

Review Article

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## Role of Monoamine Oxidase A (MAO-A) in Cardiac Aging

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### Article Info

#### Article Notes

Received: January 29, 2020

Accepted: April 21, 2020

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#### Keywords:

Monoamine Oxidase A  
Oxidative stress  
Cardiac Aging  
Cardiomyocytes  
Heart failure  
Reactive oxygen Species

### Abstract

Among different sources that contribute in the global oxidative stress, the vast majority of cellular reactive oxygen species (ROS) originate from mitochondrial compartments. Recently, monoamine oxidases (MAOs) are identified as a prominent source of ROS. Monoamine oxidases are localized in the outer membrane of mitochondria and exist as two different isoforms, MAO-A and MAO-B. MAOs are mitochondrial flavoenzymes responsible for oxidative deamination of biogenic amines and during this process,  $H_2O_2$  and aldehydes are generated as intermediate products. The role of monoamine oxidase in cardiovascular pathophysiology has only recently gained some attention as it is demonstrated that  $H_2O_2$  and aldehydes may target myocardial function and consequently cardiac function. Results obtained by different research groups showed that MAO-A plays a key role in the regulation of physiological cardiac function and in the development of acute and chronic heart diseases through two mechanisms: regulation of substrate concentration and intracellular production of ROS. In this review, we will focus on the role of MAO-A in the field of cardiac aging and related diseases.

**Abbreviations:** ROS: Reactive oxygen species; MAO: Monoamine oxidase;  $H_2O_2$ : Hydrogen peroxide; WHO: World Health Organization; TAC: Transverse aortic constriction; CLG: Clorgyline; Tyr: Tyramine; HF: Heart failure.

### Introduction

MAO-A is known as a pro-oxidative, mitochondrial membrane-bound enzyme which is widely distributed in all mammalian cell types except erythrocytes and encoded by X chromosome (Xp 11.23-11.4)<sup>1,2</sup>. This is a catalytically active flavoprotein which in the presence of molecular oxygen catalyzes oxidative deamination of biogenic and dietary amines like dopamine, nor-epinephrine, serotonin, tyramine, etc. and converts them into their corresponding aldehydes and ROS<sup>3-6</sup>. MAO-A has already been reported as a major contributor in the resolution of inflammation, cancer cell progression and metastasis<sup>7-10</sup>. It has also been reported as a signature marker of alternatively activated monocytes and macrophages<sup>8,11</sup>. Growing evidences have established the role of MAO-A in the pathogenesis of many cardiovascular disorders like myocardial injury<sup>12</sup>, heart failure<sup>13</sup>, vascular wall remodelling<sup>14</sup> and cardiac cell apoptosis. Considering the role of MAOs in the cardiac pathology we will focus on the role of MAO-A in cardiac diseases. Aging is the progressive loss of tissue and organ function with respect to time<sup>13</sup>. Growing evidences from different experiments suggest that aging process to a larger extent is related to macromolecular damage (i.e. lipids, DNA and proteins) by reactive oxygen species (ROS) mostly affecting long lived post mitotic cells such as neurons and cardiac myocytes<sup>15</sup>. The

exact mechanism of oxidative stress induced aging is still not clear but probably increased ROS levels lead to cellular senescence, a physiological mechanism, that stops cellular proliferation in response to damages that occur during replication.

Among different sources that contribute to global oxidative stress, vast majority of cellular ROS originate from (>90%) mitochondrial compartment. Recently, researchers have identified MAOs as a prominent source of ROS. Monoamine oxidase resides in the outer membrane of mitochondria. MAO-A and MAO-B these two isoforms are of great importance in the regulation of catecholamine and other biogenic amines in mammals. MAO-A appoint a FAD cofactor to catalyze oxidative deamination of various monoamines including different neurotransmitters (i.e serotonin, norepinephrine, dopamine) and some exogenous amines which are generally ingested by normal diets (tyramine) producing H<sub>2</sub>O<sub>2</sub> and relative aldehydes as by-products.

