◎原 著

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

Fumihiro Mitsunobu, Takashi Mifune, Yasuhiro Hosaki, Kozo Ashida, Hirofumi Tsugeno, Makoto Okamoto, Seishi Harada, Eiichiro Yumoto, Shingo Takata, Koji Ochi[1)], Hideo Harada[1)], and Harumi Hasegawa[2)]

Division of Medicine, Misasa Medical Branch, [1)]Department of Laboratory Medicine, Okayama University Medical School, [2)]Division of Medicine, Tottori City Hospital

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 fibrosis[1)]. 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[2,3]. The symptoms characterictic 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 leukotrines (cysLTs), LTC4, LTD4 and LTE4, are pro-inflammatory mediators, which participate in airway inflammation. LTB4 stimulates neutrophil chemotaxis[4], enhances neutrophil-endothelial interaction[5], and stimulates neutrophil activation[6]. In contrast, cysLTs contract airway smooth muscle[7], increase bronchial wall edema[8] and stimulate mucous secretion[9].

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 $\leqq$, <-930 HU), and C (-930 HU $\leqq$).

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.[10]. 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.

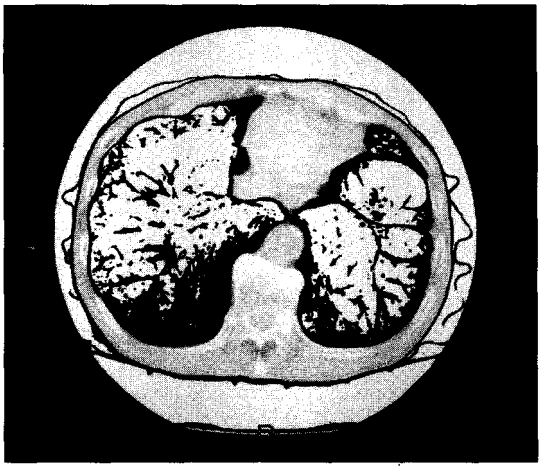

FIg.1. Low attenuation area of the lung <-950 HU on high resolution computed tomography in patient with pulmonary emphysema.
(mean CT number -953 HU, maximum %LAA 67.5%)(73 years, male, group A)

The production of leukotrienes, LTB4 and LTC4, by peripheral leukocytes was evaluated by a method previously reported[11]. Buffy coat was separated by adding a quater volume of 6% dextran and followed by being left 1h at room temperature. The number of the cells was adjusted to $5x10^6$ cells/ml in Tris ACM, and then Ca ionophore A23187 (1 μg) was added to the

cell suspension. The mixed solution was incubated for 15 min at 37℃ , 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 decompressed 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.[12]. The results were expressed as ng/5x10^6 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.[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).

Table1. Characteristics of patients with pulmonary emphysema classified by mean CT number

Group	Mean CT number	No of patients	Mean age (years)	Max %LAA
A	<-940	5	68.0	69.5%
B	-940≦, <-930	6	70.5	54.5%
C	-930≦	6	67.7	46.8%

Max %LAA;maximum % low attenuation area

Table2. Pulmonary function in patients with emphysema classified by mean CT number

Group	FVC (%predicted)	FEV1.0%	DLco (%predicted)
A	67.1(%)[a] ± 9.9	43.8(%) ± 4.8	67.9(%) ± 8.0
B	79.3(%) ±22.8	44.3(%) ± 6.5	73.2(%) ±24.4
C	82.8(%)[a] ±10.4	48.9(%) ±15.8	80.1(%) ±24.5

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-

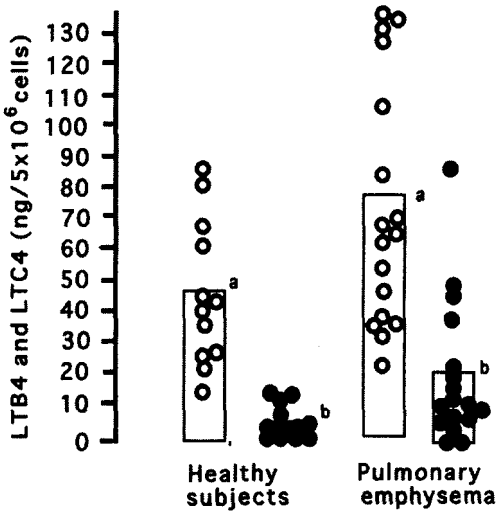

Fig.2. Generation of leukotrienes B4 (LTB4)(○) and C4 (LTC4)(●)by leukocytes in patients with pulmonary emphysema and healty subjects. a and b;p<0.05.

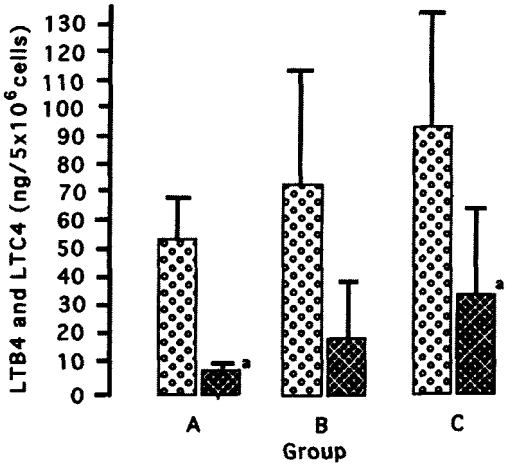

Fig.3. Generation of leucotrienes B4 (LTB4)(▨) and C4 (LTC4)(▩)by leukocytes in patients with pulmonary emphysema classified by the mean CT number: A; <-940, B; -940 ≦ , <-930, C; -930 ≦ . a;p<0.05.

