AIRWAY RESPONSIVENESS TO A BETA-ADRENERGIC AGONIST IN SMOKERS

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Background: Airway hyperresponsiveness (AHR) is the most characteristic feature of asthma, which is reported in chronic obstructive pulmonary disease (COPD) patients and smoker subjects. However, there are controversies regarding airway responsiveness to β-adrenergic agonists in asthma, as well as COPD and smoker subjects. Our previous studies have shown AHR to β-agonists in asthmatic patients. Therefore, in the present study, airway sensitivity to a β-agonist was examined in smoker subjects.

Methods: The threshold concentration of inhaled salbutamol required for a 20% increase in forced expiratory volume in 1 sec (FEV1), as PC20, or a 35% increase in specific airway conductance (sGaw), as PC35, was measured in 12 nonsmoker and 12 smoker subjects.

Results: Airway responsiveness to salbutamol was greater in smokers (PC20 = 65.83 ± 14.77 mg/L and PC35 = 27.75 ± 18.41 mg/L) than nonsmokers (PC20 = 244.17 ± 44.2 mg/L and PC35 = 90.50 ± 28.67 mg/L, P < 0.001 for both cases). There was a significant correlation between FEV1 with PC20 salbutamol (r = 0.547, P < 0.005). The relationship between the amount of smoking and PC35 salbutamol was also statistically significant (r = –0.654, P < 0.001). The slope of concentration-response curve for salbutamol was significantly greater in smokers than nonsmoker subjects, by measuring both FEV1 and sGaw (P < 0.001 for both cases).

Conclusion: The increased sensitivity of smokers to inhaled salbutamol suggests that they could also be more sensitive to their endogenous adrenaline, which may thus dilate and stabilize their airways.

Keywords: Adrenoceptors • airway responsiveness • salbutamol • smoking

Introduction

The most characteristic feature of asthma is airway hyperresponsiveness to a wide variety of inhaled physical, chemical, pharmacological, and immunological stimuli, and the level of responsiveness has been shown to correlate loosely with the severity of asthma.1 Many studies have demonstrated an increased responsiveness of bronchial tree to nonspecific constrictor stimuli in patients with asthma and chronic bronchitis.2, 3 Smoking has been regarded as one of the most important risk factors causing chronic obstructive pulmonary diseases (COPD).4

There is a widespread agreement that airway hyperresponsiveness (AHR), usually assessed as the response to inhaled histamin or methacholine, is found consistently in middle-aged male smokers with mild or moderate chronic airflow obstruction. Smoker subjects who have shown preceeding, accelerated annual decline in forced expiratory volume in one second (FEV1) also show abnormal AHR to inhaled bronchoconstrictor or bronchodilator drugs.5 Several investigators have also noted AHR due to different stimuli, even in the absence of airway obstruction in smoker subjects.6 – 11 Even parental smoking enhances airway responsiveness in children.12

However, there are controversies regarding airway responsiveness to β-adrenergic agonists in asthma. Previous studies have shown very similar
dose-response relationships to β₂-agonists in normal and asthmatic subjects. 13 - 16 Our previous studies showed an increased airway responsiveness to isoprenaline and salbutamol in asthmatics compared to normal subjects. 17, 18 There was also a close correlation between airway responsiveness to methacholine and histamine with those of isoprenaline and salbutamol in our previous studies. 17, 18

Therefore, to examine the effect of smoking on airway responsiveness to β-adrenergic agonists, in the present study the airway responsiveness to salbutamol in a group of smoker subjects with completely normal FEV₁ and no clinical sign and symptom of COPD was examined.

Patients and Methods

Subjects

Twelve nonsmoker (mean age ± SD; 28.91 ± 5.86 years, 12M) and 12 smoker subjects (mean age ± SD; 39.00 ± 9.29 years, 12M) were studied for determining airway responsiveness to salbutamol (Table 1). None had past or present history of respiratory complaints, or clinical symptom and signs of pulmonary disorders. Smoker subjects had a history of at least 10 years of smoking and 10 cigarettes per day. The salbutamol challenge test was performed on all twelve normal and smoker subjects. Experiments conducted were approved by the ethical committee of our institution and each subject gave an informed consent.

Techniques and protocol

Each subject attended the laboratory at approximately the same time of day on each occasion. 19 Subjects were prohibited from smoking and taking caffeinated drinks for 2 hours before challenge. In a random order, for two days, the following experimental procedures were performed to estimate the nonspecific airway responsiveness.

