NITRIC OXIDE: REDOX BALANCE, PROTEIN MODIFICATION AND THERAPEUTIC POTENTIAL IN CARDIOVASCULAR SYSTEM

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ABSTRACT

Heart disease is the major causes of hospitalization, morbidity and mortality worldwide. Reactive oxygen species (ROS) are proposed to contribute to the deterioration of cardiac function in patients with heart diseases. ROS are increased in the failing heart and involved in atherosclerosis, myocardial ischemia/reperfusion injury, and heart failure. Increased production of ROS directly or indirectly affect nitric oxide availability. The nitric oxide/soluble guanylatecyclase/cyclic guanosine-3',5'-monophosphate (NO/sGC/cGMP) pathway plays an important role in cardiovascular regulation by producing vasodilation, angiogenesis, inhibiting platelet aggregation and myocardial contraction, and vascular smooth muscle proliferation. However, the NO/sGC/cGMP pathway is disrupted in patients with cardiovascular disorder. Strategies are designed to make drugs that increase nitric oxide synthesis or activate NO signaling pathway and promising to show some beneficial effect. In this review, the interaction of redox balance with nitric oxide to maintain pathophysiology of cardiovascular function along with therapeutic approaches against cardiovascular diseases has been discussed.

Keywords: *Nitric oxide; cardiovascular; pharmacology; redox balance; nitrosylation*

[I] INTRODUCTION

Atmospheric air is composed of 79% nitrogen. When nitrogen is burned, it produces nitric oxide. Nitric oxide is an unstable and reactive gas especially in the presence of oxygen. It changes to nitrate and nitrite in a matter of seconds. It was also known to be present and produced by lower organisms such as bacteria and has great importance to higher organisms. However, NO was not considered as an important regulator in biological system until and unless Ferid Murad tried to analyze how vasodilation drugs act upon the cardiovascular system to achieve their pharmacological effects. In his experiments in 1977, he observed that nitroglycerin caused a release of nitric oxide, which relaxes the smooth muscle cells [1]. His work was fascinated by other scientists since gases were not known to regulate such important cellular functions. Three years after the discovery of Murad, Robert Furchgott worked on the effects of drugs on blood vessels. He found that the blood vessels dilate due to production of an unknown signal molecule which he named endothelium-derived relaxing factor (EDRF) from intact endothelium [2]. In 1979, Louis Ignarro was able to prove the effects of nitric oxide on the cardiovascular system as a vasorelaxant and NO works through a second messenger, cyclic GMP [3]. In 1983, he identified that the EDRF and NO both activated guanylate cyclase and elevated cyclic GMP. The cyclic GMP levels and the vasorelaxant effects of both EDRF and NO were blocked by methylene blue. Finally, he concluded that the effect of EDRF on vasorelaxation is through NO. Robert F. Furchgott, Louis J. Ignarro and Ferid Murad were awarded the Nobel Prize in Medicine of 1998 for their norm-breaking discoveries regarding the effects of nitric oxide on the cardiovascular system. The discovery of nitric oxide and its role on the cardiovascular system as a signalling molecule overwhelms the entire scientific community. Several applications of nitric oxide on the cardiovascular system have been developed. New drugs are being developed such as vasodilators and antiplatelet agents for the treatment of hypertension, atherosclerosis, stroke, angina pectoris, heart failure, and vascular complications of diabetes and other vascular disorders. Now nitric oxide effect is not limited to only cardiovascular system, it has widespread application on other biological system like immune, gastrointestinal, urinary and nervous system.

