The role of leukotrienes B₄ (LTB₄) and C₄ (LTC₄) in pathophysiology of asthma in the elderly. Relationship to bronchial hyperresponsiveness and FEV₁.0%

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Abstract: The generation of leukotrienes B₄ (LTB₄) and C₄ (LTC₄) by leucocytes stimulated with Ca ionophore A23187 was examined in 67 patients with asthma. 1. The generation of leukotriene B₄ (LTB₄) by leucocytes was significantly more increased in patients with asthma than in those with pulmonary emphysema (PE) and healthy subjects. The generation of leukotriene C₄ (LTC₄) was also significantly more increased in patients with asthma compared to the generation in those with PE and healthy subjects. The generation of both LTB₄ and LTC₄ was significantly more increased in patients with PE than in healthy subjects. 2. The generation of both LTB₄ and LTC₄ was larger in patients with asthma over age 70 than in those of other age groups, however, the difference was not significant. 3. The generation of LTC₄ was significantly higher in attack stage than in non-attack stage in all age groups of patients with asthma, however, the generation of LTB₄ was not significantly different between the two stages. 4. Bronchial hyperresponsiveness to methacholine in patients with asthma tended to decrease with aging, and the bronchial hyperresponsiveness was to a certain extent correlated with the generation of LTB₄, but not LTC₄. 5. The generation of LTC₄ was correlated with value of FEV₁.0%.

These results may suggest that both LTB₄ and LTC₄ participate in the pathophysiology of asthma in the elderly.

Key words: asthma, LTB₄, LTC₄, FEV₁.0%, bronchial hyperresponsiveness
Introduction

Leukotrienes (LTB4 and cysLTs) are potent pro-inflammatory mediators related to pathophysiological changes of the airways in asthma. LTB4 is mainly generated by neutrophils. LTB4 stimulate neutrophil chemotaxis and activation of the cells, and selectively increases the number and percentage of neutrophils in the human lung. It has been demonstrated that neutrophil inflammation enhances bronchial hyperresponsiveness. Our previous studies have shown that a significant correlation between LTB4 generation by leukocytes and the degree of bronchial hyperresponsiveness to methacholine is observed in patients with asthma. Cysteinyl leukotrienes (cysLTs) are mainly produced by eosinophils, and the amount of cysLTs generated is related to the eosinophil activation state. CysLTs display bronchoconstrictory effects, increase mucus formation, and bronchial wall edema.

Our previous studies have shown that enhanced leukotriene generation from peripheral leukocytes is observed in patients with COPD, and the presence of specific IgE antibodies against inhalant allergens enhances LTC4 generation, bronchial hyperresponsiveness, and the relationship between LTC4 generation and airway obstruction. Our studies have also shown that increased generation of LTC4 is suppressed by perilla seed oil (α-linolenic acid) supplementation.

In this study, the role of LTB4 and LTC4 was observed in elderly patients with asthma in relation to asthmatic cycle, bronchial hyperresponsiveness, and FEV1.0%.

Subjects and Methods

The subjects of this study is 67 patients with asthma (40 females and 37 males, mean age 62.1 years, range 27-86 years), 17 with pulmonary emphysema (all males, 68.8 years, 56-81 years) and 20 healthy subjects (11 females and 9 males, 51.7 years, 32-91 years). Asthma was diagnosed according to the criteria of the American Thoracic Society (ATS). The subjects were divided into 4 groups according to patient age: <49, 50-59, 60-69, and 70+ years.

The generation of leukotrienes, LTB4 and LTC4, by peripheral leukocytes was assessed by a method previously reported. Buffy coat was separated by adding a quarter volume of 6% dextran and followed by being left 1 hour at room temperature. After the number of the cells was adjusted to 5x10^6/ml in Tris ACM, Ca ionophore A23187 (1 μg) was added to the cell suspension. The mixed solution was incubated for 15 min at 37°C and centrifuged at 3000 rpm for 30 min after the addition of 4 times volume of pre-chilled ethanol (finally 80% ethanol). Supernatant was taken into the syringe filter (Toyo Roshi Co, Japan), and the filtrate was decompressed and dryed up to solid. The solid was dissolved with 250 μl of 50% ethanol. The HPLC analysis for LTB4 and LTC4 was performed by a method described by Lam, et al. The results were expressed as ng/5x10^6 cells.

