Acute exposure to hair bleach causes airway hyperresponsiveness in a rabbit model

T. Mensing, W. Marek, M. Raulf-Heimsoth, X. Baur

ABSTRACT: Ammonium persulphate (APS) and hydrogen peroxide (H2O2) are used as oxidants in many industrial processes and are the main constituents of standard hair bleaching products. In a previous study, it was demonstrated that aerosols of APS induce alterations in airway responsiveness. The present study examined whether exposure for 4 h to a hair bleach composition (containing APS, potassium persulphate and H2O2) or H2O2 could induce airway hyperresponsiveness and/or an obstructive ventilation pattern in a rabbit model.

Methods

A detailed description of the rabbit model and the statistical data analysis has been presented in previous papers [12, 13]. Male and female white New Zealand rabbits of the same breed and of similar age and body weight (3.5–4.0 kg) were anaesthetized with 20–30 mg·kg-1 thiopentobarbital sodium (Trapanal®, Byk Gulden, Konstanz, Germany) after premedication with 25 mg·kg-1 ketamine hydrochloride (Ketanest®; Parke-Davis, Berlin, Germany) and 5 mg·kg-1 xylazin (Rompun®, Bayer, Leverkusen, Germany) and placed in a supine position. The level of anaesthesia was kept constant by continuous infusion of 0.2 mg·kg-1·h-1 thiopenotobarbital sodium via a catheter, inserted into the femoral vein. The body temperature was maintained at 39±0.5°C by means of a thermocontroller, connected to a heating pad. The animals were intubated (3.0 mm i.d.; Mallinkrodt, Athlone, Ireland) and breathed room air spontaneously. All animals were in a healthy condition, free from signs of acute airway infections and had not previously suffered from any known infections.

Recording of respiratory and cardiovascular parameters

Respiratory air-flow (\(V^t\)) was recorded by a Fleisch's head (00; Hugo Sachs, March, Germany) attached to the animal's mouth. Tidal volume (\(V^t\)) was obtained by integration of the inspiratory flow signal. Differences in oesophageal pressure (\(\gamma P^o\)) were measured using a catheter.
inserted in the oesophagus connected to a pressure transducer. Dynamic elastance (Edyn) was calculated from ∆Poes/Vt. By catheterization of the femoral artery, cardiovascular parameters were measured and small blood samples (about 0.4 mL) were collected to examine blood gases and correlated acid–base parameters. Data were recorded on a polygraph (Graphitec Linearorder WR 3310; Graphitec, Tokyo, Japan) and, after analogue/digital conversion of the measured signals, digitized on a personal computer.

Airway hyperresponsiveness in vivo experiments

Changes in airway response to aerosols of 0.2% and 2% ACh solutions in saline, generated by a commercial nebulizer (Pari, Clinic II; Starnberg, Germany), were investigated. Solutions (0.13 mL) were nebulized in 5.7 L room air·min⁻¹ and stored in a reservoir bag. The particles had a diameter of 0.5–5.5 μm. During the challenge tests, animals inhaled for 1 min with a mean of 1.1±0.3 L from the ACh aerosol [12], which corresponded to a total dose of 0.05 and 0.5 mg ACh, respectively (0.2% and 2% solution).

Changes in airway responses were measured when respiratory and cardiovascular parameters were constant. At the beginning, inhalation of aerosolized 0.2% ACh solution did not change baseline values of Edyn in healthy rabbits. Application of nebulized 2.0% ACh solution caused a rise in Edyn of 50–150% of the baseline value. Attention was focused on the responses to aerolized 2.0% ACh solution, before and after exposure to irritating agents. The responses to ACh were transient, lasting for <15 min.

Each group, consisting of eight or nine rabbits, inhaled one concentration of hair bleach or H₂O₂, respectively. All agents were tested at three different concentrations of a nebulized hair bleach solution in air (groups E–G), corresponding to the inhalation of 230, 23 or 2.3 mg hair bleach in 4 h, respectively (for composition see table 1). The standard hair bleach composition and crude substances in the inhaled aerosols

<table>
<thead>
<tr>
<th>Hair bleach composition</th>
<th>Crude substances</th>
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<tr>
<td>H₂O₂</td>
<td>APS</td>
</tr>
<tr>
<td>Dilution 1:10</td>
<td>136</td>
</tr>
<tr>
<td>1:100</td>
<td>13.6</td>
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<tr>
<td>1:1000</td>
<td>1.36</td>
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<tr>
<td>TLV [14]</td>
<td>1.4</td>
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<td>TLV [15]</td>
<td>5</td>
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H₂O₂: hydrogen peroxide; APS: ammonium persulphate; KPS: potassium persulphate; TLV: threshold limit value.

Statistical analysis. The data represent the mean±sd of n=8 or n=9 of the respective group. The significance of the differences between the ACh response before and after exposure to the chemical compounds was tested by Students' paired t-test [14]. Differences were considered significant for p<0.05.

The experiments were performed with the written consent of the ethical commission for animal experiments.

Results

Control group A

Group A was exposed twice for 2 h to saline aerosols. The first inhalation of nebulized 2.0% ACh solution at the beginning increased Edyn by about 100% of the baseline value from 2.5±0.3 to 5.1±1.1 kPa·dL⁻¹. No significant response was found to aerosolized 0.2% ACh solution. Two 2-h exposure periods to saline aerosols did not influence the ACh responses (fig. 1).

