◎原 著

Dietary supplementation with n－3 fatty acids in bronchial asthma correlated with the generation of LTB 4 and LTC 4

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Abstract : In recent years, it has been noted that there is a close correlation between leukotrienes and late asthmatic reaction (LAR). In this study, effects of dietary supplementation with perilla seed oil rich in alpha-linolenic acid, which is speculated to affect the generation of leukotrienes through metabolism of arachidonic acid (AA), were evaluated in 6 patients with asthma. The symptoms and ventilatory function were improved after 2 -week dietary supplementation with perilla seed oil. The generation of LTB 4 and LTC4 by peripheral leucocytes stimulated with Ca ionophore A23187 was significantly suppressed by the dietary supplementation (LTB4 and LTC 4 ; $p<0.05$). Regarding the composition of fatty acids in serum phospholipids, the concentrations of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and AA tended to increase after the supplementation, accompanied with an increase in the ratio of EPA to AA. These results suggest that dietary supplementation with perilla seed oil brings beneficial effects in the treatment of asthma.

Key words : n－3 fatty acids, bronchial asthma, arachidonic acid, LTB 4, LTC 4

Introduction

It has been suggested that dietary supplementation with polyunsaturated fatty acids (PUFA) is beneficial for the treatment of various chronic diseases. Unsaturated fatty acids of marine origin, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), protect the progress of cardiovascular disease [1] by modyfying prostanoid synthesis [2]. In contrst, effects of supplementation with n－3 fatty acids on bronchial asthma are still controversial [3], however, several reports suggest beneficial effects

of n－3 fatty acids on asthma and allergic rhinitis [4-7].

It has been reported that there is a negative correlation between linoleic acid (LA) intake and serum amounts of EPA in the elderly [8]. The ratio of LA to α－linolenic acid may have important effects on the levels of the longer chain n－3 fatty acids, particularly EPA, in platelet and plasma fatty acids [9], suggesting the importance of n－3 /n－6 fatty acid ratio in serum total phospholipids.

In the present study, effects of dietrary supplementation with perilla seed oil on bronchial asthma were evaluated in relation to symptoms, ventilatory function, generation of leukotrienes B4 and C4 by leucocytes, and changes in composition of serum phospholipid fatty acids.

Subjects and Methods

The subjects of this study were 6 patients (5 females and 1 male) with asthma, including one patient with cough variant asthma (CVA). Their mean age was 62 years (range 51－72 years), and the mean of serum IgE was 178.8 IU/mℓ (range 7－720 IU/mℓ). Clinical symptoms and peak flow rate (PFR) in the early morning and evening were recorded in all subjects. Bronchial reactivity to β 2 agonist was estimated in all subjects by observing the improvement of morning PER after inhalation of salbutamol.

The subjects took 10－20 gram of perilla seed oil per day as salad dressing or mayonmaise for 2－4 weeks. The amount of oil used in diet and supplementary diet oil was recorded throughout the study periods.

The generation of leukotrienes B4 (LTB4) and C4 (LTC4) by peripheral leucocuytes was assessed by a method previously described [10, 11]. Cells were separated by counterflow centrifugation elutriation with a JE 6 B rotor (Beckman Co.) [12], as described previously [13]. After the number of the cells was adjusted to 5×10^6/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 300g for 10 min at 4℃. The HPLC analysis for extraction and quantification of LTB4 and LTC4 was performed by a method described by Lam et al.[14]. The extraction of leukotrienes was performed using a C18 Sep-Pak (Waters Associates). The concentrations of LTB4 and LTC4 were analyzed by HPLC system, Model 510 (Waters Associates), equipped with an ultraviolet detector. The column used was a 5 mm×10cm Radial-Pax cartridge (Shimazu Co.). The results were expressed as ng/5×10^6 cells.

Analysis of the composition of serum phospholipid fatty acids was performed by a method reported by Okita et al. [15].

Results

The improvement of morning PFR by inhalation of β 2 agonist was 47% before dietary supplementation, and 40% 2 weeks after the manipulation.

The clinical symptoms tended to improve in all patients with asthma 2 weeks after dietary supplementation with perilla seed oil. The mean peak flow rate (PFR) also improved after the dietary supplementation. Percent increase in morning PFR before and after inhalation of β2 agonist was 13% and 9% (n＝6), respectively, 2 weeks after the supplementation (Fig. 1).