Bronchial reactivity to methacholine was measured by an Astograph (TCK 6100, Chest Co). Different concentrations of methacholine (49, 98, 195, 390, 781, 1563, 3125, 6250, 12500 and 25000 μg/ml) were prepared for bronchial challenge according to the method used by Chai et al. The increase of total respiratory resistance (Rrs) after methacholine
inhalation was measured by the oscillation method.\(^1\) A methacholine concentration causing a significant increase in \(R_{rs}\) was assessed as \(C_{min}\) (minimum concentration). All medications were stopped 12 hours prior to examination.

Ventilatory function tests using a Box Spiror 81-S (Chest Co, Japan) were carried out in all subjects when they were attack-free.

IgE antibodies against inhalant allergens, house dust mite, cockroach, and Candida albicans, were estimated by radioallergosorbent test (RAST), and serum level of IgE was measured by radioimmunosorbent test (RIST).

Statistically significant differences of the mean were estimated using the unpaired Student's t-test. A p value of <0.05 was regarded as significant.

**Results**

Table 1 shows the characteristics of patients with asthma studied here. The mean level of serum IgE was higher in patients under age 49 compared with the level in other age groups. However, the differences were not significant. The frequency of patients with a positive RAST against inhalant allergens was more than 50% in all age groups.

<table>
<thead>
<tr>
<th>Age, years</th>
<th>No of patients</th>
<th>Mean age years</th>
<th>IgE (IU/ml)</th>
<th>positive* RAST(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;49</td>
<td>8</td>
<td>40.2</td>
<td>1201</td>
<td>6/8(75.0%)</td>
</tr>
<tr>
<td>50-59</td>
<td>20</td>
<td>55.7</td>
<td>392</td>
<td>20/15(75.0%)</td>
</tr>
<tr>
<td>60-69</td>
<td>21</td>
<td>62.6</td>
<td>339</td>
<td>11/21(52.4%)</td>
</tr>
<tr>
<td>70+</td>
<td>18</td>
<td>74.0</td>
<td>390</td>
<td>11/18(61.0%)</td>
</tr>
</tbody>
</table>

*positive RAST score against inhalant allergens

The generation of LTB4 and LTC4 was compared among healthy subjects, patients with pulmonary emphysema (PE), and those with asthma. The LTB4 and LTC4 generation was significantly larger in patients with asthma than in those with PE and healthy subjects. The generation of both LTB4 and LTC4 in patients with PE was significantly higher than the generation in healthy subjects (Fig. 1).

The generation of LTB4 by leukocytes was the highest (96.2 ±32.8 ng/5x10^6 cells) in patients with asthma over age 70, however, there were not significantly differences among four age groups (Fig. 2). The LTC4 generation by leukocytes was also the highest (57.6±48.9 ng/5x10^6 cells).
9 ng/5x10^6 cells) in elderly patients over age 70 compared with the generation in other age groups (Fig. 3).

![Graph of LTC4 generation in patients with asthma](image)

Fig. 3. Generation of LTC4 by leukocytes in patients with asthma

The generation of LTB4 during attack stages was larger than the generation in non-attack stages in patients under age 49. The same tendency as the generation in these patients was observed in other age groups. However, the difference in LTB4 generation between attack and non-attack stages was not significant in all age groups (Fig. 4). In contrast, the generation of LTC4 during attack stages was significantly larger than the generation in non-attack stages in all age groups (Fig. 5).

![Graph of LTB4 generation in patients with asthma](image)

Fig. 4. Generation of LTB4 by leukocytes in patients with asthma in relation to asthmatic cycle. A: attack stage, N: non-attack stage

Bronchial hyperresponsiveness (BH) to methacholine showed a tendency to decrease with aging, as shown in Fig. 6. A correlation between BH to methacholine and the generation of LTB4 by leucocytes was found in patients with asthma. Only 2 (11.8%) of 17 patients with BH to methacholine more than 1563 mcg/ml showed an increased generation of LTB4 more than 100 ng/6x10^6 cells. In contrast, 16 (45.7%) of 35 patients with BH to methacholine less than 390
mcg/ml showed an increased LTB4 generation more than 100 ng/5x10⁶ cells (Fig. 7). Any significant correlation was not observed between LTC4 generation and BH to methacholine, as shown in Fig. 8.

