Low levels of nitric oxide and carbon monoxide in \(\alpha_1\)-antitrypsin deficiency

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Machado, Roberto F., James K. Stoller, Daniel Laskowski, Shuo Zheng, Joseph A. Lupica, Raed A. Dweik, and Serpil C. Erzurum. Low levels of nitric oxide and carbon monoxide in \(\alpha_1\)-antitrypsin deficiency. J Appl Physiol 93: 2038–2043, 2002. First published August 30, 2002; 10.1152/japplphysiol.00659.2002.—Quantitations of exhaled nitric oxide (NO) and carbon monoxide (CO) have been proposed as noninvasive markers of airflow inflammation. We hypothesized that exhaled CO is increased in individuals with \(\alpha_1\)-antitrypsin (AT) deficiency, who have lung inflammation and injury related to oxidative and proteolytic processes. Nineteen individuals with \(\alpha_1\)-AT deficiency, 22 healthy controls, and 12 patients with non-\(\alpha_1\)-AT-deficient chronic obstructive pulmonary disease (COPD) had NO, CO, CO2, and O2 measured in exhaled breath. Individuals with \(\alpha_1\)-AT deficiency had lower levels of NO and CO than control or COPD individuals. \(\alpha_1\)-AT-deficient and COPD patients had lower exhaled CO2 than controls, although only \(\alpha_1\)-AT-deficient patients had higher exhaled O2 than healthy controls. NO was correlated inversely with exhaled O2 and directly with exhaled CO2, supporting a role for NO in regulation of gas exchange. Exhaled gases were not significantly related to corticosteroid use or lung function. Demonstration of lower than normal CO and NO levels may be useful as an additional noninvasive method to evaluate \(\alpha_1\)-AT deficiency in individuals with a severe, early onset of obstructive lung disease.

Exhaled CO is increased in \(\alpha_1\)-AT deficiency (17). Exhaled NO has been proposed as a marker of lung inflammation (17). In this context, it is surprising that exhaled NO levels of individuals with PI \(\alpha_1\)-AT deficiency are lower than NO levels of healthy controls or of individuals with PI M heterozygous phenotypes or non-\(\alpha_1\)-AT-deficient COPD (22).

Recently, exhaled carbon monoxide (CO) has also been proposed as a marker of lung inflammation (18). Produced by heme oxygenases (HO), CO is increased in the exhaled breath of individuals with smoking-related COPD (23). On the basis of this, we hypothesized that exhaled CO is increased in \(\alpha_1\)-AT deficiency and that levels may be related to the severity of lung disease. To evaluate this, the exhaled gases, including NO, CO, CO2, and O2, were measured in individuals with \(\alpha_1\)-AT deficiency compared with healthy controls and individuals with smoking-related COPD.

METHODS

Study population. The study population included individuals with \(\alpha_1\)-AT deficiency, COPD related to previous cigarette smoke exposure, and healthy controls. Individuals with PI \(\alpha_1\)-AT deficiency and serum \(\alpha_1\)-AT levels below the protective threshold value of 11 \(\mu\)mol/l were identified at an educational patient-oriented meeting organized by the Cleveland Clinic Foundation. Healthy control individuals were identified by absence of pulmonary symptoms or history of pulmonary disease. COPD diagnosis was based on American

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Thoracic Society guidelines (27a). Exclusion criteria included current cigarette smoking, asthma, recent respiratory tract infection, and exacerbation of lung disease within the previous 6 wk.

The study was approved by the Institutional Review Board, and informed consent was obtained from all volunteers.

Of line collection and measurement of exhaled gases. As previously described (20), exhaled gases were obtained by an off-line method in agreement with American Thoracic Society recommendations for exhaled NO determination. Briefly, individuals inhaled NO-free air to total lung capacity and exhaled against 10 cmH2O pressure, to meet the American Thoracic Society recommended flow rate of 3.55 l/s, into a Mylar collection bag (Physiological Measurement Systems, Bay Village, OH). All individuals were seated at rest for at least 15 min before gases were collected. Exhaled CO and CO2 were measured in the exhaled gases with a Siemens Ultramat 6 infrared analyzer (Karlsruhe, Germany) that was adapted for use in this study. The analyzer was calibrated daily by using CO-free gas and a gas with a known CO and CO2 concentration. The analyzer was sensitive to a concentration of 100 ppb for CO and 0.1% for CO2. Absorbed wavelengths for CO and CO2 are characteristic and separable to the individual gases so that CO2 interference with CO does not occur (20). NO concentrations were determined by using a chemiluminescence analyzer (Sievers Instruments, Boulder, CO). A Teledyne UPO-130 microfuel O2 sensor (City of Industry, CA) was used for determination of exhaled O2 levels. The O2 analyzer was calibrated by using zero air, followed by high-gain calibration with 100% O2 (Praxair, Cleveland, OH). Zero air was prepared by passing ultrapure nitrogen (99.999% pure nitrogen; PraxAir) through a NuPure II Eliminator room temperature purifier for inert gases (Manotick, Ontario, Canada). The gas purifier reduces gaseous impurities to concentrations of <1 part/billion for O2, CO2, CO, hydrogen dioxide, hydrogen, and methane. Purified gas was then collected and used as a zero calibration gas for the analyzers.

