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Correlation between efficacy of Pranlukast and LTC4 generation by peripheral leukocytes

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Abstract : The correlation between the efficacy of 4-weeks administration with pranlukast, leukotriene receptor antagonist, and LTs generation by peripheral leukocytes were evaluated in 18 patients with mild-persistent asthma. The efficacy of pranlukast administration was assessed by symptom, morning PEF and pulmonary function. Pranlukast were effective in 12/18(67%) patients. In those patients, LTC4 generation before pranlukast administration was significantly high, compared with that in pranlukast-ineffective patients. LTC4 generation decreased after 4-weeks administration with pranlukast in effective patients. In ineffective patients, however, LTC4 generation increased after 4-weeks administration. LTB4 had shown no significant difference between effective and ineffective patients before administration, and LTB4 decreased after 4-weeks in both groups. Proportion of peripheral eosinophils in effective patients were higher than that in ineffective patients, however not significant. After 4-weeks, proportion of eosinophils was decreased in effective patients and increased in ineffective patients. These findings suggest that pranlukast is effective for patients with high LTC4 generation and has the effect to suppress the accumulation of eosinophils in such patients.

Key words : bronchial asthma, pranlukast, leukotriene receptor antagonist, LTC4,

Introduction

Leukotriene C4 (LTC4) are membrane-derived lipid mediators derived from arachidonic acid via the 5-lipoxygenase pathway[1]. The biological activities of LTs have an important inflammatory role in several lung diseases, including asthma[2,3]. LTC4, and its bioactive metabolites, LTD4 and LTE4 cause bronchial smooth muscle contraction in vitro,

prolonged bronchial contraction in vivo, and increased vascular permeability and increased mucus production[4,5], all of which are characteristic features of the pathology of asthma. Several specific inhibitors of LT synthesis and LT receptor antagonists have been showed inhibitory effects to airway obstruction induced by allergens, exercise and hyperventilation[6,7].

Pranlukast, a selective receptor antagonist of cysteinyl leukotrienes (LTC4, LTD4 and LTE4), has been shown to protect against the bronchial obstruction induced allergens.

Pranlukast have also demonstrated efficacy in many patients with asthma[8]. However, a few asthmatics showed little or no improvement of asthma symptoms with pranlukast administrations.

The present study investigated the correlation between efficacy of pranlukast for patients with asthma and leukotriene generation by peripheral leukocyte.

Method

We studied 18 adults patients with mild-persistent asthma (13 females and 5 males ; mean age 54.3 years). Theophylline and β_2-stimulator were administered to all patients, and inhaled beclomethasone to 4 patients. No patients took systemic administration of corticosteroid.

After giving informed consent, patients received 225mg of Pranlukast (Ono Pharm. Co. Ltd., Osaka, Japan) twice daily for 4-weeks. During 4-weeks, patients were asked to record symptoms in their diaries and to monitor changes in the PEF in the morning using a peak flow meter (Mini-Wright). Pulmonary function test were measured using a dry-seal spirometer (CHSTAC-33 ; Chest, Tokyo, Japan) before and after 4-weeks administration of pranlukast.

The generation of LTs by peripheral leukocytes was assessed by HPLC method. In brief, cells were separated by aqueous two-phase partition using dextran. The number of cells was then adjusted to $5x10^6$/ml in Tris ACM and cell differentiation by Kimura-Tanizaki stains were undergone. The Ca ionophore A23187 (1 μg) was added to the cell suspension and incubated for 15 min at 37℃. LTC4 and LTB4 were extracted using a C18 Seppak (Waters Associates, Milford, Mass., USA) and their concentrations were determined by HPLC (Model 510, equipped with an ultraviolet detector ; Waters Associates). The results are expressed as nanograms per $5x10^6$ cells.

After 4 weeks, the efficacy of pranlukast were assessed by the changes of daily asthma symptoms, morning PEF and pulmonary functions. Then patients were devided into two groups, effective and ineffective group. The correlation of the efficacy of pranlukast and LTs generation by peripheral leukocytes were evaluated before and after 4 week.

