Increased Exhalation of Hydrogen Peroxide in Patients with Stable and Unstable Chronic Obstructive Pulmonary Disease

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An imbalance between oxidative stress and antioxidative capacity is thought to play an important role in the development and progression of chronic obstructive pulmonary disease (COPD). To assess the lung oxidative status in patients with COPD, we studied whether exhaled hydrogen peroxide (H₂O₂) is increased in breath condensate of patients with stable COPD (n = 12, mean FEV₁ 51% pred) and in patients with exacerbated COPD (n = 19, actual FEV₁ 36% pred) compared with a healthy control group (n = 10, FEV₁ 108% pred). Expired breath condensate during 15 min of tidal breathing was collected by cooling. The concentration of H₂O₂ was measured spectrophotometrically by means of horse radish peroxidase-catalyzed oxidation of tetramethylbenzidine. Concentrations of H₂O₂ (mean ± SEM) were significantly elevated at 0.205 ± 0.054 nM in patients with stable COPD compared with 0.029 ± 0.012 nM in the control group (p < 0.05) and were further increased to 0.600 ± 0.075 nM in patients with acutely exacerbated COPD (p < 0.001 compared with patients with stable COPD). Patients with pulmonary infiltrates on chest radiograph showed similar values compared with patients without obvious infiltrates. These findings demonstrate that patients with stable COPD exhibit increased oxidant production in the airways and that oxidant production increases further during exacerbations.
TABLE 1
CHARACTERISTICS OF PATIENTS WITH STABLE COPD

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, sex</th>
<th>Smoking*</th>
<th>PaO₂ (kPa)</th>
<th>PacO₂ (kPa)</th>
<th>FEV₁ (% pred)†</th>
<th>H₂O₂ (μM)</th>
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* Smoking: C = current, E = ex.
† FEV₁: actual value at the morning of collection of breath condensate.

3 mo (FEV₁, 108 ± 6% pred [12]). Patients with COPD were included if postbronchodilator FEV₁ was below 60% pred and/or FEV₁/V̇₆₅ was below 60%. All patients were classified as smokers or exsmokers (i.e., discontinuation of smoking for at least 3 mo) based on medical history. Exclusion criteria were pulmonary disorders at present or in the past possibly contributing to chronic airflow obstruction (e.g., tuberculosis, sarcoidosis, bronchiectasis, and asthma), clinical signs of bronchial hyperreactivity and/or acute response to an inhaled bronchodilator of more than 15% of predicted value, chronic airflow obstruction without smoking in the past, regular intake of vitamin C and E, and treatment with oral or inhaled N-acetylcysteine. Patients with stable COPD (n = 12; mean [± SEM] age, 70 ± 3 yr; mean FEV₁, 51 ± 4% pred) were defined as having no increase in symptoms and no exacerbations in the previous 3 mo. They were on maintenance therapy with inhaled bronchodilators, oral or intravenous corticosteroids, or antibiotics. In this group, 19 patients were studied (mean [± SEM] age, 69 ± 2 yr; FEV₁, measured within 6 mo before the exacerbation, 49 ± 4% pred). Patients with signs of upper respiratory tract infection were excluded. Measurements in the acute patients were performed on the first or second day after consultation at the outpatient clinic or at hospitalization. Only patients who sought medical attention within 1 wk after the start of exacerbation symptoms were included in the study. All received supplemental oxygen, which was discontinued for at least 30 min before the collection of exhaled breath condensate. The study was approved by the hospital ethics committee; informed consent was obtained from all subjects.

Collection of expired breath and measurement of H₂O₂. The samples were collected in the morning, approximately 1 h after inhalation of the patient's own bronchodilator. Current smokers were requested to refrain from smoking after midnight. First, FEV₁ was measured. Subsequently, the participants were breathing through a face mask with a two-way valve. The expired air was conducted through a tube with a col-

TABLE 2
CHARACTERISTICS OF PATIENTS WITH EXACERBATED COPD

<table>
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<th>Patient</th>
<th>Age, sex</th>
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<th>PacO₂ (kPa)</th>
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* Smoking: C = current, E = ex.
† FEV₁: actual value at the morning of the collection of breath condensate.
‡ Arterial blood gas obtained after at least 30 minutes of breathing ambient air.
n.m. = not measured.
lacting system which was placed in ice. The collecting system was con-
ected to a 2 ml sterile plastic tube. In this way, approximately 1 ml of
breath condensate was collected within 15 min of tidal breathing. The
samples were immediately placed in liquid nitrogen. Measurements of
H2O2 were performed within 6 h after sample collection, since prelimi-
nary data showed that H2O2 concentrations did not change during this
period. The method described by Gallati and Pracht was applied (13).
Briefly, 100 µl of 420 µM 3',3',5',5'-tetramethylbenzidine (dissolved in
0.42 M citrate buffer, pH 3.8) and 10 µl of 52.5 U/ml of horseradish
peroxidase (HRP; Sigma Chemicals) were added to 100 µl of the con-
densate. The reaction proceeded for 20 min at room temperature. Sub-
sequently, the mixture was acidified to a pH of 1 with 10 µl of 18 N
sulfuric acid. The reaction product was measured spectrophotometri-
cally at 450 nm using an automated microplate reader (model EL312;
Bio-Tek Instruments Inc.). The absorbance is directly proportional to
the concentration of H2O2. The detection limit was approximately 0.1
µM H2O2. All samples were measured in duplicate; mean values were
used for subsequent analysis.