In the aging process, reactive oxygen species occupy a very important position<sup>16,17</sup>. Multiple sources such as xanthine oxidase<sup>18</sup>, NADPH oxidase<sup>19</sup> and mitochondrial respiratory chain<sup>20,21</sup> can be involved in ROS generation. In case of aging process, the increase in the intracellular ROS and the oxidative stress dependent decline of cell functions are partially related to impairment of mitochondrial respiratory chain<sup>20-22</sup>. The mitochondrial flavoenzymes monoamine oxidase A and B (MAO-A & MAO-B) play a major role in oxidative de-amination of biogenic amines<sup>23</sup>. MAOs are also a major source of H<sub>2</sub>O<sub>2</sub>. Recent research has demonstrated that H<sub>2</sub>O<sub>2</sub> generated by MAOs during substrate degradation is involved in cell proliferation<sup>24,25</sup> and apoptosis<sup>26-28</sup> -both are important events associated with aging process. The increase in the MAO activity has been associated with a detrimental structural and functional process of aging in some brain region<sup>29-31</sup>. So, an imbalance in MAO levels and activity may lead to neuropsychiatric disorders and also plays a pivotal role in the regulation of neurotransmitter. As MAOs have established themselves as a prominent source of ROS, it is believed that they may contribute to 63 various cardiovascular disorders, and also they can manipulate the aging process. According to the WHO report in 2013, a wide prevalence of cardiovascular disease is more common among persons aged > 65 years.

So, in this review, we will discuss :

1. Recently discovered roles of MAO-A in cardiac aging.
2. Role of mitochondrial ROS in cardiac aging.
3. Role of MAO-A as potential driver in cardiac aging.

### **Role of MAO-A in Cardiac Aging**

In aging process, ROS play an important role<sup>16,17</sup>. Myocyte apoptosis and reactive hypertrophy contribute

to the development of cardiac failure and heart aging, and increase in ROS production has been considered as one of the most important factors involved in these processes<sup>32-37</sup>. Furthermore, in the process of cardiac aging, impairment of cardiac metabolic and functional tolerance towards oxidative stress and decrease in some cardiac scavenger enzymes have been implicated<sup>38</sup>. Monoamine oxidase A and B are enzymes of great importance in the regulation of catecholamine and other biogenic amines in mammals. They are expressed in equivalent levels in human heart but differ significantly in rodents. MAO-A is the major isoform in rat heart and MAO-B is expressed in mouse heart<sup>39,40</sup>. MAOs appoint a FAD cofactor to catalyze oxidative deamination of several monoamines like various neurotransmitters serotonin, norepinephrine, and dopamine generating H<sub>2</sub>O<sub>2</sub> and corresponding by products. Categorically, serotonin is a substrate of MAO-A but catecholamine can be oxidised by both isoforms<sup>41</sup>.

Recent researches have demonstrated that MAO-A and MAO-B are both identified as a major source of H<sub>2</sub>O<sub>2</sub> and participate in progression of cardiac injury<sup>39</sup>. It has also been demonstrated that MAO-A expression and their ability to produce ROS increases with age<sup>42</sup>. This concept has become more evident with respect to the well documented tissue specific increase in MAO-A and MAO-B with respect to age. In aging heart, MAO-A level has been shown to increase around six fold and trigger cardiac damage<sup>42</sup>. Here, Maurel *et al*<sup>42</sup> and his co-workers have studied H<sub>2</sub>O<sub>2</sub> production in heart of young (1month), adult (3 and 6 months), and old (24 months) rats. Combined results from western blotting, semiquantitative real time PCR, Chemiluminescence assay (CL) and enzyme activity demonstrated that the age dependent increase in H<sub>2</sub>O<sub>2</sub> production by MAOs is fully related to the expression of MAO-A. So, this study also demonstrates that during aging, MAO-A is an important regulator of cellular ROS.

Umbarkar *et al* (2015)<sup>43</sup> and her colleagues investigated whether MAO-A can be a potential source of ROS and contribute to development of cardiomyopathy. For detailed examination, diabetes was induced in wistar rats by single intraperitoneal injection of streptozotocin (STZ). To investigate the role of MAO-A in the development of pathophysiological features of diabetic cardiomyopathy, hyperglycaemic and age matched control rats were treated with or without MAO-A specific inhibitor clorgyline (CLG) at 1mg/kg/day for 8 weeks. Diabetes upregulated MAO-A activity. Elevated markers of oxidative stress such as cardiac lipid peroxidation, superoxide dismutase activity uncoupling protein 3 expressions, enhanced apoptotic cell death and increased fibrosis. All these parameters were attenuated by CLG treatment indicating a positive correlation of MAO-A with all these and MAO-A -derived ROS contributes in diabetic cardiomyopathy.

So, these findings provide a skeletal structure for the most unexplored role of MAO-A in the biology i.e aging heart and associated physiological condition.

