SHORT REPORT

Glutaraldehyde-induced occupational asthma: BALF components and BALF and serum Clara cell protein (CC16) changes due to specific inhalatory provocation test

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Objectives The purpose of this study was to evaluate bronchoalveolar lavage fluid (BALF) components and Clara cell protein (CC16) concentration in serum and BALF in patients with glutaraldehyde (GA)-induced asthma, before and after a specific inhalatory provocation test (SIPT) with GA, in comparison to atopic asthmatics and healthy individuals.

Methods Spirometry and bronchoalveolar lavage were performed before and after SIPT. The serum and BALF concentrations of CC16 and cytogram content in BALF were evaluated.

Results In GA-sensitized asthmatics, the level of CC16 in BALF and serum was significantly lower at 24 h after SIPT in comparison with the values recorded prior to the experiment. There was a significant increase in the proportion of eosinophils, basophils and lymphocytes in BALF of GA-sensitized asthmatics obtained after SIPT.

Conclusions The determination of CC16 either in serum or in BALF is a non-invasive test to detect Clara cell damage.

Key words Bronchoalveolar lavage; CC16; glutaraldehyde; occupational asthma.

Introduction

Clara cell protein (CC16), the product from Clara cells, has anti-inflammatory activity and therefore plays a role in controlling airway inflammation. The determination of CC16 in serum is a new non-invasive test to detect Clara cell damage or an increased epithelial permeability in various lung disorders [1].

Glutaraldehyde (GA) belongs to the growing group of low-molecular-weight (LMW) chemicals. So far, CC16 has not been evaluated in occupational asthma due to LMW compounds.

The aim of this study was to examine CC16 concentration in serum and bronchoalveolar lavage fluid (BALF) and to evaluate the cytological changes in BALF before and after a specific inhalatory provocation test (SIPT) with GA.

Methods

The study group comprised nine subjects with occupational asthma due to GA. The patients were hospitalized in a GA-free environment during the course of the study. The control groups consisted of five atopic patients with perennial asthma and rhinitis and five healthy individuals. All the controls were not occupationally exposed to GA.

At first, the subjects were challenged with placebo (0.9% saline solution) and, at least 7 days later, a challenge with 2% GA was performed according to Gannon et al. [2]. The concentration of GA during exposure was determined using a Hewlett-Packard gas chromatograph according to the National Institute for Occupational Safety and Health (NIOSH) [3].

Skin prick tests (SPTs) with common allergens were performed (Allergopharma, Germany). Total serum IgE
was evaluated using the Uni-CAP system (Pharmacia, Uppsala, Sweden). Morphological changes in BALF were analysed before and 24 h after SIPT according to Palczynski et al. [4]. Bronchial response was measured by serial monitoring of forced expiratory volume in 1 s before and 5 min, 30 min, 1 h, 5 h and 24 h after the challenge. The histamine provocation test was performed according to Cockroft et al. [5] before and 30 h after SIPT. Clara cell protein was determined according to Halatek et al. [6].

The results obtained after SIPT were compared with baseline values in the analysed groups using two-tailed paired Student’s t-test. The results obtained after SIPT in patients with GA-induced asthma were compared with those of the controls using two-tailed unpaired Student’s t-test. Pearson’s correlation coefficient was used to test for a relationship between cell percentage and CC16 concentration either in serum or BALF. P-values <0.05 were considered significant.

The regional bioethical committee approved the study protocol.

Results

The median air concentration of GA during SIPT was 0.38 mg/m³ (range 0.23–0.4 mg/m³), i.e. below the occupational exposure limit [7].

All the subjects with GA-induced occupational asthma presented shortness of breath and wheezing only after SIPT. We did not observe any changes in the intensity of dyspnoea after placebo challenge.

None of the GA-sensitized asthmatics had positive SPTs to common allergens. The median value of total serum IgE was 27 kU/l (range 10.7–136) (Table 1).

An increase in bronchial hyperreactivity to histamine was only observed in the subjects with GA-induced asthma (Table 1).

