◎原 著

The generation of leukotrienes B4 (LTB4) and C4 (LTC4) and bronchial hyperresponsiveness in patients with asthma and spa therapy

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Abstarct : Changes in the generation of leukotrienes B4 (LTB4) and C4 (LTC4) by leucocytes after spa therapy were examined in 30 patients with asthma. 1. The efficacy of spa therapy was marked in 8 (26.7%), and moderate in 16 (53.3%) of 30 patients with asthma, and slight or no efficacy of the therapy was observed in the residual 6 patients (20.0%). 2. Bronchial reactivity to methacholine was the highest in patients with slight or no efficacy of spa therapy, however, there were no significant differences among the three groups classified by clinical efficacy. 3. The generation of LTC4 was significantly higher in patients with slight or no efficacy compared with the generation in those with marked ($p<0.01$) and moderate efficacy ($p<0.001$). However, there were no signifcant differences in the generation of LTB4 among them. 4. The generation of LTC4 signifcantly decreased in patients with marked and moderate efficacy after spa therapy, but not in those with slight or no efficacy. The generation of LTB4 was not significantly different before and after spa therapy among patients with marked, moderate, and slight or no efficacy. These results show that the efficacy of spa therapy for patients with asthma is closely related to the generation of LTC4 by leucocytes.

Key words : asthma, spa therapy, bronchial hyperresponsiveness, LTB4, LTC4

Introduction

Bronchial asthma is a disease characterized by bronchial hyperresponsiveness associated with airway inflammation. In onset mechanisms of asthma, bridging of IgE receptors on mast cell membrane is caused by allergen, followed by the release of chemical mediators such as histamine and leukotrienes B4 (LTB4) and C4 (LTC4)[1,2]. These chemical mediators induce pathophysiological changes of airways such as bronchoconstriction, mucus hypersecretion and edema of mucous membrane. Thus, chemical mediators play an important role in the triggering events of asthma attacks.

Spa therapy has been reported to be effective for patients with asthma[3-5], particularly for steroid-dependent asthma[6,7]. In analysis of action mechanisms of spa therapy for asthma, it has been demonstrated that ventilatory function is improved by direct action of spa therapy[8,9], and the effects on suppressed function of adrenocortical glands[10], endocrine-autonomic nerve system[11], and psychological factors[12] have been observed as indirect action of spa therapy.

Our previous studies have shown that the generation of LTB4 by leucocytes was significantly larger in patients with spa efficacy than in those without efficacy[13]. The generation of LTC4 in patients with spa efficacy was larger compared to the generation in those without efficacy, however, the difference was not significant. These results suggest that spa efficacy for patients with asthma is related to a certain extent to the generation of LTB4 and LTC4 by leucocytes.

In the present study, changes in the generation of LTB4 and LTC4 by leucocytes after spa therapy was examined in patients with asthma in relation to spa efficacy.

Subjects and Methods

The subjects in this study was 30 patients (19 females and 11 males) with asthma. Their mean age was 59.1 years (range 27 to 74 years). The mean level of serum IgE was 555 IU/ml (range 25-5195 IU/ml). All of them had a complex spa therapy comprised of swimming training in a hot spring pool, fango therapy, and inhalation with iodine salt solution for one to three months[7].

The efficacy of spa therapy was assessed by comparing their symptoms before and after spa therapy. The efficacy was estimated in four degrees ; marked, moderate, and slight or no efficacy, and in subjects in whom marked and moderate efficacy was shown, spa therapy was evaluated as being effective. The spa efficacy was analyzed in relation to brobchial hyperresponsiveness to methacholine and the generation of leukotrienes B4 (LTB4) and C4 (LTC4) by leucocytes.

Bronchial hyperresponsiveness to methacholine was measured by an Astograph (TCK 6100, Chest Co), as previously described[14]. All medications were stopped 12 hours prior to examination. A methacholine concentration causing a significant increase in total respiratory resistance (Rrs) was assessed as Cmin (minimum concentration).

Ventilatory function test using a Box Spiror 81-S were carried out in all subjects when they were attack-free.

The amount of LTB4 and LTC4 generated by the peripheral leucocytes were assessed according to the folowing procedure[15]. A quarter volume of 6% dextran was added to 20 ml of peripheral blood, and this was left for 1 hour at room temperature. The leucocyte-rich plasma supernatant was then removed and used. The

number of cells was adjusted to $5x10^6$ cells/mL in Tris CM buffer, and 1 μg of calcium ionophore A23187 (Sigma St Louis, Mo) was then added to the cell suspension. The solution was mixed and incubated for 15 min at 37℃.

After a 4x volume of prechilled ethanol (final, 80% ethanol) was added, this was centrifuged at 3000 rpm for 30 min. A syringe filter (Toyo Roshi Co, Tokyo) was used to draw the supernatant, and the filtrate was decompressed and dried to a solid. Quantification of LTB4 and LTC4 was performed by HPLC and UV spe ctroscopy, with the method of Lam et al[16]. Quantities of LTB4 and LTC4 were expressed as nanogram per $5x10^6$ cells.