### MAO-A Activity Increases During Aging and Triggers Oxidative Stress-Mediated DNA Damage Response in Cardiac Cells

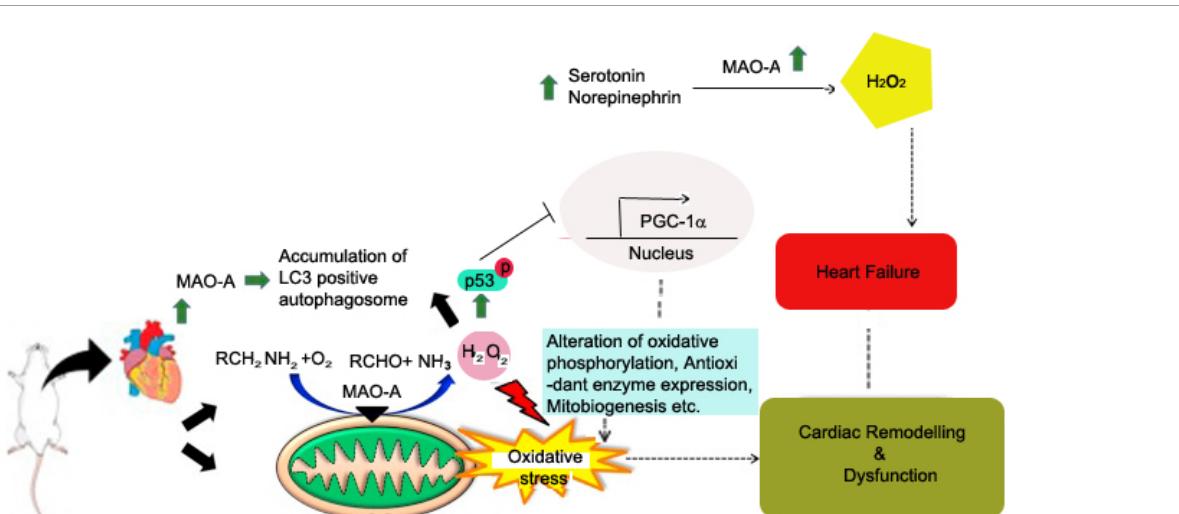
Manzella *et al.*, in 2018<sup>44</sup> analyzed the effects of aging on MAO-A expression, oxidative stress and senescent markers in adult mouse ventricular myocytes. Upregulation of MAO-A along with 4-hydroxynonenal (4-HNE), a marker of lipid peroxidation was observed in 20 months old mouse cardiomyocytes compared to 3 months old mouse cardiomyocytes. An increase in MAO-A enzymatic activity was noticed in old cardiomyocytes along with increase in ROS generation in response to MAO-A substrate tyramine (Tyr). On the other hand, classical senescent markers like p53, p21 and p15/p16 were significantly upregulated at protein level in aged cardiomyocytes. ROS generation induced by tyramine was prevented by either treatment with selective MAO-A inhibitor clorgyline, or siRNA mediated knockdown of MAO-A or treatment with antioxidant like trolox. Researchers also evaluated MAO-A induced DNA damage and the consequent activation of DNA damage response by comet assay over a 72h period of tyr stimulation. So, collectively they have demonstrated MAO-A activation in aging process results in oxidative stress and DNA damage response.

### Mechanism of Action of MAO-A in Heart

MAO-A activities are enhanced in several models of

heart failure and aging rat hearts. For investigation of the consequences of increased MAO-A activity in heart failure and aging, Villeneuve and co-workers developed *in-vitro* and *in-vivo* model of MAO-A over expression<sup>45</sup>. *In-vivo* over expression of MAO-A in young mice led to decreased level of bioamines (norepinephrine and serotonin) along with increased concentration of aldehyde metabolites generated by MAO-A catalysed amine oxidation. On the other hand, mice with cardiac-selective MAO-A over expression (Tg-MAO-A) displayed enhanced level of H<sub>2</sub>O<sub>2</sub> in heart and mitochondrial DNA. Gene expression analysis by microarray in Tg-MAO-A hearts revealed downregulation of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) pathway involved in mitochondrial biogenesis. Subsequently, Tg-MAO-A executes progressive cardiomyocyte necrosis causing premature death by heart failure at nine months of age. In *in-vitro* condition, activation of p53 by MAO-A was responsible for mitochondrial damage, PGC-1α down regulation and cardiomyocyte necrosis.

