Possible interaction between exposure to environmental tobacco smoke and therapy in children with asthma

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1. The aim of the study was to determine the carbachol and albuterol responsiveness in treated and untreated asthmatic and allergic children exposed to environmental tobacco smoke assessed by urinary cotinine measurements.

2. Forty-six asthmatic and allergic children with normal spirometric values were recruited. The doubling dose, concentration of carbachol producing a 2-fold increase in specific airway resistance (SRaw) was determined and 200 µg of albuterol were administered via a Volumatic® spacer. The percentage of bronchodilatation was defined as the difference between the largest obtained SRaw and the post-β₂ SRaw divided by the largest SRaw. Data were compared by a Mann–Whitney U-test.

3. The 23 children with a high urinary cotinine, compared with the 23 children without urinary cotinine, had a decreased doubling dose (108.2 ± 14.7 µg versus 160.9 ± 19.5 µg; P = 0.04) and an increased percentage of bronchodilatation (74.8 ± 1.4% versus 68.8 ± 1.8%; P = 0.03). A prophylactic anti-inflammatory treatment induced a weaker bronchial reactivity to carbachol and a slightly greater bronchodilatation in children exposed to environmental tobacco smoke.

4. Environmental tobacco smoke increases bronchial reactivity in asthmatic and allergic children. This effect might be reduced by anti-inflammatory therapy. The bronchodilator response may be enhanced in exposed children and may be caused by one or several direct interactions between tobacco smoke compounds and albuterol.

INTRODUCTION

Passive or involuntary smoking is defined as the exposure of non-smokers to tobacco combustion products in an indoor environment [1]. Despite a preferential exposure to the sidestream smoke, and thus a quantitatively smaller and qualitatively different exposure compared with active smokers, involuntary smokers suffer from adverse health effects [2].

A recent literature review [3] evaluates the impact of adult tobacco use on the health of children: every year, tobacco is associated with an estimated 284 to 360 deaths.
from lower respiratory tract diseases and fires initiated by smoking materials, 354,000 to 2.2 million episodes of otitis media, 14–21,000 tonsillectomies and/or adenoidectomies, 529,000 physician visits for asthma, 1.3 to 2 million visits for coughs, and, in children younger than 5 years of age, 260–436,000 episodes of bronchitis and 115–190,000 episodes of pneumonia.

Exposure to environmental tobacco smoke (ETS), particularly to maternal smoking, increases the prevalence and symptomatology of asthma with a 63% increased risk of emergency room visits for acute asthma [4–6]. Undoubtedly, even when asthma is absent, passive smoking has noxious effects on pulmonary function. Natural evolution of the pulmonary function is perturbed in infants, with a reduction in airway size and alterations in growth and/or maturation of passive mechanical properties of the respiratory system [7], and also in older children, with a reduction in growth of the vital capacity and forced expiratory volume [8,9]. Most recent studies concerning pulmonary function tests (PFTs) in children exposed to ETS demonstrate that the spirometric effect of the ETS is bronchial obstruction, with predominant involvement of small airways [10,11]. In a recent study, Corbo et al. [12] suggest an adverse effect on lung function from even low-level exposure to ETS among non-smoking children who live with reportedly non-smoking parents. The same data are reported in children with an established diagnosis of asthma and an exposure to ETS, with a more pronounced obstructive syndrome [13].

Considering bronchial reactivity (BR) in non-asthmatic and asthmatic children exposed to ETS: to our knowledge, four out of seven studies demonstrate a significant relationship between BR and ETS [14–20]. Unfortunately, although of great methodological value, these reported studies are not associated with an objective measurement of ETS exposure such as cotinine, the principal metabolite of nicotine [21]. In these same works, neither allergic nor non-allergic children, treated nor untreated patients are individualized. Thus, the aim of this study is to confirm the BR, assessed by bronchial response to carbachol, in treated and untreated asthmatic and allergic children exposed to ETS, assessed by urinary cotinine concentrations. In addition, as $\beta_2$-agonists represent another indicator of the reactivity of bronchial smooth muscle and also one of the most frequently prescribed drugs in asthma, we decided to study likewise the bronchial responsiveness to albuterol.