Statistical analysis. Quantitative data are summarized as means ± SE; categorical data are summarized by frequencies. Associations between pairs of variables are described by Pearson’s correlation coefficient and a test for nonzero correlation. Two-tailed t-test statistics, χ2, ANOVA, and ANOVA on ranks were utilized where appropriate, with the Bonferroni correction being applied to the significance criterion once pairwise comparisons were made among the study groups.

<table>
<thead>
<tr>
<th>Table 1. Clinical characteristics of study population</th>
<th>α1-AT</th>
<th>Control</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Age, yr</td>
<td>50 ± 2</td>
<td>33 ± 2</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>8/11</td>
<td>13/9</td>
<td>8/4</td>
</tr>
<tr>
<td>Serum α1-AT level, µmol/l</td>
<td>4.0 ± 1.2</td>
<td></td>
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<tr>
<td>Cigarettes, pack/yr</td>
<td>19 ± 3</td>
<td>0</td>
<td>60 ± 7</td>
</tr>
<tr>
<td>Ever received augmentation therapy (%)</td>
<td>11 (84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhaled steroids (%)</td>
<td>12 (63)</td>
<td>5 (38)</td>
<td></td>
</tr>
<tr>
<td>FEV1, %predicted</td>
<td>52 ± 8</td>
<td>53 ± 5</td>
<td></td>
</tr>
<tr>
<td>FVC, %predicted</td>
<td>83 ± 5</td>
<td>82 ± 6</td>
<td></td>
</tr>
<tr>
<td>DLCO, %predicted</td>
<td>62 ± 6</td>
<td>61 ± 5</td>
<td></td>
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</tbody>
</table>

Values are means ± SE. FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; DLCO, lung CO-diffusing capacity; AT, antitrypsin; COPD, chronic obstructive pulmonary disease. *P < 0.001.

RESULTS

Clinical characteristics. The characteristics of the study population are shown in Table 1. Patients with COPD were significantly older than individuals with α1-AT deficiency, and both groups were significantly older than healthy controls (P < 0.001). Individuals with COPD had a significantly longer smoking history than α1-AT-deficient individuals (P < 0.001). Inhaled corticosteroid use was similar in both groups (P = 0.42), as was percent predicted forced vital capacity (P = 0.92), forced expiratory volume in 1 s (P = 0.91), and lung CO-diffusing capacity (P = 0.86). Four patients were on supplemental O2 [α1-AT deficiency (n = 2) and COPD (n = 2)]. For analyses of correlation between exhaled O2 and CO2 and exhaled O2 levels, individuals on supplemental O2 were excluded.

Exhaled gases. NO levels were lower in α1-AT-deficient patients than in healthy controls or COPD patients (Table 2, Fig. 1). NO did not correlate with lung function in α1-AT-deficient or COPD patients (all P > 0.1). However, NO correlated inversely with exhaled O2 (r = -0.575, P = 0.015) and directly with CO2 in α1-AT-deficient patients (r = 0.465, P = 0.044) (Fig. 2). In contrast, NO was unrelated to exhaled O2 or CO2 in both the control and COPD groups.

The linear fit of the NO-O2 and NO-CO2 data reveals the following relationships between the exhaled gases

$$[\text{NO}] = 40 - \frac{[\text{O}_2]}{0.5}$$  \hspace{1cm} (1)

$$[\text{NO}] = 0.7 + \frac{[\text{CO}_2]}{0.6}$$  \hspace{1cm} (2)

where [NO], [O2], and [CO2] denote concentrations of NO, O2, and CO2, respectively.

Like NO, CO levels were lower in α1-AT-deficient patients than in controls or individuals with COPD (Table 2, Fig. 1). CO levels did not correlate with lung function or any other exhaled gas (all P > 0.1).