Figure 2 shows improvement of PFR in patient with asthma (56 years old, female) after the supplementation. The PFR in the

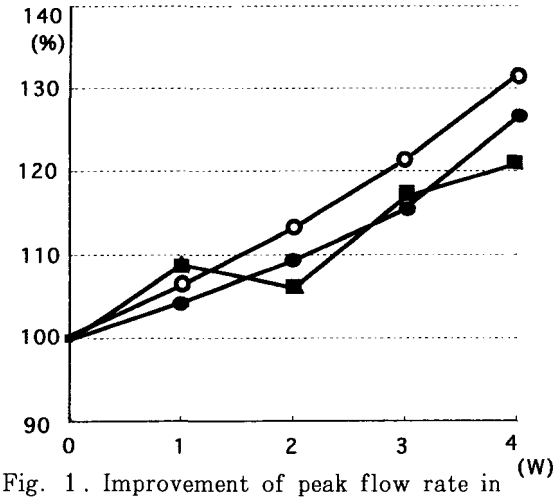

Fig. 1. Improvement of peak flow rate in asthmatics with dietary supplementation. Data represent the mean of 6 subjects. Peak flow rate (●—●) : in the early morning, (○—○) : after β 2 agonist inhalation, and (■—■) : in the evening.

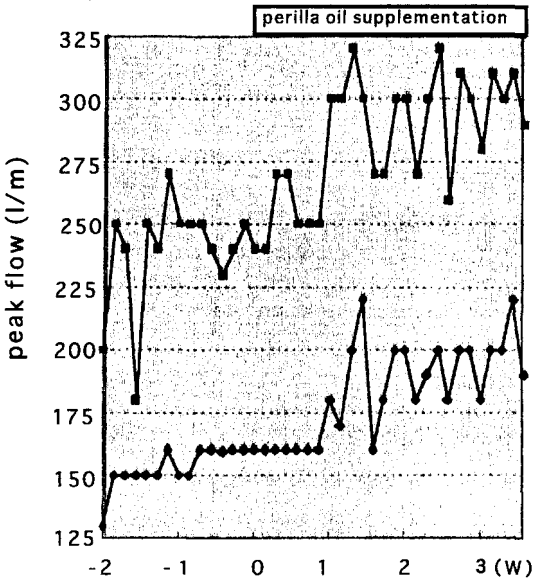

Fig. 2. Changes of peak flow rate in the early morning (◆—◆) and after β 2 agonist inhalation (■—■) in asthmatics with dietary supplementation (56 years old, female)

early morning tended to increase 1 week after the beginning of dietary supplementation. During the periods the medications were not changed in this case.

The PFR improvement was also observed in another patient with asthma (58 years old, female), who received dietary supplementation for 4 weeks. The morning PFR before and after β 2 agonist inhalation showed a tendency to increase after the supplementation despite the decrease in dose of beclomethasone inhalation. Furthermore, the rate clearly increased after consecutive dietary supplementation, accompanied with pranlukast (Fig. 3).

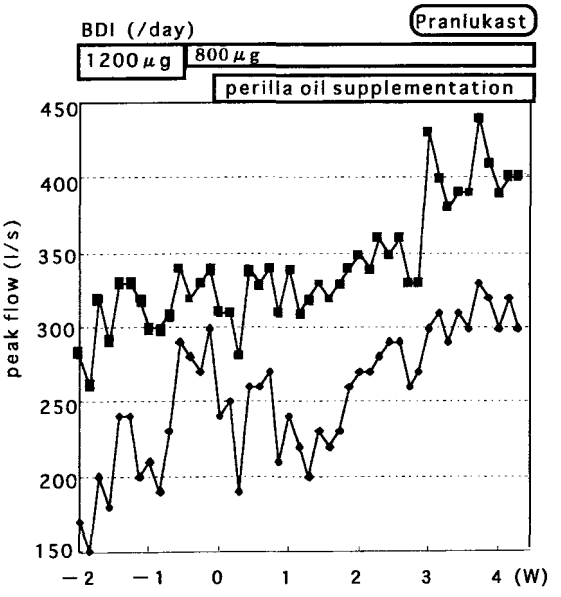

Fig. 3. Changes of peak flow rate in the early morning (◆—◆) and after β 2 agonist inhalation (■—■) in asthmatics with dietary supplementation (58 years old, female)

Ca ionophore A23187-induced generation of LTB 4 and LTC 4 by peripheral leococytes was evaluated in 5 patients (including CVA) before and after manipulation with perilla

seed oil. The mean values of LTB 4 and LT C 4 before the supplementation was 77.6 ± 24.4 and 64.0±33.5ng/ 5 ×10⁶ cells (mean± SD) respectively. After the oil manipulation the value of LTB 4 tended to decrease (41.6 ±24.9 ng/ 5 ×10⁶ cells) in 4 of the 5 subjects ($p<0.05$). The decrease of LTB 4 generation was marked in subjects 3, 4, and 5, whose generation of LTB 4 by leucocytes was relatively high (Fig. 4).

