Nitric oxide: a new concept in chronic sinusitis pathogenesis

Mohsen Naraghi, MDa,*, Armin Farajzadeh Deroee, MDa, MohammadReza Ebrahimkhani, MDb, Samira Kiani, MDb, AhmadReza Dehpour, PhDb

*Iranian Rhinology Research Society, Department of Otorhinolaryngology, Head and Neck surgery & Otorhinolaryngology Research Center, Tehran University of Medical Sciences, Tehran, Iran
bDepartment of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Purpose: Exhaled NO is produced mainly in paranasal sinuses and nasal mucosa. Nasal NO has been suggested to have a variety of effects in nasal cavity. Decreased exhaled NO is found in chronic sinusitis, and NO metabolite levels are increased in animal models of chronic sinusitis, suggesting a role for them in sinusitis pathogenesis. There was no data available on human NO metabolite level.

Materials and methods: We lavaged maxillary sinuses in a control and 2 patient groups. The control group was patients who underwent functional endoscopic sinus surgery (FESS) due to any other reason than chronic sinusitis. The patient groups had chronic rhinosinusitis with and without polyposis who underwent FESS. Maxillary sinuses were lavaged during FESS, and NO metabolites (nitrate and nitrite) were lavaged in the lavage fluid.

Results: Nitric oxide metabolite levels (mean ± SEM) were 8.085 ± 1.43 μmol/L in healthy maxillary sinus lavage fluid and 18.04 ± 3.51 and 16.78 ± 2.91 μmol/L in chronic rhinosinusitis with and without polyposis, respectively. Lavage fluid of sinuses with chronic sinusitis had elevated levels of NO metabolites, which were significantly higher than the control group. The difference between the chronic sinusitis with and without polyposis groups was not significant.

Conclusions: Nitric oxide metabolites were significantly higher in maxillary sinuses of patients with chronic sinusitis. Elevated levels of NO and NO metabolites in sinusitis might damage healthy sinus epithelium. NO metabolites may have an important role in sinusitis pathogenesis.

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1. Introduction

The presence of NO in exhaled breath of humans was first demonstrated in 1991 [1]. Later studies showed that NO found in exhaled air was produced in the upper airways [2,3], mainly by Schneiderian mucosa in the nose and paranasal sinuses [4-6]. The origin of the NO measured from the nasal airway has been controversial, and there are some indications that favor the paranasal sinuses rather than the mucosa of the nasal cavity and vice versa [7].

Nitric oxide seems to have a variety of effects on the nose and paranasal sinuses. Interestingly, some of the known functions of NO in sinuses are to create a sterile environment. It increases the mucociliary beat frequency and hence increases the mucociliary clearance [8,9]. NO is also known to have bacteriostatic and antiviral effects [10,11].

The role of NO in inflammation is far from certain, and NO synthesis is clearly enhanced locally at the sites of inflammation by inducible NO synthase (iNOS) activity [12-14]. NO has a dual function in inflammation [15] and shows pro- and anti-inflammatory effects in different situations. NO in nasal cavity seems to play a role in
Inflammation like every other part in the body. During rhinosinusitis, NO in nose and sinuses is oxidized to its more stable metabolites: nitrite (NO\(_{2}^{-}\)) and nitrate (NO\(_{3}^{-}\)) [16]. Nitrite and nitrate can then be oxidized to peroxynitrite (ONOO\(^{-}\)). When peroxynitrite is protonated in the acidic environment of sinusitis, peroxynitrous acid is formed and leads to the production of cytotoxic agents such as hydroxyl and nitric dioxide [17,18]. In a study performed on rabbits, elevated NO metabolite levels were found in chronic sinusitis and began to return to normal levels during recovery [19]. It was shown that nasal exhaled NO is decreased in patients with acute and chronic sinusitis. Interestingly, NO was not decreased in patients with common cold [20,21]. Studies performed on NO and sinusitis show a strong relation between them and suggest a role for NO in sinusitis pathogenesis. Because of the different biologic marker values in species and the absence of any data on NO metabolites in humans, which seem to be important in sinusitis pathogenesis, we felt that further investigations on NO metabolites and human sinusitis are mandatory. Our study is the first study in humans measuring NO metabolites in chronic sinusitis. Because NO generation can be indicated by the formation of stable end products of NO (nitrite and nitrate) [22], we measured NO metabolites nitrate and nitrite in maxillary sinuses of the patient and control groups. We hypothesized that NO and NO metabolites are increased in chronic sinusitis, and pathologic events that occur in chronic sinusitis may be related to elevated levels of NO and NO metabolites.

2. Materials and methods

This study was performed on 37 cases in 3 groups: group 1, the control group (n = 12); group 2, the chronic rhinosinusitis patients with nasal polyposis (n = 14); and group 3, the chronic rhinosinusitis patients without nasal polyposis (n = 11). The control group was patients who underwent functional endoscopic sinus surgery (FESS) due to any other reason than chronic rhinosinusitis. In the control group, 5 cases had concha bullosa and 7 had paradoxical middle turbinate causing contact point headaches. Paranasal sinuses of the control group had to appear normal in computed tomography scan. The patient groups were patients with chronic rhinosinusitis who underwent FESS. Chronic rhinosinusitis with or without polyposis was diagnosed through history, nasal endoscopy, and paranasal sinus computed tomography scan. None of the patients had sinus surgery before, and none had received treatment for at least 4 weeks before the operation. The patients had at least 1 involved maxillary sinus. Fungal sinusitis, history of allergic rhinitis, acute rhinosinusitis within 1 month before FESS, ciliary motility disorders, and smoking were exclusion criteria for the groups. The studied population were age and sex matched. All patients gave informed consent after the nature of the experimental procedures was explained, and the study was approved by the local ethics committee.

