2. SCOPE AND AIM OF PRESENT STUDY
2. SCOPE AND AIM OF THE PRESENT STUDY

Several lines of evidence have suggested that ANP/NPR-A/cGMP system act as an intrinsic negative regulator of cardiac hypertrophy and fibrosis. Notably, mice carrying a targeted disruption of Npr1 gene exhibit blood pressure independent, progressive cardiac hypertrophy and fibrosis. On the other hand, over expression of Npr1 gene and/or ANP in myocardial cells reduce the agonist induced fibrosis. Furthermore, in vitro studies have shown that ANP/NPR-A/cGMP system inhibits the agonist induced collagen synthesis, DNA synthesis and cell proliferation in cultured neonatal cardiac fibroblast (CF) cells. Although these studied have shown the involvement of ANP/NPR-A signalling system against the ECM collagen accumulation, but the mechanism by which of ANP/NPR-A signalling cascade inhibits fibrosis is not well understood.

Genetic mouse models with disruption of both ANP and NPRA genes have provided strong support for the role of pro-inflammatory cytokines, and enhanced matrix metalloproteinases activities in the progression of fibrosis. CF cells are the predominant source of collagen and matrix remodelling protein MMPs. The direct influence of agonist and antagonist factor against CF cells can not be studied in vivo models due to the influence of mechanical stretch and other physiological factors in CF cells. Patients with cardiac hypertrophy and heart failure have increased plasma and cardiac levels of ANP and BNP. In addition, down regulation of NPR-A gene was also reported in cardiac failure. Nevertheless, Saito et al. and other have shown the exogenous infusion of ANP during cardiac failure results an improved hemodynamics and left ventricular functions. Although the exact mechanism responsible for the beneficial effects of ANP is not much clear, this may be explained in part by the large distribution volume of α-ANP. The large distribution volume would raise the possibility that the ANP receptors are not saturated in patients with CHF, even if they are down-regulated. Excessive expression and
activity of MMP-2 and MMP-9 is considered as the key mechanism in remodelling and fibrosis. However, the effect of exogenous infusion of ANP on the activation of these enzymes during ANG II infused condition is yet to be deduced.

RAAS is deemed to play a pivotal role in the development of cardiac fibrosis. *In vivo* studies have shown that inhibitors of the RAS particularly ACE inhibitors and AT1 receptor antagonist regress hypertension induced cardiac hypertrophy and fibrosis. Besides, *in vitro* experiments also have shown the dose dependent inhibition of collagen synthesis and proliferation cardiac fibroblast in ANG II induced fibroblast culture. Binding of ANG II to its receptor stimulates multiple signal transduction pathways, including mitogen-activated protein kinases (MAPK) and reactive oxygen species (ROS). Excessive ROS generation has been suggested to be involved in a variety of cardiovascular diseases, including hypertension, cardiac hypertrophy, cardiomyopathy, ischemic heart disease and inflammation in the cardiovascular system. ANG II has been shown to activate NADPH oxidase, a precursor of ROS in number of cell, namely endothelial cells, vascular smooth muscle cells, and fibroblast. Furthermore, NADPH oxidase activation and increased ROS production are implicated in ANGII -induced effects such as vascular smooth muscle hypertrophy, hypertension and cardiac and liver fibrosis. Interstitial fibrosis of the heart induced by ANG II infusion is significantly attenuated in systemic Nox2 and p47phox (components of NADPH oxidase) knockout mice as compared to wild-type mice. Moreover, it has been shown that ANG II -induced neonatal rat cardiac myocyte hypertrophy was blocked by antioxidants.

. Hence, in the present study, we have analyzed the mechanism involved in the ANP/NPRA/cGMP signalling against ANG II induced fibrosis in CF cells using normal and
NPR-A suppressed CF cells. We used an siRNA technique to suppress the NPR-A gene. siRNA is an extensively used technique to elucidate the role of individual protein in signalling pathway.

**Specific Aim I:**

i) To determine the dose dependent effect of ANP on ANG II induced adult CF cell proliferation and collagen synthesis.

ii) To analyze the effect of ANP on the gene expression pattern of collagen-I, collagen-II, MMP-2, and MMP-9 in ANG II treated adult cardiac fibroblast cells.

**Specific Aim II:**

i) Establishment of Npr1 gene-suppressed CF cells by using siRNA nucleotide against NPR-A gene.

ii) To evaluate the effect of ANP/NPR-A signalling on the various components associated with fibrosis such as MMPs, NADPH oxidase activity, ROS generation, and NF-κB activity in normal and Npr1 gene-suppressed CF cells.

**Specific Aim III:**

i) To examine the role of exogenous infusion of ANP on collagen production, mRNA expression of MMP-2 and MMP-9 in ANG II infused adult Wistar rats.

ii) To examine the ROS and NF-κB activation in ANP, ANG II alone or co-infused rat heart (in vivo).