LITERATURE REVIEW

The role of nitric oxide in chronic rhinosinusitis

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ABSTRACT

Chronic rhinosinusitis is a very common medical condition that affects nasal and paranasal sinuses mucosa in both adults and children. Its pathology, however, still remains unclear and researchers focus more and more on the role of nitric oxide (NO), a free radical produced in normal conditions by the paranasal sinuses epithelium in healthy patients, in the development of this disease. NO's role in the upper airway disease is not completely known, but it appears to act like a first-line host defence agent, maintaining the sinuses sterile due to its antiviral and bacteriostatic properties and by increasing mucociliary clearance. NO levels in the exhaled air of patients with CRS are lower than in healthy patients. One explanation for this might be the sinus obstruction that occurs in CRS because subjects with complete sinus opacification have the lowest levels. Furthermore, NO levels decrease after CRS treatments, suggesting that its measurement might help in monitoring the patient's response to therapy. In this review, we discuss the NO synthesis in the respiratory tract, its involvement in airway pathology, its role in the pathogenesis of CRS and the current clinical uses for NO in CRS and several other airway diseases.

KEYWORDS: chronic rhinosinusitis, pathophysiology, nitric oxide, nitric oxide synthesis

INTRODUCTION

Chronic rhinosinusitis (CRS) is a prevalent medical condition in western societies, causing impaired quality of life, reduced workplace productivity and thus producing an important financial burden. CRS is defined as the symptomatic inflammation of the paranasal sinuses and linings of the nasal passages with duration of more than 12 weeks. It comprises a heterogeneous group of sinus diseases, either accompanied by polyp formation (CRSwNP) or without polyps (CRSsNP). The pathogenesis of CRS remains controversial and appears to be multifactorial. It is characterized by a great diversity of immunological mechanisms and possible etiological factors, reflected in the effector T-cell signature, eosinophilic versus neutrophilic inflammation, remodelling parameters (e.g., TGF-β), eicosanoid and IgE production, microorganisms, and epithelial barrier malfunctions[1]. Nitric oxide (NO) is an important airway mediator with various functions whose role in chronic rhinosinusitis is gaining increasingly higher interest. This paper focuses on the pathophysiological impact of NO in CRS, its importance in current clinical use and the possible future contribution in CRS treatment.

NITRIC OXIDE PATHWAY

Nitric oxide or "endothelium-derived relaxing factor" is a free radical that regulates various biological functions. Determining NO's precise physiological activity is difficult due to the many locations from which it can be produced in the respiratory tract. The endogenous biosynthesis of NO is the result of L-arginase oxidation, reaction catalyzed by specific enzymes - nitric oxide synthesis (NOS). The reaction yields L-citrulline in addition to NO and is both NADPH and oxygen-dependent (Figure 1)[2].

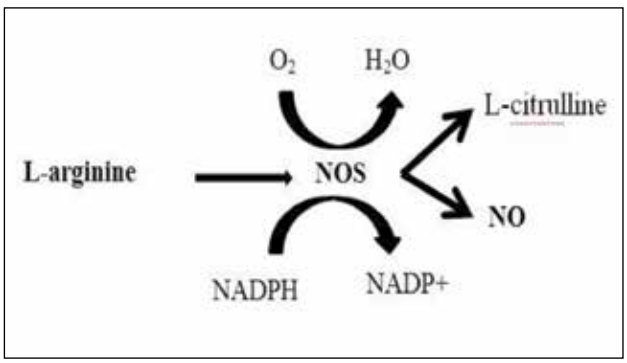

Figure 1 Nitric oxide synthesis

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NOS have three distinct isoforms that can all be found in the respiratory tract: neural (NOS I/nNOS), endothelial (NOS III/eNOS) and inducible (NOS II/iNOS). Functionally, the enzyme can be either constitutive (cNOS), Ca2+-calmodulin, which can be found in platelets and endothelial, epithelial and neuronal cells, or inducible (iNOS), which is a cytosolic protein that can be expressed in phagocytes (monocytes, macrophages and neutrophils) and epithelial, endothelial and vascular smooth muscle cells[3-5]. cNOS leads to a rapid release of femtomolar (fM) or picomolar (pM) concentrations of NO when the receptors are stimulated by agonists like acetylcholine[6]. iNOS expression is regulated at a pre-translational level, it is induced by pro-inflammatory cytokines such as TNF-α, IFN-γ and IL-1β and it leads to a sustained release of large quantities of pro-inflammatory NO several hours after exposure[5]. The NOS found in the human respiratory tract is the inducible form, constantly expressed and responsible for NO production within the sinus cavity of healthy subjects[7,8].

