THE EFFECT OF FIBEROPTIC BRONCHOSCOPY ON EXHALED NITRIC OXIDE

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Nitric oxide has been extensively studied as a noninvasive marker of airway inflammation. Assuming that bronchoscopy can produce not only systemic but also local inflammatory response, we hypothesized that bronchofiberoscopy can be responsible for an increase in nitric oxide synthesis with resulting increase in fractional concentration of exhaled nitric oxide (FE\textsubscript{NO}). Fifty five subjects (F/M-23/32; mean age 53.9 ±17.3 yr) undergoing diagnostic bronchoscopy participated in the study. The indications for bronchoscopy were as follows: interstitial lung diseases (n=13; 23.6%), lung cancer (n=11; 20.0%), hemoptysis (n=10; 18.2%), differential diagnosis of asthma/dyspnea (n=9; 16.4%), pulmonary infections (n=7; 12.7%), and others (n=5; 9.1%). During bronchoscopy bronchial washing (n=18), bronchoalveolar lavage (BAL) (n=26), and bronchial biopsies (n=24) were performed. FE\textsubscript{NO} was analyzed on-line with chemiluminescence analyzer (NIOX, Aerocrine, Sweden) according to the ATS guidelines, before and at 1, 2, 3 and 24 h after bronchoscopy. The mean FE\textsubscript{NO} before bronchoscopy was 21.0 ±3.31(SE) ppb, it decreased to 14.8 ±2.10 ppb 1 h after bronchoscopy, reached a nadir at 2 h (14.4 ±2.28 ppb; P<0.05), and was not different from baseline 24 h after bronchoscopy (22.8 ±2.90 ppb). There were no differences in the FE\textsubscript{NO} profile in BAL patients compared with those in whom only the bronchial washing was performed. We conclude that bronchoscopy leads to a decrease in FE\textsubscript{NO}. The underlying mechanisms are at present unclear.

Key words: bronchoalveolar lavage, exhaled nitric oxide, fiberoptic bronchoscopy, inflammatory response
INTRODUCTION

Following its benchmark discovery, nitric oxide (NO) is now known to play important functional roles in a variety of physiological systems. It controls smooth muscle tone in respiratory, cardiovascular, and digestive system. It also is a messenger molecule in central and peripheral nerves and plays a critical role in natural defense mechanisms against bacterial infections. Endogenous NO is synthesized from L-arginine and oxygen molecule by nitric oxide synthase (NOS). Three isoforms of NOS are known: the two constitutively expressed, dependent on high concentration of ionized calcium and calmoduline (NOS1, NOS3) and one inducible (NOS2) whose activity does not depend on intracellular calcium concentration (1).

In 1991, NO was discovered in exhaled air in guinea pigs, rabbits, and humans and this initiated a rapid increase in the number of studies on exhaled NO. Fractional concentration of exhaled nitric oxide ($\text{FE}_{\text{NO}}$) is usually measured with on-line chemiluminescence analyzers. Since nasal NO concentration is significantly higher relative to lower airways, appropriate technique of the measurement to prevent mixing of oral and nasal air is mandatory.

The cellular origin of exhaled NO is still uncertain. It appears, however, that the main source of NO is bronchial epithelial cells and not pulmonary alveolar cells or pulmonary vessels endothelium (2, 3). All three isoforms of NOS are expressed in the respiratory system. Proinflammatory cytokines (e.g. TNF$\alpha$ or IL-1$\beta$) stimulate the NOS2 expression in respiratory epithelial cells leading to an increase in $\text{FE}_{\text{NO}}$.

Endoscopic procedures can trigger excessive release of proinflammatory cytokines including TNF-$\alpha$, IL-1$\beta$, IL-6 from alveolar macrophages (4, 5). There are data suggesting that bronchoscopy and bronchoalveolar lavage (BAL) can provoke systemic inflammatory response, characterized by headache, muscle pain, and fever, in 1-30% of subjects (6, 7). We hypothesized that fiberoptic bronchoscopy can produce not only systemic but also local inflammatory response, enhancing NO production in respiratory epithelial cells and increasing exhaled NO. Therefore, we aimed to assess the influence of bronchofiberoscopy on NO concentration in exhaled air.

