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Review Article

Nitric Oxide: A Short-Living Molecule that Promises Longevity

Sema Genc and Figen Gurdol*

Department of Biochemistry, Istanbul Faculty of Medicine, Istanbul University, Capa, Istanbul, Turkey

***Corresponding Author**: Figen Gurdol, Professor, Department of Biochemistry, Istanbul Faculty of Medicine, Istanbul University, Capa, Istanbul, Turkey.

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Abstract

Endothelium-derived nitric oxide [NO] is the most potent molecule to maintain vascular homeostasis by means of a wide range of biological properties. Endothelial dysfunction that leads to atherosclerosis results from a decreased NO availability, which is associated with decreased activity of eNOS, decreased concentration of L-arginine, and increased degradation of NO. Recent research has indicated the importance of NO bioavailability in endothelial function as well as various other biological processes. Therefore, pharmacological approach focused on the development of specific therapies targeting the molecules in NO synthesis and breakdown to enhance bioactivity of NO. Better understanding of NO metabolism will help researchers to proceed in controlling the pathological processes that are associated with impaired NO bioavailability.

Keywords: *Endothelial Dysfunction; Nitric Oxide; Asymmetric Dimethylarginine; Arginine*

Introduction

The endothelial cells that cover the interior surface of all blood vessels build up a barrier between the blood and the surrounding tissues. They also provide a critical junction for vascular signaling. Various intrinsic and extrinsic stimuli such as shear stress, temperature, transmural pressure, neurohumoral and chemical agents can induce endothelial cells to produce a number of bioactive substances that control the cell growth, metabolism and contractility of intact vessels [1]. These bioactive mediators are nitric oxide [NO], endothelin [ET]-1, prostaglandins [PGI_2, PGF_2, PGE_2] and angiotensin II. The balance among the vasoactive substances originated from the endothelial cells is very important to preserve healthy functioning of the blood vessels [2,3].

Endothelium-derived nitric oxide [NO] is the most potent molecule to maintain vascular homeostasis by means of a wide range of biological properties. In this context, we aimed to provide insight to the latest approach in NO metabolism through the narrative analysis of recent studies. Additionally, we reviewed the potential therapies to protect vascular endothelial function.

Nitric oxide metabolism

Endogenous NO is synthesized from L-arginine by nitric oxide synthase [NOS] through a series of redox reactions. NOS catalyzes the degradation of L-arginine to NO and L-citrulline by a two-step process in the presence of oxygen, FAD, FMN, heme, tetrahydrobiopterin [BH_4] and NADPH [4]. BH_4, an essential cofactor for all isoforms of NOS, is synthesized in the body from GTP via the GTP-cyclohydrolase-1 [GTP-CH] pathway. Vitamin C, folate and some antioxidants enhance the BH_4 activity by chemical stabilization and scavenging reactive oxygen species [5]. Three isoforms of NOS are defined; neuronal NOS [nNOS or NOS1], inducible NOS [iNOS or NOS2] and endothelial NOS [eNOS or NOS3]. eNOS, also known as constitutive NOS [cNOS] is found in the coronary, capillary and endocardial endothelium, and in less quantity in cardiomyocytes [6]. Both NOS1 and NOS3 levels are mediated by the intracellular Ca^{2+} concentration, while NOS2 is regulated

by the proinflammatory cytokines. Information about mitochondrial form of NOS [mtNOS] is still controversial [7]. On the other hand, a non-enzymatic NO synthesis from nitrite has also been observed, particularly in ischemic tissues [8]. The rate of NO production by endothelial cells has been measured and found to be 0.8 pmol/min/mg endothelial cells, the mass of which is 1.5 kg in the whole body [9].

The cell membrane forms a barrier to NO diffusion. Therefore, the transport of NO into the cell can be possible by means of the aquaporin-1 channels [10]. The rate of NO metabolism depends on the environmental conditions such as pO_2, pH and free radical concentration. The half-life of NO is very short, and its fate is oxidative breakdown to nitrite and nitrate. These end-products reflect the amount of total endothelial NO synthesis.

The major determinants of NO breakdown are reactive oxygen species [ROS]. NO reacts with redox-activated cysteine thiols to form S-nitrosylated proteins, which represent an active storage pool for NO [11].

Physiological functions of Nitric Oxide

NO has a crucial role in various biological functions throughout the body. In the vascular bed, NO regulates vascular tonus, blood flow, cell proliferation, platelet aggregation, superoxide production and secretion of adhesion molecules [12]. NO-sensitive soluble guanylate cyclase [sGC] has a prominent role in the cardiovascular regulation by increasing intracellular cGMP levels [10]. The shear stress induces NO production while it inhibits ET-1 release from the endothelial cells, thus giving rise to endothelium-dependent vasodilatation [13-15]. NO inhibits the proinflammatory transcription factor, nuclear factor kappa B [NF-κB], and controls the formation of reactive oxygen species [ROS] by interacting with superoxide anion and suppressing the xanthine oxidase and NADPH oxidase [NOX] enzymes.