The autophagy-lysosome pathway is an important mechanism of quality control in heart for damage proteins and organelles like mitochondria but its efficiency decreases gradually during aging and heart failure<sup>46,47</sup>. A recent research by Santin *et al* (2016)<sup>48</sup> demonstrated that persistent activation of MAO-A led to the progressive accumulation of LC3 positive autophagosomes [Fig. 1], p62 ubiquitinylated proteins and damaged mitochondria. Blockage of autophagic flux was due to attenuated lysosomal acidification and biogenesis through inhibition



**Fig 1:** Model showing the role of MAO-A in cardiac pathology and heart failure.

Enhanced MAO-A activity produces more H<sub>2</sub>O<sub>2</sub> and contributes in oxidative stress and heart failure. MAO-A-generated oxidative stress triggers p53 activation leading to the downregulation of peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), a master regulator of mitochondrial biogenesis. On the other hand, MAO-A-generated oxidative stress impairs lysosome function and acidification leading to autophagic flux blockade and altered mitochondrial quality control. This figure is basically adapted and modified from following two papers:

1. Umbarkar *et al.*, 2015; Free Rad Biol and Medicine; 87: 263-273.
2. Villeneuve *et al.*, 2013; Antioxidants and Redox Signalling; 18: 5-18.

of transcription factor EB (TFEB)—a master regulator of autophagy-lysosome pathway. Interestingly, in MAO-A overexpressed mice, gene therapy with cardiomyocyte driven TFEB adenoassociated vector rescued autophagic dysfunction, cardiac remodelling and heart failure. Although, changes associated with MAO-A activation, like mitochondrial damage, p53 activation, PGC-1 $\alpha$  downregulation and autophagy blockage [Fig. 1] mimic accelerated cardiac aging detailed examination is needed to resolve this issue.

There are evidences regarding role of MAO-B in age related heart disease. It was recently shown that in a model of pressure overload, genetic deletion of MAO-B protects against oxidative stress, apoptosis and ventricular dysfunction<sup>16</sup>. The authors also demonstrated a direct link between MAO activation and ROS formation inside mitochondria and compared the accumulation of ROS in the mitochondrial and cytosolic compartment using a redox fluorescent probe and observed earlier increase in H<sub>2</sub>O<sub>2</sub> level (10 min) in mitochondria compared to cytosol (30 min) after MAO activation.

Apart from ROS, aldehydes produced during metabolism of dopamine by MAO-B were recently shown to contribute in mitochondrial dysfunction in cardiac cells. In heart tissues, aldehyde dehydrogenase 2 (ALDH<sub>2</sub>) is the most abundant isoform. *In-vitro* knockdown of ALDH<sub>2</sub> by siRNA treatment endorses dopamine induced accumulation of aldehydes through MAO-B and alteration in mitochondrial membrane potential<sup>39</sup>. *In-vivo* study proved that ALDH<sub>2</sub> deficiency in mice contributed in cardiac aging with aldehyde overload, impaired autophagic flux. So, altogether, these studies support a role for aldehydes in cardiac aging.

## Role of MAO-A in Cardiac Pathophysiology

### Increased MAO-A activity in cardiac aging and heart failure

The activity of MAO-A was measured in mouse and rat models of heart failure. Pulmonary hypertension is a known pathological situation to increase vascular pulmonary resistance resulting right ventricular resistance and ultimately right ventricular failure. Using a rat model of pulmonary hypertension, Liu *et al* (2008)<sup>49</sup> measured the protein level expression and activity of MAO-A in the right and left ventricles. In the right ventricle, MAO-A protein level expression and activity were found to be lower than in the left ventricle. These results were associated with a lower MAO-A mRNA level in the left ventricle. Petrank *et al* (2011)<sup>50</sup> identified myocardial proteins and measured the expression of MAO-A in the myocardial tissue using a proteomic analysis. Compared with sham rats, those with HF induced by a chronic aorto-caval fistula showed an up-regulation of MAO-A levels.

### Overexpression of MAO-A in cardiomyocytes leads to heart failure

A little upregulation in MAO-A activity is sufficient to trigger deleterious effects in the heart. Villeneuve *et al.* (2013)<sup>45</sup> examined the consequences of MAO-A specific over expression in cardiomyocytes, obtained by a transgenic method on cardiac morphology and function in mice. The significant reduction of fractional shortening, ventricular dilation between 3 and 7 months and pulmonary congestion in the 7-month-old transgenic mice confirmed the emergence of HF. MAO up-regulation did not induce myocardial hypertrophy but it was associated with cardiomyocyte death and dilated cardiomyopathy. On the other hand, chronic administration of clorgyline- (MAO-A inhibitor) prevented left ventricular dysfunction and dilation in transgenic mice.