MATERIAL AND METHODS

Study protocol was accepted by the Bioethics Committee of Warsaw Medical University in Poland and all patients provided informed consent. The study included 55 patients (F/M-23/32) referred for diagnostic bronchoscopy in our institution. Table 1 shows demographic and clinical characteristic of the patients. Indications for bronchoscopy are presented in Table 2.

Instrumentation

Oral fiberoptic bronchoscopy was performed with Pentax EB-1830T2, FB19-TV or Olympus BF-1T30 flexible bronchoscopes. All patients were premedicated with diazepam (5-10 mg i.m.) and
atropine sulphate (0.5 mg i.m.) 30-40 minutes prior to the procedure. To achieve appropriate local anesthesia 10% lidocaine was sprayed in the oropharynx and 2% lidocaine was directly instilled over the vocal cord, main carina, and bronchi (the total dose of lidocaine varied between 300 and 500 mg). Bronchoscopic procedures included visual macroscopic inspection of the upper airways trachea and bronchi, BAL (150-200 ml 0.9% NaCl) (n=26), small volume bronchial washing (10-20 ml) (n=18), and/or forceps endobronchial biopsies (n=24). \( \text{FE}_{\text{NO}} \) was measured with an online chemiluminescence analyzer Niox (Aerocrine, Sweden) in accordance with the ATS guidelines (8) (expiratory flow rate from 0.045-0.055 l/s). A mean value from 3 reproducible measurements (intermeasurement variability <10 %) was used for further analysis. \( \text{FE}_{\text{NO}} \) was measured before bronchoscopy and then at 1, 2, 3, and 24 h post-bronchoscopy.

### Statistical analysis

Changes in \( \text{FE}_{\text{NO}} \) were analyzed with one-way ANOVA for repeated measurements. When significant F values for time-by-condition interaction were noted, Newman-Keuls tests were used for post hoc analyses. Descriptive statistics of \( \text{FE}_{\text{NO}} \) is presented as mean ±SE, while other values are given as mean ±SD. Correlations were examined with Pearson correlation coefficient. P<0.05 was considered significant.

### RESULTS

The mean value of \( \text{FE}_{\text{NO}} \) before bronchoscopy was 21.0 ±3.3 ppb. There was a significant decrease in exhaled NO to 14.8 ±2.1 ppb (P<0.001) 1 h after bronchoscopy (Fig. 1). \( \text{FE}_{\text{NO}} \) reached its nadir at 2 h and stayed low throughout the 3\textsuperscript{rd} post-bronchoscopic hour (14.4 ±2.3 ppb; P<0.001 and 18.0 ±2.68 ppb; P<0.05, respectively) (Fig. 1). At 24 h after bronchoscopy, \( \text{FE}_{\text{NO}} \) value of 22.8 ±2.9 ppb was not different from that at baseline.

Table 1. Baseline characteristics of patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ±SD</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>53.9 ±17.3</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>25.8 ±4.1</td>
</tr>
<tr>
<td>Current smokers (n, %)</td>
<td>17 (31%)</td>
</tr>
<tr>
<td>FVC (% of predicted)</td>
<td>94.3 ±21.8</td>
</tr>
<tr>
<td>FEV(_1) (% of predicted)</td>
<td>85.1 ±22.3</td>
</tr>
<tr>
<td>FEV(_1)%FVC</td>
<td>74.6 ±12.2</td>
</tr>
</tbody>
</table>

Table 2. Indications for bronchoscopy.