The NO molecule has properties that eliminate the need for extracellular receptors or targeted NO degradation. When released by iNOS, it has host-defense action, eliminating various pathogens, inhibiting viral replication and even killing tumor cells by acting as an immune effector molecule[9]. NO is toxic to a number of bacteria, fungi and parasites and it can either inhibit their growth or kill them[10]. Inhibition of DNA synthesis by direct deamination or by ribonucleotide reductase inactivation could be one of the mechanisms involved[11,12]. A few pathways for NO breakdown and inactivation are described. NO forms the peroxynitrite anion, a potent cytotoxic molecule, by interacting with superoxide anion[6]. By reacting with molecular oxygen, it forms nitrite, which is further oxidized to nitrate in the presence of hematoproteins[13]. Furthermore, in combination with proteins like albumin, it forms S-nitrosothiols, which may act as storage or carrier forms of NO[14].

Another key enzyme involved in the NO pathway is arginase, which converts L-arginine into L-ornithine and urea (Figure 2). Since the two enzymes use the same substrate, reciprocal regulation of both L-arginine metabolic pathways has been demonstrated, so NO production can be decreased by elevated levels of arginase via substrate limitation. Two isoenzymes of arginase have been identified: arginase I, highly expressed in the liver and arginase II, mainly expressed in extrahepatic tissues[15-17]. Woo Sung Cho et al. demonstrated the presence of both isoforms in normal and allergic nasal mucosa, but the level of expression in patients with allergic rhinitis is higher than in healthy subjects[18].

The exact involvement of arginase in the physiopathology of respiratory tract diseases is yet to be determined. It is speculated that it may play a central role in airway hyper-responsiveness, airway inflammation and airway remodelling, by causing a deficiency of anti-inflammatory and bronchodilating NO and by increasing L-ornithine levels, which is known for its involvement in cell proliferation and collagen synthesis[19].

NOS/arginase balance might be involved in nasal secretion and blood flow regulation, sustaining the assumption that the decrease of NO in several sinonasal diseases such as allergic rhinitis, asthma and CRS is due to a competition between arginase and NOS for arginine[18]. The data available in the published literature regarding NOS-arginase competition for L-arginine suggests that the administration of an arginase inhibitor may be beneficial in treating these diseases.