### MAO-A inhibition prevents left-ventricular dysfunction in model of heart failure

The impact of null MAO-A activity on heart function was studied in a murine model expressing a dominant-negative MAO-A<sup>51</sup>. These mice had preserved left ventricular pressure and cardiac volumes, but also maintained their cardiac function after 9 weeks of transverse aortic constriction (TAC). Histological examination revealed that, the dominant negative mice submitted to TAC had less interstitial fibrosis<sup>51</sup>. Thus, genetic inhibition of MAO-A activity helped limiting the consequences of pressure overload and prevented the transition from hypertrophy to failure. On the other hand, pharmacological inhibition of MAO-A by clorgyline also prevented adverse cardiac structural effects.

### MAO-A and neurotransmitters: role of the serotonin and norepinephrine

MAO-A substrate serotonin (5-HT) is known to play a major role in the regulation of cardiac function<sup>52</sup>. Lairesz *et al.* found that the myocardial 5-HT was augmented in MAO-A KO mice subjected to ventricular pressure overload by aortic banding, compared to the wild-type mice<sup>53</sup>. 5-HT2A receptors of serotonin also increased in the ventricles of the MAO-A KO mice<sup>53</sup>. Their inhibition reduced left ventricular hypertrophy after aortic banding. According to these results, the regulation of peripheral 5-HT by MAO-A may play a role in ventricular remodelling, and particularly hypertrophy, through the 5-HT2A receptor. In addition, targeted MAO-A over-expression in mouse cardiomyocytes led to a significant cardiac serotonin depletion and to the rise of its metabolite 5-hydroxyindoleacetic acid (5-HIAA), due to the reaction catalyzed by MAO-A<sup>45</sup>. In conditions of pressure overload, norepinephrine (NE) catabolism is increased: the cardiac amount of the primary catabolic product of NE, dihydroxyphenyl glycol (DHPG), is elevated,

whilst the level of NE is significantly reduced<sup>54</sup>. Interestingly, addition of clorgyline prevents the rise of DHPG and cardiac NE depletion<sup>54</sup>. Furthermore, NE metabolism by MAO-A is associated with exacerbated oxidative stress, hypertrophy, chamber dilation and reduced systolic function<sup>54</sup>.

### Contribution of Other ROS Regulators in Cardiac Aging and Importance of MAO-A as a ROS Regulator in Cardiac Aging

The imbalance between antioxidant defense mechanism and ROS production are the main cause of oxidative stress in a physiological system. In case of cardiovascular diseases (CVD), in the blood vessel wall layers can produce ROS in pathological conditions<sup>55</sup>. For the majority of CVDs the enzymatic sources of ROS include NAD(P)H oxidase, lipoxygenase, cyclooxygenase (COX), Xanthine oxidase (XO), uncoupled nitric oxide synthase (NOS), Cytochrome P450<sup>56</sup>. Generally, NADPH Oxidase (NOX) commonly found on cellular membrane and overexpression of NOX<sub>2</sub> and NOX<sub>4</sub> is commonly associated with CVDs. A study by Kuroda *et al* (2010)<sup>57</sup> showed that NOX<sub>4</sub> knockout mice showed lower level of cardiac O<sub>2</sub><sup>-</sup> suggesting that NOX<sub>4</sub> is a potential source of superoxide in cardiac myocytes. On the other hand, NOX<sub>4</sub> overexpression worsened the cardiac function and induced apoptosis and fibrosis in mice with response to pressure overload<sup>57</sup>.

Cardiac aging and disease are associated with oxidative stress which can impair redox signals by altering essential cystein thiolets. Cardiac specific overexpression of catalase (Cat) an enzyme that detoxifies excess H<sub>2</sub>O<sub>2</sub>, is useful to protect from oxidative stress and endorses delayed cardiac aging in mice<sup>58</sup>. Catalase overexpression globally decreases thiol occupancy including numerous mitochondrial and contractile proteins. System Biology approach assigned the majority of the proteins with differentially modified thiols in Cat transgenic mice (Tg) in the pathways of cardiac aging including cellular stress response, proteostasis and apoptosis. Moreover, Cat Tg mice exhibited diminished protein glutathione adducts and decreased H<sub>2</sub>O<sub>2</sub> production from mitochondrial complex I and complex II- suggesting improved function of cardiac mitochondria. So, this research suggests that catalase may alleviate cardiac disease and aging by modulationg cystine thiol oxidation<sup>58</sup>.