**MATERIAL AND METHODS**

**Subjects**
The study population consisted of children referred to the Respiratory Function Laboratory with a clinical diagnosis of asthma, based on a history of recurrent episodes of wheeze, dyspnoea and coughing in response to allergens or non-allergic stimuli [22]. These children were sent to the Laboratory by their general practitioner, paediatrician or pulmonologist to perform PFTs. Only children with a clinical diagnosis of asthma and a positive skin-prick test to one or more common allergens (house dust mite, cat, cockroach or Graminaceae) were included in the study. Most of the children were followed up by the authors. In the other cases, allergy was confirmed by a phone call to the children’s physician. A questionnaire was addressed to the child and/or one of the parents on personal history of respiratory diseases, onset of asthma, aetiology and treatment of asthma and smoking habits of the family. Children with a concomitant upper airway infection or asthma exacerbation, which could have influenced the PFTs, were excluded. A spirometric test was performed and a normal baseline test, defined as a value of forced expiratory capacity (FEC) and forced expiratory volume in 1.0 s (FEV$_1$) $\geq$ 80% of the predicted value, was expected to start the bronchial challenge. Finally the children provided a urine sample for measurement of cotinine. This study was approved by the local ethics committee and informed consent was obtained from all the families.

**Bronchial challenge**
All baseline PFTs were performed between 09:30 and 12:00 hours. Parents were instructed to withhold any kind of anti-asthmatic, antihistamine or inhaled therapy for 24 h before the tests. The PFTs consisted of a flow volume spirometric test and specific airway resistance (SRaw) measurements with a constant body plethysmograph (model Master Lab JAEGER, Wurzburg, Germany), using the method of Dubois et al [23]. The mean of five reproducible measurements was used each time. The functional measurements were compared with the predicted values of Zapletal et al. [24]. For carbachol inhalation, a standardized dosimeter technique was used. Carbachol puffs were delivered by a dosimeter (MEFAR dosimeter, Elletromedicalli, Brescia, Italy): air driving pressure = 1.65 kg/cm$^2$; air flow rate = 70/75 l/min; particle size = 0.5–4 $\mu$m. Two parameters were adjusted before the test: a nebulization time of 1.2 s and a pause time of 5 s between two puffs. A carbachol solution of 2 mg/ml was used and 3 ml of the solution was placed in the nebulizer (20 $\mu$g of carbachol delivered per puff). While wearing a nose clip, the children were instructed to breathe quietly through a spacer device (Volumatic*, Glaxo-Wellcome Laboratories, Paris, France). Cumulative doses of carbachol were then administered. The doses administered were 40 $\mu$g, 60 $\mu$g, 100 $\mu$g, 100 $\mu$g and a further 100 $\mu$g if necessary in order to reach the maximal dose of 400 $\mu$g. SRaw measurements were performed 2 or 3 min after each inhaled dose of carbachol.
The doubling dose, or concentration of carbachol which produced a 2-fold increase of SRaw, was noted. At the end of the challenge corresponding to the largest measured SRaw, two separate puffs of albuterol (200 µg) were administered via the Volumatic® spacer and the post-β₂ SRaw was measured 15 min later.

**Urinary cotinine analysis**

Urine samples were collected in sterile bottles and stored at +4 °C. The method used a solid phase extraction on Extrelut® columns (Merck). An internal standard, 2-phenylimidazole, was added to 10 ml of urine. The pH was adjusted to 11 with 5 M NaOH. The compounds were eluted with methylene chloride in a glass tube containing 100 µl of glacial acetic acid, and the eluate was evaporated under nitrogen at 40 °C. The residue was dissolved with mobile phase and 20 µl was injected into the chromatograph. Cotinine analysis was performed by HPLC on a Beckman Gold System consisting of a programmable model 126 pump, a programmable model 166 multi-wavelength detector monitored at 257 nm, and a microBondapak C-18 column Inertsil ODS-3 (Interchim). The relation was linear within the cotinine concentration range of 10 to 5000 ng/ml. The recovery of cotinine measured under the extraction conditions was more than 92% within 5 to 5000 ng/ml. The limit of detection from 10 ml of urine was less than 1 ng/ml. Concentrations of creatinine were determined using the Jaffe reaction on the Dax apparatus (Bayer Diagnostic). The results of cotinine concentrations were expressed as ng/mg of creatinine. Analysis was blind to the questionnaires on smoking habits.

