6. SUMMARY AND CONCLUSION
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Several lines of evidence suggest that ANP/NPR-A/cGMP system act as an intrinsic negative regulator of cardiac hypertrophy and fibrosis. Notably, mice carrying targeted disruption of Npr1 gene exhibit blood pressure independent progressive cardiac hypertrophy and fibrosis, which eventually leads to congestive heart failure. On the other hand, over expression of Npr1 gene and/or Nppa gene in myocardial cells reduce the agonist induced fibrosis. Although these studied have shown the involvement of ANP/NPR-A signaling system in the regulation of ECM collagen remodeling, but the downstream cascade of ANP/NPR-A signaling that inhibits collagen production in diseased heart is not yet clear.

Among the various regulatory systems that impact on cardiac fibrosis, the renin-angiotensin-aldosterone system (RAAS) is deemed to play a pivotal role in regulating the deposition of extracellular matrix in the heart and causing cardiac fibrosis. ROS have been implicated in the signal transduction of ANG II. NADPH oxidase is the major source of ROS. ANG II has been shown to activate NADPH oxidase in the endothelial cells, vascular smooth muscle cells, and fibroblasts. Both ROS and NF-κB has found to be involved in the regulation of major ECM remodeling protein and, matrix metalloproteinases.

Hence, in the present study we have utilized normal and NPRA suppressed adult CF cells. ANG II, a well known fibrotic agonist was used to induce fibrosis. The involvement of ROS/ NF-κB were investigated using the normal and NPR-A suppressed CF cells

Major findings of the present study

Chapter I

- ANG II dose dependently stimulated adult cardiac fibroblast cells proliferation, and collagen production.
- ANP (10^{-8}) co-treatment dose dependently suppressed the agonist-ANG II induced cell proliferation, and collagen production in adult cardiac fibroblast cells.
Chapter II

- ANG II induced NPR-A suppressed cardiac fibroblast cells exhibited a more pronounced mRNA expression of collagen I, collagen III and collagen productional as compared with control adult cardiac fibroblast cells.
- ANG II treated NPR-A suppressed cardiac fibroblast cells showed significantly increased mRNA expression and activity of MMP-2 and MMP-9 as compared to the normal cardiac fibroblast cells.
- Oxidative stress inducer NADPH oxidase activity and gene expression of NADPH oxidase subunits NOX4 and p47phox were more significantly increased in NPR-A suppressed cardiac fibroblast cells as compared to the normal cardiac fibroblast cells.
- NPR-A suppressed cardiac fibroblast cells exhibited a more pronounced ROS generation, and NF-κB nuclear translocation as compared to the normal cardiac fibroblast cells.
- ANP treatment significantly suppressed the ANG II induced collagen production, gene expression and activity of MMP-2 & MMP-9, NADPH oxidase activity and ROS generation in cardiac fibroblast cells.

Chapter III

- Exogenous infusion of ANP $10^{-8}$ suppressed the ANG II induced MMP-2 and MMP-9 gene expression and activity (p value) in adult Wistar rat heart.
- ANG II induced ROS generation and NF-κB $(P <0.001)$ were markedly suppressed in the ANP infused rat heart.

Conclusion

The results of both in vitro and in vivo studies support the view that ANP/NPR-A signaling system critically involved in regulating cardiac ECM collagen synthesis. ANP treatment markedly suppressed the ANG II induced collagen synthesis and activity of MMP-2 and MMP-9 by suppressing ROS generation and NF-κB nuclear translocation mechanism. The schematic representation explains (Scheme-1) the anti-fibrotic mechanism of ANP/NPRA signaling system in the heart.