Another first line of defence against mitochondrial ROS is Superoxide dismutase (SODs) which plays a role to dismutate superoxide into H<sub>2</sub>O<sub>2</sub>. Among three distinct isoforms of SODs, SOD<sub>2</sub> specifically localizes in the mitochondrial matrix<sup>59,60</sup>. Different researchers have investigated the role of SOD<sub>2</sub> in cardiac aging. Deletion of SOD<sub>2</sub> gene results in early postnatal lethality in mice<sup>61,62</sup>. SOD<sub>2</sub> deficient (SOD<sub>2</sub><sup>+/+</sup>) mice are viable but demonstrate increased susceptibility to oxidative stress, diminished mitochondrial function and enhanced sensitivity to

apoptosis<sup>63,64</sup>. On the other hand, in an atherosclerosis background [(apo E) KO], SOD<sub>2</sub> deficiency results in accelerated atherosclerosis<sup>57</sup>, and endothelial dysfunction in mice<sup>65</sup>.

Zhou *et al.*, 2012 have demonstrated that SOD<sub>2</sub> deficiency over a lifetime is enough to induce aortic stiffening, decreased aortic compliance and cause cardiac dysfunction. Aortic stiffening with aging in SOD<sup>+/+</sup> mice is associated with structural changes in the aortic wall with increased collagen content and rupture in elastin laminae. SOD<sub>2</sub> deficiency also increases collagen I and MMP2 production in aged smooth muscle cells (SMC). So, they have concluded that mitochondrial oxidative stress over a lifetime causes aortic stiffening by inducing vascular wall remodelling, SMC apoptosis<sup>66</sup>.

### Importance of MAO-A as a Relevant Source of ROS in Cardiac Aging

Recently, different research papers have revealed the importance of MAO-A as a major source of H<sub>2</sub>O<sub>2</sub> in heart which may play an important role in the onset and progression of cardiac injury<sup>67</sup>. Moreover, it is well established that MAO-A's expression and its ability to produce ROS increases with age<sup>42</sup>. It is also pronounced in age associated chronic disease like hypertension, pressure overload, and diabetes. MAO-A overactivity elicits mitochondrial damage and myocardial degeneration in rodent models of pressure overload and diabetes which can effectively be inhibited by using MAO-A inhibiting drugs<sup>43,45,51</sup>. This concept has become even more relevant now a days in support of well documented tissue specific increase in MAO-A levels with age. MAO-A level has shown to increase 6 fold in the aging heart- a phenomenon proposed to be specifically enhancing the effects exerted by factors and conditions that trigger the cardiac damage<sup>42</sup>. On the other hand, a clinical study has showed that there is a correlation between MAO-A levels and postoperative arterial fibrillation, a cardiac arrhythmia often associated with aging<sup>68</sup>.

Moreover, by using gene targeted approaches in mice like cardiomyocyte specific overexpression or deletion, researchers have demonstrated the deleterious role played by MAO-A in ventricular dysfunction during chronic ischemia<sup>69</sup>. Mechanistically, the excess of ROS generated by MAO-A lead to an accumulation of 4-hydroxynonenal (4-HNE) inside the mitochondria. 4-HNE is basically a product of lipid peroxidation and reactive aldehyde that is particularly deleterious as it is more long lived than ROS and forms adducts with proteins to modify their function and conformation. It was also demonstrated that activation of MAO-A and generation of H<sub>2</sub>O<sub>2</sub> lead to cardiolipin peroxidation and accumulation of mitochondrial 4-HNE. Moreover, 4-HNE is a main contributor of MAO-A associated ventricular dysfunction<sup>69</sup>. So, overall, we

can say that during heart failure, an increase in MAO-A substrates together with enhanced MAO-A expression leads to the accumulation of  $H_2O_2$  into the mitochondria. Next, ROS mediated peroxidation of cardiolipin enhances the production of 4-HNE which binds to VDAC (Voltage dependent anion channel) and MCU (Mitochondrial  $Ca^{+2}$  uniporter). A resulting increase in  $Ca^{+2}$  uptake leads to  $Ca^{+2}$  overload and mitochondrial dysfunction with ATP depletion and loss of mitochondrial membrane potential.