<table>
<thead>
<tr>
<th>Indication</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial lung diseases</td>
<td>13</td>
<td>23.6</td>
</tr>
<tr>
<td>Suspicion of lung cancer</td>
<td>11</td>
<td>20.0</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>10</td>
<td>18.2</td>
</tr>
<tr>
<td>Differential diagnosis of asthma/dyspnea</td>
<td>9</td>
<td>16.4</td>
</tr>
<tr>
<td>Pulmonary infections (pneumonia, tuberculosis)</td>
<td>7</td>
<td>12.7</td>
</tr>
<tr>
<td>Others</td>
<td>5</td>
<td>9.1</td>
</tr>
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</table>
No significant correlations were found between the baseline or post-bronchoscopy FE$\text{NO}$, on the one side, and body temperature, measured up to 36 h after the procedure, baseline spirometry, and arterial blood gases on the other side. There were no differences in the pattern of FE$\text{NO}$ changes between the groups of patients undergoing different bronchoscopic procedures. In particular, no differences were noted in the profile of FE$\text{NO}$ changes between patients who underwent BAL or bronchial washing (Table 3).

**DISCUSSION**

Exhaled NO measurement is a simple and noninvasive marker of airway inflammation, especially in asthma patients (9). Numerous studies suggest strong correlation between FE$\text{NO}$ and the number of eosinophils in induced sputum (10), BALF (11), or biochemical markers of eosinophilic inflammation of the airways (12). There are, however, some other studies, which failed to show the relationship between FE$_{\text{NO}}$ and the activity or number of eosinophils in bronchial mucosa (13). Serial exhaled NO measurements have been used more recently to assess asthma control and adherence to the therapy in asthmatic individuals treated with inhaled corticosteroids. FE$_{\text{NO}}$ has also been reported as a useful tool
in predicting upcoming exacerbation of asthma (14, 15). FE\textsubscript{NO} has proven very useful in differentiating chronic asthmatic cough vs. cough resulting from other diseases (16). It has also been used in monitoring of chronic diseases, including COPD, bronchiectases, and others (17, 18). Decreased exhaled NO has been reported in the Kartagener syndrome (19).

The authors searched through the Medline database of medical bibliographic information and found no earlier studies on the influence of endobronchial procedures on exhaled nitric oxide. Given this lack of earlier reports, the authors expected that inflammatory response induced by bronchoscopy and BAL would rather cause an increase in FE\textsubscript{NO}. Preliminary data in 17 patients, presented earlier in the abstract form showed a decrease in FE\textsubscript{NO} following fiberoptic bronchoscopy, which was rather surprising (20). The current study, conducted in a bigger group of patients, fully confirmed earlier observations that the decrease in exhaled NO takes place irrespectively of the type of bronchoscopic procedure and the underlying lung disease being the indication for bronchoscopy. This is further reinforced by a separate analysis of BAL or bronchial washing patients (Table 3). Despite a higher baseline FE\textsubscript{NO} level in BAL compared with bronchial washings, post-bronchoscopy decrease in FE\textsubscript{NO} followed the same pattern in both groups. The most plausible explanation for a higher baseline FE\textsubscript{NO} in the BAL group is the fact that 9 out of the 26 patients in this group were diagnosed with asthma.

Both the complexity of fiberoptic bronchoscopy (e.g. premedication, local anesthesia, different endobronchial procedures) and the absence of earlier observations on FE\textsubscript{NO} and endobronchial procedures make the clear assessment of their relationship difficult. Furthermore, according to the ATS/ERS guidelines, interpretation of FE\textsubscript{NO} should consider the influence of other external factors, such as age and sex of a patient, food and beverages, smoking history, coexisting diseases, upper and lower respiratory tract infections as well as current medications (8). There were no significant correlations between transient alterations in exhaled NO and the demographic characteristic of our patients, therefore the causal relationship between any of these external factors and FE\textsubscript{NO} seems doubtful.

Our results do not provide an explanatory clue for the observed phenomenon, but several possible mechanisms should be considered. In the authors' opinion these are the following: (i) lidocaine administered locally, (ii) stress-induced hyperventilation and cough during the bronchoscopy, (iii) changes in lung function induced by bronchoscopy, (iv) "trapping" of nitric oxide particles by airway mucous and/or saline instilled during bronchoalveolar lavage, as well as "washing out" NO during aspiration of airway secretion, bronchial washing or bronchoalveolar lavage fluid.