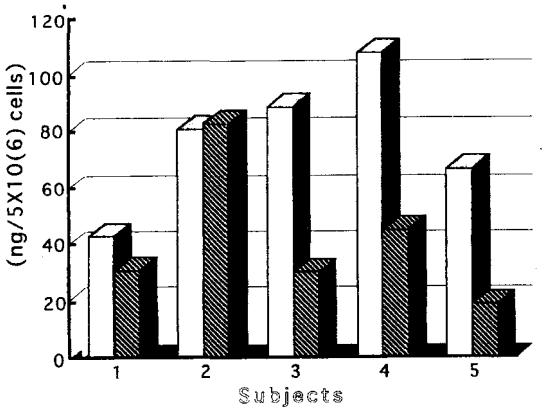

Fig. 4. Decreases in the generation of LTB 4 by peripheral leukocytes from subjects before (□) and after dietary supplementation (▨).

The generation of LTC 4 by leucocytes significantly decreased (38.8 ± 23.2ng/ 5 × 10⁶ cells) ($p<0.05$). Neither peak of LTB 5 and LTC 5 in HPLC was not observed before and after dietary supplementation (Fig. 5).

The linoleic acid (LA), AA, EPA, and DHA in plasma phospholipid were 787±103, 327± 12, 13±16, and 285±61nmol/mℓ, respectively, prior to oil supplementation. After dietary supplementation the content of these fatty acids increased; LA+105%, AA+124%, EPA+146%, and DHA+146% as compared with the values before the supplementation. The increase was larger in EPA and DHA than in AA and other fatty acids (Fig. 6).

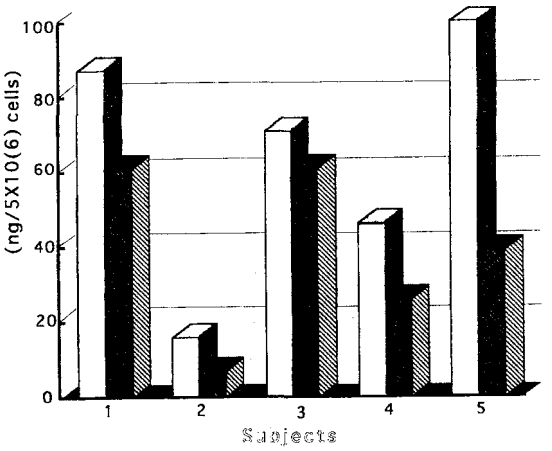

Fig. 5. Decreases in the generation of LTC 4 by peripheral leukocytes from subjects before (□) and after dietary supplementation (▨).

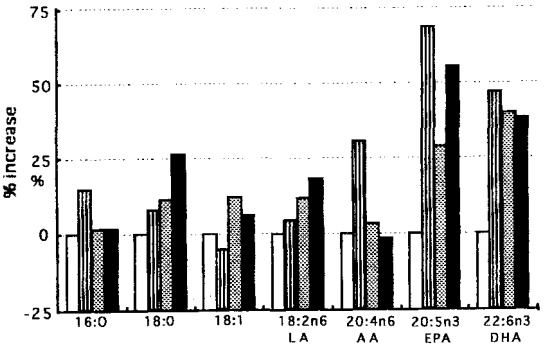

Fig. 6. Changes in the mean levels of fatty acids in serun phospholipids in patients with asthma after supplementation with perilla seed oil.
(□) : before diet supplementation and (▥) : 1 W, (▦) : 2 W, (■) : 3 W after the supplementation. 16 : 0 : palmitic acid, 18 : 0 ; stearinic acid, 18 : 1 ; oleicacid, 18 : 2n6 (LA) ; linoleic acid, 20 : 4n6(AA) ; arachidonic acid, 20 : 5 n 3 (EPA) ; eicosapentaenoic acid, 22 : 6n3 (DHA) ; docosahexaic acid.

Discussion

Bronchial allergen challenge induces immediate asthmatic reaction (IAR) within 30 min and late asthmatic reaction (LAR) which occurs at 6 to 8 hours after the challnge. The LAR, in which inflammatory cells such as lymphocytes, neutrophils, eosinophils and basophils migrate into allergic reaction sites in the airways[16-20], is closely associated with bronchial hyperresponsiveness[21, 22].