### Pathological Consequences of Exogenous Hydrogen Peroxide or Endogenous ROS on Myocardial Tissues

The synchronization of coupled oscillators plays an important role in many biological systems like heart. In case of heart disease, cardiac myocytes can exhibit abnormal electrical oscillations such as early after depolarizations (EADs) which are associated with lethal arrhythmias. Recent experiments on isolated rabbit heart in a Langerdorff perfusion system have demonstrated the role of exogenous  $H_2O_2$  at (0.2-1) mM in inducing early after depolarization and subsequent arrhythmias such as polymorphic ventricular tachycardia<sup>70</sup> confirming previously postulated role of endogenous ROS in reperfusion arrhythmias during myocardial infarction<sup>71</sup>. Among the molecular targets of ROS several non-selective cation channels of the transient receptor potential TRP family such as TRPM2 or TRPM7 may allow late calcium inflow during ischemia leading to necrosis<sup>72</sup>. Growing evidences have suggested that  $Ca^{+2}$  entry through TRP channel might play a pivotal role in cardiac function and pathology. TRP proteins are divided into six groups TRPC (Canonical), TRPV (Vanilloid), TRPM (Melastatin), TRPA (Ankyrin), TRPML (Mucolipin), TRPP (Polycystin) which are activated by different physical or chemical stimuli<sup>73</sup>. A landmark study has demonstrated convincingly that activation of neuronal TRPM7 channels by peroxynitrite happens during an *in-vitro* model of ischemia. Here, this group have shown that antiexcitotoxic therapy (AET) in anoxic neurons unmasks a lethal cation current  $I_{OGD}$  ( $OGD =$  Oxygen glucose deprivation) reported to mediate neuronal death. In OGD,  $I_{OGD}$  is activated by activated reactive oxygen/  $N_2$  species (ROS) permitting  $Ca^{+2}$  uptake that further stimulates ROS and  $I_{OGD}$  activation. Blocking OGD or suppressing TRPM7 expression prevents anoxic neuronal death even in the absence of AET, indicating that TRPM7 is an important mediator of anoxic death<sup>74</sup>.

On the other hand, TRPM7 has been evidenced in isolated ventricular cardiomyocytes of different species like rat and pig<sup>75</sup>. Apart from several TRP channels, cardiac tissue can express  $Mg^{+2}$  inhibited, non selective cation current ( $I_{MIC}$ ) that bears many characteristics of cation current channels. Researchers have used whole cell voltage clamp technique in rat and pig ventricular myocytes to characterize the blockage and permeation property of

cardiac  $I_{MIC}$  channels. They also compared  $I_{MIC}$  channels with TRP channels particularly with  $Mg^{+2}$  sensitive TRMP6 and TRMP7 channels. They observed that removing extracellular divalent cation unmasks large inward and outward monovalent current which can be inhibited by  $Mg^{+2}$ <sup>75</sup>. In an *in-vitro* model of cellular replicative senescence, higher levels of TRMP7 inward current have been recorded in human amniocytes, possibly as a result of activation of increased levels of endogenous ROS. Transient receptor potential (TRP)M7 like current density at -120mV was significantly increased in senescent amniocyte<sup>76</sup>.

### Systemic Function of MAO-A for Influencing Cardiac Function

MAO-A has been predominantly found in the brain and in noradrenergic and dopaminergic neurons. MAO-A has also been found in many peripheral tissues including cardiomyocytes. The role of MAO-A in terminating the actions of neurotransmitters/dietary amines in central and peripheral nervous system and in the extra neuronal tissue have been extensively studied and the oxidative deamination by MAO-A can influence the cardiac function both directly or indirectly. Serotonin (5-HT), released from the extra neuronal tissue and catecholamine like norepinephrine (NE) released from the intracardiac nerves, interact with their receptors and taken up through the extra neuronal monoamine transporter (EMT), present in the cardiomyocyte membrane. Once in the cytoplasm, these neurotransmitters are degraded by MAO-A and generate  $H_2O_2$  that affect cellular processes even in the physiological conditions. Limited information has been achieved till now about the products of its activity.  $H_2O_2$  is a ROS that could be toxic at high concentrations, or it could generate hydroxyl radical in the presence of  $Fe^{2+}$ . Ammonia accumulation and aldehyde intermediates are also toxic for the biological systems and a decrease in aldehyde dehydrogenase activity, due to increased oxidative stress can influence the system<sup>67</sup>. Formation of these by products of MAO-A activity in the cardiomyocytes can directly influence the heart.

