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DEFENDING THE NEONATAL HEART: MISOPROSTOL ATTENUATES HYPOXIA-INDUCED CARDIOMYOCYTE PROLIFERATION THROUGH BNIP3 AND PERINUCLEAR CALCIUM SIGNALLING

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Background:
Systemic hypoxia resulting from preterm birth, altered lung development, and cyanotic congenital heart disease impedes the developmental and regulatory pathways in the neonatal heart. Although the mechanisms are unknown, neonatal hypoxia drives aberrant cardiomyocyte proliferation, ultimately programing the heart to fail in early life. By promoting the expression of the Bnip3 isoform, sNip, the prostaglandin E1 analogue, misoprostol has previously been shown to be cardioprotective in the hypoxia-exposed neonatal heart.

Objective:
We hypothesize that misoprostol may also be able to prevent neonatal cardiomyocyte proliferation, and that this too may be a sNip-dependent mechanism.

Methods:
Using rat primary ventricular neonatal cardiomyocytes and a cardiac myoblast cell line (H9c2), in combination with environmental hypoxia (10% oxygen) and misoprostol drug treatments, we assessed myocyte proliferation and maturation by fluorescence microscopy, flow cytometry, western blot analysis, and RT-PCR (n=3). We further tested this using a rodent model of neonatal hypoxia, that combines 10% oxygen, with and without misoprostol from post-natal day 3-10 (n=6). Endpoints of interest included cell cycle gene expression (RT-PCR) and cardiac histology.

Results:
In cells, molecular markers and fluorescent indicators of proliferation were significantly elevated with hypoxia exposure, which was attenuated with misoprostol treatment ($p<0.01$). We further describe a role for sNip in the regulation of cardiomyocyte proliferation, where sNip-induced nuclear calcium accumulation reduced the expression of a proliferative MEF2C-myocardin-BMP10 pathway, and favored the expression of the cardiac maturation factors, BMP2 and MEF2A ($p<0.01$). These observations were supported by knockdown studies, where we restored hypoxia-induced proliferation in misoprostol-treated cells with an siRNA targeting sNip. Furthermore, in hypoxia-exposed rat pups we observed elevated heart weights, in combination with significant increases in left ventricular nuclei number, which was also attenuated with the addition of misoprostol ($p<0.05$).

Conclusion:
Together this data demonstrates a clear mechanism for hypoxia-induced neonatal cardiomyocyte proliferation which can ultimately be reversed pharmacologically through misoprostol.