[II] REDOX BALANCE AND NITRIC OXIDE

Redox balance is an important physiological process and plays a crucial role in cardiomyocytes, endothelial cells, platelets and vascular smooth muscle cells. The redox balance in living cells is dominated by oxygen. Reducing condition in cytosol is essential for proper function of proteins. Thus, oxygen and reactive oxygen species are a constant threat to biological systems. Cysteine, sulfur containing non-essential amino acid under normal atmospheric conditions will oxidize completely to form a disulfide bond. Thus proteins containing cysteine are affected spontaneously by molecular oxygen or reactive oxygen species. Disulfides thus form need to reduce (unoxidized) back into their sulfhydryl forms to maintain cellular redox potential [4]. Living cells have two major pathways that deal with reduction of disulfide bonds in the cytosol: the thioredoxin and the glutaredoxin pathways. Redox balance is regulated by thioredoxin (TRX) and glutaredoxin (GRX), which protect the cells from oxidative stress. The TRX system consists of TRX, NADPH, and TRX reductase (TrxR), whereas the GRX system consists of GRX, NADPH, glutathione (GSH), and glutathione reductase (GR) [5, 6]. By maintaining of redox balance, TRX and GRX also affect metabolic and cell signaling pathways. During oxidative stress, oxidized protein thiols can form intra and intermolecular disulfides that can subsequently be reduced by Trx or Grx. Oxidized Trx is reduced by Trx-reductases (TrxR) using electrons from NADPH while oxidized Grx is reduced by reduced glutathione (GSH). The oxidized glutathione (GSSG) generated from GSH, is subsequently recycled by glutathione reductase (GR) at the expense of NADPH [Figure–1]. The GSH to GSSG ratio (GSH/GSSG) in the cell is an important marker of the redox balance and the major determinant of the cellular redox potential. In different pathological condition, this redox balance is impaired, and cardiomyocytes and endothelial cells are under oxidative stress. Oxidative stress, in general, defined as a pathological condition characterized by an imbalance between reactive oxygen species (ROS) and endogenous antioxidant [7, 8]. Oxidative stress is a characteristic feature of many pathological conditions, such as atherosclerosis, hypercholesterolemia, hypertension, diabetes, and heart failure [7, 8, 9, 10]. Within the cardiovascular system, several cellular enzyme systems are potential sources of ROS and can contribute to oxidative stress. These include NADPH oxidases (Nox), the mitochondrial respiratory chain, cyclooxygenases, lipoxygenases, "uncoupled" nitric oxide (NO) synthases, cytochrome P450 reductases, and xanthine oxidase [Figure–1] [11]. Among all those sources of reactive oxygen species, the uncoupled" nitric oxide (NO) synthase and peroxynitrate play an important role in cardiovascular system. In contrary, cellular redox balance maintained by thiol systems can also regulate the biosynthesis of nitric oxide (NO) in cardiovascular system. For example, TRX induces mitochondrial manganese superoxide dismutase (Mn-SOD) and protecting endothelial nitric oxide synthase (eNOS) degradation induced by reactive oxygen species [12].
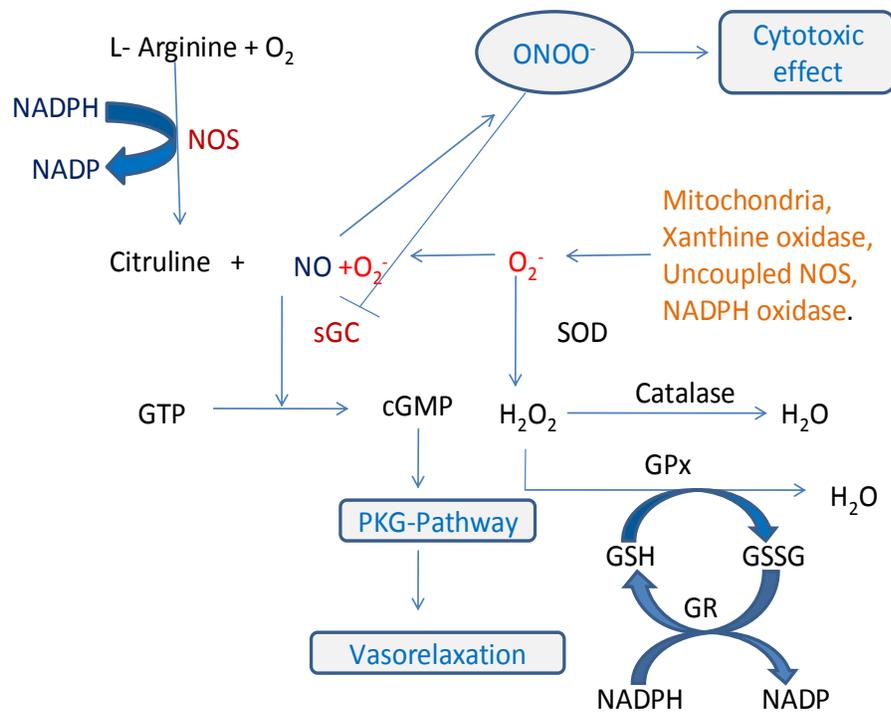

Fig: 1. Effect of oxidative stress on nitric oxide biosynthesis and vasorelaxation

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Table: 1. Different forms of NO synthase, their expression and functions

Type of NOS	Expression in the cells	Function
NOS1(nNOS)	Brain, skeletal muscle, pancreatic cells, cardiomyocytes.	Neurotransmission.
NOS2(iNOS)	Macrophages, smooth muscle cells, Myocyte, liver cells	Inflammation, Cytotoxic, septic shock
NOS3(eNOS)	Endothelium, brain, epithelial cells.	Vasorelaxation, platelet aggregations, leukocyte adhesion.

