Effects of Dietary Supplementation with n-3 Fatty Acids Compared with n-6 Fatty Acids on Bronchial Asthma

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Abstract

Objective The effects of perilla seed oil (n-3 fatty acids) on bronchial asthma were compared with the effects of corn oil (n-6 fatty acids) in relation to the pulmonary function and the generation of leukotriene B4 (LTB4) and C4 (LTC4) by leucocytes.

Methods and Subjects 14 asthmatic subjects were divided randomly into two groups: one group (7 subjects) consumed perilla seed oil-rich supplementation and the other group (7 subjects) consumed corn oil-rich supplementation for 4 weeks. Generation of LTs by leucocytes and respiratory function were compared between the two groups.

Results The generation of LTB4 and LTC4 by leucocytes tended to increase in subjects (N=7) with corn oil-rich supplementation, and decrease in subjects (N=7) with perilla seed oil-rich supplementation. Significant differences between the two groups were observed in the generation of LTB4 at 2 weeks (p<0.05) and LTC4 at 2 weeks (p<0.05) after dietary supplementation. Significant increases in the value of PEF (p<0.05), FVC (p<0.01), FEV1/0 (p<0.05) and V25 (p<0.05) were found in subjects who received perilla seed oil supplementation for 4 weeks. And significant differences in the value of FVC (p<0.05) and FEV1/0 (p<0.05) were observed between the two groups after 4 weeks of dietary supplementation.

Conclusion These results suggest that perilla seed oil-rich supplementation is useful for the treatment of asthma in terms of suppression of LTB4 and LTC4 generation by leucocytes, and improvement of pulmonary function.

(Key words: perilla seed oil, α-linolenic acid, leukotriene B4, leukotriene C4, respiratory function)

Introduction

Bronchial allergen challenge induces an immediate asthmatic reaction (IAR) within 30 minutes and a late asthmatic reaction (LAR), which occurs 6–8 hours after the challenge. The LAR, in which inflammatory cells such as lymphocytes, neutrophils, eosinophils and basophils migrate into allergic reaction sites in the airway (1, 2), is closely associated with bronchial hyperresponsiveness (3, 4). Leukotrienes (LTs) are among the major chemical mediators in asthma, particularly in the LAR, and are synthesized by inflammatory cells in large amounts during allergic reactions.

LTs are generated from arachidonic acid (AA), which is released from membrane phospholipids during cell activation, through the 5-lipoxygenase pathway (5). LTB4 and LTC4 are generated from linoleic acid (LA) through AA, and LTB5 from α-linolenic acid (α-LNA) through eicosapentaenoic acid (EPA) through the same 5-lipoxygenase pathway. However, the action of LTB5 is much weaker than that of LTB4.

Dietary supplementation with perilla seed oil, which is rich in α-LNA, has been expected to suppress the LT generation by leucocytes and increase the generation of LTB5. Conversely, supplementation with corn oil, which is rich in LA, is expected to increase the generation of LTB4 and LTC4, and decrease the generation of LTB5 by leucocytes. In a previous study (6), we showed the inhibitory effects of a diet containing perilla seed oil, a vegetable oil rich in α-LNA, on the generation of LTs by leucocytes.

In this study, we examined the effects of perilla seed oil-rich supplementation versus corn oil-rich supplementation in patients with bronchial asthma.

Subjects and Methods

The subjects of this study were 14 patients (6 men and 8 women) who were admitted to our hospital for the treatment of asthma. Seven subjects were atopic and the others were non-atopic. All subjects had moderately severe type asthma. The
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The mean duration of asthma was 15 years. All patients were treated with long-acting oral theophylline, inhaled β2 adrenergic agonists and inhaled glucocorticosteroid (beclomethasone dipropionate: BDP) regularly. The mean dose of inhaled BDP was 196.4±173.7 μg/day. Their mean age was 58.9 years (range, 22 to 84 years) and the mean of serum IgE was 1,003 IU/ml (range, 21 to 6,300 IU/ml).

Bronchial asthma was evaluated according to the criteria of the International Consensus of Diagnosis and Management of Asthma (7). All patients had reversible airway response, as indicated by a 15% or a greater increase in their forced expiratory volume in one second (FEV₁₀) after inhaled bronchodilator use. The study was approved by the Institutional Human Investigation Committee at our hospital. Informed consent for the study protocol was obtained from all patients. Peak expiratory flow (PEF) in the early morning was recorded in all subjects using a peak flow meter (Assess: Health Scan Products Inc., Cedar Grove, NJ, USA).