The surgery was performed meticulously to have minimal bleeding and eliminate multiple lavages during surgery. The involved maxillary sinuses of the groups were lavaged with sterile distilled water during FESS. The sinuses were filled with distilled water and then the lavage fluid was aspirated. The samples were taken away from light to 0°C and then stored in −27°C. The measurements were done according to a method by Miranda et al [22]. After loading the plate with samples (100 μL) and adding saturated solution of VCl\(_{2}\) (100 μL) to each well, Griess reagents (50 μL each) were rapidly added to the wells. Sulfanilamide and naphthylethylenediamine dihydrochloride were applied for preparation of Griess reagents. The plates were incubated at 37°C for 30 minutes and then the absorbance at 540 nm was measured using a standard plate reader. Fresh standard solutions of nitrate were included in each experiment. Data are presented as mean ± SD of the mean. Comparisons between the results of the groups were made using 1-way analysis of variance. Statistical significance was accepted for P < .05.

3. Results

The mean ages of the control and chronic rhinosinusitis without and with polyposis groups (mean ± SEM) were 29.8 ± 6.1, 32.1 ± 5.2, and 36.2 ± 4.7 years, respectively. The NO metabolite levels (mean ± SEM) were 8.085 ± 1.43 μmol/L in the maxillary sinus lavage fluid of group 1 and 18.04 ± 3.51 and 16.78 ± 2.91 μmol/L in groups 2 and 3, respectively. The maxillary sinus lavage fluid of the patient groups had higher levels of NO metabolites that were statistically significant (P1 vs 2 = .006 and P1 vs 3 = .008). The difference between groups 2 and 3 was not significant (P2 vs 3 = .12).

4. Discussion

In our study, NO metabolite levels in sinus lavage fluid were significantly increased in 2 major variants of chronic rhinosinusitis compared with normal sinuses, but it did not differ significantly between chronic rhinosinusitis with or without polyposis. This suggests that although these variants may have different pathogenesis, both have increased levels of NO metabolites in sinus cavity in the final stages of the disease and subsequent sinus dysfunction. In this study, contrary to the study performed on rabbit models of chronic rhinosinusitis [19], the infection was presumed to be originated from the nasal cavity and then spread to the paranasal sinuses, and the infection did not begin directly in maxillary sinuses.

It has been shown that exhaled NO from the nares is decreased in acute and chronic sinusitis. Deja et al [23] also showed that gaseous NO was markedly reduced in sinus...
cavity in sinusitis. It has also been shown that measuring NO metabolites is a method for estimating NO production. Because of the above findings, one may expect decreased levels of NO metabolites in our study, but our study showed increased levels of NO metabolites in the sinus cavity in sinusitis. This discrepancy might be explained by the following: NO production is increased by inflammatory cells, and excess secretions and thick aqueous epithelial lining in sinusitis may inhibit diffusion of gaseous NO into the air-filled sinus cavity similar to suppurative lung conditions [24]. Neutrophil-derived superoxide that favors NO metabolism is thought to be increased significantly during sinusitis, and the produced NO, which seems to be mostly originated from inflammatory cells in rhinosinusitis, metabolizes before it reaches the sinus cavity [19]. All of these may cause a decrease in sinus gaseous NO in spite of increased levels of NO production and elevated levels of NO metabolites in the sinuses. Finally, ostial obstruction does not allow the gaseous NO to reach nasal cavity, and this reason in addition to all of the above may lead to a decreased level of gaseous nasal NO in sinusitis.

Nitric oxide in nasal cavity is mainly produced by sinus ciliary epithelium iNOS in normal conditions and inflammatory cells iNOS in inflammation. The sinus epithelium iNOS activity seems to be essential for constant production of NO, which seems to be necessary for maintaining a ciliary beat frequency at a level sufficient for optimal mucociliary clearing function and a healthy sinus (Fig. 1). In sinusitis, sinus epithelium iNOS expression is decreased significantly [23]. Inflammatory cell iNOS can produce very high amounts of NO. It can be assumed during sinusitis that NO production is increased significantly by host defense cells in the sinus cavity. Because of ostial obstruction and excess secretions and aqueous environment of sinuses that do not allow NO to reach the air-filled sinus cavity and exit to the nasal cavity, this high amount of NO could possibly dissolve and metabolize in acidic aqueous environment of the sinuses. At this phase, there is an increased level of NO metabolites in the sinuses as shown in our study. Such amount of NO metabolites could be autotoxic for the surrounding epithelium (Fig. 2) as seen in pertussis and as theorized in asthma [25]. This might indicate a role for NO in sinus defense mechanisms against germs but a destructive effect on the sinus epithelium if NO and its metabolites are present at extremely high levels [19]. Because the ciliary epithelium is the main source of NO in the healthy sinus, the damaged sinus epithelium in sinusitis leads to decreased sinus epithelium mucociliary clearance. It can be hypothesized that because of decreased mucociliary clearance and ostial obstruction caused by inflammation, retention of secretions and exudates occurs. This event is followed by more intense inflammation and ostial blockage, which causes further epithelial damage due to further increase in acidity of the sinus cavity and production of peroxynitrous acid and other cytotoxic agents, aggravating the vicious cycle of sinusitis.

5. Conclusion

Our study showed significantly higher levels of NO metabolites in chronically infected sinuses of humans. NO and its metabolites might play a pivotal role in sinusitis pathogenesis, and further studies could be useful in understanding sinusitis pathogenesis and improving sinusitis, which may lead to medical management of sinusitis using NO-modulating drugs in the future.
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References