[III] ROLE OF NITRIC OXIDE IN CARDIOVASCULAR SYSTEM

NO is an important cellular signalling molecule, having a vital role in many biological processes. NO is synthesized from the amino acid L-arginine and catalysed by the enzyme NO synthases (NOS) **[Figure–1]** [13]. NOS are one of the most regulated enzymes in biology. There are three types of NOS isoforms which are encoded by three separate genes and involved in the synthesis of NO. Out of three known isoforms, two are constitutive (eNOS and nNOS) and the third one is inducible (iNOS). The different forms of NO synthase, their expression and functions have been classified in **[Table–1]**. In the cardiovascular system, NO is an important determinant of basal vascular tone, prevents platelet activation, limits leukocyte adhesion to the endothelium, and regulates myocardial contractility **[Figure–2]**. Each function of NO is discussed here.
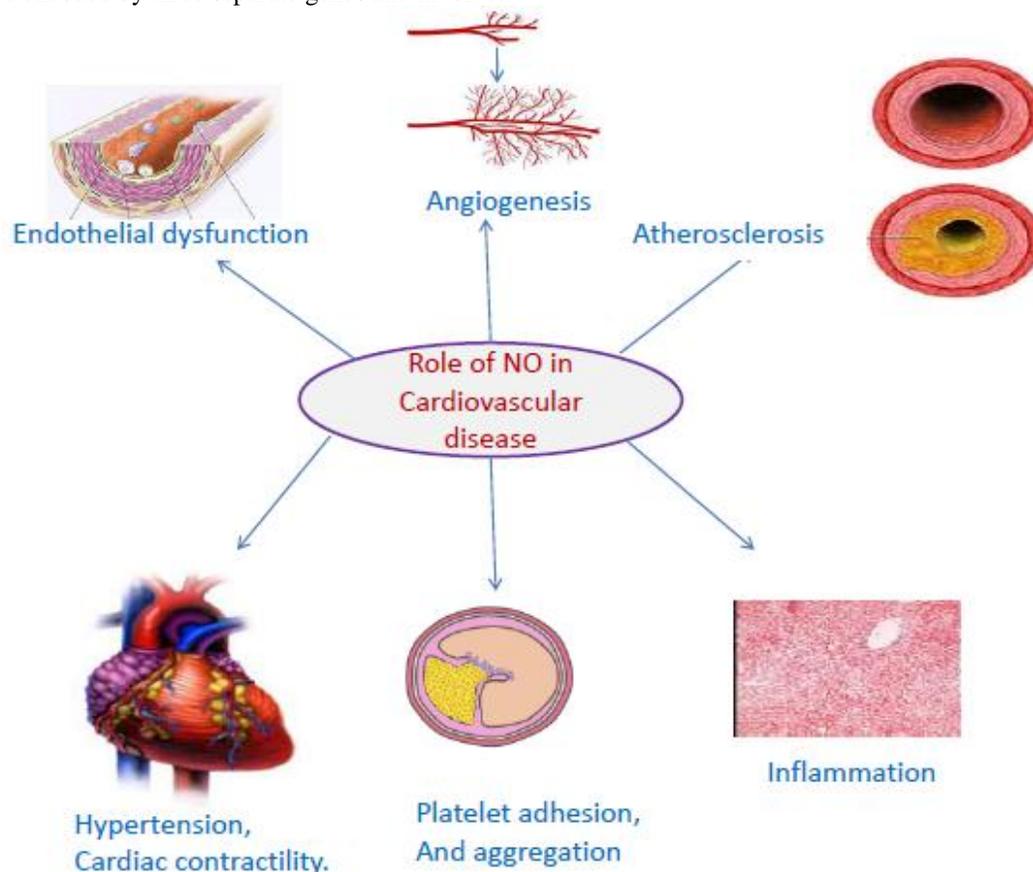