Leukotrienes, one of the major chemical mediators in asthma, play important roles in the LAR. Any beneficial effects of n-3 fatty acids were not observed in many of patients with asthma studied in relation to peak flow rate, symptoms score, and need for bronchodilators[4]. However, it has been found that there was a reduction in the LAR in subjects with dietary supplementation with fish oil. Another report showed that subjects taking MaxEPA had a blunting of the LAR to allergen challenge[5]. Dry et al. also reported that asthma patients taking fish oil chronically appeared to have a statistically significant improvement of pulmonary functions[6]. In patients with hayfever accompanied with asthma, a significant reduction in nasal symptoms during the pollen season, but not in asthma symptoms, was observed by dietary supplementation with fish oil[7].

Leukotrienes are made in large amounts during allergic reactions. Sulfid-peptide leukotrienes of the 'five series' from EPA have relatively similar properties to those of the 'four series' from arachidonate[23]. Therefore, it has been speculated that changing from production of the usual '4-series' leukotrienes to the '5-series' from EPA might not result in a large difference in allergic reaction. LTB 4 is chemotactic and recruits many inflammatory cells into allergic reaction sites in the airways. LTB 4 stimulation is reduced by n-3 fatty acids[24, 25]. Both LTB 4 from arachidonate and LTB 5 from EPA have a similar biological activities, however, the action of LTB 5 is very weak compared to LTB 4.

In contrast, cyclooxygenase products made from EPA have different biological avtivities from those from arachidonate. Thromboxane A 3 does not stimulate platelets in the way that thromboxane A 2 does. LTB 5 and thromboxane A 3 made from n-3 fatty acids are different in the degree and character of biological activities from those of LT B 4 and thromboxane A 2 from arachidonate.

A recent report has demonstrated that supplementation with EPA decreases the generation of LTB 4 and LTC 4 from leucocytes[26]. However, clinical efficacy of EPA on asthma was observed at only one time point (2 months after the begining of supplementation) during the 3-month study.

In the present study, effects of α-linolenic acid enriched perilla seed oil on bronchial asthma were examined. The results obtained here revealed that dietary supplementation with α-linolenic acid improves symptoms and ventilatory function in patients with asthma, and decreases the generation of LTB 4 and LTC 4 by leucocytes. Regarding composition of serum phospholipid fatty acids, concentrations of EPA, DHA and AA tended to increase after dietary supplementation with α-linolenic acid. Further studies are needed for detail analysis of a change in composition of serm phospholipid fatty acids by α-linolenic acid administration.

References

1. Sanders TAN : Marine oils : metabolic effects and role in human nutrition. Proc Natr Soc 52 : 457−472, 1993.
2. Ferretti A, Flanagan VP, Judd JT, et al. : Fish oil supplementation reduces excretion of 2, 3-dinor-oxo PGF1 α and 1-dehyd ro-thromboxane B 2 / 2, 3-dinor-oxo-PG F1 α excretion ratio in adult men. J Nutr Biochem 4 : 695−698, 1993.
3. Knapp HR : Omega-3 fatty acids in respiratory diseases : A review. J Am College Nutr 14 : 18−23, 1995.
4. Arm JP, Holtob CE, Mencia-Huerta JM, et al. : Effect of dietary supplementation with fish oil on mild asthma. Thorax 43 : 82−921, 1983.
5. Arm JP, Horton CE, Spur BW, et al. : The effects of dietary supplementation with fish oil on the airways response to inhaled allergen in bronchial asthma. Am Rev Respir Dis 39 : 1395−1400, 1989.
6. Dry J and Vincent D : Effect of a fish oil diet on asthma : Results of a 1-year double blind study. Int Arch Allergy Appllmmunol 98 : 156−157, 1991.
7. Thien FC, Mehcia-Huerta JM and Lee T H : Dietary fish oil effects on seasonal hayfever and asthma in pollensensitive subjects. Am Rev Respir Dis 147 : 1138−11 43, 1993.
8. Houwelingen AC, Kester ADM, Kromhout D, et al. : Comparison between habitual intake of polyunsaturated fatty acids and their concentrations in serum lipid fractions. Eur J Clin Nutr 43 : 11−20, 1989.
9. Chan JK, McDonald BE, Gerrard JM, et al. : Effect of dietary α-linolenic acid and its ratio to linoleic acid on platelet and plasma fatty acids and thrombogenesis. Lipids 28 : 811−817, 1993.
10. Tanizaki Y, Kitani H, Okazaki M, et al. : Association of asthma with serum IgE levels and aging. Bronchoalveolar cells and the release of histamine and leukotrienes, LTC4 and LTB4, from leucocytes. Jpn J Clin Immun 16 : 44−51, 1993.
11. Tanizaki Y, Kitani H, Okazaki M, et al. : Changes in the proportions of bronchoalveolar lymphocytes, neutrophils and basophilic cells and the release of histamine and leukotrienes from bronchoalveolar cells in patients with steroid-dependent intractable asthma. Int Arch Allergy Immunol 101 : 196−202, 1993.
12. Jemionek JF, Contreas TJ, French JE, et al. : Technique for increased granulocyte recovery from human whole blood by counterflow centrifugation elutriation. 1. In vitro analysis. Transfusion 19 : 120−128, 1978.
13. Tanizaki Y, Sudo M, Kitani H, et al. : Release of heparin-like substance and histamine from basophilic leucocytes separated by counterflow centrifugation elutriation. Jpn J Med 29 : 356−361, 1990.
14. Lam S, Chan H, LeRiche JC, et al. : Release of leukotrienes in patients with bronchial asthma. J Allergy Clin Immunol 81 : 711−717, 1988.
15. Okita M, Yoshida S, Yamamoto J, et al. : n-3 and n-6 fatty acid intake and serum phospholipid fatty acid composition in middle-aged women living in rural and urban areas in Okayama prefecture. J Nutr Sci Vitaminol 41 : 313−323, 1995.
16. Kirby JG, Hargreave FG, Gleich GJ, et al. : Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. Am Rev Respir Dis 136 : 379−383, 1987.
17. Wardlaw AJ and Kay AB : The role of

