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Hydrogen peroxide in exhaled air is increased in stable asthmatic children

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Hydrogen peroxide in exhaled air is increased in stable asthmatic children. Q. Jöbbsis, H.C. Raatgeep, P.W.M. Hermans, J.C. de Jongste. ©ERS Journals Ltd 1997.

ABSTRACT: Exhaled air condensate provides a noninvasive means of obtaining samples from the lower respiratory tract. Hydrogen peroxide (H_2O_2) in exhaled air has been proposed as a marker of airway inflammation. We hypothesized that in stable asthmatic children the H_2O_2 concentration in exhaled air condensate may be elevated as a result of airway inflammation.

In a cross-sectional study, 66 allergic asthmatic children (of whom, 41 were treated with inhaled steroids) and 21 healthy controls exhaled through a cold trap. The resulting condensate was examined fluorimetrically for the presence of H_2O_2 . All subjects were clinically stable, nonsmokers, without infection.

The median H_2O_2 level in the exhaled air condensate of the asthmatic patients was significantly higher than in healthy controls (0.60 and 0.15 μmol , respectively; $p < 0.05$), largely because of high values in the stable asthmatic children who did not use anti-inflammatory treatment (0.8 μmol ; $p < 0.01$ compared to controls).

We conclude that hydrogen peroxide is elevated in exhaled air condensate of children with stable asthma, and may reflect airway inflammation.

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Our present understanding of asthma as a disease of airway inflammation has been greatly enhanced by studies using bronchoalveolar lavage (BAL) [1]. In young children, BAL is generally not acceptable for diagnostic and monitoring purposes. A noninvasive method to assess airway inflammation is important for this group [2]. Exhaled air condensate has been proposed as a noninvasive means of obtaining samples from the lower respiratory tract, based on the hypothesis that aerosol particles excreted in breath may reflect the composition of the lower airway fluids [3]. Several investigators have analysed exhaled air condensate for different mediators of inflammation [3–5]; however, data on children are scanty [5].

Hydrogen peroxide (H_2O_2) is a putative marker of airway inflammation in exhaled air: inflammatory cells produce H_2O_2 [6], which causes lung inflammation and damage [7–9]; stimulated alveolar macrophages from asthmatics generate more reactive oxygen species than alveolar macrophages from healthy subjects [10]. Indeed, an increased content of H_2O_2 has been described in various inflammatory lung disorders: in exhaled air of cigarette smokers [11]; patients with adult respiratory distress syndrome (ARDS) [12, 13]; and patients with acute hypoxaemic respiratory failure [14]. DOHLMAN *et al.* [5] described an increased H_2O_2 concentration in exhaled air condensate in a small group of asthmatic children, mainly in those with acute disease. As inflammation is also likely to be present in symptom-free asthmatics [15], we hypothesized that the H_2O_2 concentration may also be elevated in exhaled air condensate of children with stable asthma, and may serve as a marker of airway inflammation.

Patients and methods

Study population

Sixty six asthmatic children (23 girls and 43 boys) attending the out-patient clinic of Sophia Children's Hospital were included in the study. Asthma was diagnosed on clinical grounds following international guidelines [16]. Appropriate therapy was prescribed by the patient's own physician, and had not been changed during the 3 months preceding the study. All 66 patients used an inhaled bronchodilator on demand, and 41 (15 girls and 26 boys) used an inhaled corticosteroid daily. The median age of those without steroids was 12.6 yrs, and of those receiving steroids was 11.1 yrs. All were lifelong nonsmokers and were clinically stable. All had bronchial hyperresponsiveness (provocative dose of inhaled methacholine that produced a 20% fall in forced expiratory volume in one second (PD₂₀) of $< 150 \mu\text{g}$) documented in the past, and had allergy as documented by radio allergosorbent test (RAST) class 2 or higher for at least one common airborne allergen. Control subjects were healthy, nonsmoking young adult volunteers (median age 25 yrs), with no history of allergy and respiratory disease, nor of any other chronic illness, and used no medication. None of the subjects had had symptoms of acute respiratory infection within the month before the condensate was collected. The study was approved by the Hospital Ethical Committee.

