REVIEW ARTICLE

NITRIC OXIDE MEASUREMENT FROM BLOOD TO LUNGS, IS THERE A LINK?

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Endothelium-derived nitric oxide (NO) is a key molecule in regulation of vascular tone and its association with vascular disease has long been recognized. NO inhibits many processes known to be involved in the formation of atherosclerotic plaque, including monocyte adhesion, platelet aggregation and vascular smooth muscle cell proliferation. Another important role of endothelial NO is the protection of the vascular wall from the oxidative stress induced by its own metabolic products and by the oxidation products of lipids and lipoproteins. Endothelial dysfunction, occurs at very early stages of atherosclerosis. It is therefore possible that deficiency in local NO availability could be a final common pathway that accelerates atherogenesis in humans. In addition to its role in the vascular endothelium, NO availability has been shown to modulate metabolism of lipoproteins. Negative correlation has been reported between plasma concentrations of NO metabolic products and plasma total and Low Density Lipoprotein [LDL] cholesterol levels while High Density Lipoprotein [HDL] improves vascular function in hypercholesterolaemic subjects. The loss of NO has considerable effect on the development of the disease. In the early stages of the disease reduced NO would leave the endothelium vulnerable to increased leukocyte diapedesis and increase the possibility of LDL oxidation. Oxidative stress and endothelial dysfunction are major contributors to development and progression of atherosclerosis in Diabetes Mellitus. Moreover, reports show that diabetics have impaired lung functions. It has been proposed that insulin resistance leads to airway inflammation. Exhaled nitric oxide (ExNO) is a recently introduced non invasive marker to measure inflammation and oxidative stress in the lung. So far no work has been done on exhaled NO levels in patients with DM. There are also no studies correlating exhaled NO to blood NO levels. We are also aiming to see if there is any relationship between exhaled NO with serum NO levels in diabetics as well as healthy individuals.

Key Words: Atherosclerosis, endothelial dysfunction, blood nitric oxide, exhaled nitric oxide, dyslipidemia

INTRODUCTION

Vascular diseases are the leading cause of death in modern era. Atherosclerosis usually occurs over years or decades. Many factors are associated with the acceleration of atherosclerosis, regardless of the underlying primary pathogenic change. The well-recognized risk factors include elevated plasma total and low-density lipoprotein (LDL) cholesterol level, high blood pressure, reduced high-density lipoprotein (HDL) cholesterol level, high blood level of L-homocysteine and diabetes mellitus (DM). Endothelium-derived nitric oxide (NO) is a key molecule in regulation of vascular tone and its association with vascular disease has long been recognized. NO inhibits many processes known to be involved in the formation of atherosclerotic plaque, including monocyte adhesion, platelet aggregation and vascular smooth muscle cell proliferation. Another important role of endothelial NO is the protection of the vascular wall from the oxidative stress induced by its own metabolic products and by the oxidation products of lipids and lipoproteins.

Endothelial dysfunction, which is defined as impaired ability of vascular endothelium for vasodilation, can be detected at very early stages of atherosclerosis. It is therefore possible that reduction in local NO availability could be a final common pathway responsible for accelerated atherogenesis in humans. Endothelial dysfunction may also initiate vascular inflammation, an important pathological feature in early atherosclerosis.

Diabetes mellitus is associated with increased rates of morbidity and mortality caused primarily by the accelerated development of atherosclerotic disease.

Patients with DM have been reported to have impaired lung functions. It has been found that impaired pulmonary function may increase the risk for developing insulin resistance or diabetes. Additional research is needed to better understand these relationships and their possible implications, especially with the promising results of treatment with intrabronchial administration of insulin. It has also been proposed that insulin resistance leads to airway inflammation. Exhaled nitric oxide (exhaled NO) is a recently introduced non invasive marker to measure inflammation and oxidative stress in the lung. It is a product of the action of local proinflammatory cytokines and has been suggested to play a role in the control of the pathophysiology of inflammatory airway diseases So far no work has been done on exhaled NO levels in patients with DM. There are also no studies correlating exhaled NO to

blood NO levels. We are also aiming to see if there is any relationship between exhaled NO with serum NO levels in diabetics as well as healthy individuals.

