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Review

The Role of Nitric Oxide Related Therapeutics in the Treatment of Cardiovascular Pathologies

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ABSTRACT

The heart is a bio pump responsible for the provision of oxygen and nutrients to all body cells. It plays a vital role in the survival of the organism. The essential function of the heart is performed through the close interaction of the cardiac myocytes with the endocardial and capillary endothelial cells. The endothelium releases nitric oxide (NO) and modulates several physiological and pathological processes. The cardiac myocyte is also capable of NO production. Although the effect of NO on cardiac responses has been examined, data regarding the effect of NO on the heart remain controversial. The discrepancies in studies can be explained by several factors, such as different animal species, preparation, redox status, and NO concentration. However, an increasing interest in the role of NO has generated significant progress in the investigation of NO-based therapies. In this review, first, the general properties of NO are described as well as the physiological functions and pathological role in the heart. Then, an evaluation is made of several important NO-related treatment options, such as NO donors, NO synthase inhibitors, phosphodiesterase inhibitors, and soluble guanylate cyclase stimulators/activators. A summary is also given of NO-based drug candidates planned for use in clinical trials in the near future.

Keywords: Heart, nitric oxide, multiple roles, mechanism, therapeutic implication

INTRODUCTION

Nitric oxide (NO), which is both a signal molecule and an effector, plays an important role in the physiological and pathological processes of organisms. The chemical structure of NO was first discovered by an English chemist, Joseph Priestley, in 1772, and as a colorless and odorless natural gas, it is found in the upper layers of the atmosphere, vehicle exhausts, and acid rain (1). Its bioactivity has been recently more fully understood. Although the first organic nitrate, glyceryl trinitrate (GTN), was originally synthesized by an Italian chemist, Ascanio Sobrero, in 1847, it was first used as a vasodilator in 1928 (2). In 1979, nitroprusside was shown to act via NO in bovine coronary artery, and it was demonstrated that NO activates guanylate cyclase, resulting in vascular relaxation through cyclic guanosine monophosphate (cGMP) production (3). However, the in vivo relaxant effect of acetylcholine (ACh) on the vessels has not always been reproducible in in vitro conditions. In 1962, Jelliffe observed that the basic effect of ACh on the rabbit aorta is relaxation rather than contraction in in vitro conditions. A milestone in the study of NO was the work by Furchgott and Zawadzki in 1980 evaluating the effects of ACh on the rabbit thoracic aorta. Careful observation in that study found that the variability of ACh in responses in the vessel was dependent on the presence of the vascular endothelium (4). At that time, it was suggested that a substance was released from the vascular endothelium by ACh and was called endothelial-derived relaxing factor (EDRF). The candidate molecule was thought to be an unstable free radical. It was proposed that EDRF and NO were the same molecule in 1986 (5). Nowadays, NO is known to have an influential function in several biological processes in various organisms.

NO has also been shown to play an important role in the modulation of cardiac functions in physiological and pathological conditions. However, diverse responses to NO have been observed in different animal species or experimental models, such as cardiac myocytes/tissues isolated from atrial–ventricular/left–right regions of the heart. Furthermore, NO signaling pathways and the mechanism of NO actions have been poorly identified in cardiovascular studies from the perfused whole heart in in vivo examination of NO concentration and redox state.

GENERAL PROPERTIES OF NO

Endogenous NO is produced in response to several substances by NO synthase (NOS) from L-arginine (Figure 1). Three different isoforms of NOS are defined as endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). eNOS and nNOS proteins are defined as constitutive NOS (cNOS) because they are expressed in the cells in basal conditions. In the physiological

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Figure 1. NO synthesis via the NOS enzyme from L-arginine. NOS converts L-arginine to L-citrulline using Ca–calmodulin, oxygen, nicotinamide adenine dinucleotide phosphate, tetrahydrobiopterin, and flavin adenine dinucleotide as cofactors