The level of total IgE in serum were measured by radioimmunosorbent test (RIST), and specific IgE antibodies against inhalant allergens were estimated by radioallergosorbent test (RA ST).

Statistically significant differences of the mean were evaluated using unpaired Student't test. A p value of <0.05 was regarded as significant.

Results

Table 1 shows the clinical effects of spa therapy on asthma in relation to patient age and the value of FEV1.0%. The efficacy of spa therapy was marked in 8 (26.7%), moderate in 16(53.3%), and slight or no efficacy in 6 (20.0%) of 30 patients with asthma. The efficacy of spa therapy was not related to age. The value of FEV1.0% was higher in patients with marked and moderate efficacy than in those with slight or no efficacy. However, this difference was not significant. The serum IgE level was not correlated with spa efficacy. The frequency of patients with positive RAST against inhalant allergens was higher in patients with slight or no efficacy of spa therapy than in those with marked and moderate efficacy, however, the difference was not significant (Table 2).

Table 1. Efficacy of spa therapy for patients with asthma, patient age, and FEV1.0%

Efficacy	No of patients	Age, years	FEV1.0%
Marked	8 (26.7%)	52.0	70.0± 9.1
Moderate	16 (53.3%)	59.7	67.1±11.0
Slight or No	6 (20.0%)	56.7	64.2±11.3

Table 2. Efficacy of spa therapy for patients with asthma and IgE-mediated allergy

Efficacy	No of patients	IgE (IU/ml)	Positive RAST*
Marked	8	327 (25-1658)	5/ 8 (62.5%)
Moderate	16	711 (36-5195)	11/16 (68.8%)
Slight or No	6	376 (108-1124)	5/ 6 (83.3%)

*Frequency of patients with positive RAST against inhalant allergens

Bronchial hyperresponsiveness to methacholine was lower in patients with slight or no efficacy compared with that in those with marked efficacy, as shown in Fig. 1.

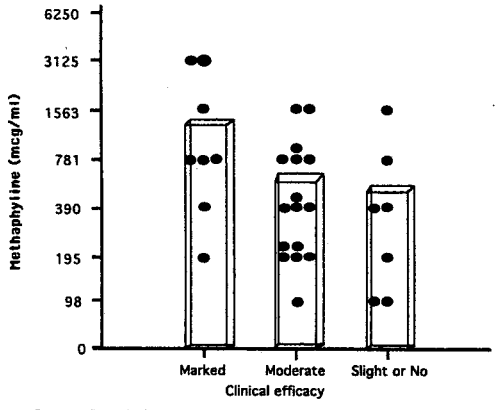

Fig.1 Bronchial hyperresponsiveness to methacholine in patients with asthma in relation to clinical efficacy

There were no significant differences in LTB4 generation among three groups classified by spa

efficacy. In contrast, the generation of LTC4 was significantly larger in patients with slight or no efficacy than in those with marked ($p<0.01$) and moderate efficacy ($p<0.001$) (Fig. 2).

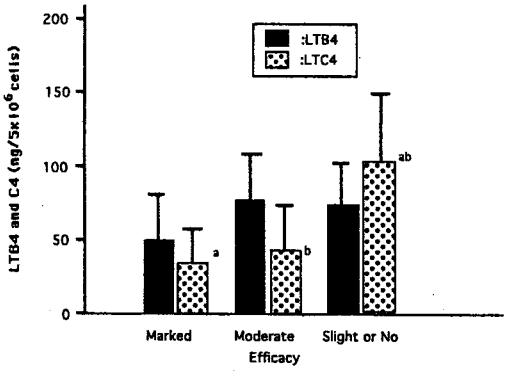

Fig.2. Generation of LTB4 and LTC4 by leukocytes in patients with asthma during non-attack stages in relation to clinical efficacy. a: $p<0.01$, b :$p<0.001$.

The generation of LTB4 by leucocytes in patients with marked efficacy was not significantly different before and after spa therapy, however, the generation of LTC4 by leucocytes was significantly larger before spa therapy compared to the generation after the therapy (Fig. 3).

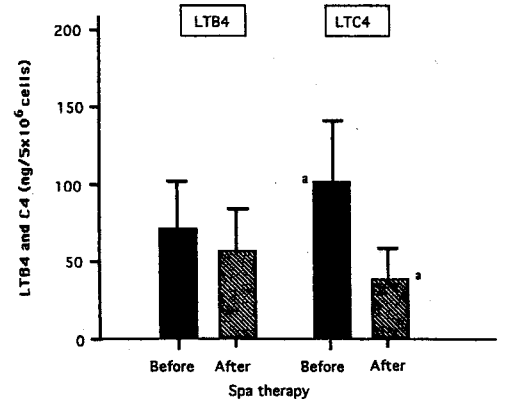

Fig.3. Generation of LTB4 and LTC4 by leukocytes in asthmatics with marked efficacy before and after spa therapy. a: $p<0.001$.

In patients with moderate efficacy of spa therapy, the generation of LTC4 before spa therapy was also significantly higher compared to the generation after the therapy ($p<0.01$), but not the generation of LTB4 (Fig. 4).