Norepinephrine and serotonin exhibit a variety of biological responses, beyond their roles as neurotransmitters in the central nervous system. The increase in sympathetic nervous system (SNS) activity is typical of chronic heart failure (HF) and is characterized by norepinephrine spillover and decreased neuronal uptake<sup>77</sup>. Physiological aging is also characterized by SNS dysfunction as shown by the increase in circulating catecholamine levels in old compared to adult individuals<sup>78</sup>. Higher serotonin levels were also associated with worse HF symptoms and systolic dysfunction<sup>79,80</sup>. Therefore, the increase in norepinephrine and serotonin levels could participate in cardiovascular dysfunction and may explain the age-associated increase in cardiovascular morbidity

and mortality<sup>81</sup>. Hence the activity of MAO-A present in the neural system shows a control mechanism to balance the supply of serotonin and norepinephrine substrate to be uptaken through the transporter present in the cardiomyocyte membrane and thereby indirectly influence the cardiac function.

## Discussion and Conclusion

Aging is a complex process where multiple factors are involved. Growing evidences have demonstrated that oxidative stress, mitochondrial dysfunction contributes in many age related diseases. CVDs are the leading causes of death in elderly person world wide. As discussed in many research articles, old age is a significant risk factor for CVDs. This aging population need to develop therapeutic strategies that prevent myocardial dysfunction in the elderly, especially LVH and diastolic dysfunction. Hypertension and old age are the most common causes of LVH, which increases the risk of coronary heart disease, congestive heart failure, stroke, and sudden death. The roles of mitochondrial ROS, insulin-IGF-PI3K, catecholamine, and nutrient signaling have been widely discussed in different research reports. Further studies are needed to elucidate the complex interactions between mitochondrial ROS, SIRTs, mTOR, Ca<sup>+2</sup>, and other cellular signalling. As clinical trials in case of antioxidant application to attenuate the progression of CVDs have shown disappointing results<sup>82</sup>, these may not be the optimal therapeutic agents. However, there are several promising mitochondrial-targeted small molecule antioxidants, including mitochondrial-targeted ubiquinone (MitoQ), and SS31 peptide antioxidants<sup>83-85</sup>. Other mitochondrial targeted mechanisms, such as cyclosporine to block the opening of mitochondrial permeability-transition pore<sup>86</sup>, are also attractive treatment strategies. Further clinical trials are necessary to study the potential application of mitochondrial targeted therapeutics in the treatment or prevention of cardiac aging, hypertensive cardiomyopathy, and heart failure.

In the past few years, researchers have uncovered that MAO's activation and ROS generation can drive mitochondrial damage and age related diseases. Since the p53/PGC-1α mitochondrial dysfunction axis has been identified as a major pathway involved in postmitotic senescence, MAO-A may constitute an important factor during cardiac aging and heart failure and it could serve as a target for drugs employing cardioprotective actions. Moreover, apart from H<sub>2</sub>O<sub>2</sub> formation, MAOs are also a source of reactive aldehydes and ammonia. Stimulation of mitochondrial ALDH<sub>2</sub> activity, the enzyme responsible for aldehyde conversion into the corresponding carboxylic acids, improves mitochondrial function and reduces cardiac damage in several models of cardiac injury. Evidence available so far suggests that MAO inhibition is beneficial for treatment of cardiovascular pathologies. From a

translational point of view a major hurdle is accepting the use of MAO inhibitors in the clinic. In fact, consumption of food rich in tyramine, such as wine and cheese, has been found to cause hypertensive crises in patients treated with irreversible MAO-A inhibitors. So, the probable solution of this problem is the introduction of a new generation of reversible MAO inhibitors, which appears to prevent this adverse effect. MAO-A inhibition is protective in the setting of different cardiac stresses such as pressure overload HF, diabetic cardiomyopathy, chronic ischemia, indicating its pivotal role in deleterious ROS production and mitochondrial dysfunction<sup>67,69</sup>. In a therapeutic point of view, researchers have found that the administration of moclobemide, a MAO-A selective and reversible inhibitor, which is the active compound of moclamine drug, widely used as an antidepressant, prevented cardiac dysfunction, lung congestion and ventricular remodeling in mice with chronic cardiac ischemia<sup>87</sup>. It would be interesting to consider the possibility of repurposing this drug for heart therapy in the future. Further studies will be required to provide more clear understanding of the role of MAO-A in the molecular mechanisms linking biogenic amine metabolism and ROS generation to accelerate disease progression in aging process. A better understanding of response to oxidative stress and mitochondrial dynamics and MAO-A regulation will lead to new therapeutic approaches for the prevention of age associated cardiac diseases.

## Conflict of Interest

The authors declare that they have no conflict of interest with the contents of this article.

## Acknowledgement

The authors would like to acknowledge all the lab members for their support during the preparation of this manuscript and finally acknowledge the Department of Biotechnology, NIT Durgapur for extending facilities for research work in the related field.

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