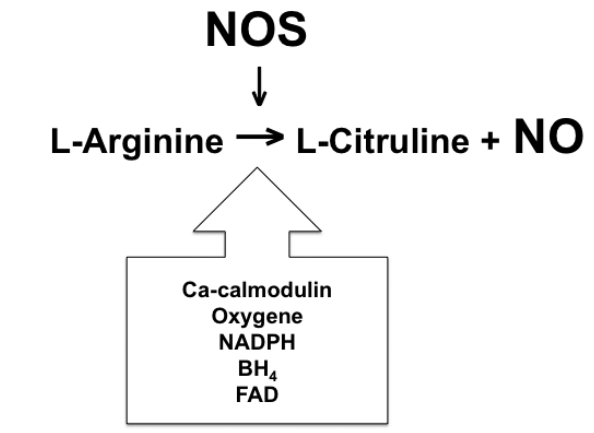
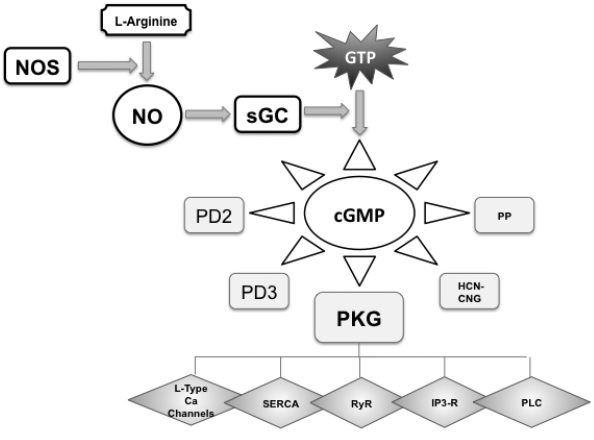


Figure 2. NO-stimulated cGMP-dependent pathway.
sGC: soluble guanylate cyclase, GTP: guanosine triphosphate, cGMP: cyclic guanosine monophosphate, PDE: phosphodiesterase enzymes, HCN-CNG: hyperpolarization-activated cyclic nucleotide-gated ion channels, PKG: protein kinase G, SERCA: sarcoplasmic reticulum Ca-ATPase, RyR: ryanodine receptor, IP3R: inositol trisphosphate receptor, PLC: phospholipase C



condition, a low level of NO is produced by the cNOSs. Several immunological agents stimulate the iNOS that generates a higher amount of NO. Basically, nNOS is found not only in the central nervous system and peripheral neurons but also in other cells. eNOS is mainly found in the endothelium, whereas iNOS is mostly found in the immune cells. Although iNOS is known to play a role in immune defense against microorganisms and tumor cells, findings have also been found that it contributes to the formation of some pathological processes, such as chronic inflammation and organ rejection (6).

In addition to NO synthesis by the NOS enzyme, NO production can also occur in the body by conversion of NO metabolite nitrite via the nitrate reductase enzyme. NO may also form spontaneously from nitrite in low pH medium (7).

Another source of NO is nitrate contained in foods. High levels of inorganic nitrate are found, especially in green leafy vegetables in daily diet. In many studies, dietary nitrate has been shown to have significant cardiovascular effects. Another foodborne group that has been proven to be related to the NO system is polyphenols that affect the reduction of nitrite to bioactive NO, thereby enhancing NO bioactivity. A low alcohol intake leads to NOS activation and increased expression of eNOS. Conversely, the chronic use of higher amounts of alcohol disrupts endothelial function and leads to a decrease in NO bioavailability (8).

Nitric oxide is metabolized to nitrite and nitrate within seconds after production. Its biological half-life may vary from approximately 6 to 50 s. Most of nitrate in the blood is excreted in the urine, but a certain amount of nitrate is converted to nitrite by oral bacteria. Nitrate in the gut is reduced to ammonia that is excreted in the urine after absorption and conversion to urea. However, in cases of oxidative stress (OS), high concentrations of the superoxide radical ($O_2^{\cdot-}$) interact with NO, and peroxynitrite anion ($ONOO^-$) is synthesized. This reaction also diminishes NO from the environment, and this NO-reducing pathway is called nitrosative stress (NS) (9).

Mechanisms of NO Action

In the target cell, NO activates soluble guanylate cyclase (sGC) and then increases the cGMP level. The main target of cGMP in the cells is protein kinase G (PKG), which regulates the activities of various proteins (Figure 2). Another important intracellular target of cGMP is phosphodiesterase (PDE) enzymes that metabolize cyclic adenosine monophosphate (cAMP) and cGMP. While cGMP activates PDE2, it inhibits PDE3, thus affecting intracellular cAMP levels. This mechanism constitutes the "cross-talk" pathway of cGMP with cAMP. In addition, cGMP can activate cyclic nucleotide-gated ion channels in the cell (10).

For many years, the effect of NO has been explained by mechanisms dependent on cGMP. However, it later became clear that there are also cGMP-independent ways. It has been demonstrated that NO primarily causes three basic chemical reactions: nitrosylation, nitrosation, and nitration (Figure 3) (11).