Collection of exhaled air condensate

Exhaled air condensate was obtained by passing expired air through a 50 cm double-jacketed glass tube cooled to a temperature of 0°C by means of countercurrent circulating iced water. The subjects breathed through a mouthpiece and a two-way nonrebreathing valve (Rudolph, Kansas City, USA), which also served as a saliva trap. They were asked to breathe at a normal frequency and tidal volume, wearing a noseclip, for a period of 10–15 min. The condensate, at least 1 mL, was collected on ice.

Lung function

All subjects underwent flow-volume measurements immediately after collection of the condensate. Flow-volume curves were obtained in triplicate, using a Lilly-type pneumotachograph (Masterlab Jaeger, Würzburg, Germany) before and after inhalation of 1 mg of terbutaline powder (Turbohaler®). Results were expressed as percentage predicted [17].

Hydrogen peroxide

The concentration of H₂O₂ in exhaled air condensate was determined by means a fluorimetric assay based on the reaction of H₂O₂ with horseradish peroxidase to form a compound which oxidizes p-hydroxyphenylacetic acid to a fluorescent product [18]. Briefly, 400 µL of condensate was mixed with 1.5 mM (10 µL) p-hydroxyphenylacetic acid and 100 µg·mL⁻¹ (4 µL) horseradish peroxidase (both Sigma Chemical Co., St. Louis, MO, USA) immediately after collection, and frozen at -20°C. The fluorescent product of the condensate and of standard solutions of H₂O₂ were measured with a fluorimeter (model 3000; Perkin-Elmer, Norwalk, USA) at an excitation wavelength of 295 nm and an emission wavelength of 405 nm. The lower limit of H₂O₂ detection was 0.1 µM. Concentrations of H₂O₂ in condensate were obtained by linear interpolation of a standard curve. Preliminary observations in a limited number of young healthy adults indicated that, with this method, the within-subject between-days variations were within 0.1 µmol.

Data analysis

Results are expressed as median and range because of a nonsymmetrical distribution and because some values were below the detection limit. Since the anti-inflammatory action of inhaled steroids could be a confounding factor influencing H₂O₂ in exhaled air condensate as marker of airway inflammation, subgroups with and without steroids were analysed separately. Comparisons between groups were made by the Mann-Whitney test for two unpaired independent samples. A two tailed p-value of less than 0.05 was considered significant. There were insufficient data to perform a formal power calculation, but we reasoned that with a group size of 20 subjects we should be able to detect a 1 SD difference (our own preliminary observations suggested this to be about 20%) at a two-sided alpha of 0.05 with a power of 90%.

Results

All subjects completed the protocol without difficulty. The time to collect at least 1 mL of condensate varied between 10–15 min.

Lung function measurement in asthmatic children without steroids showed near normal values (mean forced vital capacity (FVC) 93% pred, forced expiratory volume in one second (FEV₁) 100% pred, increase in FEV₁ after 1 mg inhaled terbutaline 7%). Asthmatics with steroids were slightly better (FVC 100% pred, FEV₁ 107% pred, increase in FEV₁ after terbutaline 6%; but not significantly different from the children without steroids). Controls all had a normal lung function (mean FVC 108% pred, FEV₁ 111%, increase in FEV₁ after terbutaline 3%).

The median H₂O₂ concentration in condensates of all asthmatic children was significantly higher than in condensate from healthy control subjects (0.60 and 0.15 µmol, respectively; p<0.05). The median H₂O₂ concentration in the asthmatics who used steroids (0.45 µmol) was higher than in healthy control subjects, but the difference was not significant. There was a highly significant difference in median H₂O₂ concentration between asthmatics without anti-inflammatory treatment and healthy controls (0.8 µmol and 0.15 µmol, respectively; p<0.01). Individual data are shown in figure 1. There was no correlation between the concentration of exhaled H₂O₂ and the change in FEV₁ before and after bronchodilation in control subjects or in asthmatic patients. Furthermore, no correlation was found between the daily dose of inhaled steroids and H₂O₂ concentration in the condensate (data not shown).