The subjects were divided randomly into two groups: one group (7 subjects) consumed 10–20 grams of corn oil per day as salad dressing and/or mayonnaise instead of other oils for 4 weeks and the other group (7 subjects) consumed the same amount of perilla seed oil per day for 4 weeks. Other dietary components were not changed, and the amount of oil used in the diet and supplemented diet were recorded throughout the study period.

Pulmonary function tests, forced vital capacity (FVC), forced expiratory volume in one second (FEV₁₀) and V₂₅ were performed when patients were attack free using a Chestac 33 (Chest Co., Tokyo) linked to a computer.

The generation of LTB₄ and LTC₄ by peripheral leucocytes was assessed by a method previously described (8, 9). Cells were separated by counterflow centrifugation elutriation with a JE 6B rotor (Beckman Co., Geneva, Switzerland) (10), as described previously (11). The number of the cells was then adjusted to 5x10⁶/ml in Tris ACM (composition: 1 ml of 0.1 mol/l Ca²⁺, 0.5 ml of 0.1 mol/l Mg²⁺ and 98.5 ml Tris A buffer; Trizma preset crystal, pH 7.7; Sigma Chemical Co., St. Louis, Mo, USA). The Ca ionophore A23187 (1 μg) was added to the cell suspension. The mixed solution was incubated for 15 minutes at 4°C. Quantification of LTB₄ and LTC₄ was performed by a method described by Lam et al (12). The extraction of LTs was performed using a C18 Seppak (Waters Associates, Milford, MA). The concentrations of LTB₄ and LTC₄ were analyzed by HPLC system Model 510 (Waters Associates, Milford MA), equipped with an ultraviolet detector. The column used was a 5mmx10cm Radial-Pax cartridge (Shimazu Co., Kyoto). The results were expressed as ng/5x10⁶ cells.

The changes in LTB₄, LTC₄ and ventilatory parameters (PEF, FVC, FEV₁₀ and V₂₅) values after dietary supplementation were expressed as the ratios of values before and after the supplementation.

The results were expressed as mean±standard deviation (SD). Statistically differences between means were estimated using the Student’s t-test. A p value of <0.05 was regarded as significant.

Results

The generation of LTB₄ by leucocytes tended to increase in subjects with corn oil-rich supplementation, and to decrease in subjects with perilla seed oil-rich supplementation. The difference in the changes of LTB₄ generation between the two groups was significant at two weeks after the supplementation (p<0.05), as shown in Fig. 1. The generation of LTC₄ by leucocytes also
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increased in subjects with corn oil-rich supplementation. In contrast, LTC4 generation showed a tendency to decrease in subjects with perilla seed oil-rich supplementation, and significant differences in the LTC4 generation were observed between the two groups at 2 weeks after each supplementation (p<0.05) (Fig. 2).

The values of PEF in the morning increased in the two groups at 4 weeks after dietary supplementation. A significant increase in PEF was observed at 4 weeks after perilla seed oil-rich supplementation (p<0.05) (Fig. 3). A significant increase in the value of FVC was observed at 4 weeks after perilla seed oil-rich supplementation (p<0.01) and significant difference was observed between the two groups at 4 weeks after dietary supplementation (p<0.05) (Fig. 4). The FEV10 value was also significantly increased at 4 weeks after dietary supplementation with perilla seed oil and a significant difference was observed between the two groups at 4 weeks after dietary supplementation (p<0.05) (Fig. 5). The V25 value was significantly

Figure 3. Changes of PEF in the morning after dietary supplementation for 4 weeks. A significant increase in PEF value was observed 4 weeks after perilla seed oil-rich supplementation. □: perilla seed oil-rich supplementation group. ■: corn oil-rich supplementation group. *: p<0.05, PEF: peak expiratory flow (before inhalation of β2 adrenergic agonists).

Figure 5. Changes of FEV10 value in the two groups after dietary supplementation. A significant increase in FEV10 value was observed at 4 weeks after perilla seed oil-rich supplementation. A significant difference was also observed between the two groups at 4 weeks after dietary supplementation. □: perilla seed oil-rich supplementation group. ■: corn oil-rich supplementation group. *: p<0.05, a: p<0.05, FEV10: forced expiratory volume in one second.

Figure 4. Changes of FVC in the two groups. A significant increase in the FVC value was observed at four weeks after perilla seed oil-rich supplementation. A significant difference was also observed between the two groups at 4 weeks after dietary supplementation. □: perilla seed oil-rich supplementation group. ■: corn oil-rich supplementation group. **: p<0.01, a: p<0.05, FVC: forced vital capacity.