**Statistical analysis**

The results were expressed as means ± S.E.M. The bronchodilator response was measured as a percentage change, so-called percentage of bronchodilatation, from the largest measured SRaw values according to the following formula: [(largest SRaw value minus post-β₂ SRaw value)/largest SRaw value] × 100. The results were compared by a Mann–Whitney U-test. A test of correlation was performed to study the relationship between the doubling dose and the value of urinary cotinine and between the percentage of bronchodilatation and the value of urinary cotinine. Statistics were performed on a Macintosh computer using StatviewII® (Abacus concept, 1992, Berkeley, CA). A P value ≤ 0.05 was considered significant.

**RESULTS**

No child or family refused to participate in the study. Fifty-four children were eligible. Eight children were excluded because of a diagnosis of allergy established only on an increased blood concentration of IgE (n = 2), a concomitant upper airway infection (n = 2), a FEV₁₀ below 80% of the predicted value (n = 3) or absence of a urine sample (n = 1). Thus, 46 asthmatic and allergic children were included. The characteristics of the population are presented in Table 1. The mean age of the children (26 boys and 20 girls) was 8.3 years. The mean standing height and weight for children exposed to ETS (129.6 cm, 29.1 kg) was not significantly different from that for unexposed children (129.4 cm, 27.8 kg). The mean duration of suffering from asthma was 3 years. The number of crises per year was similar between groups. The proportion of symptoms between crises (cough or spastic episode with laugh, anger or sport) was also similar between groups. However, in most cases (65%), the diagnosis of asthma was recently advanced. This fact explained the small proportion of children with asthma treated with a prophylactic inhaled anti-inflammatory treatment like inhaled steroids (n = 7) or sodium cromoglycate (n = 7). Specific H1 antagonists were prescribed for eight children. None of the children required long-acting β₂-agonists or theophylline or immunotherapy. There was no difference between the two groups in terms of biometric data, duration, aetiology, severity and treatment of asthma. Fifty percent of the children were exposed to ETS assessed by the urinary cotinine measurement. Because of relatively low concentrations of urinary cotinine (Table 1), no child was presumed to be an active smoker. One child had no detectable urinary cotinine despite a recognized maternal smoking (less than 8 cigarettes per day, outside). This child was included in the non-exposed group. When an elevated urinary cotinine was found, exposure to ETS was due to the father in 10 cases (6 boys and 4 girls), the mother in 3 cases (1 boy and 2 girls) and both parents in 10 cases (6 boys and 4 girls). In 85% of cases the parents smoked indoors more than 10 cigarettes per day. The parental smoking habits were not gender dependent.

The results of the spirometric test, expressed as a percentage of the predicted value [24], were similar in the non-exposed and exposed groups: FVC = 101.7 ± 2.3 and 106.6 ± 2.2%, P = 0.13; FEV₁₀ = 101.9 ± 2.3 and 106.5 ± 2.6%, P = 0.20; peak expiratory flow rate = 145

**Table 1 Characteristics of the asthmatic and allergic children**

<table>
<thead>
<tr>
<th></th>
<th>No urinary cotinine (n = 23)</th>
<th>Elevated urinary cotinine (n = 23)</th>
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<tbody>
<tr>
<td>Symptom</td>
<td></td>
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<tr>
<td>Symptoms between crises</td>
<td>n = 14 (60.9%)</td>
<td>n = 10 (43.5%)</td>
</tr>
<tr>
<td>Anti-inflammatory treatment</td>
<td>n = 8 (34.8%)</td>
<td>n = 6 (26.1%)</td>
</tr>
<tr>
<td>Cotinine (ng/mg of creatinine)</td>
<td>0</td>
<td>10.3 (1–98)</td>
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</tbody>
</table>
Table 2 Carbachol and albuterol challenges in asthmatic and allergic children according to their urinary cotinine

Results are expressed as means ± S.E.M. with 95% confidence interval and compared by a Mann–Whitney U-test.