eosinophil in the pathogenesis of asthma. Allergy 42 : 321−335, 1987.

18. Beasley RM, Roche WE, Roberts A : Cel lular events in the bronchi in mild asthma and after bronchial provocation. Am Rev Respir Dis 139 : 806−811, 1989.
19. Wenzel SE, Wescott JY and Larsen GL : Bronchoalveolar lavage fluid mediator levels 5 minutes after allergen challenge in atopic subjects with asthma : Relationship to the development of late asthmatic responses. J Allergy Clin Immunol 87 : 540−548, 1991.
20. Walker C, Kaegi MK, Braun P, et al. : Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. J Allergy Clin Immunol 88 : 934−942, 1991.
21. Kelly CA, Ward C, Stenton SC, et al. : Numbers and activity of inflammatory cell in bronchoalveolar lavage fluid in asthma, and their relationship to airway hyperresponsiveness. Thorax 43 : 684−692, 1988.
22. Pauwels R : The relationship between airway inflammation and bronchial hyperresponsiveness. Clin Exp Allergy 19 : 395−3398, 1989.
23. Dahlen SE, Hedqvist P and Hammarstrom S : Contractile activities of several cystein-containing leukotrienes in the guinea pig lung strip. Eur J Pharmac 866 : 207−215, 1983.
24. Lee TH, Mencia-Huerta JM, Shih C, et al. : Effects of exogenous arachidonic, eicosapentaenoic, and docosahexaenoic acids on the generation of 5 -lipoxygenase products by ionophore-activated human neutrophils. J Clin Invest 75 : 1922−1933, 1984.
25. Prescott SM : The effect of eicosapentaenoic acid on leukotriene B production by human neutrophils. J Biol Chem 259 : 7615−7621, 1984.
26. 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 Thorc Dis 33 : 395−412, 1996.

気管支喘息におけるエゴマ油と白血球のロイコトリエンＢ４，Ｃ４合成

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近年，ロイコトリエンと遅発型気管支反応（LAR）との密接な関連が注目されている。本論文では，アラキドン酸（AA）代謝を通してロイコトリエン合成に関与すると推定される，αリノレン酸を多く含むエゴマ油による食事療法の臨床効果を，気管支喘息を対象に検討した。臨床症状および換気機能は，２週間のエゴマ油投与で明らかな改善差傾向を示した。Ca ionophore A23187刺激時の白血球のLTB４およびLTC４産生は，エゴマ油投与により，投与前に比べ有意の減少を示した（$p<0.05$）。血中脂肪酸に関しては，イエイコサペンタエン酸（EPA），ドコサヘキサエン酸（DHA）およびAA濃度は，エゴマ油投与により増加傾向を示し，同時にEPA／AA比も増加する傾向が見られた。以上の結果より，エゴマ油による食事療法は，治療上有用であると考えられた。

索引用語：n-３系脂肪酸，気管支喘息，アラキドン酸，LTB４，LTC４