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Single breath exhaled nitric oxide in lung transplant patients: a preliminary clinical study

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Abstract Exhaled nitric oxide is considered as a marker of airway inflammation. We report here our preliminary experience with single-breath exhaled nitric oxide measured in lung transplant patients with and without bronchiolitis obliterans syndrome and in cardiac transplant patients. Peak and end-expiratory nitric oxide concentrations did not differ between groups, but single-breath exhaled nitric oxide recordings were strikingly different in patients suffering from bronchiolitis obliterans syndrome, with a slower decrease from peak to end-expiratory nitric oxide concentration. Further studies are required in order to determine whether theses abnormalities reflect the inflammatory process of bronchiolitis obliterans syndrome.

Key words Nitric oxide · Breath test · Bronchiolitis obliterans · Lung transplantation

Introduction

Nitric oxide (NO) is present as gaseous molecule in the exhaled air of many species [6]. In humans, high exhaled NO concentrations have been reported in some broncho-pulmonary inflammatory disorders such as asthma, bronchiectasis or infection [2].

Lung transplantation is now recognized as a valuable therapeutic modality for patients with chronic end-stage respiratory insufficiency. To date, bronchiolitis obliterans (BO) is the major mid- and long-term complication of lung transplantations [1, 14]. It is a chronical inflammatory disorder affecting predominantly the terminal and respiratory bronchioles, causing an obstructive syndrome subsequently leading to graft failure. We report here our preliminary results of the assessment of exhaled NO in lung transplant patients with and without bronchiolitis obliterans.

Materials and methods

Population

From November 1996 through May 1997, 11 lung transplant- and 7 cardiac transplant patients were enrolled in our clinical study. After approval from the local Ethics Committee, all patients gave their informed consent to participation in the study.

Lung transplant patients (7 females; age: 21.2 yrs ± 4.1(SEM)) were evaluated 22.4 months ± 7.8 after having undergone transplantation. Pre-transplant diseases were cystic fibrosis (9 patients), histiocytosis and primary pulmonary hypertension (1 patient each). Seven bilateral lung transplantations, 3 heart and lung transplantations, and 1 double-lung and liver transplantation were carried out.

Bronchiolitis obliterans syndrome (BOS) was diagnosed in three lung transplant patients (BOS group) based on the established international criteria [8]. All had a persistent and significant decrease in FEV_1 not related to infection, acute rejection or bronchial stenosis. BO was not confirmed by transbronchial biopsies. Two BOS patients were grade 3, and one was grade 2 [8]. In each case, decrease of lung spirometry was progressive. Of the unaffected lung transplant patients (non-BOS group; $n = 8$), none suffered from acute rejection, infection or BOS in a 3-month period following exhaled NO measurements.

Forced expiratory volume in 1 s (expressed as percentage of the mean of the 2 highest post-operative FEV_1 values) were