Nitrosylation is the binding of NO to the metal core of the functional proteins (metal nitrosylation). NO can regulate some enzyme activities by reacting with transition metals, such as iron, copper, and zinc, in the prosthetic group of proteins in the cell. The attachment of NO to heme is particularly important for the activation of the sGC enzyme. The activity of sGC is influenced by the cell's redox status. Oxidation of the heme group may render the enzyme insensitive to NO (10). NO-induced inhibition of cytochrome C enzyme is also the result of interaction with the copper core of the enzyme (11).

The nitrosation and nitration reactions of NO occur in an OS that appears in elevation of reactive oxygen species (ROS). The reaction of NO with $O_2^{\cdot-}$ gives rise to highly reactive nitrogen species, producing nitrosation and nitration of target molecules (12).

Figure 3. Brief summary of the NO-induced chemical reactions in the target cells. Interaction of NO with superoxide radical is concentration dependent. O2−•: superoxide radical

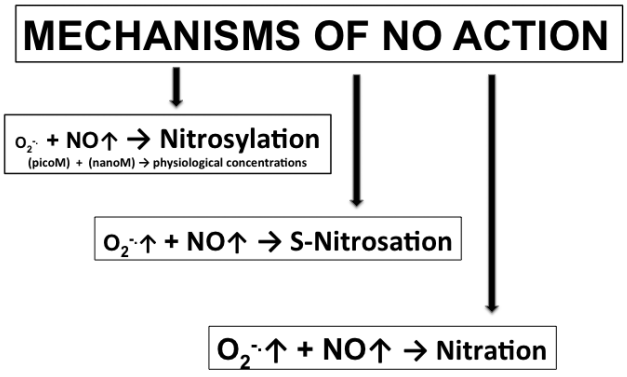
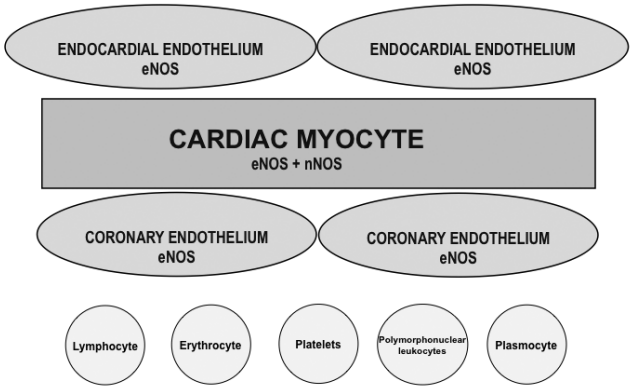


Figure 4. Close anatomic neighborhood between cardiomyocytes and endothelial cells



Nitrosation is the interaction of NO with the free thiol groups of protein cysteine residues and causes the formation of S-nitrosothiols. This mechanism is also called S-nitrosylation, and its roles have been shown with >1000 proposed targets in the heart. The inhibition of sGC by S-nitrosation may also occur, and intracellular cGMP levels can be reduced (13).

The binding of a nitroso group to the aromatic carbon of the target proteins is known as nitration. Nitration of the tyrosine residues of proteins is important for cell functions. In the case of an excessive increase of NO and $O_2^{\cdot-}$, different reactions and nitroso radicals may occur depending on the amount of oxygen and carbon dioxide and the pH in the tissue. The dominance of these three NO-induced signal transduction mechanisms depends on the redox state of the medium. In the physiological condition, NO concentration is around nanomolar level, and its increase leads to metal nitrosylation at low superoxide radical concentration (around picomolar), whereas NO formation at high $O_2^{\cdot-}$ levels causes nitrosation and nitration reactions in pathological conditions (14).

NO ACTIONS ON CARDIAC FUNCTIONS

The heart contains different cells that have different functions. While the contractile cardiomyocytes are responsible for the pumping function of the heart, pacemaker cells produce spontaneous electrical activity. The endothelial cells on the endocardium and capillary bed regulate the functions of the cardiomyocytes, and their interaction with the cells in the blood circulation is important for the critical functions of the heart (Figure 4).

The effects of the endothelium on cardiac function were first demonstrated by Brutsaert et al. (15). Both endocardial and capillary endothelial cells were shown to be directly affected by the contractile performance of the underlying cardiomyocytes.