Nitric Oxide and Cardiovascular Disease

Endothelium-derived nitric oxide (NO) is a key molecule in regulation of vascular tone and homeostasis, and its association with vascular disease has long been recognized. In blood vessels, endothelial NO is produced from L-arginine and molecular oxygen by a constitutive, low output endothelial NO synthase (eNOS), a calcium-dependent enzyme, which is located in microdomains of cellular membranes called caveolae, and which requires several cofactors, including tetrahydrobiopterin (BH_4), for its actions.[1] After synthesis endothelial NO diffuses to the vessel lumen and to the underlying smooth muscle cells, where it stimulates their relaxation and vasodilation by activation of the soluble guanylate cyclase and generation of cyclic guanosine *5*-monophosphate. In addition, endothelial NO inhibits many processes known to be involved in the formation of atherosclerotic plaque like monocyte adhesion, platelet aggregation and vascular smooth muscle cell proliferation.[2] Another important role of endothelial NO is the protection of the vascular wall from the oxidative stress induced by its own metabolic products and by the oxidation products of lipids and lipoproteins.[3]

The half-life of endothelial NO is determined by its quick binding with oxyhemoglobin and with thiol groups in plasma proteins.[4,5] The oxyhemoglobin-bound NO is rapidly metabolized to stable oxidative end products, nitrite and nitrate (NO_x), which diffuse into the circulation and are excreted in the urine. The reaction between NO and plasma thiols leads to the reversible formation of *S*-nitrosothiols. These compounds are relatively stable, and are considered as a storage/transport form of NO.[6]

Endothelial dysfunction, which is impaired ability of vascular endothelium for vasodilation, can be detected at very early stages of atherosclerosis.[7] It is therefore possible that deficiency in local NO availability could be a final common pathway that accelerates atherogenesis in humans. There are two general mechanisms by which endothelial NO deficiency might cause endothelial dysfunction.[8] First, the synthesis of endothelial NO can be diminished due to a reduced expression or activity of eNOS. Second, the breakdown of eNOS-derived NO may be increased due to oxidative stress. The inactivation of eNOS enzyme may be related to a relative deficiency of NOS substrate, L-arginine, or its cofactor, BH_4, or to a decreased clearance of eNOS endogenous competitive inhibitor, asymmetric N^G N^G-dimethyl-L-arginine (ADMA). The accelerated NO decomposition occurs probably due to enhanced formation of free radicals such as superoxide anion.

The superoxide anion reacts rapidly with endothelium-derived NO, forming a powerful oxidant, peroxynitrite, which accelerates atherosclerosis by initiating lipid peroxidation in LDL and also by inactivating eNOS by directly attacking the endothelium. The superoxide anion levels are normally maintained low by superoxide dismutase, which is the major antioxidant enzyme in the blood vessel wall. In patients with CVD superoxide dismutase activity is down regulated.[9]

Endothelial dysfunction might initiate vascular inflammation, another important feature in early atherosclerosis. Inflammation in vessel wall leads to cytokine-induced activation of the high-output NOS isoform, called inducible NOS (iNOS). Inducible NOS is widely distributed in tissues and is also expressed in atherosclerotic plaque. The large quantities of NO produced by this enzyme in the inflammed blood vessels could favour the formation of peroxynitrite, contributing to cytotoxicity and tissue injury. Thus, although NO produced by eNOS may protect against atherosclerosis, NO derived from iNOS appears to have deleterious effects.[10]