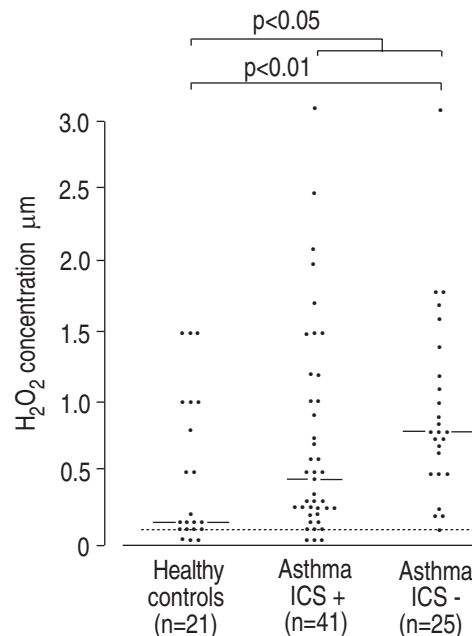


Fig. 1. — Concentration of H₂O₂ in exhaled air condensate of stable asthmatic children and healthy control subjects. The asthmatics are divided into a subgroup using inhaled corticosteroids (ICS+), and a group without anti-inflammatory treatment (ICS-). Each dot represents one patient. Median values are indicated by a horizontal line (—) and the detection limit by a horizontal broken line (-----). The differences between median values of healthy subjects and all asthmatic subjects was significant (0.15 and 0.60 µmol, respectively; p<0.05), the difference between healthy subjects and the asthmatics without steroids was highly significant (0.15 and 0.80 µmol, respectively; p<0.01).

Discussion

This study is the first to demonstrate a significantly increased concentration of H_2O_2 in exhaled air condensate from stable asthmatic children compared to healthy controls. That the H_2O_2 concentrations are lower in asthmatics who use anti-inflammatory medication supports the hypothesis that exhaled H_2O_2 reflects the presence of airway inflammation.

The present results seem different from those of an earlier study by DOHLMAN *et al.* [5], describing increased H_2O_2 concentration in exhaled air condensate in a small group of asthmatic children with acute respiratory disease, whereas exhaled H_2O_2 from stable asthmatics was not different from control subjects. Both studies used similar control groups. We think that the much larger groups in the present study, and the separation of asthmatics with and without anti-inflammatory treatment explain the different outcomes.

The present study shows that inhaled steroids are associated with lower exhaled H_2O_2 concentrations in asthmatics. A study in ARDS patients treated with corticosteroids showed a tendency towards lower levels of H_2O_2 in the exhaled air condensate as compared to ARDS patients not receiving corticosteroids [14]. The present cross-sectional study cannot demonstrate a causal relationship between steroid use and lower exhaled peroxide; prospective controlled intervention studies are needed for that purpose.

An increased content of H_2O_2 has also been reported in other inflammatory respiratory diseases [11–14]. This means that exhaled H_2O_2 is probably not a specific diagnostic test for asthma. Rather than a diagnostic test, exhaled peroxide may be used to guide anti-inflammatory treatment and estimate disease severity over time within subjects.

It can be argued that the control subjects in the present study were not age-matched, and that age-dependent changes in exhaled peroxide might partly explain the results. This seems unlikely: there are no data suggesting that healthy children produce more reactive oxygen species in their airways than healthy young adults. However, we do feel that, in the absence of such data, establishing normal values over a wide age range is an important goal for the near future.

In conclusion, this cross-sectional study has shown that the concentration of H_2O_2 in exhaled air condensate is increased in stable asthmatic children, and lower in patients receiving anti-inflammatory treatment, suggesting that airway inflammation increases exhaled peroxide. Correlation of H_2O_2 in exhaled air condensate with invasive measures of airway inflammation, such as bronchial biopsies, is needed to validate the hypothesis that exhaled peroxide reflects airway inflammation. On the basis of the present findings, we believe that the concentration of hydrogen peroxide in exhaled air is a potentially useful marker of airway inflammation in asthmatic children, especially since the procedure is quick and easy to perform. Further studies should explore its value as a noninvasive test, *e.g.* for monitoring effects of anti-inflammatory therapy.

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