceptible to oxidative stress due to increased perinatal exposure and lack of antioxidant defenses (2). Oxidative stress also causes heart failure in adults and contributes to cardiovascular disease (3). Deranged oxidant signaling is a common pathway implicated in heart failure both in adults and in the perinatal period. We here report a heart failure model that permits dynamic regulation of reactive oxygen species (ROS) both in adults and in the developing heart.

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We utilized a chemogenetic approach exploiting a yeast D-amino acid oxidase (DAAO) that generates hydrogen peroxide (H₂O₂) upon provision of D-alanine (4). We previously used AAV9 gene transfer to target DAAO expression to cardiomyocytes in vivo and showed that chronic generation of H₂O₂ causes heart failure (4-6). Here we generated a cardiomyocyte-specific transgenic mouse line (DAAO-TG^{Car}) expressing DAAO as a fusion protein with the H₂O₂ biosensor HyPer allowing for simultaneous generation (DAAO) and detection (HyPer) of H₂O₂. We validated cardiac-specific DAAO expression and confirmed production of H₂O₂ upon addition of D-alanine (Supplemental Figure 1). Adult DAAO-TG^{Car} mice developed heart failure after in vivo treatment with D-alanine. Cardiomyocytes from DAAO-TG^{Car} mice showed higher baseline H₂O₂ levels after in vivo D-alanine treatment, indicating a higher intracellular oxidized state. D-alanine feeding increased mitochondrial superoxide (O₂⁻⁻) in cardiomyocytes from adult DAAO-TG^{Car} animals, along with lower mitochondrial membrane potential, decreased respiratory capacity, and increased baseline glycolysis, providing evidence of mitochondrial dysregulation as a characteristic of cardiac dysfunction (Supplemental Figure 2 A-D).

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For the neonatal model, we generated heterozygous DAAO-TG^{Car} pups by crossing homozygous male DAAO-TG^{Car} sires with WT females so that all pups express DAAO. We induced *in utero* oxidative stress by providing D-alanine or L-alanine (control) in the mothers' drinking water from embryonic day 8.5 until birth. Neonates exposed to D-alanine *in utero* have a decrease in cardiac function compared to controls,

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accompanied by decreased cardiac wall thickness and increased end-systolic volume. Neonatal cardiomyocytes isolated following *in utero* D-alanine showed deranged redox balance, elevated H₂O₂ and mitochondrial O₂⁻⁻ Levels, along with mitochondrial dysfunction (Figure 1). D-alanine-exposed neonates showed increased cardiomyocyte apoptosis and proliferation (Figure 1 D-F), decreased cardiomyocyte size, and disrupted architecture (Supplemental Figure 3 C-F).

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Proteomic analyses of heart tissues from animals exposed to oxidative stress as adults or after *in utero* exposure revealed marked alterations in the cardiac proteome. There were 594 and 441 differentially-expressed proteins in the neonates and adults exposed to cardiac H₂O₂ compared to controls, respectively (Figure 1G, Supplemental figure 2E,F). In both adult and neonatal hearts exposed to H₂O₂, highly enriched terms related to energy metabolism, mitochondrial organization, cardiac function, cell death and oxidative stress. In neonatal hearts exposed to H₂O₂, uniquely enriched terms included proteins involved in cardiac development (TNNI1, SGCD, TNNC1), protein translation (SARS, TARS1, EARS2) and mitochondrial biogenesis (TOMM70, OPA1, TIMM13). The adult cardiac proteome was uniquely enriched for terms related to inflammation (PDIA3, STIP1, HSPD1), cardiomyocyte death (ATP2A2, HSPB6, EIF5A) and glucose metabolism (GPI, PGAM2, ENO3), demonstrating both striking similarities and marked differences in the response to oxidative stress between adult and neonatal myocardium (Supplemental figure 4).