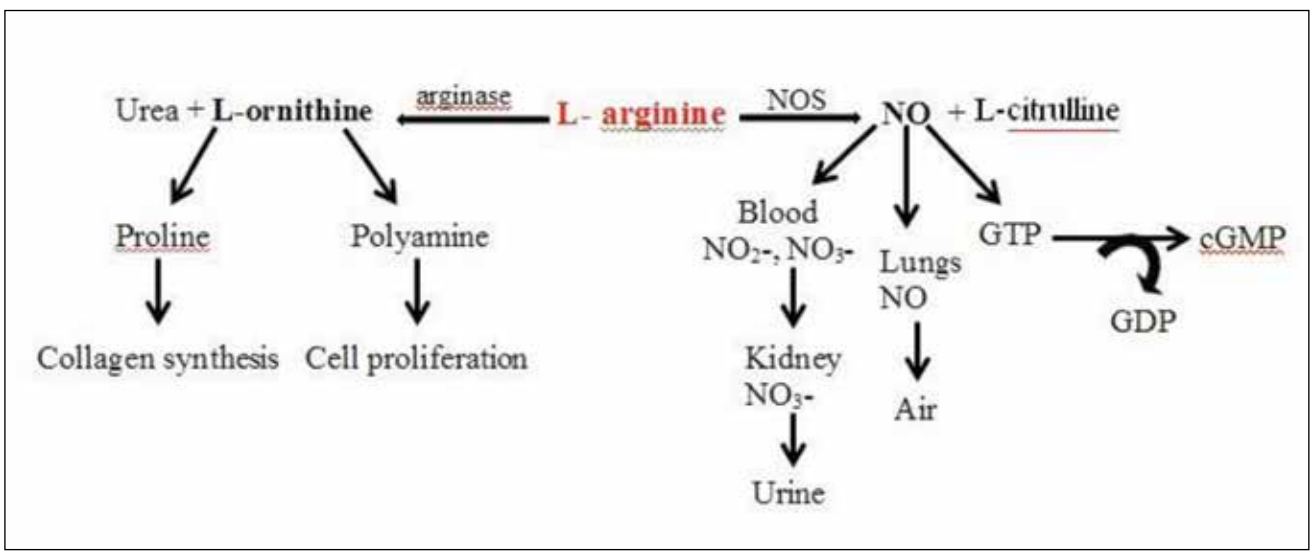

Figure 2 L-arginine pathways

NITRIC OXIDE AND THE RESPIRATORY TRACT

The NO presence in the exhaled air of patients was first documented in 1991. Later, studies have shown that most of the exhaled NO is originating from the nasal mucosa and paranasal sinuses. Its concentration in the lower airway is 20-fold lower than in the upper airway in healthy patients[20,21]. Nasal nitric oxide can be determined directly or indirectly. The direct method is based on the photochemical reaction between NO and ozone so NO is measured by chemiluminescence. The indirect method determines the products of NO metabolism and NOS expression[23]. Kharitonov et al. demonstrated the existence of two types of calcium-independent NOS by observing a decrease in nasal NO levels in patients with allergic rhinitis treated with topically applied glucocorticoids. Steroids are known to inhibit classical iNOS expression, but paranasal sinuses should not be affected by nasal sprays, so the iNOS expressed in sinus cavities is not the classical iNOS, but a permanent, normal, first line host defense[24]. Furthermore, in experimental studies on animals exposed to an allergen, an increase in iNOS expression was observed, while no change in eNOS or nNOS was noted[25,26]. Other factors like bacterial lipopolysaccharides[27], Pseudomonas aeruginosa[28] and respiratory syncytial virus[29] have been proven to increase epithelial iNOS expression. Due to its bacteriostatic and antiviral properties, NO maintains the sinuses sterile. Some bacteria are sensitive to a concentration of 100 parts per billion (ppb) NO, whereas the local concentration of NO in the paranasal sinuses can reach 23.000 ppb[30]. Using L-arginine analogues to inhibit NO production leads to increased susceptibility to parasitic infections with Leishmania, Mycobacteria and Plasmodium[31-33].

NO increases the mucociliary clearance by up-regulating ciliary motility and a correlation between impaired mucociliary function and low levels of NO in the upper airway has been demonstrated[34,35]. Runer et al. proved there is an increase in ciliary beat frequency when a NO donor is applied in the nasal mucosa[36]. Lundberg et al suggested that NO is an "aerocrine messenger" by observing that nasal breathing increases oxygen uptake and reduces pulmonary vascular resistance in patients with healthy lungs[20,21]. Also, the negative pressure created in the sinuses during inhalation forces the NO containing gas out, thereby increasing nasal NO levels[37]. Some authors speculate that, when inhaled from a proximal source, NO could modulate lung function and may improve ventilation-perfusion matching[21]. Asthmatic patients have high levels of fractional exhaled NO (FeNO) probably due to the eosinophilic airway inflammation that occurs in most patients with this disease[38]. In addition, researchers who study sleep disorders have found that NO also plays a role in regulation of neuromuscular pathways in the pharyngeal muscles, maintaining muscle tone, spontaneous respiration and sleep regulation[39].

Depending on the type and phase of the inflammation, its local concentration and the individual response, nitric oxide may have pro- or anti-inflammatory effects[40]. Colantonio et al. and Bommarito et al. reported that in patients with nasal polyposis nasal NO levels are reduced corresponding to the severity of the disease and a rise was observed when therapeutic measures were taken[41,42]. Nasal NO levels are higher in patients with allergic rhinitis than in those with nasal polyposis without allergies and a similar degree of sinus alteration[36].

Exogenous administration of NO might have antiproliferative effects on the airway smooth muscle; thus it may become important in preventing airway remodelling in patients with chronic obstructive pulmonary disease (COPD) or chronic asthma[43]. NO may play an important part in the pathogenesis of lung disorders characterised by hypersecretion of airway surface liquid due to its role as a transepithelial ion movement regulator[44,45].

NITRIC OXIDE IN RHINOSINUSITIS

After demonstrating that paranasal sinuses are the main source of exhaled NO and that it is continuously produced in healthy subjects, a renewed interest in the pathogenesis of rhinosinusitis was evoked. Many studies have shown that NO in the exhaled air of patients with acute and chronic RS is decreased compared with those who have healthy sinuses, but an exact explanation for this was not offered[46,47]. One can speculate that the increased production of cytotoxic agents observed in chronic inflammation can damage the NO producing sinus mucosa, thus a decreased quantity of NO is synthesized. Another explanation could be a decreased iNOS expression caused by certain cytokines, such as IL-4, IL-6 and TGF-β, found in the sinus mucosa of patients with CRS[22,47]. In contrast, Arnal et al. found that nasal NO levels are not significantly low in subjects with CRS but there is an inverse correlation between the extent of CT-scan alterations and nNO levels[36]. The degree of sinus pathology measured using CT scores correlates with the nasal NO levels; subjects with complete sinus opacification on CT scans have the lowest levels[47].

