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Exhaled nitric oxide: A non-invasive method to assess airways inflammation in asthmatics

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RESUMEN

El objetivo de este artículo es presentar las mejores evidencias con relación a la aplicación del óxido nitroso para evaluar la inflamación de las vías aéreas. La inflamación de las vías aéreas juega un papel fundamental en asma y es considerada como la causa más importante de exacerbaciones y persistencia de cambios estructurales de las vías aéreas. La evaluación de la inflamación de las vías aéreas es importante en la investigación de los mecanismos básicos de la enfermedad. Además, la evaluación de la inflamación de las vías aéreas puede demostrar alteraciones de las vías aéreas no detectada por síntomas, evaluación clínica, pruebas de función pulmonar o hipersensibilidad de las vías aéreas. La inflamación de las vías aéreas ha sido medida en forma tradicional a través de secreciones y biopsias usando broncoscopia flexible, pero estos métodos son muy invasivos y no son apropiados para el monitoreo en forma repetida de la inflamación de las vías aéreas. En los últimos años, investigadores han buscado otras alternativas menos invasivas como el uso de secreciones inducidas o la medición de óxido nitroso en aire exhalado como marcadores de la inflamación. Usando mediciones del óxido nitroso exhalado como un índice de inflamación aun es un asunto complicado, ya que éste puede ser afectado por factores biológicos o técnicos además de la inflamación de las vías aéreas. Sin embargo, dentro de sus limitaciones, este método parece ser una alternativa rápida y no invasiva en la evaluación de la inflamación de pacientes asmáticos.

Palabras clave: Asma, óxido nitroso, inflamación, corticosteroides.

ABSTRACT

The aim of this article is to summarize the best evidence available about the applications of nitric oxide (NO) to evaluate airway inflammation. Airway inflammation plays a fundamental role in asthma and is considered to be a major cause for exacerbation and persisting structural changes of the airways. Evaluation of airway inflammation is an important sign for investigating the underlying mechanisms of asthma. Measurement of airways inflammation may reveal diseased airways not detectable by symptoms, clinical examination, lung function or airways hyperresponsiveness. Airways inflammation has been measured in secretions and biopsies obtained during flexible bronchoscopy, but these methods are too invasive and not appropriate for monitoring airways inflammation repeatedly. Over the last few years research has looked into other less invasive alternatives such as induced sputum and evaluation of exhaled NO (ENO) as markers of inflammation. Using measurement of ENO as an index of lung inflammation remains a complex matter, as it is affected by many biological and technical factors other than airways inflammation. However, within limits, it still offers the promise of being a quick and non-invasive approach for evaluation of inflammation in subjects with asthma.

Key words: Asthma, nitric oxide, inflammation, corticosteroids.

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INTRODUCTION

Over the last two decades, substantial progress has been made in the understanding of the pathogenesis of asthma. Asthma is now defined as a disease of chronic airways inflammation and no longer as a reversible airways obstruction disorder.[1] Inflammation in asthma is characterized by cellular infiltration, vascular leakage, and hypertrophy and/or hyperplasia of resident cells and smooth muscles. Structural changes of the airways walls occur early in the course of the disease.[2] despite recent advances, airways regulation and responsiveness in asthma are rather complex phenomena whose origin is large unknown. Increased levels and activities of a number of inflammatory mediators have been suggested as important factors involved in the pathogenesis of the exaggerated airways response in asthma. Of those, nitric oxide (NO) seems to play a major role. NO is a freely diffusible molecule that is found in high levels in exhaled air in asthmatic patients.[3] NO is important to lung smooth muscle relaxation, neurotransmission, and host defence mechanisms.[3] Therefore, measurement of exhaled NO (ENO) seems to be a promising non-invasive method to assess inflammation. When asthma therapy is adequate, inflammation can be reduced over the long-term, symptoms can usually be controlled, and most asthma-related problems prevented. The benefits of inhaled corticosteroids as a preferred long-term control in children with asthma has been emphasised recently.[4] Thus, ENO evaluation of the asthmatic patient could include follow-up assessment of the degree of airways inflammation to monitor the therapeutic success.