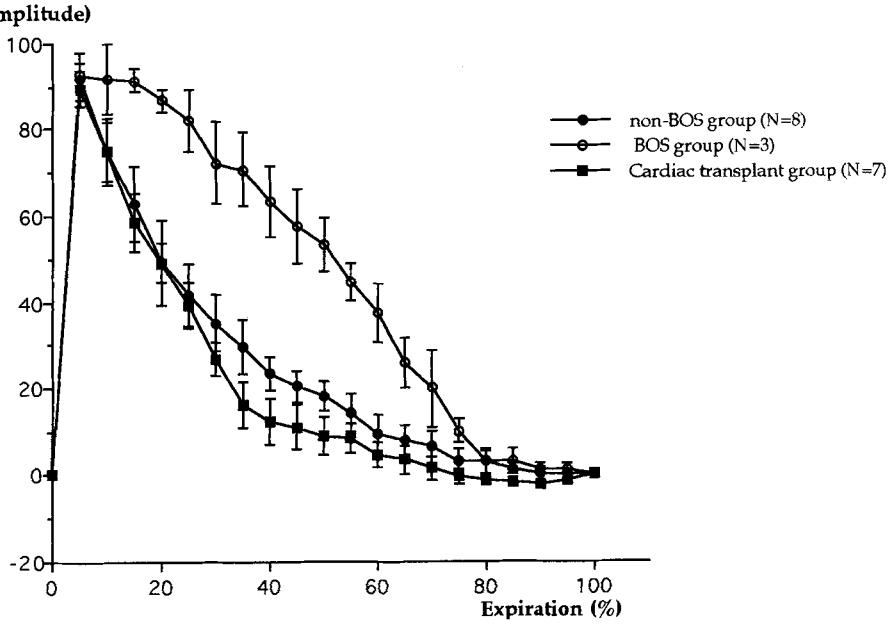

Fig. 1a

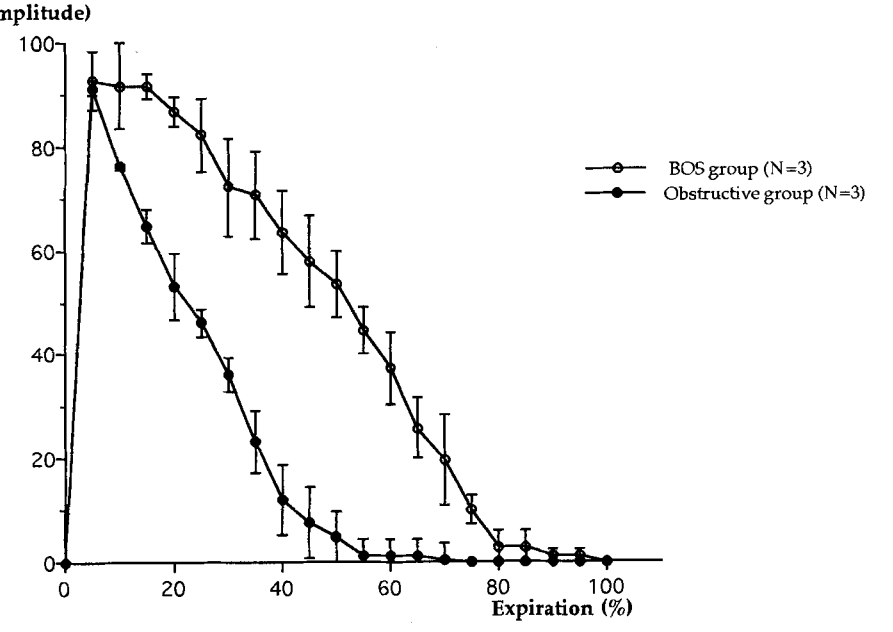

Fig. 1b

Fig. 1 a, b Single-breath exhaled nitric oxide concentration, expressed as percentage of the amplitude (see text) in lung- and heart transplant patients. **a** Single-breath exhaled NO in lung transplant patients with and without bronchiolitis obliterans (BOS), and in cardiac transplant patients. **b** Single-breath exhaled NO in lung transplant patients with BOS and in transplant patients with an obstructive syndrome. Expiration is expressed as percentage of exhalation duration

41.9 % ± 7.7 and 99.9 % ± 1.4 in patients with and without bronchiolitis obliterans.

Seven cardiac transplant patients (5 males; age: 46.4 yrs ± 4.9) were assessed 45.4 months ± 15.5 after transplantation. Pre-transplant diseases consisted of ischemia (2 cases), dilated cardiomyopathy (4 cases) and tetralogy of Fallot (1 case). None of the patients were smokers, and none suffered from respiratory infection at the time of exhaled nitric oxide measurements.

Exhaled NO measurement

Exhaled NO was measured using a chemiluminescence analyzer (NOX 4000, Seres, France). Samples were introduced into the analyzer at a rate of 1 L/min, and responses were recorded on a chart recorder. The NO analyzer was calibrated daily with NO calibration gas at a concentration of 100 ppb. Response time of the analyzer with the recorder was measured at 4.2 ± 0.1 s, from the initial

