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Misoprostol Regulates Bnip3 Activity in the Heart to Prevent Hypoxia-Induced Neonatal Cardiometabolic Dysfunction

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Background:

Systemic hypoxia affects more than 60% of preterm infants and is associated with both impaired cardiac metabolism and the development many diseases of prematurity. While the mechanism of injury remains elusive, it is clear that the hypoxia-inducible death gene, Bnip3, plays a central role.

Objective:

We hypothesize that with the addition of prostaglandin signaling through Misoprostol, the cellular activity of Bnip3 can be inhibited, thereby protecting the neonatal heart from hypoxia-induced cardiometabolic dysfunction.

Methods:

Cardiometabolic dysfunction was assessed in primary neonatal cardiomyocytes that were exposed to environmental hypoxia (1% oxygen) and treated with Misoprostol. Following treatment, both mitochondrial membrane potential and free radicle production were measured via fluorescence microscopy (n=3). Concurrently, flow cytometry was used to determine cardiomyocyte viability, which was compared to control treatments [normoxia (21% oxygen) and/or drug vehicle] (n=3). To explore the underlying mechanism, a cardiac myoblast cell line (H9c2) was used in gain-of-function transfection experiments in combination with epiflorescent imaging and biochemical assays.

Results:

In primary neonatal cardiomyocytes, environmental hypoxia reduced mitochondrial membrane potential by more than 61% and caused a 47% increase in cytotoxic free radicle production ($p<0.01$). These observed changes in mitochondrial function were also accompanied by significant reductions in cardiomyocyte viability ($p<0.01$). Importantly, all three measures of myocyte function were restored to control levels with the application of Misoprostol ($p<0.01$). Consistent with the hypoxia studies, Bnip3 expression in H9c2 cells had significant deleterious effects on mitochondrial structure and membrane potential, as well as cellular viability ($p<0.01$), which were all blocked with the addition of Misoprostol ($p<0.01$). However, when a functionally important Bnip3 phosphorylation-motif was neutralized, and when PKA was inhibited, Misoprostol-induced cardiometabolic protection was completely lost ($p<0.01$).

Conclusion:

This work demonstrates that prostaglandin-induced modulation of Bnip3 is advantageous to cardiomyocyte protection and may further serve to prevent hypoxia-induced cardiometabolic dysfunction in the neonatal heart.