Blood Nitric Oxide And Dyslipidemias

Dyslipidemia and atherosclerosis are associated with impaired endothelium-mediated vasodilatation in laboratory animals and in humans suggesting a loss of NO activity.[11] Previous studies have indicated that under these conditions, the bioavailability of endothelial NO is impaired, due to both reduced synthesis and increased breakdown. In hypercholesterolemia, the infusion of L-arginine improves endothelium-dependent vasodilation caused by acetylcholine or by serotonin.[12,13] In contrast, in healthy humans L-arginine does not produce vasodilatory responses. In addition to diminishing NO synthesis, hypercholesterolemia may affect the endothelial NO breakdown and enhance the production of superoxide anion.[14,15]

In addition to its role in the vascular endothelium, NO availability has been shown to modulate metabolism of lipoproteins. There is a well defined negative correlation between plasma concentrations of NO metabolic products, (NO_x), and plasma total and LDL cholesterol levels.[16]

Atherosclerosis is a progressive disease that begins with fatty streaks and through a process of lipoprotein deposition and cellular dysfunction progresses to complicated plaques. NO has both pro- and anti-atherosclerotic effects. The anti-atherosclerotic effects rely upon enough amounts of NO to regulate platelet function, leukocyte adhesion and extravasation, inhibit LDL oxidation and prevent smooth muscle cell (SMC) proliferation.[17] The reduced bioavailability of NO is thought to occur prior to the development of atherosclerosis. The loss

of NO has considerable effect on the development of the disease. In the early stages of the disease reduced NO would leave the endothelium vulnerable to increased leukocyte diapedesis and increase the possibility of LDL oxidation in the subendothelial space. Furthermore, the smooth muscle cell (SMC) proliferation associated with neointimal thickening would proceed unchecked. In the final stages, loss of NO could aggravate platelet activation, leading to thrombosis and myocardial infarction. Hence reduced bioavailability could contribute to the progression of atherosclerosis at all stages of the disease process. In hypercholesterolaemia the production of NO actually increases, and may be due, to an up regulation of NOS expression in the endothelium.[18] Other lipoproteins also have important effects on the bioavailablity of NO either by decreases biosynthesis or enhanced formation of superoxide. LDL, VLDL and chylomicron remnants have all been shown to have negative effects.[19,20] Although the studies highlighting that eNOS expression and activity may be altered in atherosclerosis, the most common feature of the disease is oxidative stress, and there is strong evidence that NO plays a central role in this process. As highlighted earlier the increased production of O^{2-} is closely associated with the loss of NO. The dysfunctional arteries found in atherosclerotic and hypercholesterolaemic animals produce larger quantities of O^{2-} than do healthy vessels.[21] Animal models of early atherosclerosis have shown that hypercholesterolaemia causes a twofold increase in NADPH-derived O^{-},[22] while in late atherosclerosis this increases to threefold.[23]

Exhaled NO

It is now 12 years since it was first reported that exhaled nitric oxide (ExNO) levels are increased in bronchial asthma.[24] This discovery followed a period of intense interest in the biology of NO during the late 1980s.[25] The numerous roles of NO in respiratory pathophysiology have been extensively reviewed. NO is an endogenous messenger with a diverse range of effects including non-adrenergic, non-cholinergic neurotransmission, vascular and non-vascular smooth muscle relaxation. There is contradictory evidence regarding the exact function of NO in lung disease. In pathological situations NO is a pro-inflammatory mediator with immunomodulatory effects.[26]

ExNO measurements have been useful in asthma, COPD, cystic fibrosis and lung transplantation. In patients with chronic and/or severe asthma, ExNO levels are helpful to determine whether or not eosinophilic airway inflammation is currently active. For the time being at least, it is reasonable that upper limits of normal for healthy adults and school age children should be set at 33 ppb and 25 ppb, respectively. Both high (50 ppb) and low (25 ppb) ExNO levels may be used to predict outcomes in patients with a definite history of asthma currently in remission, and in whom withdrawal of steroid therapy is being undertaken.[27]

Exhaled NO and Diabetes Mellitus

There have been many studies on the pulmonary function in diabetic patients, showing impairment of lung function in terms of Forced Vital Capacity (FVC) and Forced Expiratory Volume in 1st s (FEV_1);[28] and Total Lung Capacity (TLC),[29,30] and Carbon monoxide (CO) transfer factor. FEV_1 and FVC have been shown to be significantly and inversely associated with the incidence of diabetes,[31,32,33] whereas decreased lung function was related to increased risk of disease.