For the salbutamol (Sigma Chemical Ltd, Poole, Dorset, UK) challenge, the cumulative concentration-response method was used as previously recommended. 20 Salbutamol sulfate (molecular weight = 576.7), dissolved in 0.9% NaCl solution, was delivered as an aerosol from a Wright nebulizer with an airflow of 8 L/min, 2 min for each concentration.

Subjects were instructed to breath normally during nebulization of different concentrations of drugs. The volume of solution delivered for each concentration was 0.2 mL. The aerosol had a mass median aerodynamic diameter (MMAD) of 3.0 µm, as determined by laser light scattering (Malvern Instruments 2,600 HSD Analyzer, Malvern, UK). The same nebulizer was used throughout the experiment. At the beginning of each challenge, baseline forced expiratory volume in one second (FEV₁), and specific conductance (sGaw) were measured using a body plethysmograph with a pneumotachograph sensor (Model V max 6,200, Sensor Medics, California, USA). Prior to the measurement of FEV₁ and sGaw, the required maneuver was demonstrated by the operator, and subjects were encouraged and supervised throughout the test performance. Measurements of FEV₁ and sGaw were performed using the acceptability standards outlined by the American Thoracic Society (ATS), 21 with subjects in a standing position inside the box and wearing

Table 1. Characteristics of subjects included in this study.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex - Age</th>
<th>Height (cm)</th>
<th>FEV₁ % pre</th>
<th>Sex - Age</th>
<th>Height (cm)</th>
<th>FEV₁ % pre</th>
<th>Smoking P/Y</th>
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<td>97</td>
<td>M-29</td>
<td>183</td>
<td>85</td>
<td>10</td>
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</table>

Mean 28.91 174.66 104.27 39.00 173.17 91.25 12.00

SD 5.86 4.97 5.97 9.29 8.65 9.08 3.64

FEV₁ = forced expiratory volume in 1 second; % pre = % predicted value; P/Y = pack per year; M = male.
nose clips. The challenge began with inhalation of 0.9% NaCl (control diluent) aerosol, followed by 2 minutes of salbutamol inhalation, 2 minutes of resting, and a final 2 minutes for measuring the FEV<sub>1</sub> and sGaw. It was followed by progressively doubling drug concentrations and the challenges were terminated when a 20% rise in FEV<sub>1</sub> and 35% rise in sGaw was recorded. The starting concentration of salbutamol was 2 mg/L, and the maximum concentration was 120 mg/L in nonsmoker and smoker subjects.

**Measurements**

A cumulative log concentration-response curve was constructed from which we determined the concentration of agonist producing a 20% change in FEV<sub>1</sub> (PC<sub>20</sub>) and 35% change in sGaw (PC<sub>35</sub>). In all 12 nonsmoker subjects, with salbutamol, the maximum increase in FEV<sub>1</sub> was less than 20%. Therefore, the FEV<sub>1</sub> at the level of achieving a 35% increase in sGaw was determined. The increase in FEV<sub>1</sub>, due to the administration of salbutamol in nonsmoker subjects, was between 12 – 19%.

**Statistics**

Mean values for FEV<sub>1</sub>, maximum response, and slope were quoted as arithmetic mean ± SD. For PC<sub>20</sub> geometric mean was also used. Variables were correlated using the least square regression test. When comparing values of FEV<sub>1</sub>, maximum response, and slope of concentration-response curves, unpaired t-test was used, but for comparison of PC<sub>20</sub> and PC<sub>35</sub> between normal and asthmatic subjects, both unpaired t-test and nonparametric Mann-Whitney U-test were used. Significance was accepted at P < 0.05.

**Results**

**Values of FEV<sub>1</sub>**

Mean values for baseline FEV<sub>1</sub> is shown in Table 2. Although, mean values of FEV<sub>1</sub> in smoker subjects were within the normal range, but it was significantly lower than the nonsmoker subjects. (P < 0.001, Table 2).

**Responsiveness to salbutamol**

Mean values for PC<sub>20</sub> and PC<sub>35</sub> salbutamol in nonsmoker and smoker subjects are shown in Table 2. The geometric mean PC<sub>20</sub> and PC<sub>35</sub> for salbutamol in nonsmokers were 3.71 and 3.69 times greater than the smoker subjects, respectively (P < 0.001 for both cases; Figure 1 [a and c]).

**Maximum response to salbutamol**

Maximum response to salbutamol in smokers was nonsignificantly greater than nonsmoker subjects, by measuring both FEV<sub>1</sub> and sGaw (Table 2).