For both α1-AT-deficient and COPD patients, exhaled CO2 levels were lower than controls (Table 2, Fig. 1). Exhaled O2 levels were only higher in α1-AT-deficient patients (Table 2, Fig. 1). Exhaled CO2 correlated inversely with O2 in all study groups, which reflected the relationship between O2 uptake and CO2 release from the lung (all P < 0.001) (Fig. 3). Lung

<table>
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<th>Table 2. Exhaled gases in study groups</th>
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<tr>
<td>α1-AT Deficiency</td>
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<td>------------------</td>
</tr>
<tr>
<td>NO, ppb</td>
</tr>
<tr>
<td>CO, ppm</td>
</tr>
<tr>
<td>CO2, %</td>
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<tr>
<td>O2, %</td>
</tr>
</tbody>
</table>

Values are means ± SE. \*P < 0.01 vs. control. †P < 0.01 vs. COPD.

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function did not correlate with levels of either of the two gases (all $P > 0.1$). Age did not correlate with exhaled gas values or with lung function in any of the study groups (all $P > 0.05$), which is consistent with previous observations (7).

Interestingly, the linear fit of the $O_2$-$CO_2$ data reveals different relationships between the exhaled gases among the different groups (analysis of covariance, $P = 0.001$).

For controls

$$[O_2] = 19.6 - \frac{[CO_2]}{1.6} \quad (3)$$

For $\alpha_1$-AT deficiency

$$[O_2] = 20.7 - \frac{[CO_2]}{0.9} \quad (4)$$

For COPD

$$[O_2] = 21.3 - \frac{[CO_2]}{0.8} \quad (5)$$

The different slope of the fitted lines for control and obstructive lung disease populations suggests that $O_2$ uptake and $CO_2$ release from the lungs of individuals with obstructive lung disease maybe less efficient than in controls or that metabolic consumption of $O_2$ is altered in obstructive lung disease.

**Effect of treatment on exhaled NO and CO.** Previous studies have shown that inhaled corticosteroids (ICS) lower exhaled NO (19) and CO (31). To determine whether the lower levels of NO and CO in individuals with $\alpha_1$-AT deficiency were related to steroid use, we...
evaluated individuals by type of therapy. NO levels were similar in α1-AT-deficient individuals irrespective of inhaled corticosteroid use [NO (ppb): −ICS 4.7 ± 0.8; +ICS 5.9 ± 1; P = 0.4]. Similarly, COPD individuals on ICS had NO levels similar to those not receiving steroids [NO (ppb): −ICS 13 ± 3.2; +ICS 16.5 ± 1.6; P = 0.41]. CO was similar in the α1-AT-deficient group with or without ICS use [CO (ppm): −ICS 0.6 ± 0.2; +ICS 0.3 ± 0.06; P = 0.22] and in the COPD group with or without ICS use [CO (ppm): −ICS 1.6 ± 0.4; +ICS 1.5 ± 0.6; P = 0.95]. It is possible, however, that, because of the small number of patients in each group, a lack of effect may be due to lack of statistical power. Although there was no difference in the exhaled gases of the steroid-naive vs. the steroid-treated groups, reanalysis of data was performed, excluding those individuals receiving corticosteroids. The results were similar to those seen in the whole study population [NO (ppb): α1-AT deficient 4.7 ± 0.8; control 8.6 ± 0.6; COPD 13 ± 3.2; P = 0.002; CO (ppm): α1-AT deficient 0.6 ± 0.2; control 1.3 ± 0.11; COPD 1.6 ± 0.4; P = 0.014].

Exhaled NO and CO levels of α1-AT-deficient individuals receiving augmentation therapy were similar to levels of those who were not [NO (ppb): augmentation 5.7 ± 1; no augmentation 5.2 ± 1; P = 0.73; CO (ppm): augmentation 0.3 ± 0.05; no augmentation 0.6 ± 0.2; P = 0.19].

DISCUSSION

The results of this study show that exhaled gases of α1-AT-deficient individuals are markedly altered compared with healthy controls, and, unexpectedly, compared with individuals with non-α1-AT-deficient COPD. Exhaled CO and NO are lower in patients with α1-AT-deficiency than in healthy controls and individuals with COPD. Furthermore, CO2 levels are lower than controls in both α1-AT-deficient and COPD patients, whereas exhaled O2 in α1-AT-deficient patients is higher than in controls or COPD patients. Alterations in airflow or minute ventilation in COPD and α1-AT-deficient patients may be the cause of the decreased CO2 seen in these patients, which had the same degree of pulmonary dysfunction, compared with healthy controls. In contrast to the parallel change in CO2, derangements of CO and NO were distinct between COPD and α1-AT-deficient groups. Hence, minute ventilation or alterations in airflow are not likely causes of the changes seen.