Lung function may also predict the development of insulin resistance or diabetes.[32,33] In the Normative Aging Study[32], FVC, FEV_1 and maximal mid-expiratory flow rate (MMEF) were associated with insulin resistance and another study found that impaired lung function predicted the development of diabetes.[33]

Although these studies have found that impaired pulmonary function may increase the risk for developing diabetes, additional research is needed to better understand these relationships and their possible implications, especially with the promising results of treatment with intrabronchial administration of insulin. A limitation of some studies is that type 1 diabetics were not distinguished from type 2 diabetics.[28,31,33]

It has also been seen that decreased lung function was greater in diabetic subjects treated with insulin than in subjects treated with oral hypoglycaemics and/or diet.[28]

Disease-related changes in the pulmonary microvasculature are thought to be a reason for some of these changes in type 1 patients.[29] Type 1 diabetic patients showed reduced TLC and CO transfer factor, indicative of a pulmonary restrictive dysfunction [29,30] and also in a study where the authors suspect most patients were type 2 diabetics.[31] From work in type 1 diabetic children,[34] there is evidence that supports the view that the lung is functionally involved early on in the course of the disease, and this could suggest a causal relationship or at least both abnormalities, pulmonary and endocrine could have similarities in their underlying pathophysiology; these associations could reflect childhood exposures which affect lung growth and also programme insulin resistance.[35]

Interestingly, lung function has been shown to be decreased before the onset of diabetes and it decreases longitudinally more than physiological age related decreases.[36] These authors propose that insulin resistance leads to low lung function possibly by the actions of leptin and resistin, which may affect pulmonary mechanics and airway inflammation. ExNO is closely related to airway inflammation and would be an ideal tool to assess this phenomenon in diabetic patients.

Exhaled nitric oxide (ExNO) is a recently introduced marker to measure inflammation in the lung and we are conducting research on patients with respiratory diseases and also with diabetes mellitus. This study will promote its clinical and research use in the Kingdom of Saudi Arabia. Exhaled NO is a reactive and volatile free radical gas detectable in the exhaled air of human subjects.[37] Exhaled NO is a product of the action of local proinflammatory cytokines and has been suggested to play a role in the control of the pathophysiology of inflammatory airway diseases.[38] Exhaled NO is increased in inflammatory airways disease, including bronchiectasis[39] and asthma.[40,41] There is less information on exhaled NO in other chronic lung diseases associated with airflow obstruction and inflammation such as emphysema, chronic bronchitis and COPD.[42,43,44] Therefore the measurement of exhaled NO represents a new method for the non-invasive monitoring of airway inflammation and oxidant stress in patients.

Measurement of Exhaled Nitric Oxide

Exhaled Nitric oxide (ExNO) is measured non-invasively with a chemiluminescence nitric oxide gas analyser. The American Thoracic Society recommends single-breath exhaled nitric oxide measurements from the mouth, for online measurements, from expiration from TLC. American Thoracic Society standardized procedures are used for ExNO measurement. The instrument is calibrated before use over the range of 0–100 ppb with dilutions of a known NO source, and care is taken that atmospheric levels do not exceed 30 ppb because this is known to affect ExNO.[45,46]

CONCLUSIONS

Endothelial NO has emerged as a prominent protector against cardiovascular disease. ExNO measurements offer a step forward in the assessment of airways disease. As an 'inflammatory marker', ExNO provides the clinician with status of airway inflammation, thus complementing conventional physiological testing. We do not know until now if there is a link between blood and ExNO levels? Patients with DM have been reported to have impaired lung functions along with endothelial dysfunction and ExNO may be of great clinical value in diabetics. ExNO measurements are easy to perform, reproducible, and technically less demanding than other traditional tests of pulmonary pathology. Much more work needs to be done before we can interpret the results with complete confidence.

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