**Slope of salbutamol concentration-response curves**

Slope of concentration-response curves for salbutamol in smoker subjects, by measuring FEV<sub>1</sub> (0.92 ± 0.29) and sGaw (1.66 ± 0.49), were significantly greater than nonsmokers (0.21 ± 0.03 and 0.45 ± 0.11, respectively, P < 0.001 for both cases; Table 2, Figure 2 [a and b]).

**Relationship between PC<sub>20</sub> salbutamol and FEV<sub>1</sub>**

There were close correlations between baseline values of FEV<sub>1</sub> and PC<sub>20</sub> salbutamol (Figure 3).

**Table 2.** Mean values of forced expiratory volume in one second (FEV<sub>1</sub>), specific airway conductance (sGaw), airway responsiveness (PC<sub>20</sub> and PC<sub>35</sub>), slope of concentration-response curves (slope), and maximum response (Max Resp) to salbutamol in nonsmoker and smoker subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonsmoker subjects</th>
<th>Smoker subjects</th>
<th>Statistics</th>
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</thead>
<tbody>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; (%) pre</td>
<td>Arithmetic mean ± SD 104.25 ± 5.97</td>
<td>89.91 ± 11.30</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>PC&lt;sub&gt;20&lt;/sub&gt; (mg/L)</td>
<td>Arithmetic mean ± SD 244.17 ± 44.2</td>
<td>65.83 ± 14.77</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Geometric mean</td>
<td>240.67</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Slope</td>
<td>Arithmetic mean ± SD 0.21 ± 0.03</td>
<td>0.92 ± 0.29</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Max Resp</td>
<td>Arithmetic mean ± SD 116.50 ± 1.97</td>
<td>119.42 ± 3.23</td>
<td>NS</td>
</tr>
<tr>
<td>sGaw</td>
<td>Arithmetic mean ± SD 1.76 ± 0.47</td>
<td>1.55 ± 0.90</td>
<td>NS</td>
</tr>
<tr>
<td>PC&lt;sub&gt;35&lt;/sub&gt; (mg/L)</td>
<td>Arithmetic mean ± SD 90.50 ± 28.67</td>
<td>27.75 ± 18.41</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Geometric mean</td>
<td>86.57</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Slope</td>
<td>Arithmetic mean ± SD 0.45 ± 0.11</td>
<td>1.66 ± 0.49</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Max Resp</td>
<td>Arithmetic mean ± SD 136.20 ± 4.49</td>
<td>138.67 ± 4.33</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data of FEV<sub>1</sub> maximum response and slope of concentration-response curves were quoted as arithmetic mean ± SD and those of PC<sub>20</sub> as both arithmetic mean ± SD and geometric mean. The number of smoker and nonsmoker subjects for salbutamol and methacholine challenge test were 12.
The relationship between the amount of smoking and PC 35 salbutamol was also statistically significant ($r = –0.656$, $P < 0.001$).

**Discussion**

This study showed increased airway responsiveness to salbutamol in smokers compared to nonsmoker subjects. The results of this study are similar to those of our previous study, which indicated airway hyperresponsiveness of asthmatic patients to salbutamol and isoprenaline,$^{17, 18}$ indicating increased airway responsiveness to β-agonists in asthma. Thus, based on the findings of the present study it seems that the phenomenon of hyperresponsiveness in smoker subjects should include β-agonists drugs.

The results of the present study showed that smokers are 3.9 times more sensitive to salbutamol than nonsmokers. The differences between smoker and nonsmoker subjects in airway responsiveness to salbutamol were smaller than those of asthmatic and normal subjects. Asthmatic subjects were 17.2 times more sensitive to salbutamol.$^{18}$ These differences in airway hyperresponsiveness (AHR) between asthmatic patients and smokers indicate that although there is AHR to β-agonists in smoker subjects, this phenomenon in this group of subjects is less than asthmatic patients.$^{18}$ In fact, previous studies have demonstrated an increased airway responsiveness to different stimuli in smokers.$^{6–11}$ Even parental smoking may cause AHR in their children.$^{12}$

The results of this study showed that the airways of smokers are hypersensitive to β-agonists, by using two different methods of measurement. Although, measurement of airway specific conductance (sGaw) and determination of

**Figure 1.** Individual values and geometric mean (big symbols) of airway response to salbutamol, (a) by measuring FEV$_1$ (PC$_{20}$) and (c) by measuring sGaw (PC$_{35}$) in nonsmokers (open symbols) and smoker subjects (filled symbols), and the statistical differences between two groups; $** = P < 0.001$.

The relationship between the amount of smoking and PC$_{35}$ salbutamol was also statistically significant ($r = –0.656$, $P < 0.001$).