Relationship between NO and inflammation of the airways

A part from the oxygen and carbon dioxide, exhaled air contains a large number of volatile substances. These compounds in human breath have been studied intensely for the diagnosis of disease in recent years. NO has been identified as an important mediator with a wide spectrum of actions. The molecule of NO originates from L-arginine which is converted to L-citrulline by NO-synthase (NOS), an enzyme with several isoforms.[5] These isoforms can be induced by cytokines (iNOS) to produce large amounts of NO, which are likely to be responsible of elevated ENO in asthmatic patients.[6] Studies suggest that in asthmatic patients the release of NO might be a physiologic mechanism counteracting the bronchoconstriction caused by various stimuli.

Synthesis of nitrogen oxides (NOx) in the lung requires at least one isoform of NOS, enzyme substrates and cofactors. Of the NOS isoforms active in the lung, the types I and III (constitutive) are activated by mediator-signalled calcium influxes leading to calmodulin binding. These are present primary in subepithelial neurons (type I) and vascular endothelium (type III).[7-9] In contrast, type II NOS (inducible) or iNOS is tightly bound to calmodulin after translation. It is inducible in airways epithelial, vascular endothelial, and inflammatory cells. Pro-inflammatory cytokines can enhance its activity in the airways epithelial cells.[7] In contrast, corticosteroids inhibit the transcription and the activity of iNOS, but have little effect on the constitutive isoforms. There is evidence that in the asthmatic airways expression of iNOS is upregulated.[8] Therefore, in view of their important biologic functions, changes in the expression of NOS type I and III may have important pathophysiologic consequences.

The generation of cytokines from macrophages and other inflammatory cells is considered to be important in the maintenance of the inflammatory response, particularly since asthma exacerbation is thought to be caused by a worsening of airway inflammation. Cytokines IL-4, INF-, TNF-, and IL-1 have been implicated in the regulation of type II NOS.[10-11] Studies in humans have shown an increased level of ENO following antigen challenge.[8] The inflammatory response in the airways is not directed by a single mechanism, but rather an orchestrated process where various factors interact in a controlled and regulated process. For example, NO has a direct chemotactic effect to eosinophils, neutrophils and monocytes.[10] Studies in mice have shown that NO might inhibit the Th1 responses and thus favour the development of Th2 responses with subsequent eosinophilia. Studies have shown that type I NOS knockout mice present about 60% of the ENO of wild-type mice, although type II NOS knockout mice do not differ from wild-type mice in airways responsiveness after ovalbumin challenge.[12]

NO metabolites such as peroxynitrate (OONO-) cause airway hyperresponsiveness and airways epithelial damage, enhance inflammatory cell recruitment, and inhibit pulmonary surfactant. For example, studies have shown that there is increased peroxynitrate formation in the airways of asthmatics, which may contribute to airways obstruction, hyperresponsiveness, and epithelial damage.[13] Interaction between NO and oxygen will form nitrite (NO_2-); NO_2- accumulation in cell culture supernatants is commonly used to estimate NO production. NO_2- is stable for several hours in water and plasma but is rapidly converted to nitrate (NO_3-) in whole blood. Additional research has indicated that elevated levels of NO_2- or NO_3- (which are also increased in sputum in asthmatic patients) correlate with the increased level of NO in asthma.[14]

The NOS enzyme can be inhibited by steroids and due to this, NO concentration in exhaled air decreases after steroid treatment, an effect that could possibly be used to monitor the compliance of patients and treatment effectiveness. NO is not only a marker but may

have anti-inflammatory and pro-inflammatory effects. The physiological responses of asthma exacerbation are associated with acidification of lung water. Acute asthmatic pH changes (acidification) are associated with a fall in lung water NO_2- concentrations that parallel increased ENO. A recent study by Hunt et al,[15] showed that the pH of deaerated exhaled airways vapour condense is over two log orders lower in patients with acute asthma than in control subjects and this normalises with corticosteroid therapy. In addition, at these low pH levels, the endogenous NO_2- is converted to NO in quantities sufficient to account for the concentrations observed in asthma. This information suggest that airways pH may be an important determinant of ENO and airways inflammation.

