Association of the mean CT number by high resolution computed tomography (HRCT) with generation of leukotrienes B4 (LTB4) and C4 (LTC4) in patients with pulmonary emphysema

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Summary: The generation of leukotrienes B4 (LTB4) and C4 (LTC4) by peripheral leukocytes stimulated with Ca ionophore A23187 was examined in 17 patients with pulmonary emphysema. They were divided into three groups according to the mean CT number; A (<-940 HU), B (-940 ≤, <-930 HU), and C (-930 HU ≤ ).

1. The values of FVC (%predicted), FEV1.0% and DLco (%predicted) showed a tendency to decrease as the mean CT number was smaller. The value of %FVC was significantly lower in patients with group A than in those with group C (p<0.05).

2. The generation of both LTB4 and LTC4 was significantly higher in patients with emphysema compared to the generation in healthy subjects (LTB4; p<0.05, LTC4;P<0.05).

3. The generation of LTB4 and LTC4 in patients with emphysema was higher as the mean CT number was larger (severity of the disease become less). The LTC4 production was significantly higher in patients with group C (mild or moderate type of emphysema) than in those with group A (advanced type of emphysema).

These results suggest that the generation of LTB4 and LTC4 is higher in patients with mild or moderate type of emphysema than in those with advanced type of the disease.

Key words: emphysema, mean CT number, LTB4, LTC4,

Introduction

Pulmonary emphysema is characterized by abnormal permanent enlargement of air spaces distal to the terminal bronchioles, accompanied by destruction of alveolar walls without obvious fibrosis1). The diagno-
sis of emphysema is based on clinical findings related to pathologic changes. In recent years, it has been shown that the relative area of the lung with low attenuation on high resolution computed tomography (HRCT) scans obtained at full inspiration is an objective measure of the extent of pulmonary emphysema. The symptoms characteristic of patients with pulmonary emphysema are various degrees of dyspnea on exertion. It is sometimes accompanied by wheezing, suggesting that large and medium airways are involved in pulmonary emphysema.

Leukotriene B4 (LTB4) and cysteinyl leukotrienes (cysLTs), LTC4, LTD4 and LTE4, are pro-inflammatory mediators, which participate in airway inflammation. LTB4 stimulates neutrophil chemotaxis, enhances neutrophil-endothelial interaction, and stimulates neutrophil activation. In contrast, cysLTs contract airway smooth muscle, increase bronchial wall edema and stimulate mucous secretion.

In the present study, the generation of LTB4 and LTC4 by leukocytes stimulated with Ca ionophore A23187 was examined in patients with pulmonary emphysema in relation to the mean CT number on HRCT.

**Subjects and Methods**

The subjects of this study were 17 patients (all males, mean age 65.2 years) with pulmonary emphysema. All of them complained of dyspnea on exertion. The diagnosis of emphysema was performed by clinical symptoms and findings including chest X-ray, and pulmonary function test. The diagnosis was also evaluated by low attenuation area on HRCT. The results from patients with emphysema were compared with the results from 12 healthy controls (7 females and 5 males, mean age 56.3 years). The subjects were divided into three groups according to the mean CT number in Hounsfield Unit (HU): A (<-940 HU), B (-940 HU ≤, <-930 HU), and C (-930 HU ≤).

CT scans were carried out on a Toshiba Xpeed scanner (2.7s, 200mAs, 120Kvp) without infusion of contrast medium, using 2mm collimation (HRCT) in patients breathholding at full inspiration. The lungs were scanned as preselected three anatomic levels: (1) top of the aortic arch, (2) origin of the lower lobe bronchus, (3) three cm above the top of the diaphragm, as reported by Miniati, et al. Inspiratory HRCT scans were evaluated quantitatively by measuring the percentage of lung area with CT number < -950 HU (%low attenuation area; %LAA)(Fig.1). The average of the CT numbers in three anatomic levels was expressed as the mean CT number. The maximam %LAA among the three anatomic levels of the lung was expressed as representative %LAA in each patient with pulmonary emphysema.

![Fig.1. Low attenuation area of the lung < -950 HU on high resolution computed tomography in patient with pulmonary emphysema.](image)

(mean CT number -953 HU, maximum %LAA 67.5%)(73 years, male, group A)

The production of leukotrienes, LTB4 and LTC4, by peripheral leukocytes was evaluated by a method previously reported. Buffy coat was separated by adding a quarter volume of 6% dextran and followed by being left 1h at room temperature. The number of the cells was adjusted to 5x10⁶ cells/ml in Tris ACM, and then Ca ionophore A23187 (1µg) 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. Supernatant was taken into the syringe filter (Toyo Roshi Co, Japan), and the filtrate was de-compressed and dried up to solid, which 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.\textsuperscript{[12]}. The results were expressed as ng/5x10\textsuperscript{6} cells.