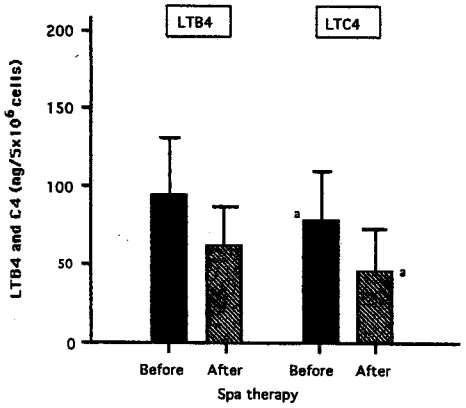

Fig.4. Generation of LTB4 and LTC4 by leukocytes in asthmatics with moderate efficacy before and after spa therapy. a: $p<0.01$.

In contrast, the generation of both LTB4 and LTC4 did not significantly change before and after spa therapy in patients with slight or no efficacy (Fig. 5).

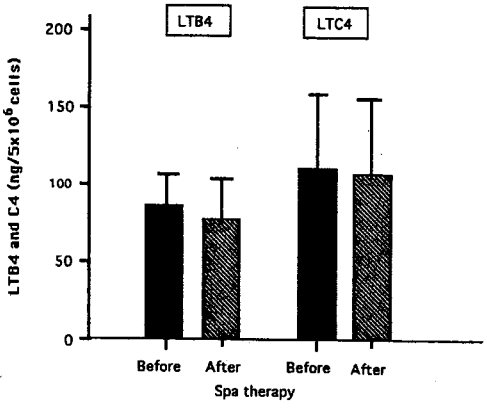

Fig.5. Generation of LTB4 and LTC4 by leukocytes in asthmatics with slight or no efficacy before and after spa therapy.

Discussion

Our previous studies have shown that spa therapy is effective for patients with asthma[3-7]. The direct actions of spa therapy improve clinical symptoms[3-5] and ventilatory function[8], and suppress bronchial hyperresponsiveness[17] in patients with asthma. The indirect actions of spa therapy improve the function of the adrenocortical glands[18].

Bronchial asthma is characterized by transient bronchoconstriction, accompanied with hypersecretion and edema of mucous membrane. Chemical mediators such as histamine, LTC4 and LTB4, which are released from tissue mast cells during the time of immediate asthmatic reaction (IAR)[19,20], and from inflammatory cells during late asthmatic reaction (LAR), play an important role in the onset mechanisms of asthma attacks[21,22].

Effects of spa therapy on asthma are often affectd by degrees of bronchial hyperresponsiveness, release of chemical mediators such as histamine and leukotrienes B4 and C4, and airway inflammatoion[23].

Our previous studies have shown that the generation of LTC4 in patients with spa efficacy was larger compared with that in those without efficacy, however, this difference was not significant, and that the generation of LTB4 by leucocytes was significantly larger in patients with spa efficacy than in those without efficacy[13]. The results also suggested that the release of histamine from leucocytes was significantly higher in patients without spa efficacy than in those with efficacy. These results suggest that spa efficacy is associated with the release of histamine, LTB4 and LTC4 from leucocytes.

In the present study, a correlation between the generation of LTB4 and LTC4 by leucocytes and spa efficacy was examined in patients with asthma in relation to asthmatic cycle. The results revealed that bronchial hyperresponsiveness was higher and the generation of LTC4 was significantly larger in patients without spa efficacy than in those with marked and moderate efficacy, but not the generation of LTB4. These results do not agree with the results previously obtained, suggesting LTB4 was significantly higher in patients with spa efficacy than in those without efficacy. The difference might be due to the different population of patients studied; more atopic type asthma patients were included in this study. Large amount of LTC4 generation before spa therapy significantly decreased in patients with marked and moderate efficacy, but not in those without efficacy. The results shows that spa therapy is less effective in patients with large amount of LTC4 generation by leucocytes.

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気管支喘息におけるロイコトリエンＢ４およびＣ４産生，気管過敏性と温泉療法．

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気管支喘息30例を対象に，温泉療法によるロイコトリエンＢ４（LTB４），Ｃ４（LTC４）産生の変動および気道過敏性と臨床効果との関連について検討を加えた。１．温泉療法の臨床効果は，著効８例（26.7%），有効16例（53.3%），やや有効あるいは無効６例（20.0%）であった。２．メサコリンに対する気道過敏性は，やや有効あるいは無効例で高い傾向が見られたが，著効，有効例と比べ有意の差は見られなかった。３．LTC４産生はやや有効あるいは無効例において著効例（$P<0.01$）や有効例（$P<0.001$）に比べ有意に高い値が示された。しかし，LTB４産生では臨床効果との関連は見られなかった。４．著効例および有効例では，温泉療法後にLTC４産生の有意の低下傾向が見られたが，無効例では有意の変動は見られなかった。また，LTB４産生に関しては温泉療法の臨床効果にかかわらず有意の変動は見られなかった。これらの結果より，気管支喘息に対する温泉療法の臨床効果は，LTC４産生とある程度関連していることを示唆しているものと考えられる。