Inflammatory factors affecting nitric oxide

There is clearly evidence that ENO increases several fold in patients with asthma, particularly during exacerbations.[16] Asthmatic patients treated with corticosteroids present a rapid fall in ENO levels.[17,18] A case-control study conducted by Baraldi et al in 16 children (aged 6 to 13 years) with acute asthma, showed a decrease in levels of ENO by 46% from baseline following 5 days of treatment with prednisone. However, the levels of ENO on the asthma group remained significantly higher than the control group. No difference in the levels of ENO were observed before and after bronchodilator test.[19]

In a study by Chatkin et al, levels of ENO were used to discriminate between chronic cough among asthmatics, and nonasthmatics.[20] They studied 38 adult patients with chronic cough (> 3 weeks), 44 asthmatics and 23 healthy controls. In the chronic cough group, 79% (30/38) were considered nonasthmatics. The levels of ENO were significantly higher in those with chronic cough attributable to asthma as compared to those with chronic cough not attributable to asthma. These findings suggest that ENO may have a role in the evaluation of both asthma and chronic cough.

Methods to assess ENO

Exhaled NO has been shown to correlate with other parameters in mild asthma such as induced sputum eosinophilia[14] and airways hyperreactivity in non-steroid-treated subjects.[17] Recently, the American Thoracic Society (ATS) published recommendations for standard procedures in the assessment of ENO.[21] This document recommends excluding the large amounts of NO that are produced in the upper airways (e.g. paranasal sinus) by using positive mouth pressure.[22] Additionally, levels of ENO can vary markedly and inversely with the exhalation flow rate. Studies have shown that alveolar NO levels are low (less than 5 ppm) because of avid binding by haemoglobin in the alveolar capillary bed.[21] Thus, if the exhalation is fast this will cause a decrease in levels of ENO. Therefore, exhalation flow rates must be constant for reproducibility.

Two methods have been used to collect ENO. One is an online or real-time method, where an exhalation is performed and ENO is directly measured into a photomultiplier tube (chemiluminescence). This technique requires only few minutes to complete and little training. The other technique is the bag-collection method where air is stored into a balloon for further analysis. This type of analysis allows measurements to be performed at remote sites from the analyser (e.g. work place, school, home, or emergency department). Studies have shown stability of the NO levels for up to 48 hours.[21] This offers an enormous opportunity to conduct field epidemiological investigations.

Other factors that can affect the levels of NO

Levels of ENO can be influenced by several factors. In addition to asthma, diseases with elevated ENO include viral respiratory infections,[23] lupus,[24] liver cirrhosis,[25] and acute lung allograft rejection.[26] Low levels of ENO have been found in cystic fibrosis,[27] HIV infection,[28] and pulmonary hypertension.[29] During the assessment of ENO, levels of ambient NO may be elevated (higher than endogenous); therefore, it is essential to quantify the present levels in the environment. A diet rich in foods containing nitrates should also be investigated.[21] NO should be measured before spirometry and bronchodilator administration, since levels may be affected.[30] In addition, since NO is a major constituent in cigarette smoke, patients should avoid smoking or exposure to tobacco smoke prior to the test.[31]

CONCLUSIONS

Measurement of ENO represents a non-invasive approach for assessing airway inflammation and the response to anti-inflammatory therapy in patients with asthma. Using measurement of ENO as an index of lung inflammation remains a complex matter, as it is affected by many biological and technical factors other than airway inflammation. However, within limits, it still offers the promise of being a quick, easy and non-invasive approach for evaluation of inflammation in subjects with asthma. Despite the large amount of research and publications in this field in recent years, this still remains a research tool that perhaps, in the near future, will have a more wide application. Currently no standard “normal” values are available for asthmatics or nonasthmatics. Future studies should be directed to determine these levels using representative populations for age, gender, and race as in the case for spirometry.

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