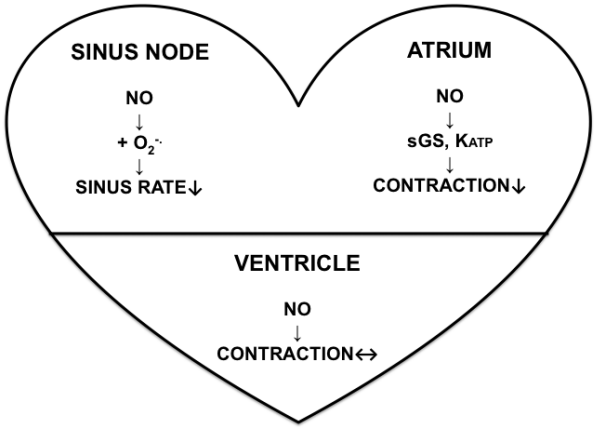
Nitric oxide is produced from the cardiac endothelial cells as well as the cardiac myocytes in the heart and regulates heart functions. While the endocardial and capillary endothelium express eNOS, both eNOS and nNOS are expressed in the cardiac myocytes. The eNOS is located in the caveolae and T-tubes, whereas the nNOS is located in the sarcoplasmic reticulum. The iNOS can be expressed in pathological conditions in both the endothelial cells and the cardiac myocytes. A high concentration of exogenous NO derived from iNOS of the immune cells may also expose the heart in pathological conditions (16).

Regulation of Cardiac Contractility by NO

Diverse responses to NO, such as positive or negative inotropy, biphasic effect, or no response, have been observed. The contribution of endogenous NO derived from the heart on cardiac contractility has been examined using selective and non-selective NOS inhibitors. The activity of eNOS inhibits beta-adrenergic inotropy, whereas the activity of nNOS may exhibit a positive inotropic effect by stimulating calcium release via the ryanodine receptor (RyR). It has been hypothesized that the negative effect of eNOS on inotropy can be compensated by nNOS. However, nNOS-derived NO may cause relaxation of the cardiomyocytes via the activation of the sarcoplasmic reticulum Ca-ATPase via the serine/threonine protein phosphatase (17).

It has also been shown that contractions of the heart change in response to the NO donors in a different manner. The NO donor, 3-morpholino-sydnonimine (SIN-1), caused a negative inotropic effect in the rat left ventricular papillary muscle (18), whereas it showed a positive inotropic activity in the cat papillary muscle. Sodium nitroprusside (SNP) caused a positive inotropic effect in the cat papillary muscle while causing a decrease in the contraction of the guinea pig heart myocytes. In some studies, it has been observed that NO and cGMP increase myocardial contractility at low concentrations, whereas a negative inotropic effect occurs at high concentrations. The amount of active NO in the target tissue can be different with different NO donors. It has been observed that organic nitrates induce a moderate increase in cGMP in the rat cardiomyocytes, leading to protein kinase A (PKA) activation and increased contraction, whereas spontaneous NO donors trigger a high level of cGMP increase, leading to PKG activation and decreased contractile response (19).

Figure 5. Region-dependent differentiation of NO action in the heart. Different mechanisms are responsible in the effects of NO on the atrium, ventricle, and sinoatrial node



It has been shown that there are histological, functional, and molecular differences between atrial and ventricular or left and right sides of the heart (20). It has been observed that NO donor diethylamine (DEA) decreases contractions of the right atrium, but there is no effect on the papillary muscles (Figure 5) (20). In addition, the PDE activities of the heart vary according to species and heart region. Thus, the PDE isoforms of the host cell can determine whether the cellular effect of NO is on the increasing or decreasing side of the cAMP (21).

The presence of pathology in the heart disrupts the association of eNOS with caveolin and nNOS with RyR, causing deviations in physiological responses. In addition to all these factors, ROS in the cardiac myocytes can reduce the effective concentration of NO in the pathological condition (9).

As can be understood from all these results, the effects of NO on cardiac contraction can be seen as both negative and positive inotropy depending on the animal species, heart preparation, and cellular situation. In addition, NO has a concentration and OS-dependent increasing or decreasing effect on heart contractions.

The negative inotropic effect of NO is explained by an increased level of cGMP. An increased cGMP activates PKG and reduces cAMP concentration by stimulating PDE2. The positive inotropic effect of NO has been explained by different mechanisms. NO-elevated cGMP inhibits the PDE3 enzyme, leading to increased intracellular cAMP concentration and hence PKA activation (22).