While an increased LTC4 generation was to a certain extent related to the value of FEV1.0% in patients with asthma: 10 (83.3%) of 12 asthmatics with an increased generation of LTC4 more than 80 ng/5x10⁶ cells showed the value of FEV1.0% less than 70% (Fig. 10).

The LTB4 generation was not related to the value of FEV1.0% in patients with asthma (Fig. 9).

**Discussion**

It has been well known that leukotriene B4 and cysLTs, LTC4, LTD4 and LTE4, play an important role in pathophysiology of the airways of bronchial asthma. LTB4, which is mainly produced by neutrophils, has a chemotactic action for neutrophils as well as interleukin 8 (IL8).
In contrast, LTC4, which is almost exclusively generated by eosinophils, has bronchoconstrictory action, increase mucus formation, and induces accumulation of eosinophils into the airways.

In the present study, the role of LTB4 and LTC4 in the elderly patients with asthma was examined in relation to asthmatic cycle, bronchial hyperresponsiveness, and the value of FEV1.0%. The generation of both LTB4 and LTC4 by leucocytes was significantly higher in patients with asthma and in those with PE than in healthy subjects. The results show the possibility that both LTB4 and LTC4 participate in the pathophysiology of asthma and pulmonary emphysema.

The generation of LTB4 by leucocytes was the largest in patients over age 70 compared to the generation in other age groups. Although the difference in the LTB4 generation among four age groups was not significant, the generation of LTB4 was considerably large even in patients over age 70. The LTC4 generation in patients over age 70 was also considerably large compared to the generation in those under age 49. These results demonstrate that both LTB4 and LTC4 participate in the onset mechanism of asthma in the elderly. Regarding the LTB4 and LTC4 generation relating to asthmatic cycle, a significant difference was found in the LTC4 generation between attack and non-attack stages: a significant increase in LTC4 generation was observed during asthma attacks. However, there was no significant difference in LTB4 generation between attack and non-attack stages. The results suggest that LTC4 is closely related to asthma attacks associated with bronchoconstriction.

Bronchial hyperresponsiveness to methacholine was related to a certain extent to the LTB4 generation by leucocytes in patients with asthma, but not to the LTC4 generation. Our previous studies have shown that leukotriene C4 production by leucocytes is associated with immunoglobulin E-mediated allergy and asthma exacerbations, and further that generation of LTB4 is closely related to bronchial hyperresponsiveness to methacholine in patients with asthma. The results suggest that an increased production of LTB4 by leucocytes is closely related to an increase in bronchial hyperresponsiveness.

The FEV1.0% value was related to a certain extent to LTC4 generation in patients with an increased production of LTC4, but not to LTB4 production, suggesting that LTC4 is correlated with bronchoconstriction. These results obtained here show that LTB4 and LTC4 play an important role in asthma in the elderly.

References


高齢者気管支喘息の臨床病態におけるロイコトリエン B4、C4 の役割．気道過敏性と FEV1.0% との関連

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気管支喘息67例を対象に，Ca ionophore A 23187刺激時の白血球からのロイコトリエン B4 （LTB4）およびC4 （LTC4）の産生を観察し，
高齢者喘息の臨床病態におけるLTB4，LTC4の役
割について検討した。1．LTB4の産生は，気管
支喘息において肺気腫および健康人に比べ有意に
高い値を示した。LTC4の産生も同様に気管支喘
息において，肺気腫，健康人に比べ有意に高い値
を示した。LTB4, LTC4いずれの産生も，肺気腫
症例において健康人と比べ有意に高い値が観察さ
れた。2．LTB4，LTC4の産生は，70才以上の喘
息症例において，他的年齢層の症例に比べより高
い値が示されたが，推計学的な有意差は見られな
かった。3．高齢者を含めずすべての年齢層の気管
支喘息においても，LTC4の産生は発作時に，非
発作時に比べ有意の亢進が見られたが，LTB4に
関しては発作時，非発作時の間に有意の差は見ら
れてなかった。4．気道の過敏性は，年齢が高くな
るにつれて低下する傾向が見られた。そして，こ
の気道の過敏性はLTB4産生とある程度関連して
いることが示されたが，LTC4との関連は見られ
なかった。5．FEV1.0%値は，ある程度LTC4産
生亢進と関連があることが示された。

以上の結果より，70才以上の喘息においても，
LTB4，LTC4がその発作時病態に重要な役割を果
していることが示唆された。