Dietary supplementation with n-3 fatty acids in bronchial asthma correlated with the generation of LTB4 and LTC4

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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 LTB4 and LTC4 by peripheral leucocytes stimulated with Ca ionophore A23187 was significantly suppressed by the dietary supplementation (LTB4 and LTC4; 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, LTB4, LTC4

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 by modifying prostanoid synthesis. In contrast, effects of supplementation with n-3 fatty acids on bronchial asthma are still controversial, 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 \(\alpha\)-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 \(\div\) n-6 fatty acid ratio in serum total phospholipids.

In the present study, effects of dietary 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/m\(\ell\) (range 7-720 IU/m\(\ell\)). Clinical symptoms and peak flow rate (PFR) in the early morning and evening were recorded in all subjects. Bronchial reactivity to \(\beta\) 2 agonist was estimated in all subjects by observing the improvement of morning PFR after inhalation of salbutamol.

The subjects took 10-20 gram of perilla seed oil per day as salad dressing or mayonnaise 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 leucocytes was assessed by a method previously described\(^11\). Cells were separated by counterflow centrifugation elutriation with a JE 6 B rotor (Beckman Co.)\(^12\), as described previously\(^10\). After the number of the cells was adjusted to \(5 \times 10^8\) in Tris ACM. Ca ionophore A23187 (1 \(\mu\)g) was added to the cell suspension. The mixed solution was incubated for 15 min at 37\(^\circ\)C and centrifuged at 300g for 10 min at 4\(^\circ\)C. 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 \(\times\) 10 cm Radial-Pax cartridge (Shimazu Co.). The results were expressed as ng/5 \(\times\) 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 \(\beta\) 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 \(\beta\)2 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
n-3 fatty acids and asthma

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 β2 agonist inhalation, and (●●): in the evening.

Fig. 2. Changes of peak flow rate in the early morning (●●) and after β2 agonist inhalation (■■) in asthmatics with dietary supplementation (56 years old, female)

Fig. 3. Changes of peak flow rate in the early morning (●●) and after β2 agonist inhalation (■■) in asthmatics with dietary supplementation (58 years old, female)

Ca ionophore A23187-induced generation of LTB4 and LTC4 by peripheral leucocytes was evaluated in 5 patients (including CVA) before and after manipulation with perilla

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 β2 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).
seed oil. The mean values of LTB4 and LTC4 before the supplementation was 77.6 ± 24.4 and 64.0 ± 33.5 ng/5 × 10^6 cells (mean ± SD) respectively. After the oil manipulation the value of LTB4 tended to decrease (41.6 ± 24.9 ng/5 × 10^6 cells) in 4 of the 5 subjects (p<0.05). The decrease of LTB4 generation was marked in subjects 3, 4, and 5, whose generation of LTB4 by leucocytes was relatively high (Fig. 4).

![Fig. 4. Decreases in the generation of LTB4 by peripheral leukocytes from subjects before (□) and after dietary supplementation (■).](image1)

The generation of LTC4 by leucocytes significantly decreased (38.8 ± 23.2 ng/5 × 10^6 cells) (p<0.05). Neither peak of LTB5 and LTC5 in HPLC was not observed before and after dietary supplementation (Fig. 5).

![Fig. 5. Decreases in the generation of LTC4 by peripheral leukocytes from subjects before (□) and after dietary supplementation (■).](image2)

The linoleic acid (LA), AA, EPA, and DHA in plasma phospholipid were 787 ± 103, 327 ± 12, 13 ± 16, and 285 ± 61 nmol/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. 6. Changes in the mean levels of fatty acids in serum phospholipids in patients with asthma after supplementation with perilla seed oil.](image3)
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 challenge. The LAR, in which inflammatory cells such as lymphocytes, neutrophils, eosinophils and basophils migrate into allergic reaction sites in the airways\(^{21,22}\), 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\(^{20}\). 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\(^{24-26}\). 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 activities 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 LTB\(_4\) 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\(^{20}\). However, clinical efficacy of EPA on asthma was observed at only one time point (2 months after the beginning of supplementation) during the 3-month study.

In the present study, effects of \(\alpha\)-linolenic acid enriched perilla seed oil on bronchial asthma were examined. The results obtained here revealed that dietary supplementation with \(\alpha\)-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 \(\alpha\)-linolenic acid. Further studies are needed for detail analysis of a change in composition of serum phospholipid fatty acids by \(\alpha\)-linolenic acid administration.
n-3 fatty acids and asthma

References

17. Wardlaw AJ and Kay AB: The role of...


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）代謝を通じ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に改善の傾向を示した。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の傾向を示し、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