Lundberg et al. observed that nasal NO levels increase dramatically if the patient was humming during measurement, most likely due to the speed up of gas exchange over the sinus ostium. The nasal NO peak after humming could not be objectified in patients with CRS and CT-proven sinus obstruction[48], so this

test could be useful in evaluating ostial patency and in identifying patients at risk of developing sinusitis[20]. Ragab et al. showed that, in CRS patients, exhaled nasal NO levels increase after either medical or surgical treatment, so it could be used to evaluate CRS therapy[49]. Likewise, in children with acute maxillary sinusitis, nasal NO levels improved substantially after antibiotic therapy[50].

In chronic rhinosinusitis, NO metabolites levels measured in the sinus cavity follow a similar trend to those of NO. When measuring NO metabolites levels in sinus lavage fluid, Naraghi et al.[46] have shown higher levels in patients with CRS compared with healthy subjects. No difference could be observed between NO metabolite levels within the sinus cavity in CRS with and without polyposis, suggesting that although the two types of sinusitis have different pathogenesis, both lead to a similar degree of sinus dysfunction[46]. They also observed what happens to the NO metabolites levels after functional endoscopic sinus surgery (FESS) and found that there is a significant increase, probably because this procedure restores the sinus physiological function[51]. A study on rabbits in which NO metabolites levels were measured after the infectious process had started in maxillary sinuses showed that the increased levels observed in CRS began to return to baseline levels during recovery[52].

Deja et al. found that, in patients with sinusitis, gaseous NO was reduced in sinus cavity if the infection started in the nasal cavity and then spread to paranasal sinuses[53]. They speculated that this phenomenon appears either because the NO is metabolized before it reaches the sinus cavity due to an increase in the neutrophil-derived superoxide expression during inflammation[53] or because the excess secretions and thick aqueous epithelial lining inhibit NO diffusion from nasal cavities into the sinuses[54]. In CRS, due to local factors such as excess secretions and aqueous environment of sinuses, NO cannot diffuse into the nasal passage, so it is metabolized in the acidic environment within the sinus cavities. Extremely high levels of NO and its metabolites are toxic to the sinus epithelium[55], leading to further impairment of the mucociliary function and increasing the inflammatory process, thus creating the vicious circle of sinusitis (Figure 3).

Lately, many specialists support the united airway concept. According to this, there is a united allergic airway that links allergic rhinitis, CRS and asthma, all three arising from a common atopic entity[56]. A link between CRS and asthma has already been demonstrated and patients with sinusitis should be evaluated for a possible concomitant asthma and vice versa[57].