There are reports of the influence of lidocaine on NO synthase activity. Shiga et al (21) showed that lidocaine decreased NO production by inducible NO synthase in murine activated macrophages at multiple levels after transcription. The authors emphasized that lidocaine inhibited NO synthesis at concentrations significantly larger (toxic concentrations) than those found in plasma. However, the authors did not exclude the possibility that lidocaine in lower concentration
could also attenuate NOS2 activity. Despite many studies on local anesthetics, the influence of these medications on exhaled nitric oxide has not been systematically examined by far (22). Our preliminary observations in 4 healthy, non-smoking volunteers (data not published) showed a decrease in FE\textsubscript{NO} from 13.7± 2.7 to 8.4 ±2.3 ppb 30 min after local lidocaine anesthesia carried out according to the routine bronchoscopic protocol. By contrast, Qian et al (23) did not observe any significant effect of lidocaine or tetracaine applied on nasal mucosa on nitric oxide exhaled through the nostrils.

The relationship between stress-induced hyperventilation or cough during bronchoscopy and FE\textsubscript{NO} alterations should also be analyzed in the light of reports on the influence of spirometric maneuver or exercise-induced hyperventilation on FE\textsubscript{NO}. The link between lung function tests and transient changes in FE\textsubscript{NO} has been intensively elucidated in recent years (24-25). Some studies showed a substantial decrease in FE\textsubscript{NO} after forced expiration in both healthy and asthma subjects (27). However, in a most recent study Tee at al (28) did not find any significant differences in mean NO levels before and after spirometry and maximal or submaximal inspiratory effort. The authors concluded that in clinical practice NO measurements obtained with a NO monitoring system are not significantly affected by prior spirometry maneuvers, the use of a nasal clip, and submaximal inspiratory effort and, thus, questioned the necessity of maintaining the recommended order of respiratory measurements, i.e., first the FE\textsubscript{NO} measurement and then lung function tests. St. Croix et al (29) studied exhaled NO at rest and during three intensities of cycling exercise and two levels of hyperventilation at constant expiratory flow of 46 ml/s (which is very similar to expiratory flow used in our protocol) and did not observe significant augmentation of systemic and/or airway NO production. In contrast, Gabriele et al (30) reported a substantial decrease in FE\textsubscript{NO} 5-15 min after a 6-min walk test in children with asthma. However, after a 30-min recovery, exhaled NO was not different from baseline in the majority of the subjects. Nevertheless, it is highly improbable that hyperventilation during bronchoscopy was similar to the ventilatory response to exercise. Therefore, causal relationship between hyperventilation and post-bronchoscopic decrease in FE\textsubscript{NO} seems very unlikely.

Belen et al (31) showed a decrease in vital capacity, forced expiratory volume in 1 second and midmaximal expiratory flow immediately after the procedure in 33 patients undergoing diagnostic bronchoscopy. By 4 h after bronchoscopy, most of the spirometric parameters were not different from baseline values. It seems that because the main source of exhaled NO remains airway epithelium, any decrease of airway surface releasing NO can lead to a decrease in FE\textsubscript{NO}. Since spirometry was not systematically performed after bronchoscopy, we cannot state whether our patients experienced changes in lung volumes that could theoretically lead to a decline in FE\textsubscript{NO}.

Some authors suggest that the secretion accumulated in the bronchial tree could inhibit NO diffusion from bronchial epithelial cells and trap NO particles
with resulting decrease in FE\textsubscript{NO} (32, 33). As we did not observe any differences in the pattern of FE\textsubscript{NO} changes between BAL and bronchial washing patients, such a relationship also seems unlikely.

In conclusion, bronchoscopy leads to a transient decrease of NO in exhaled air irrespectively of the type of endoscopic procedure. The underlying mechanism of this phenomenon remains unclear. However, it appears that these alterations may have to do with locally applied lidocaine and/or changes in lung volumes elicited by bronchoscopy. In order to describe the causative mechanisms, further studies, including measurement of other inflammatory mediators, are warranted.

REFERENCES


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