Results

Pranlukast were effective in 12/18(67%) patients, and ineffective in 6/18(33%) patients with asthma. No significant difference was seen between age or duration of asthma between effective and ineffective group. In effective group, morning PEF before pranlukast administration was lower than that in ineffective group, however there was no significance. After 4-weeks, the PEF value increased in effective group. On the other hand, ineffective group had shown decrease of morning PEF (Fig. 1). Regarding pulmonary function test, FVC were improved in effective and ineffective group after 4-

weeks. In ineffective group, however, increase of FVC was little. %FEV(predicted) had shown improvement in effective group after 4-weeks. On the other hand, in ineffective group, %FEV(predicted) had shown decrease after 4-weeks (Fig.2).

Before pranlukast administration, the amount of LTC4 in effective group was significantly higher than that in ineffective group. After 4-weeks administration, LTC4 decreased in effective group. On the other hand, LTC4 increased in ineffective group(Fig.3). LTB4 had shown no significant difference between two groups before administration. After 4-weeks, LTB4 was decreased in both groups.

Blood eosinophils in effective group were higher than that in ineffective group before administration, however not significant. After 4-weeks, eosinophils were decreased in effective group and increased in ineffective group(Fig. 4).

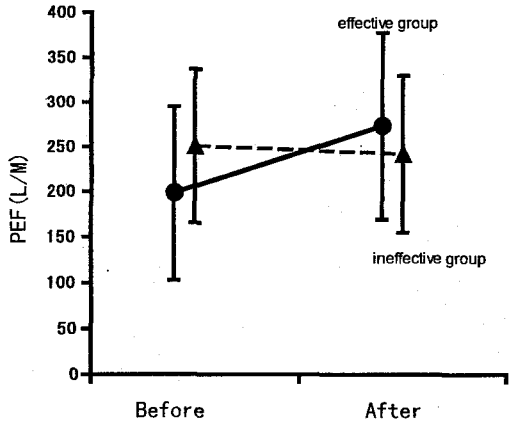

Fig.1. Changes of morning PEF after pranlukast administration. PEF in effective group increased after 4-weeks administration. In ineffective group, PEF decreased after 4-weeks.

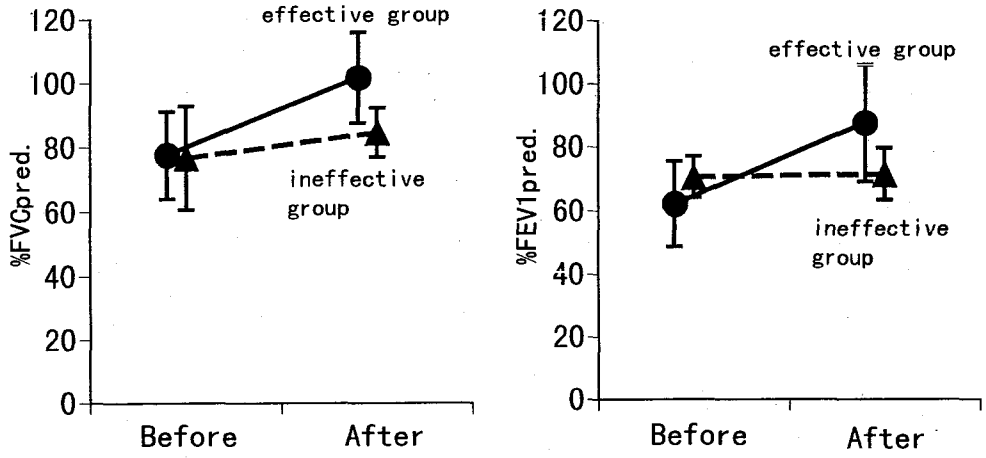

Fig.2. Changes of FVC and FEV1 before and after pranlukast administration. %FVC increased in effective and ineffective group after 4-weeks administration. %FEV1(predicted) increased in effective group after 4-weeks adminitration, and decreased in ineffective group.

