Table 1. Assignment of ^{1}H-Nuclear Magnetic Resonance Spectra

Compound	*Chemical Group*	*Chemical Shift (ppm)*	*Splitting Pattern*	*Number of Protons*
GABA	CH2 (C2)	2.31	Triplet	2
	CH2 (C3)	1.91	Quintet	2
	CH2 (C4)	3.01	Triplet	2
Inosiplex				
Inosine	CH (C5′)	3.84–4.90	Multiplet	2
	CH (C2′, C3′, C4′)	4.06–4.71	Multiplet	(3)
	CH (C1′)	6.03	Doublet	1
	CH (C2)	8.11	Singlet	1
	CH (C8)	8.28	Singlet	1
PAcBA	CH3 (C9)	2.15	Singlet	9
	CH (C3, C5)	7.39–7.49	Multiplet	6
	CH (C2, C6)	7.79–7.89	Multiplet	6
DIP	CH3 (C3)	1.23	Doublet	9
	CH3 (C4, C5)	2.89	Singlet	18
	CH2 (C1)	3.04–3.14	Quartet	(6)
	CH (C2)	4.06–4.71	Multiplet	3

DIP = 2-hydroxypropyldimethylammonium; GABA = γ-aminobutyric acid; PAcBA = *p*-acetamidobenzoate.
Standard: 3-(trimethylsilyl)-1-(propansulfonic) acid as 0.00 ppm.

taking 4.8 g of inosiplex, thus being sufficient to contribute to the in vivo magnetic resonance spectroscopic spectra.

The data above confirm that the previously unidentified resonance of the brain should have come from 2-hydroxypropyldimethylammonium, although magnetic resonance spectroscopy after the cessation of inosiplex could not be performed because we could not obtain informed consent from the patient and her family. The obtained spectra from the brain of the patient should be a combination of the 2-hydroxypropyldimethylammonium spectra and the spectra of the brain itself. In addition, we previously described the reduction of *N*-acetylaspartate on both the frontal and occipital lobes of the patient; possible contamination of the resonance of CH3 (C9) in the = *p*-acetamidobenzoate signal could have led us to underestimate the reduction.[1] Magnetic resonance spectroscopic study can provide useful information and can be used for monitoring disease progression, but one should be aware of the possibility of contamination from drug resonances when evaluating magnetic resonance spectroscopy from a patient with subacute sclerosing panencephalitis undergoing treatment.

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Received February 6, 2005. Revised March 4, 2005. Accepted for publication March 22, 2005.

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Autologous Peripheral Blood Stem Cell Transplantation in a Patient With Relapsed Pleuropulmonary Blastoma

Hideo Kaneko, Koji Isogai, Masashi Kondo, Kaori Hosoi, Tsutomu Asano, Michinori Funato, and Naomi Kondo

Summary: Pleuropulmonary blastoma (PPB) is a rare and aggressive primary intrathoracic neoplasma of children. The prognosis is extremely poor with frequent metastasis to the brain and bone. We present a 4-year-old girl with a tumor mass in the right hemithorax initially diagnosed as pneumoniae. Tumor resection was performed and the histologic report indicated the diagnosis of PPB. The patient received chemotherapy comprising vincristine, actinomycin D, doxorubicin, cisplatin, and cyclophosphamide. Irradiation was performed with total 45 Gy at the right lower pulmonary lobe. She relapsed 29 months later at the pleura between the right middle and lower pulmonary lobe. Tumor resection and total 45 Gy of irradiation were performed again. High-dose chemotherapy comprising cisplatin, adriamycin, and cyclophosphamide was performed followed by autologous peripheral blood stem cell transplantation (PBSCT). The patient achieved complete hematologic recovery. Thirty-one months after PBSCT, no signs of relapse have been observed. Although it might be that the patient could have been cured with second surgery alone or by the surgery and subsequent chemotherapy, high-dose chemotherapy and PBSCT should be considered for the treatment of relapsed PPB.

Key Words: pleuropulmonary blastoma, relapse, high-dose chemotherapy, PBSCT

(*J Pediatr Hematol Oncol* 2006;28:383–385)

Pleuropulmonary blastoma (PPB), a rare dysembryogenic neoplasma of the thoracopulmonary mesenchyma, is characterized by primitive blastema and malignant mesenchymal stroma, often showing multidirectional differentiation.[1–3]

Three types have been described: cystic (I), mixed (II), and solid (III). Histologically, it is characterized by a primitive, variably mixed blastematous, and sarcomatous appearance. It has been documented that many PPB patients had preexisting or concurrent congenital pulmonary cystic lesions (cystic adenomatoid malformation or isolated pulmonary cysts). The exclusively cystic (type I) PPB appears at an early age, even in newborns; bilateral forms have been recently described.[4] Some authors have suggested that solid areas may subsequently arise from a previous cystic lesion,[5] thus indicating tumor transition and progression to a solid form. As a general opinion, pulmonary cysts can represent a predisposing condition for later PPB.

This tumor has been treated with a multimodality approach including surgery, chemotherapy, and in some cases radiotherapy.[5–10] Prognosis remains poor. The overall survival rate at 5 years was 45%.[5] In this report, we describe a 4-year-old girl with relapsed PPB treated with high-dose chemotherapy and autologous peripheral blood stem cell transplantation (PBSCT).