<table>
<thead>
<tr>
<th></th>
<th>Without urinary cotinine</th>
<th>With elevated urinary cotinine</th>
<th>Mann–Whitney U-test</th>
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<tbody>
<tr>
<td></td>
<td>(n = 23)</td>
<td>(n = 23)</td>
<td>P</td>
</tr>
<tr>
<td>Baseline SRaw (cmH2O·s)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(µg)</td>
<td>7.4 ± 0.4 (6.5–8.3)</td>
<td>7.6 ± 0.3 (6.9–8.3)</td>
<td>0.30</td>
</tr>
<tr>
<td>Doubling dose (µg)</td>
<td>160.9 ± 19.6 (120.3–201.5)</td>
<td>108.3 ± 14.7 (77.7–138.8)</td>
<td>0.04</td>
</tr>
<tr>
<td>Largest SRaw (cmH2O·s)</td>
<td>23.8 ± 1.6 (20.3–27.2)</td>
<td>25.9 ± 1.2 (23.4–28.4)</td>
<td>0.16</td>
</tr>
<tr>
<td>Post-/β2 SRaw (cmH2O·s)</td>
<td>7.1 ± 0.4 (6.2–7.9)</td>
<td>6.3 ± 0.3 (5.7–7.0)</td>
<td>0.26</td>
</tr>
<tr>
<td>Percentage of bronchodilatation</td>
<td></td>
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</tr>
<tr>
<td>([Largest SRaw – post-/β2 SRaw]/largest SRaw]</td>
<td>68.8 ± 1.8 (65.0–72.5)</td>
<td>74.8 ± 1.4 (71.9–77.6)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

85.6 ± 5.0 and 89.1 ± 3.2 %, P = 0.56; forced expiratory flow rate at 25 and 75% of FVC (FEF 25–75) = 104.3 ± 8.7 and 94.0 ± 6.9 %, P = 0.34. The results of the bronchial challenges are presented in Table 2. The SRaw values were chosen as the major criteria for the bronchial challenge because about one-third of the population studied (n = 15) was a pre-school population with a well-known difficult reproducibility of spirometric measurements [26]. Baseline SRaw values were not statistically different between the two groups. Clinical tolerance was excellent for each child and all of them performed the bronchial challenge. The doubling dose was statistically decreased in children with an elevated urinary cotinine (P = 0.04) but no correlation was found between the doubling dose and the value of cotinine (P = 0.19; Figure 1). The level of carbachol-induced bronchoconstriction tended to be greater in children exposed to ETS but the highest obtained values of SRaw were not significantly different between the two groups. Fifteen minutes after albuterol therapy, all the patients recovered at least their initial SRaw values with, in about 60 % of cases, a lower value than the baseline SRaw. There was no statistical difference between the two groups in the post-/β2 SRaw values. Therefore, the percentage of bronchodilatation was more pronounced in children with an elevated urinary cotinine than in children without urinary cotinine (P = 0.03). A positive correlation, albeit of borderline significance (P = 0.07), was found between the percentage of bronchodilatation and the concentrations of urinary cotinine in children (Figure 2). No statistical link was demonstrated between the doubling dose and the percentage of bronchodilatation (P = 0.12).