Statistical analysis. Data between the three groups were compared
using one-way analysis of variance followed by Duncan's multiple range
test. A Wilcoxon test was performed to test differences between patients
with or without pulmonary infiltrates. Spearman correlation tests were
performed to detect a correlation between the concentration of exhaled
H2O2 and clinical markers of severity of the disease such as FEV1 and
Pao2. All values are presented as mean ± SEM; statistical significance
was assumed at p < 0.05.

RESULTS

Normal subjects had the lowest concentration of H2O2 in breath condensate (0.029 ± 0.012 µM) (Figure 1). No H2O2 was detected in
6 of 10 healthy subjects. In contrast, patients with stable COPD exhibited an increased concentration of exhaled H2O2 (0.205 ± 
0.054 µM, p < 0.05 compared with normal subjects). Hydrogen
peroxide was not detected in 2 of these 12 subjects. Finally, H2O2
was detected in all samples of the patients with exacerbated COPD and was elevated in comparison with patients with stable
COPD (0.600 ± 0.075 µM, p < 0.001).

Patients with an exacerbation with pulmonary infiltrates on chest radiograph (n = 5) showed similar values compared with
patients without obvious infiltrates (n = 14) (0.601 ± 0.166 µM and
0.599 ± 0.087 µM, respectively). In the group of patients
with stable COPD, those who still smoked (n = 4, FEV1, 61 ± 1%
pred) exhaled 0.077 ± 0.044 µM, whereas the exsmokers (n =
8, FEV1, 47 ± 6% pred) exhaled 0.269 ± 0.068 µM (current versus
exsmokers, p = 0.094). No significant correlations were found
between the levels of H2O2 and FEV1 (r = 0.13) or Pao2 (r =
0.16) in patients with stable and unstable COPD.

DISCUSSION

This study shows increased H2O2 concentrations in exhaled breath condensate in patients with stable COPD, providing direct evi-
dence of increased production of ROS in the airways of these patients. A further increase was observed in patients with an ex-
acerbation.

Concentrations of H2O2 measured in exhaled breath condensate
may be considered as the net result of production versus scavenging of H2O2. An increased production of H2O2 may be
caused by an increased number of lung inflammatory cells and/or
increased production of H2O2 by these cells (7). An increased
number of these cells have been found in the lungs of patients
with stable COPD. Both bronchial lavage and bronchoalveolar
lavage fluid obtained from smokers with COPD contained more
neutrophils than found in fluid obtained from healthy nonsmok-
ers (14, 15). Increased numbers of macrophages and T lympho-
cytes were present in the bronchial mucosa of patients with
chronic bronchitis and airflow obstruction compared with healthy
nonsmokers (16). Exacerbations of chronic bronchitis in patients
with mild COPD (mean FEV1, approximately 66% pred) were
associated with increased numbers of eosinophils, neutrophils,
and T lymphocytes in the bronchial wall (6).

Indications of increased activation and production of ROS
by these cells have been observed in smokers (3, 4, 7, 17). Bron-
choalveolar lavage fluid from smokers contained increased num-
bers of AM with higher densities, which produced more superox-
dide anion radicals (17). In addition, AM from smokers with a
recent lower respiratory tract infection released increased quanti-
ties of H2O2 (7).

The production of ROS may be reduced, at least in vitro, by
medications such as β2 sympathomimetic drugs and theophyl-
line (18). Whether this effect also occurs in vivo is unknown. Na-
tural protection against an increased concentration of H2O2 is
provided by catalase and—of particular importance in the lungs—
glutathione. Only a few studies have addressed the antiox-
dative capacity of the lungs of smokers (4, 15, 19). Reduced
activities of superoxide dismutase, glutathione S-transferase, and
glutathione peroxidase were found in the AM of elderly smokers
(4). In contrast, enhanced activities of superoxide dismutase and
catalase, but not of glutathione peroxidase, were shown in the
AM of younger smokers (19). Linden and colleagues (15) demon-
strated an increased concentration of total glutathione (free and
disulfide bound) in bronchoalveolar lavage fluid obtained from
patients with COPD who were current smokers. It is unknown
whether this increase in total glutathione is caused by continued
smoking or by COPD itself (i.e., also occurring in exsmokers).

In contrast to our findings, Sznajder and colleagues (10) found
significantly higher concentrations of exhaled H2O2 in patients
with acute respiratory failure and pulmonary infiltrates compared
with those without. These authors also noted that central nerv-
ous system involvement in these patients increased exhaled H2O2
levels compared with those patients without involvement; more-
over, patients with infiltrates and sepsis exhaled higher levels of
H2O2 than those without sepsis. Thus, the differences observed
in the study by Sznajder and colleagues may not be attributed
solely to the presence or absence of infiltrates.

The current smokers in the group with stable COPD tended
to exhale lower concentrations of H2O2 compared with the ex-
smokers. The number of patients studied, however, was too small
to make any conclusions about the effects of smoking in this
respect. In addition, bias may have occurred in our separation.
References