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This study reports the development and characterization of a DAAO-TG^{Car} transgenic mouse model that permits manipulation of H_2O_2 in the heart. We induced cardiac oxidative stress in both adult and neonatal mice and demonstrated substantial impairment in cardiac function, associated with striking changes in cardiomyocyte redox balance and mitochondrial function, along with alterations in the proteome, suggesting a shared pathogenic mechanism. We found marked differences after in vivo oxidative stress across these two developmental stages. In vivo cardiac oxidative stress in adult DAAO-TG^{Car} mice recapitulated pathological findings we reported in adult rats (4-6), showing cardiac dysfunction over a

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markedly impacted developing hearts following only days of exposure, underscoring a potential predisposition for cardiac complications later in life. Oxidative stress impairs cardiac regenerative capacity (7), and this model can be utilized to examine effects of *in utero* versus postnatal oxidative stress on 75 cardiomyocyte maturation. Our neonatal model represents an informative platform to deepen our understanding of the implications of prematurity for the developing myocardium. We propose this chemogenetic approach as a comprehensive model for examining oxidative stress-induced cardiac injury across developmental stages, from *in utero* to adult exposures. Our model provides insights into the differential effects of oxidative stress on adult and neonatal hearts, establishing a foundation for

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developmental states.

References

1. Crump C, et al. Association of Preterm Birth With Long-term Risk of Heart Failure Into Adulthood. JAMA Pediatr. 2021;175(7):689-97.

understanding divergent pathophysiological pathways affected by redox stress across different

period of several weeks in response to D-alanine feeding. In contrast, in utero cardiac oxidative stress

- 2. Pena-Bautista C, et al. Non-invasive assessment of oxidative stress in preterm infants. Free Radic Biol Med. 2019;142:73-81.
 - 3. Steinhorn B, et al. Chemogenetic Approaches to Probe Redox Pathways: Implications for Cardiovascular Pharmacology and Toxicology. Annu Rev Pharmacol Toxicol. 2022;62:551-71.
- 4. Steinhorn B, et al. Chemogenetic generation of hydrogen peroxide in the heart induces severe cardiac dysfunction. Nat Commun. 2018;9(1):4044.
 - 5. Sorrentino A, et al. Reversal of heart failure in a chemogenetic model of persistent cardiac redox stress. Am J Physiol Heart Circ Physiol. 2019;317(3):H617-H26.
 - 6. Spyropoulos F, et al. Metabolomic and transcriptomic signatures of chemogenetic heart failure. Am J Physiol Heart Circ Physiol. 2022;322(3):H451-H65.
 - Puente BN, et al. The oxygen-rich postnatal environment induces cardiomyocyte cell-7. cycle arrest through DNA damage response. Cell. 2014;157(3):565-79.

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100 Figure 1. A chemogenetic/transgenic model of neonatal heart failure. (A) Breeding strategy for heterozygous DAAO-TG^{Car} pups treated in utero. E=Embryonic Day; PN=Postnatal Day. Created with BioRender.com (B) M-mode images of the left ventricle, (C) echocardiographic parameters of ejection fraction (EF), fractional shortening (FS), end-systolic (ESV), end-diastolic (EDV) volume, anterior (AWTd) and posterior (PWTd) wall thickness in neonates exposed to D-alanine (0.4M) (red squares) or L-105 alanine (0.4M) (blue circles) from E8.5 to PNO. (D) Baseline HyPer ratio, (E) HyPer and TMRM (tetramethylrhodamine methyl ester perchlorate) images of cardiomyocytes from neonates (PN0) exposed to D-alanine or L-alanine. Ouantification of (F) Hyper ratio, (G) mitoSOX and (H) TMRM fluorescence in arbitrary units (a.U.). (I) Correlation between TMRM and HyPer (r = 0.857, P = 0.0004), dashed lines represent 95% CI. (J) Quantification of TUNEL staining (apoptosis) and Ki67 (proliferation). *p <0.05, ***p < 0.001, ****p < 0.0001 by unpaired t-test. Values shown as mean \pm SEM. (K) Volcano plot showing 110 protein abundances in D- versus L-alanine exposed neonatal hearts; fold changes and P values are logtransformed. Differentially-expressed proteins (p=0.05; FDR=0.1) displayed as upregulated (red) and downregulated (blue). Proteins not significantly changed indicated in grey.