In the central nervous system, angiogenesis, cell immunity, neuronal survival and the blood flow as well as the behavioral and cognitive functions are also maintained by the NO activity [16,17]. Additionally, NO exerts physiological functions in the immune system, contributes to the regulation of gastrointestinal motility, and defense mechanisms against infectious disease and tumors [18].

Nitric Oxide and Endothelial Dysfunction

Endothelial dysfunction is characterized by impaired endothelium-dependent vasodilation. It is triggered by proinflammatory conditions and oxidative stress, both of which affecting the synthesis, degradation and bioavailability of NO [19]. The factors such as cold and mental stress may also alter the balance in NO metabolism. High levels of low-density lipoprotein [LDL] cholesterol, hyperglycemia, obesity, and smoking may trigger endothelial dysfunction by causing restricted NO availability through oxidative stress. In patients with cardiovascular risk factors such as obesity, hyperlipidemia, hypertension, diabetes, and coronary artery diseases, increased generation of ROS have been reported [20,21]. Increased ROS levels are closely associated with impaired NO bioavailability. Increased generation of ROS in above-mentioned conditions may diminish the activities of NO synthase and superoxide dismutase [SOD], blocks L-arginine uptake, and increases oxidized-LDL [ox-LDL] cholesterol levels [22]. In addition, oxidative stress disrupts the balance between *de novo* synthesis of BH_4 and its oxidation/degradation, thus leading to excessive depletion [23]. Low cardiac output in conditions such as heart failure or other vascular injury may also contribute to disturbed NO bioavailability and varying degrees of endothelial dysfunction [24,25]. Adhesion molecules, chemokines and cytokines released from vascular endothelial cells following injury increase the permeability of the vessel wall to ox-LDL [26,27]. Oxidized lipids trigger the secretion of various growth factors that cause the proliferation of vascular smooth muscle cells and thickening of intimal layer, thus leading to the progress of atherosclerotic lesions [28].

Arginine and ADMA in NO metabolism

Arginine transporters mediate the availability of arginine for the NO synthesis and other processes [4]. The cationic transporter -1 [CAT-1] and NOS3, coexisting in plasma membrane, deliver arginine into the cell [29]. Arginine is a substrate for both arginase and NOS. The competition between arginase and NOS also controls the NO concentration in endothelial cells. Upregulation of arginase redirects the arginine metabolism from nitric oxide [NO] synthesis to the cleavage of arginine to urea and ornithine. The amount of arginase in serum

after myocardial infarction has been found increased in association with the low arginine/ADMA ratio [30]. Arginine metabolism may be altered secondarily in some diseases such as malabsorption, renal deficiency and sepsis [31]. In these cases, the intestinal-renal pathway for the *de novo* arginine synthesis is impaired [32]. During the acute phase reaction, arginine utilization is increased, leading to decreased arginine availability and altered NOS activity [33].

Asymmetric dimethylarginine [ADMA], a non-standard amino acid formed by the post-translational modification, is an endogenous inhibitor of NOS. It is produced through the action of protein-arginine methyl transferases [PRMTs] on arginine residues and released following intracellular proteolysis. The intracellular ADMA concentration together with arginine regulates the NOS activity. ADMA can inhibit all three isoforms of NOS by uncoupling the enzyme, and generates superoxides [34]. Because of the structural similarity with arginine, ADMA competes with arginine for binding NOS, and blocks NO synthesis [35]. ADMA is catabolized by the activity of dimethylarginine dimethylaminohydrolase [DDAH] 1 and 2. DDAH-1 is highly expressed in kidney and liver, which are major sites for the metabolism of ADMA. The inhibition of DDAH may result in increased ADMA concentrations in plasma and tissues. It has been shown that incubation of endothelial cells with tumor necrosis factor-alpha [TNF-α] or ox- LDL inhibits DDAH activity [36].

The relation between ADMA levels and atherosclerotic risk factors has been investigated. It has been reported that hyperglycemia, hyperhomocysteinemia and oxidative stress decrease DDHA activity leading to high ADMA levels [37,38]. The patients having at least one of the atherosclerotic risk factors such as obesity, hypertension, hypercholesterolemia, diabetes mellitus, and hyperhomocysteinemia, also had high ADMA levels in blood that caused restricted NO generation and created deleterious effects on the vessel wall [39,40]. In a large population study, a significant association was observed between plasma ADMA and homocysteine levels [41]. In an experimental study performed in animals fed a high cholesterol diet, homocysteine and ADMA concentrations were increased whereas the DDAH activity, and arginine/ADMA ratio was decreased together with oxidative stress indices such as nitrotyrosine and malondialdehyde [42]. There are studies showing an increase in ADMA concentrations in patients with type 2 diabetes or insulin resistance [43,44]. Since ADMA also competes with arginine for CAT-2, the arginine/ADMA ratio seems to be a good marker for the evaluation of NO bioavailability and the estimation of cardiovascular disease risk [45].