Figure 6. Changes of V25 value in the two groups after dietary supplementation. Significant increase in V25 value was observed at 4 weeks after perilla seed oil-rich supplementation. □: perilla seed oil-rich supplementation group. ■: corn oil-rich supplementation group. *: p<0.05.
increased at 4 weeks after dietary supplementation with perilla seed oil (p<0.05) (Fig. 6). However, no significant increase in the values of these ventilatory parameters were found after corn oil-rich supplementation and no correlation between LTs and these ventilatory parameters was observed. Eosinophil numbers of the two groups did not change significantly during this study.

**Discussion**

Asthma is characterized by airway inflammation, bronchial hyper responsiveness to non-specific stimuli, and episodic and reversible airflow obstruction. Airway inflammation is the main patho-physiological manifestation of asthma. Leukotrienes (LTs) are among the most important chemical mediators released from inflammatory cells, which are involved in the pathogenesis of asthma. LTB4 acts by recruiting and activating inflammatory cells, particularly neutrophils, and promoting edema. The mediators play an important role in the asthmatic response by recruiting leukocytes to allergic reaction sites in the airway. The mediators have a bronchoconstrictor action and participate in the onset of asthma attacks (1, 9).

It has been reported that the generation of LTB4 is reduced by n-3 fatty acids (13, 14). LTB4 generated from LA and LTB5 generated from α-LNA have similar biological activities. However, the action of LT B5 is much weaker than that of LT B4. The cysteinyl LTs (LTC4, LTD4 and LTE4) are implicated in the pathogenesis of allergen-induced airway responses as potent contractile agonists for airway smooth muscle and they mediate a later part of immediate airway obstruction; a fall in FEV1,0 after allergen exposure (15, 16).

Polyunsaturated fatty acids (PUFAs) of the n-3 series, EPA and docosahexaenoic acid (DHA), suppress the production of LTs by antagonistic metabolism, which occurs at the level of LT hydrolase through the 5-lipoxygenase pathway, and therefore they have the potential to alter LTs generation by leukocytes (17). These PUFAs have been reported to show anti-inflammatory effects in patients with chronic inflammatory diseases such as rheumatoid arthritis, psoriasis, and chronic inflammatory bowel disease (18–24). Several reports have focused on the beneficial effects of EPA or fish oil on bronchial asthma (25–29).

In the present study, the effects of α-LNA rich perilla seed oil on asthma were examined in comparison with the effects of LA-enriched corn oil. The results revealed that α-LNA-rich perilla seed oil supplementation suppressed the generation of LTB4 and LTC4 more strongly than LA-rich corn oil supplementation at two weeks of dietary supplementation. Although the exact reason for the disappearance of the perilla seed oil superiority is unknown, possible reasons are thought to be follows: the sample size was rather small to detect a true effect and increase in LA production in compensation for decreased levels of LTB4 and LTC4. Furthermore, the subjects given perilla seed oil-rich supplementation showed significantly higher increases of PEF, FVC, FEV1,0 and V25 compared with the subjects given corn oil-rich supplementation. PEF values were increased in both two groups during the study. This was thought to be caused by the other therapies (drugs and respiratory rehabilitation) accompanying the diet therapy. During the improvement of the chronic obstructive pulmonary disease, the improvement of the PEF values tended to precede that of FEV1,0 (30, 31). That might be the reason for the discrepancy in the PEF values and FEV1,0 values.

Several reports have failed to demonstrate a beneficial effect of EPA in patients with bronchial asthma (32, 33). This study revealed the efficacy of the perilla seed oil on asthma. Perilla seed oil is metabolized to EPA. Therefore, the action of perilla seed oil might include the effects of perilla seed oil itself in addition to the action of EPA.

We did not examine the change of serum IgE value and n-3 fatty acid/ n-6 fatty acid ratio during this study. Some researchers documented significant influences of dietary PUFAs on the serum IgE and n-3 fatty acid/ n-6 fatty acid ratio (34, 35).

The present study suggests that dietary supplementation with perilla seed oil, rich in α-LNA, is more significantly beneficial to bronchial asthma than that with corn oil, rich in LA, by the suppressive effects on the generation of LTs by leukocytes.

The present study may be important to develop a diet therapy for asthmatic patients. Further studies are needed to develop a nutritionally balanced diet therapy for bronchial asthma.

**References**

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