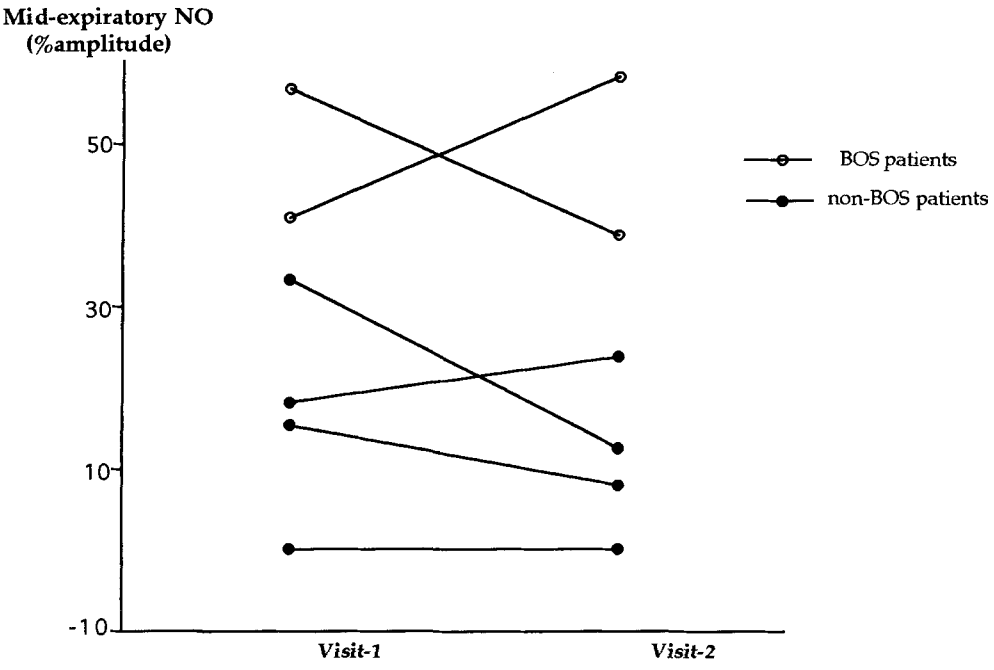

Fig. 2 Mid-expiratory nitric oxide concentration (expressed as percentage of amplitude) in lung transplant patients with and without bronchiolitis obliterans syndrome (BOS) at 2 successive visits

deflection to 90% of the plateau value when using the calibration gas at 100 ppb.

While seated and wearing nose clips, patients breathed room air. Single-breath exhaled NO was measured. Patients were asked to perform a slow vital capacity (over 10–20 s) through a wide bore tube connected via a side port to the NO analyzer. Three successive measurements were taken, allowing a 1-min period of normal breathing between each measurement. The recording with the highest amplitude (eg, peak NO- minus end-expiratory NO concentration) was used for analysis.

As our NO chemiluminescence analyser did not have a flowmeter, patients were asked to exhale as regularly as possible during the measurements. In order to allow a comparison between patients, exhalation duration was normalized, i.e, expressed as a percentage of the duration of exhalation.

Statistics

Data are reported as mean ± SEM unless otherwise mentioned. Non-parametric data were assessed by Kruskal-Wallis and Mann-Whitney's tests; Bonferoni's correction was used for multiple comparisons. Significance was defined as a P value less than 0.05.

Results

Duration of expiration was 14.9 s ± 0.6, 12.5 s ± 1.6 and 15.3 s ± 1.5 in BOS, non-BOS and cardiac transplant patients (NS). Peak NO concentration (24.9 ppb ± 3.8 in BOS patients; 19.6 ppb ± 1.8 in non-BOS patients; 25.6 ppb ± 5 in cardiac transplants) and end-expiratory NO concentration (8.9 ppb ± 1.5 in BOS patients; 6.9 ppb ± 1.7 in non-BOS patients; 7.3 ppb ± 1 in cardiac transplants) did not differ significantly between the three groups. Peak NO was dependent on room air NO concentration ($r = 0.62$, $P = 0.006$), whereas end-expiratory NO was positively correlated with expiration duration ($r = 0.7$; $P = 0.001$). The interval from the initial deflection to the peak value (expressed as a percentage of duration of expiration) was 5.9% ± 2.3, 4.4% ± 1.1 and 3.6% ± 0.9 in BOS, non-BOS and cardiac transplant patients (NS).

Single-breath exhaled NO records were markedly different between BOS and non-BOS lung transplant patients (Fig. 1 a). In BOS patients, the decrease from peak to end-expiratory NO concentration was slower. At mid-expiration, NO concentration (expressed as percentage of the amplitude of variation, i.e, peak less end-expiratory NO values) was approximately 3-times higher in the BOS group (53.3% ± 6.4 vs 18.2% ± 3.3; $P = 0.02$). This increase in mid-expiratory NO concentration did not seem to be related to the obstructive syndrome itself since 3 transplant patients (2 males; 2 heart transplant patients with chronic obstructive pulmonary disease and 1 double-lung transplant patient with a bronchial anastomotic stenosis) with an obstructive syndome (FEV1/VC (%) = 56.7 ± 6) had similar recordings to non-BOS lung transplant patients (Fig. 1 b).