Regulation of Heart Rate by NO

The intrinsic activity of the sinoatrial node generates the heart rate that is regulated by the autonomic nerves and hormones in the body. The negative and positive chronotropic effects have been described using different exogenous NO donors. In the guinea pig heart, a positive chronotropic effect of NO was recorded (23). On the other hand, the intracoronary infusion of nitroprusside has been observed to cause a decrease in the rate of human heartbeat. In a study of nine heart transplant recipients, bradycardia was observed with the NOS inhibitor, whereas the NO donor SNP caused dose-dependent tachycardia (24). It has been reported that NO donor SIN-1 induces a negative chronotropic effect on the rat right atrium, and it was not reversed by sGC inhibition. Redox modulation and PDE activity have been shown to be responsible for NO action on the sinus rate (20). The regulation of NO action on heart rate by PDE3 activity has only been implemented in the right atrium of guinea pigs. In contrast, heart rate suppression induced by NO in the rat atrium was regulated by PDE1 and PDE4 activities (21).

In recent years, it has been suggested that periodic fluctuations of Ca^{2+} in the pacemaker cell may serve the spontaneous depolarization of the membranes. NO and $O_2^{\cdot-}$ increase the sinus velocity by facilitating Ca^{2+} release from the sarcoplasmic reticulum at moderate doses, but opposite effects are seen at higher doses (25).

Regulation of Coronary Vessel Tone by NO

Nitric oxide is known to be an important molecule in the regulation of the basal coronary vascular tone as well as the peripheral vascular network. Coronary vascular tone is thought to be regulated by NO secretion from the coronary endothelial cells in physiological conditions. It is understood that significant amounts of NO are released from the coronary circulation in the heart. It has been shown that sGC activation and cGMP increase in the vascular smooth muscle are the results of transient NO release from the endothelial cells. The shear stress-induced and flow-stimulated vasodilation also occurs via NO in the coronary vascular bed (26). Chronic inhibition of NO synthesis in the pig heart has been shown to impair endothelium-dependent functions of the coronary arteries (27). It has been observed that NOS inhibitor enhances basal coronary vascular resistance in isolated perfused heart preparations. Impaired cardiac angiogenesis and capillary development in studies conducted in eNOS knockout mice have also been shown (28).

ROLE OF NO IN CARDIAC PATHOLOGIES

Ischemic Heart Diseases

The main etiology for ischemic heart disease is coronary atherosclerosis originating from endothelial dysfunction of the coronary vascular bed. The endothelial cell releases important mediators that regulate vascular tone, platelet aggregation, thrombus formation, vascular smooth muscle proliferation, permeability, immune regulation, and angiogenesis in normal or pathological conditions. Endothelial dysfunction has been correlated with decreased synthesis/release or effect of NO. It is thought to be a major risk factor for cardiovascular diseases and is thought to be the earliest detectable symptom of atherosclerosis. It has been suggested that OS is closely related to the endothelial dysfunction in coronary artery disease (29). Drugs, such as statins, spironolactone, aspirin, and angiotensin-converting enzyme inhibitors, have been shown to improve endothelial function and reduce mortality.

Arginase is an important enzyme that catalyzes the conversion of arginine to ornithine and urea. The arginase enzyme competes directly with eNOS for arginine substrate, resulting in a reduction in NO production, and induces vascular dysfunction and deterioration. The inhibition of arginase activation may increase NO bioavailability and reduce OS and thus may alleviate hypertension, diastolic function, and atherosclerosis that originate from endothelial dysfunction (26).

Nitrite and nitrate levels, which are indicative of plasma NO level, have been found to be increased in studies performed in patients with coronary artery disease (30). In patients with coronary artery spasm, deterioration of both basal and stimulated NO release has been observed. The risk of myocardial infarction is thought to be increased in persons with an impaired NO-stimulated cGMP-dependent pathway (31). Spontaneous myocardial infarction and death were examined in all NOS isoform-knockout mice (32). In addition to the beneficial effects of NO, a huge amount of NO produced by iNOS in the post-ischemia–reperfusion period expands the infarct area and contributes to left ventricular dysfunction.

In organs exposed to short periods of ischemia, the increase in resistance to severe ischemia–reperfusion that develops later is known as "ischemic preconditioning." Preconditioning in the dog myocardium has been shown to relate to the arrhythmia-reducing effects of NO. A similar study was performed in pigs with NO donor pirsidomine. However, there is no increase in cGMP with NO donors as in the dog myocardium. In a similar study in the rat heart, neither NO donor nor endogenous NO could prevent ischemia-induced arrhythmias (33).