Pulmonary function tests, forced vital capacity (FVC) and forced expiratory volume in one second (FEV 1.0), were carried out in all patients using a Chestac 33 (Chest Co, Japan) linked to a computer when they were attack-free. The diffusing capacity for carbone monoxide (DLco) was measured by the single-breath technique using a Chestac 33 (Chest Co, Japan). The actual DLco values were expressed as percent of the predicted value of Nishida et al.\textsuperscript{[13]}

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

**Results**

Table 1 shows mean age and the mean of maximum %LAA of subjects classified by the mean CT number. The mean age was not related to the value of the mean CT number. The value of maximum %LAA was closely related to the mean CT number. The values of %FVC and FEV1.0% were lower as the mean CT number was smaller. The %FVC value was significantly lower in patients with mean CT number under -940 HU than in those with mean CT number over -930 HU (p<0.05). However, the FEV1.0% value was not significantly different among three groups classified by the mean CT number. The value of DLco (%predicted) was also lower as the mean CT number was smaller, however, these differences were not significant (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>FVC (%predicted)</th>
<th>FEV1.0%</th>
<th>DLco (%predicted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>67.1(%)±9.9</td>
<td>43.8(%)±4.8</td>
<td>67.9(%)±5.0</td>
</tr>
<tr>
<td>B</td>
<td>79.3(%)±22.8</td>
<td>44.3(%)±6.5</td>
<td>73.2(%)±24.4</td>
</tr>
<tr>
<td>C</td>
<td>82.8(%)±10.4</td>
<td>48.9(%)±15.8</td>
<td>80.1(%)±24.5</td>
</tr>
</tbody>
</table>

a;p<0.05.

The production of leukotriene B4(LTB4) by peripheral leucocytes was significantly larger in patients with pulmonary emphysema than in healthy subjects (P<0.05). The production of leukotriene C4(LTC4) was also significantly higher in patients with emphysema compared to the production in healthy subjects (P<0.05), as shown in Fig.2.

The generation of LTB4 was larger as the mean CT number was smaller, however, these differences among three groups were not significant. The LTC4 generation was significantly larger in patients with the mean CT number over -930 HU than in those with the mean CT number under -940 HU (P<0.05)(Fig.3).

**Discussion**

In recent years, it has become increasingly clear that high resolution computed tomography (HRCT) is the most accurate imaging method for diagnosing em-
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An objective measure of the extent of pulmonary emphysema. In contrast, the mean CT number is lineally related to the fraction of air in the lung. Thus, it is generally agreed that CT scanning; both the mean CT number and the percent of lung area with attenuation values <-950 HU, is a sensitive technique capable of detecting emphysematous lesions.

In this study, the generation of LTB4 and LTC4 by leukocytes was examined in patients with pulmonary emphysema, who were classified into three groups according to the mean CT number. The mean CT number was closely related to the maximum %LAA <-950 HU; the mean CT number was smaller as the maximum %LAA was larger. The values of %FVC, FEV1.0% and DLco (%predicted) were also related to the mean CT number. These values were lower in patients with smaller mean CT number, suggesting that they had larger emphysematous lesions (advanced stage of emphysema).

It has been well known that leukotriene B4 and cysteinyl leukotrienes (cysLTs), LTC4, D4, and E4, play an important role in pathophysiology of the airways of bronchial asthma. LTB4 has a chemotactic action for neutrophils as well as interleukin 8 (IL8), which induces bronchial hyperresponsiveness and airway neutrophil accumulation. In contrast, LTC4 production is mainly due to eosinophils. Accumulation of eosinophils into the airways often associated with increased production of LTC4. Schauer et al. have reported that the generation of LTC4 by leukocytes stimulated with Ca ionophore A23187 was significantly higher in asthmatic children than in healthy controls. They also demonstrated that granulocytes from patients with a history of severe asthma displayed a higher LTC4 formation than granulocytes from patients with less severe disease.

However, there are few reports about the generation of LTB4 and LTC4 by leukocytes in patients with pulmonary emphysema. In this study, the generation of LTB4 and LTC4 by leukocytes stimulated with Ca ionophore A23187 was examined in patients with pulmonary emphysema in vivo. The relative areas of the lungs that have attenuation values lower than -950 HU on high-resolution CT scans obtained at full inspiration is...
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emphysema. The results revealed that the production of both LTB4 and LTC4 by leucocytes was significantly higher in patients with emphysema than in healthy subjects. Furthermore, the production of LTC4 was significantly larger in patients with emphysema showing the mean CT number more than -930 HU (mild or moderate type of emphysema) than in those with the mean CT number smaller than -940 HU (advanced type of emphysema). The results may suggest that airway inflammation is more intensive in mild or moderate type of emphysema than in advanced type of the disease.

References
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肺気腫患者におけるロイコトリエン B4, C4 産生能と高分解能 CT による平均 CT 値との関連

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宿野 浩史, 岡本 誠, 原田 義之, 湯本英一郎,
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肺気腫患者 17 名を対象として, カルシウムイオノフォア A23187 による末梢血白血球からのロイコトリエン B4(LTB4), ロイコトリエン C4(LTC4) の産生能の検討を行った。対象は高分解能 CT による平均 CT 値によって A(<940HU), B(940≦, <930HU), C(930HU≦) の 3 群に分類した。

1. FVC, FEV1.0%, %DLCO が低下するにつれて平均 CT 値が低下する傾向が見られた。
2. FVC 値は C 群に比較して, A 群において有意に低値を示した (p<0.05)。
3. 肺気腫患者における LT4 と LTC4 の産生能は肺機能と有意に高値を示した (LT4;p<0.05, LTC4;p<0.05)。

以上のように肺気腫における LT4, LTC4 産生能は進行例に比較すると, 軽・中等症例において高値であることが示唆された。