Despite a pronounced trend to a lower doubling dose in cases of boys exposed to ETS (88.3 ± 14.4 µg compared with 165.0 ± 24.6 µg for unexposed boys) or maternal smoking (98.4 ± 14.4 µg compared with 147.2 ± 16.7 µg for children whose father smoked or whose parents did not smoke), the statistical tests were not significantly different. In unexposed children without prophylactic anti-inflammatory treatment (n = 15) the doubling dose was higher than in exposed and untreated children (n = 17) [150.0 ± 27.1 µg and 92.9 ± 14.5 µg (P = 0.06) respectively]. When a prophylactic treatment was prescribed in exposed children (n = 6) the values of the doubling dose (151.6 ± 35.2 µg) were similar to those of treated (181.3 ± 24.8 µg) or untreated (150.0 ± 27.1 µg) unexposed children. We also observed a slightly greater bronchial reactivity to carbachol in untreated exposed children.
compared with treated exposed children ($P = 0.09$). The anti-inflammatory treatment seemed also to intensify the percentage of bronchodilatation, albeit not significantly ($P = 0.08$), in the children exposed to ETS (78.2 ± 1.9%) compared with unexposed children (73.6 ± 1.6%). In unexposed children the percentage of bronchodilatation was identical with or without treatment (67.9 ± 3.9% and 69.2 ± 1.9% respectively). No influence of gender or parental smoking habits on the percentage of bronchodilatation was found in this study.

**DISCUSSION**

Relatively few studies have been published about the relationship between BR in children with an established diagnosis of asthma and exposure to ETS. Initially, Murray and Morrison [14], in a cross-sectional study, reported a 4-fold increase in responsiveness to aerosolized histamine in children with asthma whose mothers smoked compared to children with asthma whose mothers did not smoke. However, their results need to be cautiously interpreted because only a low percentage of the total sample was effectively tested (41 subjects of 94 recruited, and only 10 children exposed to passive smoking). Thereafter, O’Connor et al. [15] reported a relationship of borderline significance between maternal smoking and BR, assessed with eucapnic hyperpnoea in subfreezing air, in 21 children with asthma among whom only 9 were exposed to maternal smoking. Murray and Morrison [16] showed an increased BR to histamine in 415 children with asthma, with 22% exposed to maternal smoking, which was especially significant in boys and in older children (12–15 years of age) exposed to ETS. Finally, a study conducted by Martinez et al. [17] in 166 unselected 9-year-old schoolchildren showed a significantly increased BR, assessed by a carbachol challenge, in exposed children, with a stronger relationship in boys and in exposed boys and in cases of maternal smoking, we found no relationship between BR to carbachol and gender or parental smoking habits. However, the more pronounced effect of ETS on boys is often recovered, which may be explained by different physiological responses to similar triggers caused by the gender difference growth [10,16,17]. Perhaps more interesting is the possible influence of the prophylactic anti-inflammatory treatment. Our results, albeit of borderline significance, suggest that the lowest value of the doubling dose, i.e. the maximal BR to carbachol, is obtained in exposed untreated children, while the maximal bronchodilatation is obtained in exposed treated children.

The mechanism by which ETS affects BR is unknown. The most advanced theory is certainly that of bronchial-induced inflammation because of an irritant effect of ETS involving the parasympathic receptors or because of direct damage to the respiratory epithelium resulting in an inflammatory–immune process [27,28]. Some studies in vivo demonstrated, in exposed animals [29] or in human smokers [30], greater concentrations of thromboxane A$_2$ or neutrophils, macrophages, pro-inflammatory mediators (interleukin-1β and interleukin-6) and chemokine (the monocyte chemoattractant protein-1, MCP-1, and the neutrophil chemoattractant, interleukin-8) in the products of bronchoalveolar lavage. Additionally, the concentrations of macrophages, neutrophils, interleukin-1β and interleukin-8 are elevated in a cigarette dose-dependent manner [31]. In the same way, the concentration of hydrogen peroxide (H$_2$O$_2$), a putative marker of airway inflammation, is increased in the exhaled air of cigarette smokers [31]. This airway inflammation may be maintained and/or again increased in children exposed to ETS because of the particular tobacco-induced sensibility of the bronchial tree to viruses, especially respiratory syncytial virus [32]. Thus, one can imagine that ETS may induce an excess of inflammation in an inflammatory disease such as asthma. Our results support this hypothesis. Indeed, asthmatic and allergic children given anti-inflammatory treatment and exposed to ETS need more carbachol to obtain a bronchoconstriction than the exposed children without preventive treatment.