In ischemia–reperfusion injury, NO generated from eNOS and nNOS is generally positive, whereas NO produced from iNOS generally has a negative effect. In transgenic mouse models where nNOS is overexpressed, it has been shown that nNOS reduces the infarct area (34).

Heart Failure

Nitric oxide has not been shown to have a significant effect on the power–frequency relationship in healthy subjects and patients with moderate heart failure. However, a decrease in the amount of basal NO has been observed in the coronary circulation of patients with heart failure. It has been reported that this reduction may result from lack of production and/or release of NO. In patients with heart failure, an increased iNOS-mediated cardiac NO production attenuates positive inotropy in response to beta-adrenergic stimulation and accelerates relaxation. An increase in eNOS and iNOS expressions has been observed in the cardiac cells of patients with end-stage heart failure, whereas in another study, there was a decrease in eNOS expression and an increase in iNOS expression. In a congestive heart failure model created with transgenic mice, the myocardial iNOS protein levels were increased (35). In rat models of isoproterenol-induced heart failure, an increase in NO synthesis capacity was observed. Despite this increase, there was no positive effect on the contractile function of the failed heart.

Arrhythmias

It has been reported that a decreased activity of nNOS and eNOS enzymes contributes to the formation of atrial fibrillation. Therefore, it is thought that these enzymes may play a regulatory role in the electrical activity of the heart. In the presence of long-term atrial fibrillation or atrioventricular block, an increased atrial OS has been observed due to NOS uncoupling and mitochondrial oxidase activity (36). The production of atrial $O_2^{-\bullet}$ and $ONOO^-$ has been associated with an increased risk of developing atrial fibrillation. Plasma nitrite and nitrate concentrations and platelet cGMP levels have been found to be low in studies performed in patients with atrial fibrillation. The reduction in plasma NO levels is thought to be associated with hemostatic abnormalities in patients with atrial fibrillation. In the pig atrial fibrillation model, reduced atrial NOS activity and NO bioavailability have also been observed (37).

Cardiomyopathies

It has been reported that an iNOS-derived NO increase is correlated with cardiomyopathies and an increase susceptibility to complications, such as thromboembolism. An iNOS-induced NO contributes to the pathogenesis by reducing dilatation and reducing contraction in peripartum cardiomyopathies and inflammatory dilated cardiomyopathy. The elevated plasma levels of endogenous NOS inhibitors have been measured in patients with hypertrophic cardiomyopathy. In a mouse cardiomyopathy model generated by mutation of the dystrophin proteoglycan gene, the local tissue injury is the predominant characteristic of this disease. In these damaged areas, an increase in eNOS expression has been observed (38).

Inflammatory Heart Disease

Microorganism-induced or autoimmune activation of the immune cells causes an inflammatory reaction in the heart. In viral myocarditis models generated in mice and rats, viral infection has been shown to increase iNOS activation in the heart. The NO produced has an antiviral effect by inhibiting viral replication. On the other hand, iNOS causes myocardial damage through NS-induced nitrosation and nitration reactions. An increased expression of iNOS in the area of viral pericarditis and myocarditis has been found in a pig model. In autoimmune rat myocarditis models, NO derived from iNOS has been shown to react with $O_2^{-\bullet}$ and increased myocardial damage. iNOS accumulation in the lesion area in the experimental autoimmune myocarditis of rats has been observed (39).

NO-RELATED DRUGS IN CARDIOVASCULAR DISEASES

In the physiological condition, cardiovascular functions are regulated by NO, which is known as a key molecule for cardiac contraction and rate, vascular tone, and platelet aggregation. Although NO deficiency appears in some cardiovascular diseases, excess NO production causes NS and tissue damage in another group of cardiovascular pathologies. The pharmacological implication is the normalization of the NO system in the target tissues. This strategy does not work very well in all cardiac pathologies,

but with more selective inhibitors and local active NO applications, better therapeutic interventions could be achieved.

Gas NO and NO Donors

As a selective pulmonary vasodilator, gaseous NO may show a facilitating effect on postnatal pulmonary circulation adaptation. The direct use of NO inhalation is licensed as a drug to be used in the treatment of hypoxic respiratory insufficiency in newborns. Inhaled NO used in the treatment of pulmonary hypertension appears to be more advantageous due to its cheaper, less side effect profile, and ease of use (40).