Fig: 2. Role of nitric oxide in different cardiovascular diseases

3.1. Cardiac contractility

In the heart, NO is released from the endothelium of coronary vasculature, cardiomyocytes, and nerve terminals. 20% of cardiac endothelial nitric oxide synthase (eNOS) is associated with cardiomyocytes and mainly localized to caveolae [14]. Sympathetic stimulation activates eNOS in cardiomyocytes and attenuates the inotropic and tachycardia effects. Inducible nitric oxide synthase (iNOS) can be induced by specific cytokines or stress and is found in cytosol and other subcellular compartment of cardiomyocytes [15, 16]. Neuronal nitric oxide synthase (nNOS) is also present in cardiomyocytes and mostly localized to the sarcoplasmic reticulum (SR) [15]. Contraction of cardiomyocytes is inhibited by NO-derived from nNOS through two mechanisms. First one is through the inhibition of Ca^{2+} influx via L-type Ca^{2+} channels and the second one is through stimulation of SR Ca^{2+} uptake via phospholamban phosphorylation [17].

3.2. Angiogenesis

Angiogenesis is defined as the formation of new capillaries from pre-existing blood vessels. Angiogenesis is essential to provide oxygen and nutrients to growing tissues as well as hypoxic tissues. It involves proliferation of endothelial cells and production of extracellular matrix by vascular smooth muscle cells (VSMC). NO is one of the key signalling molecules for angiogenesis. Vascular endothelial growth factor (VEGF) is another strong angiogenic factor and release NO from endothelial cells [Figure–3] [18]. The release of NO through VEGF is crucial for its ability to stimulate angiogenesis. Previously it was shown that human endothelial cells grown in a 3-D matrix of fibrin gel form capillary-like structures in response to VEGF and is blocked by the inhibition of NOS [19].
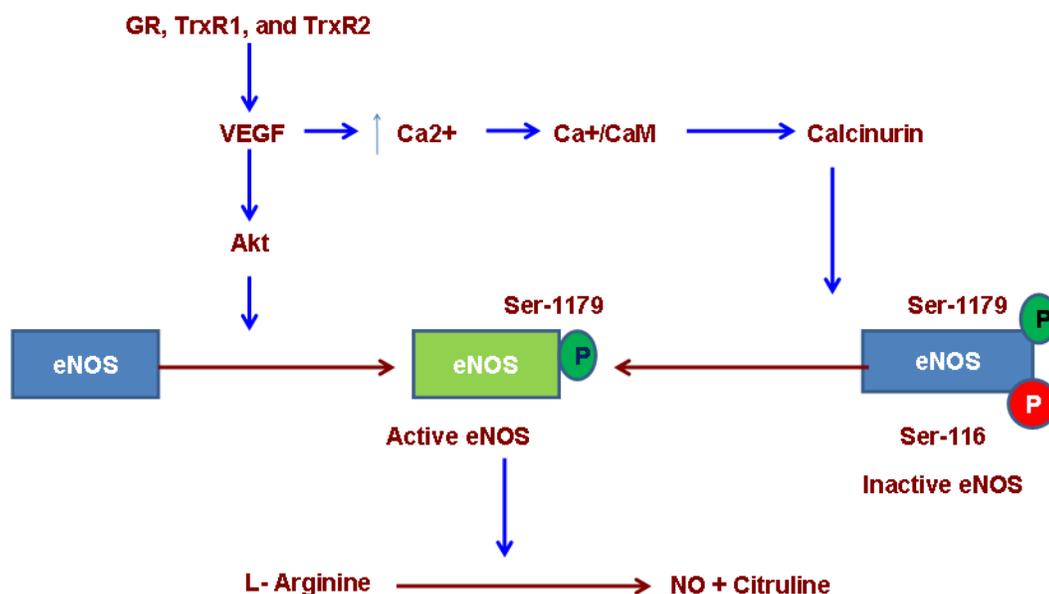

Fig: 3. VEGF mediated eNOS phosphorylation and dephosphorylation through different signalling pathways

3.3. Atherosclerosis

Atherosclerosis (also known as arteriosclerotic vascular disease) is a progressive disease of an arterial wall that thickens as the result of a build-up of fatty streaks through a process of lipoprotein deposition and cellular dysfunction. Thus atherosclerosis is the developmental process of atheromatous plaques. Nitric oxide plays a pivotal role in regulating vessel wall homeostasis. NO can have both pro- and anti-atherosclerotic effects. The anti-atherosclerotic effect of NO depends on its effect to inhibit platelet aggregation, leukocyte adhesion and extravasation, and also rely upon inhibition of LDL oxidation and prevention of smooth muscle cell proliferation. On the other hand, endogenous NO can convert to peroxynitrite in the areas of atherosclerosis due to oxidative stress [20]. The effects of peroxynitrite formation in areas of atherosclerosis reduce the availability of bioavailable NO, and increase the formation of secondary and tertiary pro-atherosclerotic oxidants. While, the

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loss of NO would abrogate its anti-platelet and anti-leukocyte actions, the pro-oxidant effects of peroxynitrite formation are for more damaging. Peroxynitrite has the capacity to induce the oxidation of LDL to develop atherosclerotic plaques [21].