Six patients were available for two follow-up measurements, 2 BOS and 4 non-BOS lung transplant patients; the interval between the 2 visits was 2.1 months ± 1 and 3.1 months ± 1 in BOS and non-BOS patients, respectively. As shown in Fig. 2, mid-expiratory NO concentration remained elevated in the BOS subset.

Discussion

In our preliminary clinical study, we did not observe any difference in peak- and end-expiratory exhaled NO concentrations between lung transplant patients with or without BOS, and cardiac transplant patients. However, single-breath exhaled NO recordings were markedly different, with a lower decrease from peak NO to end-expiratory NO values in BOS patients.

While NO measurement in exhaled air seems to be an interesting modality in the assessment of patients with bronchopulmonary inflammatory disorders, one must realize this method has some limitations. Firstly, approximately 50% of NO in exhaled air from oral expiration originates in the upper airways [10]. Contamination of exhaled air with NO from the upper airways seems to affect the begining and the end of expiration in healthy volunteers, too [17]. However, asthmatic patients have shown that the lower airways account for the increase in exhaled NO concentration [13]. Secondly, the exhaled NO measurement is dependent on experimental conditions. Nasal expiration, breathholding, and a low flow of expiration are associated with high exhaled NO concentrations [11, 15]. These effects of experimental conditions on the measurement of exhaled NO concentration have recently lead some investigators to propose a standardized protocol for exhaled NO evaluation [9]. However, we do not know today whether this standardization will efficiently assess patients with multi-focal bronchopulmonary inflammatory lesions (such as bronchiolitis obliterans).

Bronchiolitis obliterans is currently the main cause of mid- and long-term graft failure in lung transplantation. An increase in pulmonary calcium-independent NO synthase activity has been observed in this chronic bronchopulmonary inflammatory disorder [3]. Recently, an overexpression of inducible NO synthase has been described in bronchial epithelial and endothelial cells in lung transplant patients with BO [12]. Since inducible nitric oxide synthase (NOS) produces higher amounts of NO than constitutive NOS, it was tempting to speculate that elevated exhaled NO would be present in BOS patients.

Three studies dealing with exhaled NO measurement in BOS have been reported to date. Silkow [16] did not observe any difference in exhaled NO concentrations between lung transplant patients with or without BOS, whereas Dromer [4] noticed a 3-fold increase in the exhaled NO rate in BOS patients. This discrepancy may be attributed in part to the differences in their methodology. Finally, Fisher et al. [5] reported an increase in mean exhaled NO in lung transplant patients with an active BOS, whereas inactive BOS was not associated with elevated exhaled NO concentration. To our knowledge, our report is the first clinical study dealing with the morphology of single-breath exhaled NO recordings. Despite the small size of our population, a marked difference between lung transplant patients with and without BOS was noticed. Our preliminary data suggested that the observed difference (a higher mid-expiratory NO concentration in BOS patients) was not associated with the obstrucive syndrome itself, since immunosuppressed control patients without BOS, but with an obstructive syndrome, had no elevated mid-expiratory NO concentration.

At the beginning of our study, the influence of expiratory flow on exhaled NO concentration was unknown. However, measuring exhaled NO at a constant flow is probably not the panacea. Recently, Horvath [7] observed that exhaled NO concentration was not elevated in corticosteroid-treated asthmatic patients with daily symptoms of asthma and, in some instances, evidence of increased bronchial inflammation (ie, increased hydrogen peroxide concentration in breath condensates). Silkow, likewise [16] did not find any increase in exhaled NO in lung transplant patients with BOS, despite much evidence of increased broncho-pulmonary NO production [3, 12]. The reasons for the ineffectiveness of the 'standardized' protocol in assessing broncho-pulmonary inflammation under these circumstances are not fully understood.

It is possible that other protocols (with or without a constant expiratory flow) of exhaled NO measurements might be sensitive enough to distinguish between lung transplant patients with and without BOS. The single-breath analysis could be one of them. The marked differences we noticed between BOS and non-BOS patients despite the small size of our population is promising. However, further and larger studies are needed to confirm our results, to determine whether these abnormalities are related to the pulmonary inflammatory process, and whether or not these abnormalities precede the occurence of a decline in lung function in BOS.

In summary, single-breath exhaled nitric oxide measurements might prove an interesting non-invasive diagnostic tool in the assessment of bronchiolitis obliterans in lung transplant patients.

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