Influence of hair bleach on the development of airway hyperresponsiveness

During exposure, the baseline respiratory and cardiovascular parameters remained unaltered. Exposure to an aerosol containing 1,200 mg·m⁻³ of a hair bleach solution in air (group E) caused a significant increase in the response to 2.0% ACh after 2 and 4 h of exposure (p<0.05 and p<0.005, respectively). In the groups exposed to 12 mg·m⁻³ (group H) or 120 mg·m⁻³ (group G), AR to ACh increased significantly after 4 h (p<0.05) (fig. 2).

![Fig. 1. – Changes in dynamic elastane (Edyn) after challenge with 2.0% acetylcholine hydrochloride before and 2 h and 4 h after exposure to saline (n=9).](image-url)
Influence of hydrogen peroxide on the development of airway hyperresponsiveness

Baseline values of respiratory mechanical parameters were not significantly altered during and after H2O2 aerosol inhalation. The responses of Edyn after ACh challenge were not significantly altered after exposure to the three different H2O2 concentrations (groups C–E; fig. 3). However, exposure to 37 mg·m⁻³ H2O2 in air, there was a small but nonsignificant trend towards an increased contractile response to 2% ACh.

Discussion

This study examined the influence of a standard hair bleach composition and the main constituents of hair bleach on the development of AHR in rabbits in vivo. APS [11] as well as the standard hair bleach (12 mg·m⁻³), but not H2O2 (37 mg·m⁻³), caused significant increases in airway contractile responses to ACh.

In the control group, exposed to saline aerosols, AR remained stable. In previous experiments, it was demonstrated that APS at concentrations ≤50 mg·m⁻³ in air caused AHR, after an exposure of only 4 h [11]. However, exposure to hair bleach containing 11 mg·m⁻³ persulphate (4 mg·m⁻³ APS) also caused AHR after 4 h of exposure. Moreover, 5 mg·m⁻³ pure APS resulted in an increase in eosinophil-released mediators in bronchoalveolar lavage (BAL) fluid in rabbits [11, 15]. It seems likely that chronic exposure to APS at a concentration near the TLV (5 mg·m⁻³), which may occur in some working environments (e.g., hairdressing salons, APS production), might cause airway inflammation. Mergé et al. [7] measured APS concentrations of 3.6 mg·m⁻³ in a chemical production plant of persulphates.

The present results are partially in accordance with the experiments of Last et al. [16]. These authors demonstrated that exposure to an APS concentration of 4 mg·m⁻³ for 7 days, 23.5 h·day⁻¹, caused a decreased body weight and an increased fresh lung weight. The discrepancy with the present results at 5 mg·m⁻³ might be due to the longer exposure effects not evaluated in these experiments.

The persulphate concentrations in the hair bleach mixture and in the pure APS aerosol were different, so it is difficult to compare the two groups. However, no statistical difference can be obtained between the groups exposed to pure persulphate or hair bleach.

In the animals exposed to different concentrations of the strong oxidant H2O2, no enhanced responses to ACh were found. The reason for this outcome has not yet been clarified. The presence of glycoproteins or enzymes such as catalase or glutathione peroxidase, which metabolize H2O2 in the lung, may prevent the damage of airway epithelium function [17, 18] and hence the development of AHR.

Although there was a trend towards an increased responsiveness to 2% ACh after exposure to 37 mg·m⁻³, H2O2 had no effects on AR in the concentrations used in hair bleach solutions. An effect of H2O2 at higher concentrations cannot be excluded. Moreover, the choice of the species and the model may influence the effect of H2O2 [19–21].

The hair bleach solutions contained neither perfumes nor stabilizing agents, the presence of which may vary in different commercial products, to avoid any influence of these compounds. Because of the very high concentration of persulphates in the hair bleach mixture, diluted solutions containing concentrations of the crude substances near the TLV were used.

The basic mechanisms of APS and hair bleach-induced AHR are still unclear and need further investigation. However, several mechanisms of action could explain the effect of persulphates on AR.

Although persulphates are stable in aqueous solutions, a small proportion decays to oxygen radicals [22]. A reaction with organic materials is mediated by the radicals SO₂· or ·OH. Inhaled oxidants damage epithelial cells [23, 24], and this may result in a loss of prostaglandin inhibitory factors [25]. Oxidation of unsaturated fatty acids would result in an increased concentration of prostaglandins (PGE₂), which could also influence airway smooth muscle tone [23, 25].

Induced epithelium damage would favour airway contractile mechanisms. This is supported by the results of Mena et al. [26], who showed that AR increased in the absence of an intact epithelium. Another explanation for the development of AHR may be the release of histamine from mast cells, as demonstrated by Parsons et al. [27].

Further investigations into the cell and mediator profile in the BAL fluid demonstrated the participation of inflammatory cells and their mediators in the development of...
AHR after exposure to the examined substances in the lowest concentrations [15]. It is well known that eosi-
nophils and their mediators, such as eosinophilic peroxi-
dase, major basic protein and leukotrienes C₄, D₁ or E₂, contribute to the development of AHR. A release of reac-
tive oxygen species from inflammatory cells might cause damage to the epithelial cells [28]. Koller et al. [29]
showed that APS may increase the generation of leukot-
rienes from human neutrophil granulocytes.

In summary, exposure to hair bleach containing about 10 mg·m⁻³ persulphates increased airway hyperresponsive-
ness after 4 h. Hydrogen peroxide in a 26-fold concentra-
tion of the German threshold limit value neither altered respiratory parameters nor influenced airway hyperres-
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