3.4. Hypertension

NO is crucial to the maintenance of normal blood pressure. It is most widely studied for the functional aspect of vascular tone in clinics. Risk factors for endothelial damage may influence the bioavailability of endothelial NO and adversely affect the functional properties of the endothelium. Impairment of endothelial dysfunction is reported in essential hypertension and associated with a blunted response to NO-mediated effects. Several studies have showed the impairment of NO-mediated vasodilatation in brachial [22], coronary [23] and renal arteries [24] in patients with essential hypertension.Thus NO-donors may help to restore the endothelial function and show vasodilator effect in hypertensive patients.

3.5. Platelet Aggregation

Platelets are generally activated by contact with exposed collagen and aggregate together at the wound sites to initiate clotting and stop bleeding. However, adhesion and activation of platelets to the arterial wall also initiates an inflammatory response and cause vascular complications during thrombosis, premature heart disease, myocardial infarcts or strokes, and diabetes. To prevent this vascular complication, platelets produce and secrete chemicals that directly inhibit platelet aggregation. One of the key agents is the free radical gas nitric oxide (NO). Platelet derived NO plays an important role in attenuation of thrombosis. NO released by activated platelets markedly inhibits the recruitment of platelets into aggregates [25]. Platelet adhesion and aggregation are inhibited by both endogenous and exogenous NO, as well as cGMP analogs [26]. In mice deficient of soluble guanyl cyclase (sGC), the inhibitory effect of NO on agonist-induced platelet aggregation was totally blunted [27]. The importance of NO-cGMP-PKG pathway as potent inhibitors of platelet activation has been well established by many studies in human and animal platelets. Human platelet aggregation induced by von Willebrand factor (vWF) or low-dose thrombin was inhibited by cGMP-dependent Protein Kinase (PKG) inhibitors [28].

[IV] REGULATION OF NITRIC OXIDE MEDIATED CARDIOVASCULAR FUNCTION THROUGH PROTEIN MODIFICATION

Nitrosylation is a protein modification in which a nitrosyl group is post-translationally added to a protein. However, S-nitrosylation (RSNOs) is an important biological reaction of nitric oxide and refers to the addition of NO group to the thiol group of cysteine in the protein molecule. S-nitrosylation is a mechanism for dynamic, post-translational regulation of most

major classes of protein [29, 30]. S-nitrosylation affects the function of different proteins responsible for cardiovascular function. Proteins which affect by S-nitrosylation include soluble guanylyl cyclase (sGC), cGMP phosphodiesterase, eNOS, some ion channel proteins and several receptor proteins [31, 32, 33]. This process is reversible by the help of denitrosylases. S-nitrosoglutathione (GSNO) reductase (GSNOR) is involved in the denitrosylation process. GSNOR metabolizes GSNO to glutathione S-hydroxy sulfenamide (GSNHOH) and this further converted into oxidized glutathione (GSSG).Later glutathione reductase is involved in the reduction of GSSG into GSH by using NADPH reducing agent [Figure–4]. Physiological roles of both GSNO and protein S-nitrosylation were explored in GSNOR knockout mice. GSNOR knockout mice have markedly increased levels of SNO proteins and demonstrated the role of GSNO/GSNOR in SNO protein homeostasis. These mice exhibit increased SNO protein levels, endotoxic shock and mortality. Increased mortality was attenuated by administration of iNOS inhibitors [34]. By contrast, GSNOR knockout mice were protected from myocardial infarction due to S-nitrosylation-mediated stabilization of hypoxia-inducible factor HIF-1a and increased angiogenesis [35]. Similar to GSNOR, thioredoxin (Trx), which is present in cytoplasm and mitochondria, also act as denitrosylase [36, 37] and involved in the denitrosylation of SNO proteins. Trx system uses Trx-reductase (TrxR) and NADPH to regenerate reduced Trx following denitrosylation [Figure–4]. Recent examples demonstrated that denitrosylation by Trx/TrxR can be stimulus coupled, substrate specific and spatially restricted (compartmentalized) during cell signalling process [38].