Other theories have been advanced, e.g., an atopy-mediated effect or exposure in utero to maternal smoking, to explain the increased BR in the children exposed to ETS. It is well known that passive smoking enhances the blood concentrations of IgE and eosinophils and induces an allergen sensitization to common respiratory allergens [33]. On the other hand, the level of BR is directly proportional to the concentrations of IgE [34]. However, in the present study, all the children have a positive skin-prick test to one or more of the common aeroallergens and the difference in BR persists as shown by urinary cotinine concentration. As for the effect of maternal smoking mediated by exposure in utero, our study may present a bias because duration of parental smoking, including exposure in utero, was not evaluated. Soyseth et al. [20] studied 529 children and reported no significant association between BR and parental smoking, including maternal smoking during pregnancy.

The BR to albuterol is increased in our study. This effect is surprising because it is preserved and even increased when an inhaled anti-inflammatory treatment is administered. The influence of ETS on bronchodilatation was already suspected by Ekwo et al. [35] who administered inhaled isoproterenol in 89 non-asthmatic
6–12-year-old children and found a statistically significant increase in the mean values of FEV1.0 and FEF25–75 for children whose parents smoked compared with those whose parents did not smoke. The simplest explanation for a better bronchodilatation in our exposed population is that the more bronchoconstricted children can also become more bronchodilated, particularly with similar post-β2 SRaw values in both groups. However, despite a small difference between the largest SRaw values between groups, they are not statistically significant. Moreover, no link was found between the doubling dose and the bronchodilator response. On the other hand, we also found a dose-dependent relationship of borderline significance between the percentage of bronchodilatation and the concentration of urinary cotinine. Thus, a direct interaction between tobacco smoke compounds and albuterol may be suspected. It is well known that nicotine binds stereospecifically to acetylcholine receptors, that the gas-phase cigarette smoke is one of the greatest exogenous sources of nitrogen monoxide, and that cigarette smoke induces multiple damage to the bronchial epithelium [28,36]. Each of these tobacco-induced effects may be influenced by the β2-agonists: they modulate the cholinergic neurotransmission via prejunctional β2-receptors on post-ganglionic cholinergic nerves; they inhibit the excitatory non-adrenergic non-cholinergic bronchoconstrictor responses; they have intrinsic properties including an increased ciliary beating or mucociliary clearance or a decreased mediator release from mast cells, eosinophils or T-lymphocytes [37]. One result of these interactions may be, for example, an increased concentration in nitrogen monoxide which is the mediator of the bronchodilator system in humans [38]. Finally, we must consider the functional antagonism described between acetylcholine and β2-agonists [37]. The acetylcholine-induced constriction brings about a functional heterologous desensitization of the β2-receptors and so a decreased activity of the β2-agonists. As the children with an elevated urinary cotinine need a smaller quantity of carbachol to induce a bronchoconstriction compared with children not exposed to ETS, the albuterol may be more active in exposed children. Further studies are necessary to confirm our results without bronchoconstrictor challenge and to understand better the underlying mechanisms of the best ETS-induced bronchodilatation.

In conclusion, our study confirms the abnormal increased BR in asthmatic and allergic children exposed to ETS, assessed by the measurement of urinary cotinine, with an exaggerated response to bronchoconstrictor (carbachol) and bronchodilator (albuterol) agents. This excessive reactivity of the smooth muscle in asthmatic and allergic children exposed to ETS may respond to an inflammatory effect, as suggested by the possible protective effect of an anti-inflammatory treatment in our population. These data need to be confirmed in carefully designed studies. The greatest effect of albuterol observed in children exposed to ETS is unclear and needs further investigations. Our results are of borderline significance but they suggest a possible interaction between ETS and therapy in children with asthma.

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