**Figure 2.** Normalized Log concentration-response curves of salbutamol (a) by measuring FEV$_1$ and (b) by measuring sGaw in nonsmokers (open symbols) and smoker subjects (filled symbols).
PC$_{35}$ is a slightly more sensitive method in assessing airway responsiveness to salbutamol, both methods yield approximately similar results. The results of the present study also showed that slopes of concentration—response curves for salbutamol in smokers were significantly more than the normal subjects, indicating airway hyperreactivity to this agent in smokers, which was very similar to the result of our previous study in asthmatic patients. However, the maximum response to salbutamol was not significantly different between smokers and nonsmokers. Although in some normal subjects a 20% increase in FEV$_1$ due to salbutamol administration could not be obtained, in most normal subjects a plateau in concentration-response curves for salbutamol was achieved. Conversely, in all smoker subjects a 20% increase in FEV$_1$ due to salbutamol administration was obtained, but a plateau in concentration-response curves for salbutamol was not achieved. This is an exactly characteristic difference in concentration-response curves between normal and asthmatic subjects as previously described.

We have to consider the possibility that hyperresponsiveness is due to baseline airway narrowing. Several previous studies have also shown reduction of different values of pulmonary function tests among smokers compared to normal subjects. The obstruction to airflow, which develops in 15 to 20% of heavy smokers, is thought to be due to abnormalities in airways less than 2 mm in internal diameter. Previous studies from several laboratories have shown that this airway obstruction is associated with a chronic inflammatory process in the membranous and respiratory bronchioles. The results of the present study showed that FEV$_1$ values in smokers were significantly lower than those of nonsmoker subjects. The absence of correlation between airway responsiveness to salbutamol (PC$_{35}$) with values of sGaw could indicate the dependence of sGaw on airway (small airways), which is different from that of FEV$_1$. In our previous study, asthmatic subjects were hyperresponsive to salbutamol and methacholine. In fact, the airways of asthmatic patients were significantly narrowed, compared to normal subjects. Although, the values of FEV$_1$ in all smoker subjects in the present study were within the normal range, there was a significant difference in FEV$_1$ between smoker and nonsmoker subjects. There was also a significant correlation between baseline FEV$_1$ values and the values of airway responsiveness (PC$_{20}$) to salbutamol in the present study. Therefore, even a very small reduction in airway caliber could cause AHR against salbutamol in smokers.

One possible way in which airway narrowing may promote hyperresponsiveness is by causing central airway deposition of inhaled aerosol particles. In a previous work Gillett et al could show no relationship between central deposition of aerosol and methacholine responsiveness. Therefore, this mechanism is of limited importance in determining agonist responsiveness. In addition, while the density of muscarinic receptors are greater in central airways, the density of $\beta_2$-adrenoceptors increases with decreasing the diameter of airways. Therefore, the effect of central deposition of inhaled $\beta_2$-agonists on enhanced $\beta_2$-agonists responsiveness in smokers is of minor value. The significant correlation between PC$_{35}$ salbutamol and the amount of smoking could be explained as follow: smoking affects mainly small airways, which is mainly reflected by measuring the sGaw and calculation of PC$_{35}$. On the other hand the density of $\beta_2$-adrenoceptors is increased in smaller airways.

The other possible mechanism responsible for increased airway responsiveness in smokers is airway inflammation. The association between airway inflammation and AHR in asthma is well documented. The existence of airway inflammation in animals exposed to cigarette smoke, smokers, and COPD has also been
demonstrated. In fact, the increased airway epithelial permeability to different agents has been shown in animals exposed to cigarette smoke\(^3\) and in smokers.\(^3\) Thus airway inflammation could cause epithelial damage; and this, in turn, can result in better access of ligands to the active sites in the airways.

Although, several studies showed AHR in smokers,\(^6\)\(^-\)\(^1\) the novel finding of the present study is increased airway responsiveness of smokers to salbutamol. In a previous study, we have shown a significantly greater increase in airway caliber of smokers than nonsmoker subjects, which somehow supports the finding of the present study.\(^4\) The results of the present study are also supported by the finding of Pride et al.\(^5\)

In conclusion, the results of the present study showed an increased airway responsiveness to salbutamol in smokers, which was similar to those of asthmatic patients. These results indicated the reversibility of a mild bronchoconstriction of smoker subjects. The increased sensitivity of smokers to inhaled salbutamol suggests that they may also be more sensitive to their endogenous adrenaline, which may thus dilate and stabilize their airways.

References


Airway responsiveness to salbutamol in smokers

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