In many cardiovascular diseases, endothelial cells are mostly affected by intracellular redox state, and oxidative stress [39, 40] which may cause endothelial dysfunction. Several studies reported that phosphorylation and glutathionylation modification of eNOS cause endothelial dysfunction.

eNOS activity is also highly regulated by lipidation, direct protein-protein interactions and O-linked glycosylation. NOS can be self-inhibited by continuous high concentrations of NO [41]. Antioxidant molecules, such as intracellular reduced glutathione critically regulate intracellular redox status and eNOS activity, and thus NO bioavailability [42].

Phosphorylations of different amino acids of eNOS affect its activity differently. While phosphorylation of eNOS at Ser-1179 activates eNOS, phosphorylation at Thr-497 or Ser-116 is link with inhibition of eNOS activity [43, 44, 45]. VEGF potentially promotes eNOS activity by increasing intracellular Ca^{2+} and activating kinase Akt to phosphorylate at Ser-1179. VEGF stimulation of eNOS also involves the dephosphorylation of Ser-116 in a different signaling pathway that involves the Ca^{2+}/calmodulin-dependent phosphatase, calcineurin [Figure–3] [45]. As phosphorylation of eNOS at Ser-116 inhibits its enzymatic activity, dephosphorylation at Ser-116 by calcineurin-dependent pathways lead to increase in eNOS activity. Altering redox

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balance in endothelial cells can affect the NOS activity and physiological response. VEGF-stimulated phosphorylation of Akt or eNOS at the stimulatory serine residue 1179 was completely blocked after TrxR1 knockdown by siRNA. However, VEGF-promoted dephosphorylation of eNOS at inhibitory residue Ser116 was largely unaffected by siRNA-mediated knockdowns, either of GR, TrxR1, or TrxR2. eNOS function is not only dependent on phosphorylation, it is also dependent on a cofactor, tetrahydro-L-biopterin (BH4). Beside produsing NO, eNOS can also become "uncoupled" to produce superoxide and H_2O_2. In endothelial cells, BH4 oxidation has been shown to be associated with the production of superoxide by eNOS [46, 47, 48, 49, 50]. It has been recently reported that

BH2 binds eNOS with an affinity equal to that of BH4 in murine endothelial cells [46]. Sugiyama et al., 2009 [51], showed that simple depletion of endothelial BH4 GTP cyclohydrolase-1 is not sufficient to promote endothelial dysfunction. However, the concentration of intracellular oxidized biopterin (BH2) and the ratio of BH4 and BH2, play important role in the redox regulation of eNOS mediated endothelial responses. They showed that siRNA-mediated knockdown of GR or TrxR1 (but not TrxR2) significantly decreased the intracellular BH4 concentration and the BH4-to-BH2 ratio in endothelial cells. These effects on biopterin redox state which is sufficient to decrease eNOS activity and the reduction of NO production.
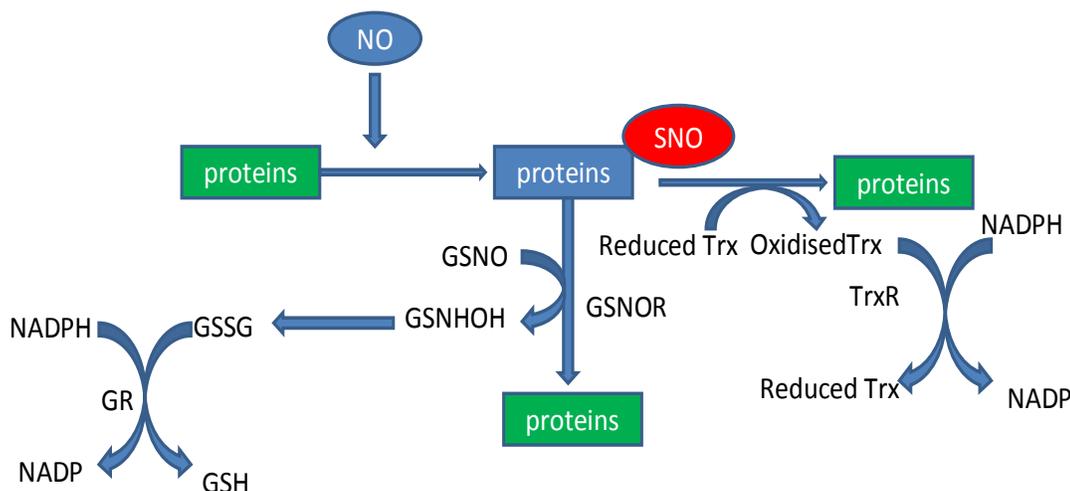

Fig: 4. S-nitrasylation and denitrosylation of proteins through redox signaling pathways.