Several potential mechanisms may account for the decreased NO in α1-AT deficiency, including increased consumption or decreased production of NO. NO is consumed by its interaction with superoxide produced during neutrophil activation (17), which has been proposed as one mechanism for the low exhaled NO of patients with cystic fibrosis (1, 4, 29). Neutrophil dominance in α1-AT-deficient airways with increased superoxide production may increasingly consume NO. In addition, neutrophil enzymes such as myeloperoxidase consume nitrite (13), which is considered a storage pool of NO in the airway and another source of exhaled NO. Depletion of nitrite may thus contribute to the decrease in exhaled NO (9). The combination of NO with superoxide or nitrite consumption by myeloperoxidase both lead to reactive nitrogen species formation, e.g., peroxynitrite, which may further worsen inflammation and lung injury. Individuals with COPD also have neutrophilic influx and higher neutrophil numbers in the airways (28). However, it is possible that because of the relatively unopposed effects of neutrophilic proteolytic activity seen in α1-AT deficiency, a higher degree of NO consumption may still occur. Interestingly, polymorphisms in the NOS III gene have been associated with severity of lung disease in α1-AT-deficient patients (24). Although mutation in this site of NOS III has not been shown to affect the activity or turnover of protein (8), other unrecognized mutations may affect the activity of the enzyme and consequently NO production. In contrast to low NO in α1-AT deficiency, COPD patients have high levels of NO. Thus the finding of lower than control values of NO in individuals with obstructive lung disease may suggest a genetic cause for airway limitation, including α1-AT deficiency, cystic fibrosis, or primary ciliary dyskinesia (18). On the other hand, there are many potential explanations for the difference in NO levels, including distribution of lung destruction, inflammatory cell concentration, and/or type and extent of airways disease, which may have little direct relationship to α1-AT deficiency.

Although evidence supports a primary airway source of exhaled NO, there is considerable evidence suggesting that alveolar production is a significant source of exhaled CO (18). Tissue expression of inducible HO-1, predominantly in alveolar macrophages, and constitutive HO-2, predominantly in lung parenchyma, is increased in cigarette smoke-exposed lungs irrespective of COPD compared with non-smoke-exposed lungs (21).
This suggests that HO is one source of the increased exhaled CO seen in COPD. CO levels decrease in asthma immediately after experimental antigen challenge, perhaps due to decreased diffusion into gas space from lung tissues, which also supports an alveolar source for the gas (20). In this context, impaired diffusion and alveolar destruction are likely mechanisms for the low exhaled CO in patients with emphysema due to α1-AT deficiency. This may not be as prominent in the COPD population, a more heterogeneous group in terms of their pathological manifestations ranging from chronic bronchitis to emphysema. On the other hand, a polymorphism in the HO-1 gene promoter is associated with susceptibility to the development of cigarette smoke-related emphysema (30). The polymorphism leads to a diminished HO-1 response to oxidative stress (e.g., as with cigarette smoke exposure). The relationship of HO polymorphisms to lung disease in general is unknown, but CO administration has been shown to exert protective anti-inflammatory effects in experimental models of lung injury, suggesting that a decrease in CO production could contribute to oxidative stress and development of emphysema (25, 26).

Lower exhaled CO2 levels in α1-AT deficiency and COPD likely reflect the impairment in gas exchange associated with obstructive lung disease, although we cannot exclude some degree of hyperventilation secondary to the presence of obstructive lung disease causing a decrease in exhaled CO2. Exhaled O2 is significantly higher in α1-AT-deficient patients than in controls or COPD patients and is inversely related to NO. Parenchymal destruction associated with emphysema may be a less likely cause for the decreased O2 uptake because COPD individuals had similar lung function to those with α1-AT deficiency. However, the lower than normal NO in α1-AT deficiency may be detrimental to O2 uptake. NO promotes pulmonary arterial vasodilatation and plays a central role in ventilation-perfusion matching (3, 6). Furthermore, NO may play an important role in O2 uptake and delivery to peripheral tissues by regulating vascular tone in response to tissue O2 tension (10, 27). Thus diminished NO in α1-AT deficiency may contribute to the derangements in ventilation-perfusion matching and to tissue oxygenation leading to less O2 uptake and higher exhaled O2.

In conclusion, individuals with α1-AT deficiency have low exhaled levels of NO and CO compared with healthy controls and patients with non-α1-AT-deficient COPD. Although the precise mechanisms responsible for these findings remain unclear, the effects do not seem to be related to lung function or inhaled corticosteroid use.

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