NO Bioavailability in Insulin Resistance and Diabetes Mellitus

People with insulin resistance have higher rates of hypertension, dyslipidemia, and impaired glucose tolerance that contribute to development, progression and complexity of atherosclerosis [46]. Endothelial function is also attenuated in both type 1 and type 2 diabetes mellitus [47]. Glucose, ADMA, LDL-cholesterol levels and the ratio of ADMA/SDMA appeared to be significantly high in the patients with poor glycemic control [43]. Low plasma arginine levels together with high arginase activity have also been demonstrated in the patients with diabetes mellitus [48].

It has been reported that even short-term increase in blood glucose concentration may reduce the NO bioavailability and endothelium- dependent vasodilation [49]. Decreased eNOS activity and NO production have been reported in patients with insulin resistance and diabetes mellitus due to the selectively impaired insulin signaling [50,51]. The physiological actions of insulin on the vascular endothelium is regulated by phosphoinositide 3-kinase-protein kinase B/Akt [PI3K-PKB/Akt] pathway, leading to the activation of eNOS and the expression of cytokines [52,53].

Blood glucose levels and glycolysis rate are known to closely associate with ROS generation in the electron transport chain in mitochondria. Excess formation of ROS results in mitochondrial dysfunction as evidenced by incomplete beta-oxidation and decreased ATP synthesis [54]. Furthermore, it results in decreased NO production through oxidative modification of eNOS by reversible S-glutathionylation on several reactive cysteine residues [55]. Additionally, oxidation of tetrahydrobiopterin, one of the cofactors in NO production, leads to uncoupling of eNOS, switching production from NO to superoxide radical. Superoxide radical can react with NO to form peroxynitrite, preventing the vasodilatory effect of NO [56].

Improving the NO bioavailability: A promising era for drug studies

In recent years, BH_4 has been introduced as a new therapeutic agent for maintaining the NO bioavailability and vascular function. The supplementation of BH_4 has been reported to improve endothelial dysfunction in hyperlipidemic patients [57,58]. Dihydrofolate reductase activity is necessary to obtain optimum BH_4/BH_2 ratio. However, some studies failed to demonstrate beneficial effects of BH_4 supplementation in cardiovascular diseases. BH_4 treatment increased the BH_2 level that has no cofactor activity, while it possesses inhibitory effect on eNOS [59,60]. Nevertheless, BH_4 has antioxidant potency, which helps to scavenge the superoxide radicals and to control the physiological media of endothelial cells. Statins and vitamin C administration are the other alternative approaches to restore NO bioavailability and endothelial function [61,62].

Arginine supplementation in order to improve NO bioavailability has also been studied. Increased NO concentration and total antioxidant effect were observed with 2g of daily arginine supplementation [63]. Hoang., *et al.* have observed an increased transcription of guanosine triphosphate [GTP] cyclohydrolase I following arginine supplementation [64]. There are studies to demonstrate the preventing or delaying effect of long-term oral arginine use in patients with impaired glucose tolerance [65]. Supplementing a patient's diet with arginine, at least theoretically, may efficiently reverse the endothelial dysfunction caused by the high levels of ADMA. Several agents that increased insulin sensitivity also exhibited ADMA-lowering effect [66-68]. Metformin and rosiglitazone decreased ADMA levels and replenished arginine/ADMA ratio in animal experiments [69-71]. Additionally, antioxidant molecules such as betaine, taurine and melatonin lowered ADMA levels in experimental animals [42,67]. Yet, there is no specific therapy for counteracting the deleterious effects of ADMA in humans.

Pharmacological agents as inhibitors of DDAH or arginase have been considered for the therapeutic strategies to increase NO bioavailability [72]. Treatment with arginase inhibitor N-hydroxy-nor-L-arginine [nor-NONA] ameliorated the microvascular coronary functions and exerted cardioprotective effect in diabetic rats.

Conclusion

Besides being a vital signaling molecule throughout the body, NO is especially crucial for the maintenance of vascular health. The researchers who deal with cardiovascular disorders as well as other metabolic diseases have focused on the molecules and signaling pathways that affect NO bioavailability. Although the pharmacological trials for the development of specific agents to control NO metabolism are not fully efficient at present, the results from animal experiments might provide novel therapeutic targets.

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