[V] PHARMACOLOGICAL MODULATOR OF NITRIC OXIDE IN CARDIO VASCULAR SYSTEM

Several studies suggest an association of defective NO production with cardiovascular risk factors, coronary arteriosclerosis, and myocardial infarction (MI) in humans. Reduction of plasma and/or urinary NOx levels, which are markers of NO production derived from all three NOSs, has been reported in patients with cardiovascular risk factors and in those with coronary arteriosclerosis [52, 53, 54, 55]. Similarly, elevation of an endogenous NOS inhibitor, asymmetric dimethylarginine (ADMA), has also been shown in patient's plasma with cardiovascular risk factors, with arteriosclerosis, and with risk of MI [56]. Gene polymorphisms of NOS are associated with low plasma NOx levels in case of arteriosclerosis and those patients with risk of MI [57]. Oxidative stress, a risk factor for several cardiovascular diseases, interferes with the NO/sGC/cGMP signaling pathway through reduction of endogenous NO and formation of the reactive oxidant species (ROS), peroxynitrite. Increase peroxynitrite level can develop

endothelial and vascular dysfunction and causes cardio-renal and pulmonary-vascular diseases [58]. All of the above data indicate the importance of nitric oxide in pathogenesis of cardiovascular diseases. Several research works has been done to modulate the nitric oxide level by administration of different pharmacological agents and to reverse the disease progression **[Table–2]**.

Importance of NO was highly explored in NOS−/− mice.In NOS−/− mice (missing of all three NOS), the renin–angiotensin system, as measured by tissue levels of angiotensin-converting enzyme (ACE) and angiotensin II type 1 (AT1) receptor and plasma levels of renin and angiotensin II, was activated [59]. Beneficial effect was observed when angiotensin receptor blocker (ARB) was administered in NOS−/− mice. Similarly, ACE blocker, captopril protected the heart against pathological left ventricular remodelling induced by continuous light and L-NAME (NO blocker) treatment [60].

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Table: 2. List of drugs or pharmacological agents which shows cardiovascular effect through direct or indirect effect of nitric oxide pathway

Drugs/Chemical compounds	Pharmacological activity	Mechanism of action related to NO
Statin (Atorvastatin)	Preventing myocardial infarction, stroke, and sudden cardiac death	Up-regulates the vascular expression of all NOS isoforms [57].
Aspirin, Indomethacin	Inhibition of platelet activation and aggregation, and reduction of atherothrombotic risk in myocardial ischemia	Enhancing NO production in vascular smooth muscle cells [63].
Nitroglycerin	Relieves an angina attack, reduce infarct size and improve cardiac function, cardiac remodeling and mortality	Act as NO donors and thus release NO in-vivo [64, 65, 66].
Nicorandil	Reduces cardiovascular death and the occurrence of myocardial infarction in patients with stable angina pectoris.	Act as both NO donor and an ATP-sensitive K+ channel opener [69].
Cinaciguat and Ataciguat	Effective in acute decompensated heart failure (ADHF), reducing pre- and afterload and increasing cardiac output.	Direct haem-independent sGC activators [56].
Nebivolol	Reductions in heart rate and blood pressure (BP), reduction in peripheral vascular resistance and improvements in systolic and diastolic function. Reduction of heart failure.	Beta-1 adrenergic receptor antagonist causes additional vasodilatation via interaction with the endothelial nitric oxide (NO) pathway [70].
Captopril	Protection against left ventricular remodelling,	Inhibition of renin-angiotensin activation due to decrease NO bioavailability [58].
NO-donor antioxidants (containing the phenol vitamin E substructure and furoxan moiety)	Reduction of ischemia-reperfusion injury in heart.	Favour an appropriate balance between NO-donor and antioxidant properties and that these two actions are synergic [71].