L-Arginine appears to be a natural NO donor and is tested in clinical trials. However, the administration of L-arginine in patients with coronary artery disease with increased plasma arginine levels has not been shown to alter endothelial NO release. Intracoronary L-arginine infusion has not been determined to have any effect on vessel diameter. When L-arginine was administered to patients with organic erectile dysfunction, recovery of sexual function was observed in some patients (36). L-Arginine administration in rats with diabetes appears to be beneficial with respect to reducing impaired vascular responses and OS (41).

Although not recognized as an NO donor until the 1980s, GTN, an organic nitrate, has been used clinically for 150 years to relieve acute attacks of angina pectoris. Organic nitrates, such as GTN, pentaerythritol tetranitrate, isosorbide dinitrate, isosorbide 5-mononitrate, and nicorandil, are transformed into NO by bioactivation. Nitroglycerin is used effectively in the treatment of angina pectoris. SNP is used in hypertensive crisis because it causes systemic vasodilatation by reducing preload and afterload in the heart (42).

Molsidomine can be deacetylated in the liver to yield SIN-1 by successive enzymatic and non-enzymatic steps. This active metabolite SIN-1 has potent vasodilator and antithrombotic effects although molsidomine has only poorly vasoactive in vitro conditions. The main mechanism of these effects is spontaneous release of NO. Additionally, it has been shown that molsidomine decreases the venous return, cardiac output, ventricular work, and myocardial oxygen consumption in animal and human studies (42).

NONOate is a DEA/NO compound consisting of DEA and NO. Owing to their similar effects to endogenous NO, NONOates are often used as an NO donor in experimental models. It has been shown that DEA/NO decreases systemic and pulmonary arterial pressure in animals. DEA/NO and spermine (SPER)/NO inhibit platelet aggregation. Moreover, SPER/NO, dipropylenetriamine/NO, and diethylenetriamine/NO inhibit the proliferation of the vascular smooth muscle cells. Furthermore, NONOates appear superior to gas NO in pulmonary hypertension and asthma with regard to the long duration of action, stability in solid form, and no need for monitoring (43).

S-nitrosothiols are accepted as natural NO donor. They release NO when they are destroyed by enzymatic and non-enzymatic routes. They inhibit platelet activation and regulate vascular tone. However, the use of NONOates may be advantageous because NONOates are direct NO donor, but S-nitrosothiols release NO after a reduction reaction (44).

Conditions of OS can lead to an NO-resistant condition in which both endogenous and therapeutic NO effects are inactivated. This mechanism of NO insensitivity is claimed to be the reason for the reduction in the clinical effectiveness of NO treatments, such as administration of inhaled NO or direct NO donors (45).

Hybrid NO Donors

Hybrid NO donors are created by adding NO release moiety to existing drugs. Such drugs are of interest because they increase the effectiveness of the parent drug or reduce their undesirable effects. To date, the major drug group used for producing NO hybrids has been nonsteroidal anti-inflammatory drugs (NSAIDs), and preclinical studies of NO-NSAID hybrids are underway (45). A lesser known NO hybrid is latanoprost-NO, synthesized to reduce intraocular pressure.

While NO production occurs in a precisely regulated manner in natural cells, NO donors may provide an irregular dose to the entire tissue or vasculature. Therefore, maximum care should be taken when NO donors are considered as a treatment option (46).

PDE5 Inhibitors

Nitric oxide is secreted from both the endothelial cells lining the inner surface of the corpus cavernosum and the nitrergic autonomic nerve endings and causes relaxation of the corpus cavernosum smooth muscle. The enzymes responsible for the degradation of cGMP in the corpus cavernosum are PDE enzymes. PDE2, PDE3, and PDE5 have been detected in human corpus cavernosum, but the major PDE activity is linked to PDE5 (47).

Erectile dysfunction is associated with inadequate NO release in this system. The inhibition of PDE5 appears to be a reasonable approach in the treatment of erectile dysfunction. In clinical trials of patients with erectile dysfunction, an increase in erectile response has been achieved with the PDE5 inhibitor sildenafil. However, the apparent hypotensive effect of sildenafil limits its use. Another PDE5 inhibitor, vardenafil, has a more hypotensive effect than sildenafil, especially on first use. The hypotensive side effect of PDE5 inhibitors becomes even more pronounced and life-threatening when PDE5 inhibitors are used in combination with NO donors or alpha antagonists. The newest PDE5 inhibitor, avanafil, comes to the forefront in terms of its ability to initiate fast erection and fewer side effects (47). Sildenafil is also used as an alternative to conventional therapy for pulmonary hypertension for children and adults (40).

sGC Stimulators and Activators

Soluble guanylate cyclase stimulators work synergistically with NO on the heme group of sGC, whereas sGC activators work when the heme group is oxidized. Thus, they can also activate the enzyme even when there is no response to NO (48).