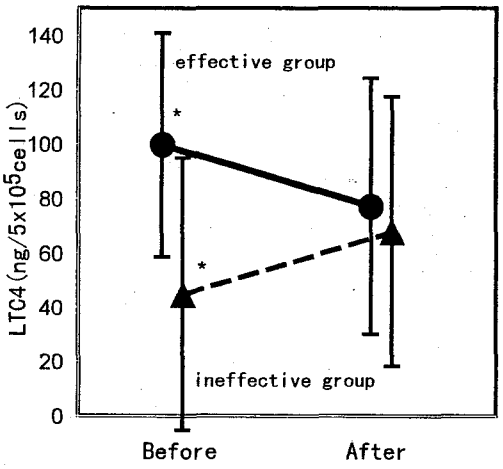

Fig.3 LTC4 generation by peripheral leukocytes. LTC4 in effective group before pranlukast administration was significantly high, compared to that in ineffective group. after 4-weeks administration, LTC4 decreased in effective group and increased in ineffective group.

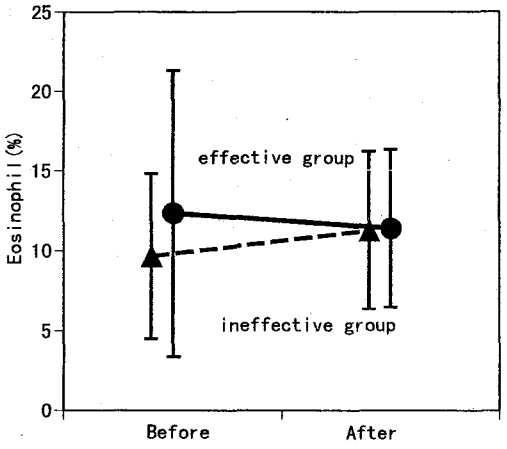

Fig.4. Changes of eosinophils proportion in peripheral leukocytes. Eosinophil proportion was high in effective group, compared to that in ineffective group, however not significant. Eosinophils in effective group decreased after 4-weeks and increased in ineffective group.

Discussion

Bronchial asthma is widely recognized as a chronic inflammatory disease[9]. Leukotrienes are potent proinflammtory mediators that appear to contribute to pathophysiological features of asthma. That is to say, contraction of airway smooth muscle, increase of microvascular permiability, stimulation of mucus secretion, decrease of mucociliary clearance were observed by LTs[10]. Our results showed that 4-weeks administration with pranlukast improved asthma symptoms, morning PEF and pulmonary functions in 67% of patients with asthma (effective group). This result suggests that LTs are important factors for majority of patients with asthma. However, 33% of patients had shown no improvements by pranlukast administration in our study (ineffective group). In ineffective group, LTC4 generation by peripheral leukocytes was significantly lower than that in effective group. This finding suggests that pranlukast has low efficacy for patients with asthma whose LTs generation are weak and other chemical mediators, such as histamine,PAF and TXA2, have major roles in their asthma reaction. LTs are synthesized by inflammatory cells during an asthma reaction. LTC4 was generated mainly by mast cells and eosinophils[11]. In peripheral blood, LTC4 was mostly generated by eosinophils. Therefore, it is supposed that LTC4 generation by peripheral leukocytes reflect the population of eosinophils. In our study, the population of eosinophils in effective group was higher than that in ineffective group before pranlukast admini-

stration. However, no significant difference was observed between two groups. Even now, measurements of LTs generation need many complicated processes. On the other hand, proportion of eosinophils is easily measurable. However, our results suggest that it may be difficult to predict the efficacy of pranlukast by the population of peripheral eosinophils, because eosinophil population in effective group and that in ineffective group overlapped largely. After 4-weeks administration, population of eosinophils decreased in effective group. This result meant that LTC4 and other chemical mediators which were generated or released by eosinophils might decrease by pranlukast administration. A major contributor to the damage in the airway of asthmatic patients is the eosinophil, which, upon activation, releases granule-associated cytotoxic, cationic proteins, including the major basic protein and eosinophil peroxidase, and membrane-derived de novo-synthesized bioactive lipid mediators, including LTC4, LTD4 and LTE4, as well as PAF. Accumulating evidence suggests that leukotriene receptor antagonists including pranlukast may influence the accumulation and maintenance of eosinophilic responses at the site of inflammation. Inhalation of LTE4 induced an increase in number of eosinophils and neutrophils in lamina propria of the airway in patients with asthma. The number of eosinophils were 10-fold greater than those of neutrophils. There was no significant change in numbers of lymphocytes, plasma cells, mast cells or macrophages[12]. Other studies showed that leukotrienes induced specific migration of eosinophils in vitro[13]. In our study, however, population of eosinophils increased after 4-weeks in ineffective group. In such patients, other eosinophil-chemotactic factors are supposed to exist.