A number of clinical trials have demonstrated the useful ness of statin for preventing cardiovascular events, such as myocardial infarction, stroke, and sudden cardiac death [61, 62]. Although statins believe to exert this vasculoprotective effects mainly through reduction of plasma lipid profile, several evidences suggested that they also have non-lipid-lowering actions [61, 62]. These include enhancement of NOS expression in endothelial cells [63] and VSMCs [64]. NOS knockout mice were utilised to find the effect of statins on vascular NOS expression and NOx production [59]. In the isolated aortas of the wild-type mice, atorvastatin significantly enhanced the protein expression of all three NOSs and NOx accumulation in a culture medium. A significant increase in atorvastatin-induced NOx accumulation in the culture medium was seen in isolated aortas of the doubly i/eNOS−/− (expressing nNOS only), the n/eNOS−/− (expressing iNOS only), and the n/iNOS−/− mice (expressing eNOS only), and the extent of the increase was ~25%, 25%, and 50%, respectively, as compared with the wild-type mice. However, no increase in atorvastatin-induced NOx accumulation in the culture medium was seen in the isolated aortas of the triply NOS−/− mice. This study suggest that atorvastatin up-regulates the vascular expression of all NOS isoforms, and that eNOS account for most of the atorvastatin induced NOx production. Sodium salicylate, aspirin, and indomethacin dose-dependently enhanced nitrite production in vascular smooth muscle cells (VSMCs). Increased nitrite production by aspirin-like drugs was accompanied by increased iNOS expression and protein accumulation in VSMCs. In addition to the direct inhibition of platelet function, aspirin-like drugs also contribute to the reduction of athero-thrombotic risk in myocardial ischemia via enhancing NO production [65].

Drugs which release NO in-vivo were employed in the treatment of ischemic heart disease. Sublingual administration of nitroglycerin relieves an angina attack, and intravenous administration of NO donors during MI has been shown to reduce infarct size and improve cardiac function, cardiac remodeling and mortality [66, 67, 68]. However, patients become resistant to NO when administered for long time. Some clinical studies showed no improvement of mortality rate in patients with acute MI [69, 70]. However, long-term oral treatment with nicorandil which has actions of both a NO donor and an ATP-sensitive K+ channel opener significantly reduces cardiovascular death and the occurrence of MI in patients with stable angina pectoris [71]. The development of nitrate tolerance limits clinical application of NO-releasing drug. Under oxidative stress and during increased formation of peroxynitrite, the desired therapeutic effect of NO is abrogated. To overcome these obstacles, direct haem-independent sGC activators have been developed, such as BAY 58-2667 (cinaciguat) and HMR1766 (ataciguat). Both of them have unique biochemical and pharmacological properties. The sGC activator BAY 58-2667 has

showed efficacy in acute decompensated heart failure (ADHF), reducing pre- and afterload and increasing cardiac output [58]. Nebivolol, a third-generation beta (1)-adrenergic receptor antagonist, causes additional vasodilatation via interaction with the endothelial nitric oxide (NO) pathway. This dual mechanism of action is responsible for the improved haemodynamic properties of nebivolol, which include reductions in heart rate and blood pressure (BP), reduction in peripheral vascular resistance and improvements in systolic and diastolic function. Additional haemodynamic effects include beneficial effects on pulmonary artery pressure, exercise capacity and left ventricular ejection fraction. These beneficial haemodynamic effects of nebivolol are reflected by improved clinical outcomes in patients with hypertension or heart failure [72].

Recently, novel compounds with more than one property have been developed for improved therapeutic efficacy. Di Stilo et. al., 2009 [73] conducted a study to observe the cardioprotective effect of a novel compound which has both nitric oxide (NO) donor and antioxidants properties. He looked the effect of new NO-donor antioxidants (containing the phenol vitamin E substructure and furoxan moiety) on ischemia-reperfusion injury in heart. From the results it appears that the limitation of the infarct area is favoured by an appropriate balance between NO-donor and antioxidant properties and that these two actions are synergic.

[VI] CONCLUSION

Over the past years, our understanding regarding the pathophysiology of cardiovascular diseases has emphasizes the key role of NO during disease evolution. Understanding the NO biology may ultimately form the basis for future therapeutic intervention. An inverse correlation between bioavailability of NO and reduced risk of cardiovascular disease has been reported by several scientific literatures. Pharmacologic and physiologic modulation of the NO pathway with various interventions will help to attenuate several cardiovascular disease processes. However, further research should be carried out to identify specific compounds with nitric oxide donors or activator of NO signaling pathways with proper doses and duration for most of its biological effects in cardiovascular system.

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