physema in vivo[10,14]. The relative areas of the lungs that has attenuation values lower than -950 HU on high-resolution CT scans obtained at full inspiration is an objective measure of the extent of pulmonary emphysema[2,3]. 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 leukotrieneB4 and ctsteinyl 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[15]. In contrast, LTC4 production is mainly due to eosinophils[16-18]. Accumulation of eosinophils into the airways often associated with increased production of LTC4[19]. Schauer et al. have reported that the generation of LTC4 by leucocytes stimulated with Ca ionophore A23187 was significantly higher in asthmatic children than in healthy controls[17]. 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 leucocytes in patients with pulmonary emphysema. In this study, the generation of LTB4 and LTC4 by leucocytes stimulated with Ca ionophore A23187 was examined in patients with

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

1. Snider GL, Kleinerman J, Thurlbeck WM, et al.:The definition of emphysema:report of a National Heart, Lung and Blood Institute, Division of Lung Diseases Workshop. Am Rev Respir Dis 132:182-185,1985.
2. Genenois PA, de Maertelaer V, De Vuyst P, et al.:Comparison of computed density and macroscopic morphology in pulmonary emphysema. Am J Respir Crit Care Med 152:653-657,1995.
3. Gevenois PA, De Vuyst P, de Maertelaer V, et al.:Comparision of computed density and microscopic morphology in pulmonary emphysema. Am J Respir Crit Care Med 154:187-192,1996.
4. Palmer RM, Stepney RJ, Higgs GA, et al.: Chemokinetic activities of arachidonic and lipoxygenase products on leukocytes of different species. Prostaglandins 20:411-418,1980.
5. Hoover RL, Karnovsky MJ, Austen KF, et al.: Leukotriene B4 action on endothelium mediators augmented neutrophil/endothelial adhesion. Proc Natl Acad Sci USA 81:2191-2193,1984.
6. Sha'afi RI, Naccache PH, Molski P, et al.:Cellular regulatory role of leukotriene B4:its effects on cation homeostasis in rabbit neutrophils. J Cell Physiol 108:401-408,1981.
7. Barnes NC, Piper PJ, and Costello JF:Comparative actions of inhaled leukotriene C4, leukotriene D4 and histamine in normal human subjects. Thorax 39:500-504,1984.
8. Lewis RA and Robin JL:Arachidonic acid derivatives as mediators of asthma. J Allergy Clin Immunol 76:259-263,1985.
9. Marom ZJ, Shelharmer MK, Bach DR, et al.:Slow-reactiong substances, leukotriene C4 and D4, increase release of mucus from human airways in vitro. Am Rev Respir Dis 136:449-451,1982.
10. Miniati M, Filippi E, Falashi F, et al.:Radiologic evaluation of emphysema in patients with chronic obstructive pulmonary disease:chest radiography versus high resolution computed tomography. Am J Respir Crit Care Med 151:1359-1367,1995.
11. Sakakibara H, Hirose K, Matsushita K, et al.:Effect of supplementation with eicosapentaenoic acid ethyl ester, MND-21, on generation of leukotrienes by Calcium ionophore-activated leukocytes in bronchial asthma. Jpn J Assoc Thorax Dis 33:396-412,1996.
12. Lam S, Chan H, LeRiche JC, et al.:Release of leukotrienes in patients with bronchial asthma. J Allegy Clim Immunol 81:711-717,1988.
13. Aritomi T, Toyoshima H, Yoshida M:Pulmonary diffusing capacity in japan. Kokyuu 13:261-267 (in Japanese).
14. Hruban RH, Meziane MA, Zerhouni EA, et al.:High resolution computed tomography of inflation-fixed lungs. Pathologic-radiologic correlation of centrilobular emphysema. Am Rev Respir Dis 136:935-940,1987.
15. Fujiwara M, Xiu Q, Tsujiura M, et al.:Role of leukotriene B4 in bronchial hyperresponsiveness induced by interleukin-8. Eur Respir J 11:306-311, 1998.
16. Shaw RJ, Cromwell O, and kay AB:Preferential generation of leukotriene C4 by human eosinophils. Clin Exp Immunol 56:716-722, 1984.
17. Schauer U, Eckhartt A, Muller R, et al.:Enhanced leukotriene C4 production by peripheral eosinophilic granulocytes from children with asthma. Int Arch

Allergy Appl Immunol 60:201-206,1989.
18. Weller PF, Lee CW, Foster DW, et al.:Generation and metabolism of 5-lipogenase pathway leukotrienes by human eosinophils:Predominant production of leukotriene C4. Proc Natl Acad Sci USA 80:7626-7630,1983.
19. Underwood DC, Osborn RR, Newsholme SJ, et al.:Persistent airway eosinophilia after leukotriene (LT) D4 administration in the guinea pigs. Am J Respir Crit Care Med 154:850-857,1996.

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

光延 文裕，御舩 尚志，保崎 泰弘，芦田 耕三，柘野 浩史，岡本 誠，原田 誠之，湯本英一郎，高田 真吾，谷崎 勝朗，越智 浩二[1)]，原田 英雄[1)]，長谷川晴巳[2)]，

岡山大学医学部三朝分院内科
臨床検査医学[1)]
鳥取市立病院内科[2)]

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。%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 ($p<0.05$)。2．LTB4 と LTC4 の産生能は健常人に比較して，肺気腫患者において有意に高値を示した (LTB4;$p<0.05$，LTC4;$p<0.05$)。3．肺気腫患者における LTB4 と LTC4 の産生能は平均 CT 値が上昇するにつれて（軽症症例ほど）高値を示した。また LTC4 産生能は C 群（早期肺気腫症例）において A 群（進行肺気腫症例）に比して有意に高値を示した。

以上より肺気腫における LTB4，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。