For asthmatics whose LTC4 generation increased, our Results suggest that pranlukast is a effective agent in improvements of daily asthmatic symptoms and pulmonary functions, and have effect to suppress the accumulation of eosinophils.

References

1. Samuelsson B. Leukotrienes : mediators of immediate hypersensitivity. Science 1983 ; 220 : 568−75.
2. Robinson C, Holgate ST. New perspectives on the putative role of eicosanoids in airway hyperresponsiveness. J ALLERGY CLIN Immunol. 1985 ; 76 : 140.
3. Henderson WR Jr. Eicosanoids and lung inflammation. Am Rev Respir Dis 1987 ; 135 : 1176−85.
4. Soter NA, Lewis RA, Corey EJ, Austen KF. Local effects of synthetic leukotrienes LTC4, LTD4, LTF4, and LTB4 in human skin. J Invest Dermmatol 1983 ; 80 : 115.
5. Holroyde MC, Altounyan RE, Cole M, Dixon M, Elliot EV. Bronchoconstriction produced in man by leukotrienes C and D. Lancet 1981 ; 2 : 17.
6. Ford-Hutchinson AW : Leukotriene antagonists and inhibitors : Clinical applications. Adv prostagrandins Thoromboxane Leukotriene Res 1995 ; 23 : 69.
7. Dahlen B, Dahlen S-E : Leukotrines as mediators of airway obstruction and inflammation in asthma. Clin Exp Allergy 1995 ; 25 : 50.
8. Miyamoto T, Takishima T, Makino S, Shida T, Nakajima M : Efficacy of a selective leukotriene C4, D4, E4 antagonist, ONO-1078, on adult patients with bronchial asthma. Igaku no Ayumi 1993 ; 164 : 225.

9. Chai H, Farr RS, Froelich LA et al. Standardization of bronchial inhalation challenge procedures. J Allergy Clin Immunol 1975 ; 56 ; 323−7.
10. Busse WW. Leukotrienes and inflammation. Am J Respir Crit Care Med 1998 ; 157 : S210−S3
11. Lundgren JD, Shelhamer JH. Pathogenesis of airway mucus hypersecretion. J Allergy Clin Immunol 1990 ; 85 : 399−417.
12. Laitinen LA, Laitinen A, Haahtela T, Vilkka V, Spur BW, Lee TH. Leukotriene E4 and granulocytic infiltration into asthmatic airways. Lancet 1993 ; 341 : 989.
13. Spada CS, Nieves AL, Krauss AH, Woodward DF. Comparison of leukotriene effects on human eosinophil and neutrophil motility in vitro. J Leukoc Biol 1994 ; 55 : 183−91.

気管支喘息症例における末梢血白血球のロイコトリエンC４産生能とロイコトリエン受容体拮抗薬プランルカストの効果に関する検討

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軽症気管支喘息18例にロイコトリエン受容体拮抗薬プランルカストを４週間投与し，その効果と末梢血白血球からのLTC4，LTB4産生能の関係を検討した．プランルカストの効果は臨床症状，起床時ピークフロー値，肺機能の変化によって判定し，効果群，非効果群の２群に分類した．18例中12例(67%)の症例がプランルカスト投与により，臨床症状の軽減，ピークフロー値の増加，肺機能の改善が認められた．効果群におけるプランルカスト投与前のLTC4値は，非効果群のLTC4値に比較して有意に高値であった．４週間の投与後には効果群ではLTC4値は減少し，非効果群では増加した．両群のLTB4値はプランルカスト投与前で有意な差は認められず，投与後には両群で減少した．投与前の好酸球分画は，効果群において非効果群に比べ高値であったが，有意な差は認められなかった．４週間の投与後，効果群においては好酸球は減少し，非効果群においては増加した．以上の結果より，プランルカストは末梢血白血球のLTC4産生能が高い症例において効果的であり，好酸球集積を抑制する作用を有すると考えられる．