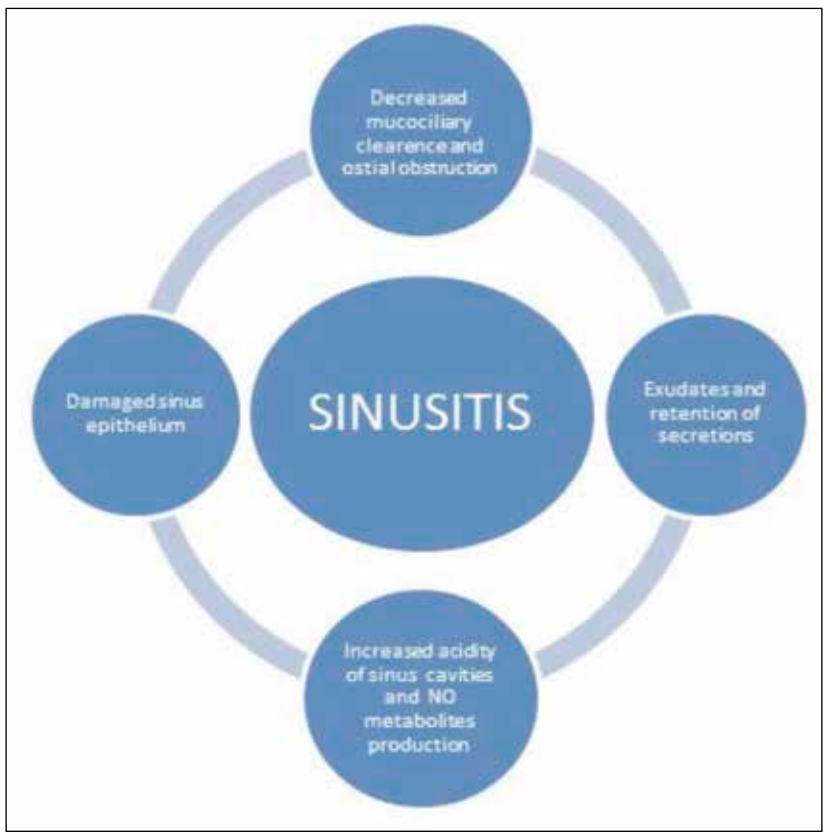

Figure 3 The vicious circle of sinusitis

CURRENT CLINICAL USES FOR NO

Although the exact role of nitric oxide in the respiratory tract is not completely elucidated, altered NO levels have been found in several airway diseases. When discussing the clinical use of NO levels, one must differentiate between exhaled NO and nasal NO. The levels of exhaled NO (eNO) are considerably lower than those of nasal NO (nNO) and are more helpful in diagnosing eosinophilic airway inflammation in both adult and paediatric patients[58]. Eosinophilic inflammation is the underlying mechanism in allergic airway inflammation, so eNO could be used in diagnosing asthma, allergic rhinitis, COPD and CRS. In asthma diagnosis, an increased eNO level despite the correct treatment suggests a persistent inflammation, and studies have shown that eNO can also be used to identify the risk of exacerbations[59, 60]. This measurement has the advantage that it is safer than bronchial provocation tests and it provides rapid results[61]. Studies have demonstrated that in asthmatic patients, eNO testing in combination with lung function tests is superior to lung function testing alone[62]. An association between lower asthma exacerbation rates and the use of eNO levels in conjunction with clinical parameters was also observed[63]. Atopy is associated with increased levels of eNO, so one should be cautious when using this measurement as diagnostic or monitoring tool for patients with asthma[64]. Since eosinophilia correlates with the response to inhaled steroids treatment, eNO can also be used to predict the patient's response to steroid treatment and to guide steroid dosing[23,65]. In paediatric patients with on-going "asthmatic" symptoms, normal or low exhaled NO levels raise the suspicion of an alternative diagnosis such as primary ciliary dyskinesia, cystic fibrosis or sinusitis[58].

Nasal NO has a potential role in the diagnosis and treatment of CRS and it could be used as screening tool for patients with CRSwNP that may eventually need surgical treatment[66]. Since researchers have discovered that nNO levels increase after CRS treatment, this might become a useful tool in monitoring the efficiency of medical or surgical therapy. Furthermore, nNO could prove a reliable non-invasive biomarker for ostial patency and sinus ventilation.[41,42,46,49,51] In patients with acute bacterial sinusitis, a significant improvement of nNO levels was noted after appropriate antibiotic treatment[50].

The utility of nasal NO measurement in patients with cystic fibrosis (CF) is controversial. Some studies have reported significantly lower levels than in healthy subjects and, in patients with upper respiratory tract symptoms, nasal NO may prove to be a useful diagnostic tool[58]. In primary ciliary dyskinesia, nNO levels are very low; nasal NO testing has a high sensitivity and specificity and it could be used as a screening tool for this disease[20]. Mucociliary structure and function should be investigated if nNO levels are lower than 100 ppb[67].

Some studies found increased nNO levels in subjects with allergic rhinitis, while others demonstrated the opposite, so using nNO to diagnose AR is not recommended[58]. Although increased NO aggravates nasal obstruction, rhinorrhea and sneezing, no correlation between the severity of symptoms and nNO levels in patients with allergic rhinitis was found. Lee et al. suggested that for cases in which allergic tests or invasive procedures can not be performed, nNO should be used to predict AR[68].

Recent studies have used humming to increase the sensitivity of nasal NO measurements. This was useful in patients with CF but not in those with primary ciliary dyskinesia (PCD)[69,70]. Also, the absence of the NO peak during humming could be useful in identifying patients with allergic rhinitis associated with sinus obstruction[20,71], but further studies are necessary to sustain this assumption.

CONCLUSIONS

Measuring nitric oxide levels in the respiratory tract is a step forward in the assessment of airway disease. Both exhaled and nasal NO could become useful tools in the screening and diagnosis of several sinonasal diseases (such as AR, CRS, polyposis), PCD and eosinophilic asthma. NO metabolites play an important part in sinusitis pathogenesis. Knowing the exact role and pathway of NO could open novel therapeutic perspectives for the management of chronic rhinosinusitis. The combined management of upper and lower respiratory tract disease could be improved by eNO and nNO measurements, but research for the standardization of test procedures is required.

Acknowledgement. This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/138776.

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