The first developed sGC stimulator is called "riociguat." It has been developed for the treatment of pulmonary hypertension and is a potent compound known for its powerful effects on pulmonary hemodynamics. It is licensed for the treatment of pulmonary hypertension and chronic thromboembolic pulmonary hypertension (48).

NOS Inhibitors

Studies on NOS inhibitors that can be used as drugs have focused on novel molecules that will not act on eNOS when inhibiting nNOS and/or iNOS. Several patented nNOS inhibitors have been examined for treatment of nNOS-induced NO overproduction in neurodegenerative diseases and post-stroke neuronal damage. NO produced by iNOS is involved in the pathogenesis of many diseases, such as sepsis, hemorrhagic shock, heart failure, stroke, rheumatoid arthritis, irritable colon, and even cancers (6). iNOS selective inhibitors are being designed, and there are many patents targeting iNOS inhibition. However, the side effect profile and low selectivity remain as the main problems.

Asymmetric dimethylarginine (ADMA) is an amino acid that is produced endogenously and emerges during protein degradation. It inhibits NO formation in the cardiovascular system in disorders, such as septic shock, which is the pathological excess of NO production by inhibiting NOS. Endogenous ADMA levels are metabolized by the dimethylarginine dimethylaminohydrolase (DDAH) enzyme, which has been proposed as a target for the regulation of NO bioavailability. There are continuing studies on the potential strategies to upgrade the NO levels and the methods to be used to activate DDAH (45).

New Horizons in NO-Based Treatments

As an alternative to NO donors or NOS inhibitors, the development of new treatment approaches, such as NO-donating nanoparticles and iNOS gene therapy, has recently attracted significant attention due to their interesting advantages NO-donating nanoparticle systems could localize high concentrations of NO in a sustained manner to the desired site alone to protect other organs from the effects of systemic toxicity (49). Local expression of the iNOS gene can be achieved by the use of different vectors for iNOS gene delivery. iNOS-based suicide gene therapy, which produces a huge amount of NO by this method, is a promising approach to cancer therapy (50). Studies are being planned on the use of similar methods in the cardiovascular system. However, it should be kept in mind that each of these systems should be predictive of possible effects and side effects that may arise when used.

CONCLUSION

It is well established that NO is an important biological messenger and triggers several physiological and pathological responses in numerous tissues. In the heart, NO modulates cardiomyocyte contraction, nodal rate, and tone of the coronary vascular bed in a physiological condition. In addition, the role of NO is critical in heart pathologies, such as ischemic heart diseases, arrhythmias, heart failures, cardiomyopathies, and inflammatory heart diseases.

However, there is much controversial data regarding the effect of NO on the heart. The most important reason for these contradictory results is that the effect of NO on the heart varies between species.

Experimental preparations alter the effect of NO on the heart. While hormonal and nervous systems are involved in the cardiac effect of NO in in vivo examinations of the heart, the NO action on the coronary vessels is blended with the myocardial effect of NO in the perfused whole heart. The direct effects of NO on the myocardial functions can be observed using isolated cardiac tissues, such as spontaneous beating right atrium, ventricular or atrial strips, and papillary muscles. Isolated cells are used to investigate NO actions on cellular and molecular functions of the cardiac myocytes.

An effective NO concentration in the target medium is dependent on the NOS activity and redox state and the NO-releasing capacity of the NO donors. Endogenous NOS activity can be altered by the expression of NOS variants, elevation of endogenous NOS inhibitors, NOS uncoupling, and OS. The effects of exogenous NO on the heart have been investigated using different NO donors, although the stability, lipophilicity, kinetics, and extent of the released NO and metabolites have been different among these agents. In addition to all these factors, different expressions/activities of proteins in the NO signal transduction pathways may also change the NO action on the heart.

It is believed that a detailed elucidation of the molecular mechanisms responsible for these differences will make a significant contribution to both understanding the role of NO in the progression of cardiovascular diseases and developing more effective drugs to act on the NO system.

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