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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 (H_2O_2) is increased in breath condensate of patients with stable COPD ($n = 12$, mean FEV_1 51% pred) and in patients with exacerbated COPD ($n = 19$, actual FEV_1 36% pred) compared with a healthy control group ($n = 10$, FEV_1 108% pred). Expired breath condensate during 15 min of tidal breathing was collected by cooling. The concentration of H_2O_2 was measured spectrophotometrically by means of horse radish peroxidase-catalyzed oxidation of tetramethylbenzidine. Concentrations of H_2O_2 (mean \pm SEM) were significantly elevated at $0.205 \pm 0.054 \mu M$ in patients with stable COPD compared with $0.029 \pm 0.012 \mu M$ in the control group ($p < 0.05$) and were further increased to $0.600 \pm 0.075 \mu M$ 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. **Dekhuijzen PNR, Aben KKH, Dekker I, Aarts LPHJ, Wielders PLML, Van Herwaarden CLA, Bast A. Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease.**

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Reactive oxygen-derived species (ROS) have been implicated in the pathogenesis and pathophysiology of tobacco smoke-induced chronic obstructive pulmonary disease (COPD) (1). This contention is supported by several findings. Cigarette smoke is a rich source of oxidants. Smokers have increased numbers of macrophages and neutrophils in their alveoli (2), and these cells are activated and produce increased amounts of ROS (3, 4). Oxidative inactivation of methionine residues of α_1 -proteinase inhibitor may contribute to the onset and progression of emphysema (5).

Production of ROS is likely to increase further during an acute exacerbation because of the accompanying increased numbers of inflammatory cells in the lower airways (6). In addition, alveolar macrophages (AM) in smokers with a recent lower respiratory tract infection were found to release increased numbers of ROS (7).

Direct *in vivo* evidence of increased concentrations of ROS in patients with COPD has not been provided, however. In this

respect, exhalation of hydrogen peroxide (H_2O_2) is of potential interest. Hydrogen peroxide is a harmful ROS because it is relatively stable, it can cross membranes due to its small size and its lack of charge, and it can generate the highly reactive hydroxyl radical in the presence of superoxide anions and iron (8). Clinically, increased levels of exhaled H_2O_2 have been demonstrated in children with asthma (9) and in patients with adult respiratory distress syndrome and acute hypoxemic respiratory failure (10, 11).

We hypothesized that an increased oxidative burden in the lungs of patients with COPD would be reflected in increased levels of exhaled H_2O_2 . The present study was undertaken to answer two questions: 1) Is the concentration of exhaled H_2O_2 increased in patients with stable COPD compared with normal subjects, and 2) Are levels of exhaled H_2O_2 increased even further during exacerbations of COPD?

METHODS

Subjects. Three groups of subjects were studied (Tables 1 and 2): normal control subjects, patients with stable COPD, and patients with acutely exacerbated COPD. Normal control subjects ($n = 10$; mean [\pm SEM] age, 53 ± 4 yr) were never-smokers with no pulmonary disorders and no signs of upper or lower respiratory tract infection in the previous

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TABLE 1
CHARACTERISTICS OF PATIENTS WITH STABLE COPD

Patient	Age, sex	Smoking*	PaO ₂ (kPa)	Paco ₂ (kPa)	FEV ₁ (% pred) [†]	H ₂ O ₂ (μM)
1	63, M	C	9.2	5.8	63	0.000
2	72, M	E	8.9	5.6	36	0.196
3	55, M	C	8.3	4.8	57	0.157
4	73, M	E	6.6	5.8	38	0.052
5	86, M	E	7.2	5.2	55	0.210
6	74, M	E	9.7	6.3	18	0.052
7	58, M	C	9.4	4.8	61	0.000
8	64, F	C	8.6	4.6	63	0.151
9	82, F	E	6.2	5.8	62	0.571
10	60, M	E	10.7	4.0	70	0.212
11	74, M	E	9.3	5.7	49	0.512
12	80, F	E	8.2	5.3	45	0.345
Mean	70		8.5	5.3	51	0.205
SEM	3		0.4	0.2	4	0.054

* 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/vital capacity ratio 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; one used inhaled corticosteroids, and none received oral steroids. An exacerbation was defined by an acute deterioration of breathlessness, mostly with increased coughing and production of purulent sputum, for which additional medication was indicated (in-

haled 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

Patient	Age, sex	Systemic steroids	Smoking*	PaO ₂	Paco ₂	FEV ₁ (% pred) [†]	H ₂ O ₂ (μM) [‡]
1	65, M	x	E	6.7	5.6	25	0.148
2	75, M	x	E	8.5	5.7	26	0.276
3	73, M	x	E	8.2	4.9	55	0.591
4	62, M	x	E	8.7	4.5	32	0.307
5	72, M		E	8.5	5.3	20	1.111
6	59, F		C	8.4	4.6	28	0.719
7	83, M	x	E	8.4	4.9	29	0.559
8	51, F	x	E	8.8	4.9	46	0.837
9	60, M	x	E	7.3	6.8	21	0.636
10	82, F	x	E	8.3	5.7	78	0.343
11	76, M	x	E	8.9	5.1	23	1.023
12	79, F	x	E	6.2	5.8	n.m.	0.396
13	70, F	x	E	6.3	8.4	20	0.761
14	57, M	x	E	7.1	5.2	22	0.558
15	60, M	x	E	9.6	5.6	31	0.326
16	76, F	x	E	7.6	5.3	63	0.329
17	63, M	x	C	9.7	5.7	29	0.204
18	64, M	x	E	8.5	5.3	70	1.063
19	74, M	x	E	7.0	5.6	35	1.208
Mean	69			8.0	5.5	36	0.600
SEM	2			0.2	0.2	4	0.075

* 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.

of smokers and exsmokers, which was based solely on medical history.

Hydrogen peroxide is produced not only in the lower but also in the upper airways. Therefore, patients with clinically suspected upper airway infection were excluded from the study in order to avoid a major contribution of these airways to total H_2O_2 exhalation. Differences in minute ventilation and breathing pattern may have occurred among the different groups. This, however, was not likely to account for the differences observed in exhaled H_2O_2 levels, as changes in minute ventilation and breathing pattern did not alter H_2O_2 exhalation in animal experiments (10).

In conclusion, our data show that increased H_2O_2 occurs in subjects with stable COPD and even more so in patients with exacerbated COPD. These data are consistent with the concept that ongoing airway inflammation with elevated production of ROS increases lung oxidative stress in patients with stable COPD.

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