Chapter - 5

Discussion
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Diabetes is a metabolic disorder which affects a large number of people worldwide. The presence of pathological conditions such as CAD and hypertension put diabetic patients at higher risk of heart failure. Myocardial damage that occurs irrespective of these conditions has been referred to as DCM. The process leading to the development of DCM is complex and multifactorial. Bulk of evidences obtained from experimental and clinical data have suggested a positive correlation between cardiovascular complications and oxidative stress. Efforts have been taken to alleviate cardiovascular complications by targeting oxidative stress with the help of antioxidants. However, erratic performance of the antioxidant therapy at clinical levels has failed to provide data supporting involvement of oxidative stress in development of pathological conditions. Multiple reasons have been listed for this discrepancy including the absence of knowledge about baseline oxidative stress in patients enrolled in these studies. One can hypothesize that application of antioxidant therapy at stage when oxidative stress mediated damage is still reversible, would yield favorable results. Monitoring such condition, with the use of existing biomarkers may give an idea about severity of oxidative damage. Indeed, identifying sources of ROS under particular pathological condition will provide a targeted approach and help to obtain conclusive evidence for the therapeutic use of antioxidants in diabetes.

MAOs, the enzyme responsible for oxidative deamination of amines, are emerging as important sources of ROS in the failing heart. It was demonstrated that MAO-A derived ROS play deleterious role under cardiac duress in events where the heart is subjected to hemodynamic overload or I/R injury. In order to verify whether MAO-A contributes significantly to increased ROS load under diabetic condition, we used a STZ induced diabetic rat model. We found that MAO-A activity increases in the hearts of STZ induced diabetic rats and administration of MAO -A specific inhibitor CLG prevented diabetes induced oxidative stress. We can thus predict that a reduction in MAO-A mediated H$_2$O$_2$ generation, as a direct consequence of CLG administration, lead to the observed reduction in oxidative stress. We further confirmed our theory by excluding the possibility that other H$_2$O$_2$ scavengers such as Catalase, Glutathione peroxidase and Peroxiredoxins were involved in decreasing oxidative stress, as CLG administration had no effect on their levels.

Heart being a dynamic organ has a highly aerobic mode of operation and thus relies on constant supply of oxidative energy from mitochondria. In order to satisfy this demand, heart is endowed with a large number of mitochondria as compared to other organs. Cardiac mitochondria are found to be well-organized in bundles which are regularly placed between myofilaments. Considering the reliance on mitochondrial metabolism and bioenergetics,
cardiac muscles are more prone to structural and functional disparities of mitochondria. In this regard it is important to mention that in diabetes, despite an increase in FFA oxidation, mitochondrial uncoupling reduces ATP production eventually leading to decrease in cardiac efficiency. This was further attributed to ROS mediated activation and/or elevation of mitochondrial uncoupling protein UCP3. Indeed, excessive production of mitochondrial ROS is responsible for oxidative damage to mitochondrial proteins and DNA. In the present study, diabetic rats showed increase in myocardial UCP3 protein levels, mtDNA content, mitochondrial fission and decrease in ETC complex I activity. All these observations support the existence of mitochondrial uncoupling and damage. MAO-A inhibition by CLG could successfully reverse these effects, indicating involvement of MAO-A derived ROS in mitochondrial damage.

Most of the studies have been focused on the oxidative damage caused by MAO-A derived ROS, however Nina et al. demonstrated that reactive aldehydes generated during MAO-mediated oxidative deamination is also responsible for mitochondrial dysfunction in cardiac myocytes. In the heart these reactive aldehydes are detoxified by another mitochondrial enzyme ALDH2. Moreover ALDH2 is susceptible to oxidative stress mediated inactivation and may lead to accumulation of reactive aldehydes such as those formed during lipid peroxidation process e.g. 4HNE. We observed decrease in activity of ALDH2 and increase in 4HNE levels in the heart of diabetic rats. MAO-A inhibition was also instrumental in bringing about an increase in ALDH2 activity and decrease in 4HNE levels. Drawing from our observation we can postulate that the cumulative effects of MAO-A mediated inactivation of ALDH2, particularly in the mitochondria, along with increased levels of MAO-A derived H$_2$O$_2$ could be involved in setting the stage for mitochondrial dysfunction.

Cardiac cell death strongly correlates with the events leading to diabetic cardiomyopathy. Previous study has shown that in STZ induced diabetic rats, ROS leads to cardiac cell death via mitochondria dependent apoptotic pathway. Consistent with this report we observed myocardial apoptosis in the STZ induced diabetic rats as evidenced by decreased Bcl2/Bax ratio, cytochrome c release, activation of caspase cascade and increased TUNEL positive nuclei. These observations could be reversed by MAO-A inhibition indicating that MAO-A derived ROS triggers myocardial apoptosis via the intrinsic pathway.

Oxidative stress causes myocardial apoptosis and triggers series of cardiac remodeling process. Hypertrophy and fibrosis are the hallmark of a remodeled heart which occurs as compensatory response to cardiac damage. However, when this condition persists for a longer time it can affect heart function. In our study we did not observe cardiac hypertrophy in diabetic rats. This could be attributed to hypoinsulinemia observed in STZ induced diabetic
Nevertheless myocardial fibrosis was evident in STZ induced diabetic rats and was prevented by CLG treatment. This further supports beneficial effect of MAO-A inhibition in preventing excess accumulation of extracellular matrix proteins, which may impede LV contractility.

Diastolic dysfunction is one of the earliest manifestations in progression of diabetic cardiomyopathy (Schannwell et al., 2002). -dp/dt, a cardiac diastolic index is widely used to assess diastolic function. Since LVSP was demonstrated to be a hemodynamic determinant of -dp/dt_{min}, several studies have implemented (-dp/dt_{min})/LVSP to evaluate left ventricular diastolic function more accurately (Weisfeldt et al., 1974; Slama et al., 2005; Grousset et al., 1984; Ogata et al., 2004). Thus we selected both -dp/dt_{min} and (-dp/dt_{min})/LVSP to determine whether MAO-A inhibition could restore diastolic function in diabetic rats. STZ induced diabetic rats developed diastolic dysfunction as indicated by significant increase in LVEDP and reduced (-dp/dt_{min})/LVSP. MAO-A inhibition in turn restored each of these parameters. Diabetes also affects electrical activity of the heart and ECG offers a non-invasive screen to assess these changes (Howarth et al., 2005). QTc interval, an electrocardiographic parameter, is of particular clinical significance as it is a prominent predictor of stroke and mortality in patients with diabetes (Christensen et al., 2000; Cardoso et al., 2003). In our model of type I diabetes, ECG showed QTc prolongation, an observation that is consistent with previous work (Howarth et al., 2005; Badole et al., 2014). CLG treatment significantly reduced this QTc dispersion. All these observations indicate that increased MAO-A activity contributes to development of cardiac contractile dysfunction in diabetic cardiomyopathy.

In the current study, we demonstrated that pharmacological inhibition of MAO-A restored cardiac dysfunction in an animal model of type I diabetes. This was accompanied by decreased cardiac oxidative stress, apoptosis and fibrosis. All these beneficial effects of MAO-A inhibition were observed despite persistent hyperglycemia, thus excluding the possibility of potential cardioprotective effects due to clorgyline-elicited global metabolic benefits.