

## **Ineffectiveness of mitoTEMPO on Cardiomyocyte S-phase Activity in TNNI3K-expressing Mice**

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**Background and Hypothesis:** The limited regenerative capacity of the mammalian adult myocardium is a significant roadblock for therapeutic approaches in cardiovascular disease. Cell cycle arrest following S-phase is widely considered a primary contributor to the reduced proliferative capacity of adult cardiomyocytes. Recently, expression of troponin I-interacting kinase (Tnni3k) was shown to increase cardiomyocyte S-phase activity in mice. Tnni3k was previously shown to enhance ROS formation and adverse cardiac remodeling following injury. Our primary hypothesis was that TNNI3K-induced cardiomyocyte DNA synthesis resulted from enhanced ROS signaling. To test this, cardiomyocyte S-phase activity in TNNI3K-expressing mice was compared between those treated with the ROS scavenging agent mitoTEMPO and untreated mice.

**Project Methods:** Transgenic mice expressing TNNI3K were subjected to 14 days infusion with mitoTEMPO (experimental group) or vehicle (control group). The mice were also subjected to 14 days infusion with bromodeoxyuridine (BrdU) to identify DNA synthesis during S-phase (all mice carried a cardiomyocyte-restricted nuclear-localized transgenic reporter to aid in cardiomyocyte nuclei identification). The proportion of cardiomyocytes in S-phase was determined and mean S-phase activity was compared between treatment groups. Ploidy analysis was also conducted to determine if cardiomyocytes completing S-phase progressed through karyokinesis.

**Results:** The percentage of cardiomyocytes in S-phase in the control and mitoTEMPO treated group were  $0.819\% \pm 0.163\%$  and  $0.855\% \pm 0.138\%$ , respectively (mean  $\pm$  SEM,  $p=0.873$ ). Ploidy analysis revealed no overt difference in DNA content in S-phase-positive cardiomyocyte nuclei between the groups. Hence, we have shown that there is no appreciable difference in cell cycle induction or progression in cardiomyocytes from control vs. mitoTEMPO treated mice expressing TNNI3K.

**Conclusion and Potential Impact:** These data suggest that (a) TNNI3K-induced cardiomyocyte S-phase activity is not secondary to elevated ROS activity, and (b) reduction of ROS activity does not relax the cell cycle block between S-phase and karyokinesis in adult cardiomyocytes.