Table 1. Demographic data, clinical and laboratory findings of patients with occupational asthma and rhinitis due to GA (N = 9)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Job</th>
<th>Duration of exposure to GA (years)</th>
<th>Results of SPT with common allergens</th>
<th>Total IgE level (kU/l)</th>
<th>Asthmatic response during SIPT with GA</th>
<th>PC_{20}H pre- and post-SIPT with GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Theatre nurse</td>
<td>6</td>
<td>–</td>
<td>45</td>
<td>Dual</td>
<td>4.2, 1.0</td>
</tr>
<tr>
<td>2</td>
<td>Nurse</td>
<td>3</td>
<td>–</td>
<td>60.5</td>
<td>Late</td>
<td>1.08, 0.67</td>
</tr>
<tr>
<td>3</td>
<td>Radiological technician</td>
<td>10</td>
<td>–</td>
<td>10.7</td>
<td>Late</td>
<td>1.9, 0.35</td>
</tr>
<tr>
<td>4</td>
<td>Laboratory technician</td>
<td>6</td>
<td>–</td>
<td>27</td>
<td>Late</td>
<td>0.83, not done</td>
</tr>
<tr>
<td>5</td>
<td>Nurse at endoscopy unit</td>
<td>3</td>
<td>–</td>
<td>24.9</td>
<td>Late</td>
<td>5.71, 0.67</td>
</tr>
<tr>
<td>6</td>
<td>Nurse</td>
<td>2</td>
<td>–</td>
<td>11.8</td>
<td>Late</td>
<td>3.7, 2.2</td>
</tr>
<tr>
<td>7</td>
<td>Ward attendant</td>
<td>6</td>
<td>–</td>
<td>17.5</td>
<td>Dual</td>
<td>6.4, 0.26</td>
</tr>
<tr>
<td>8</td>
<td>Nurse</td>
<td>10</td>
<td>–</td>
<td>40.1</td>
<td>Dual</td>
<td>4.8, 0.15</td>
</tr>
<tr>
<td>9</td>
<td>Nurse</td>
<td>10</td>
<td>–</td>
<td>136</td>
<td>Late</td>
<td>5.16, 1.11</td>
</tr>
</tbody>
</table>

PC_{20}H = provocation concentration of histamine producing a 20% fall in forced expiratory volume in 1 s.

Table 2. CC16 concentration in serum and in bronchial washings before and after the specific challenge with GA, and the proportion of eosinophils, basophils and lymphocytes in bronchial washings before and after the specific challenge with GA

<table>
<thead>
<tr>
<th>GA—serum</th>
<th>GA—BALF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>24 h after</td>
</tr>
<tr>
<td>CC16 (µg/l)</td>
<td></td>
</tr>
<tr>
<td>Patients with occupational asthma due to GA</td>
<td>1.82 ± 0.13^{a}</td>
</tr>
<tr>
<td>Patients with atopic non-occupational asthma</td>
<td>0.66 ± 0.11</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>0.56 ± 0.11</td>
</tr>
</tbody>
</table>

Proportion of cells (%)

| Patients with occupational asthma due to GA | | |
| Eosinophils | 1.67 ± 1.94 | 15 ± 10.4^{b} |
| Basophils | 0.22 ± 0.44 | 2.89 ± 1.17^{b} |
| Lymphocytes | 7.88 ± 5.34 | 17.8 ± 11.9^{b} |

Values are given as mean ± SD.

^{a}Significant difference between CC16 in GA-sensitized asthmatics and controls, P < 0.05.

^{b}Significant decrease versus baseline value, P < 0.05.
The mean values of CC16 in serum and BALF of all the patients before and after SIPT are presented in Table 2.

There was a significant increase in the proportion of eosinophils, basophils and lymphocytes in BALF of GA-sensitized asthmatics obtained after SIPT ($P < 0.05$) (Table 2).

No significant increase in the proportion of neutrophils and monocytes was found after SIPT in these patients. No statistically significant changes in the morphological response were observed in controls after the challenges.

In subjects with GA-induced asthma, a positive correlation could only be observed between the proportion of basophils in BALF and the level of CC16 in BALF after SIPT ($r = 0.69, P < 0.05$).

Discussion

In our study, all the subjects with GA-induced asthma presented isolated late or dual asthmatic reaction, similar to those observed with other LMW chemicals [8].

Inhalation of GA caused an inflammatory response characterized by eosinophil, basophil and lymphocyte recruitment to the lower respiratory tract that persisted for up to 24 h after the challenge. We observed a significant relationship between the proportion of basophils and CC16 concentration in BALF after SIPT, supporting the concept that basophils may release mediators that contribute to airway responsiveness.

The observed significantly higher serum and BALF CC16 concentrations in subjects with GA-induced asthma compared to the controls before SIPT might be the result of regeneration of this protein after cessation of occupational exposure. An identical phenomenon has been described after smoking cessation [9].

An explanation for the difference in the CC16 levels between GA-sensitized asthmatics and atopic controls may be postulated in agreement with CC16 gene polymorphisms. The mutation of the CC16 gene may be associated with a decrease in the serum concentration of CC16 [10].

It seems likely that the observed lower concentration of CC16 in BALF after SIPT may be a result of the decreased production/secretion of this protein. GA exposure may induce a dose- and time-dependent decrease of CC16 in serum, similar to that observed in BALF [6].

Additionally, the decrease in CC16 concentration after SIPT might reflect a combined mechanism of GA activity: allergic and non-specific of a toxic nature.

Acknowledgements

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Conflicts of interest

None declared.

References