CASE REPORT

A 4-year-old Japanese girl initially diagnosed as having pneumonia was referred to our hospital. A chest radiograph showed a large mass in the right lung deviating the mediastinum. Computed tomography (CT) and magnetic resonance imaging (MRI) (Fig. 1) confirmed a tumor mass in the right hemithorax.

She showed dyspnea due to the tumor. Resection of the tumor mass was urgently performed. A histopathologic review indicated type III PPB. A work-up for metastsis included a bone marrow aspirate and biopsy, bone scan and computed tomography of brain, showing no metastaic signs. Initially, we used Intergroup Rhabdomyosarcoma Studies (IRS)-III, regimen 36[11] as the protocol for the patient. The dose of CDDP, ADR, CY, VCR, and ACTD in the original chemotherapy in this patient was 525 mg/m^2, 160 mg/m^2, 2400 mg/m^2, 13.5 mg/m^2, and 0.45 mg/kg, respectively. After the chemotherapy, total dose of 45 Gy (1.8 Gy per fraction per day) was irradiated for total 25 days (5 times a week). Irradition was performed on the region of the lower pulmonary lobe and the diaphragm. These treatments caused tumor reduction and (CT) showed no tumor mass at the time of discharge.

When a periodic chest x-ray examination was carried out, a coin lesion in the right hemithorax was observed 29 months after discharge. CT and MRI showed a round mass in the pleura between right middle and lower pulmonary lobe (Fig. 2). The recurrence of the PPB developed in the right hemithorax other than the initial irradiation field. Second tumor resection was performed and a histopathologic examination indicated PPB. The histologic appearances of recurrent PPB were identical to those of the initial PPB.

Considering the poor prognosis we scheduled irradiation, high-dose chemotherapy, and PBSCT for this relapsed PPB. Before the high-dose chemotherapy, the previously used dose in

Received for publication September 22, 2005; accepted April 7, 2006.
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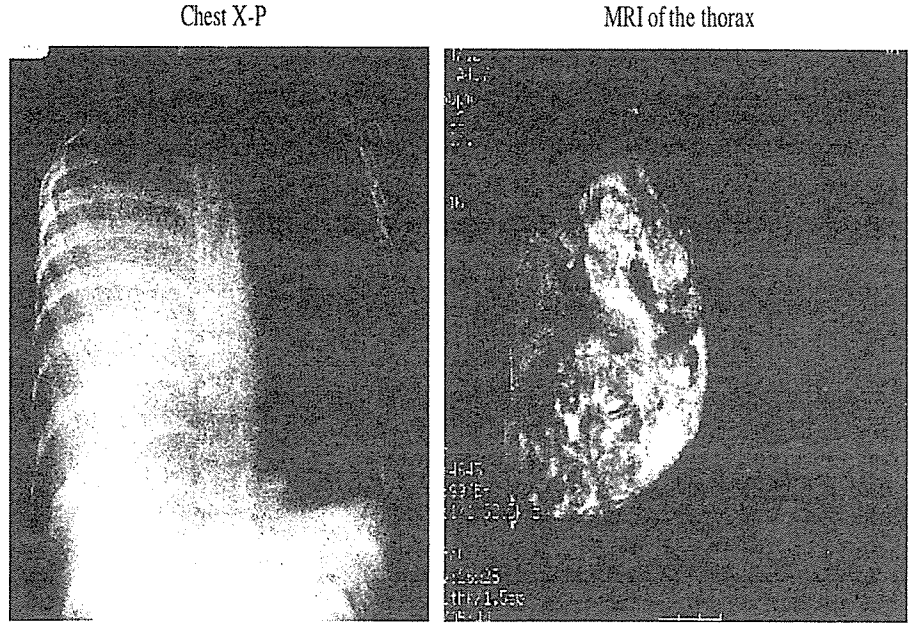

FIGURE 1. Chest x-ray and MRI of the thorax showing a tumor mass in the right hemithorax.

this patient was as follows: CDDP, 400 mg/m^2; ADR, 270 mg/m^2; and CY, 7800 mg/m^2. Total 45 Gy of irradiation was also performed in the other region of previous irradiation. There was no overlap in the irradiation fields between initial and relapse treatments. The patient received high-dose chemotherapy and PBSCT as shown in Figure 3.

There were no major complications during the procedure, and the patient reached 500 neutrophils/μL on day 10 after cell infusion and 20,000 platelets/μL on day 63. Imaging revealed no evidence of disease and the patient achieved complete hematologic recovery. Twelve months after PBSCT, she developed right optic neuritis and loss of strength in the lower limbs, which was caused by multiple sclerosis. The multiple sclerosis was treated by steroid. We consider these symptoms as caused by multiple sclerosis, because the view of cerebral MRI showed multiple white lesions in the CNS separated by time and location. However, we can not rule out the relation between right optic neuritis and loss of strength in the lower limbs and the previous treatments. Thirty-one months after PBSCT, no signs of relapse have been observed.

DISCUSSION

PPB is an uncommon primary malignant tumor of the lung. Surgery is the most important part of the treatment. Despite the use of chemotherapy with or without radiotherapy, the prognosis is poor.

In 1988, Manivel and associates[12] distinguished PPB as a separate diagnostic entity from pulmonary blastoma on the basis of age at presentation and its histologic, biologic, and clinical characteristics. PPB is confined to childhood whereas pulmonary blastoma occurs mostly in adults.

Although several of the previously reported cases have received multiagent chemotherapy, typically

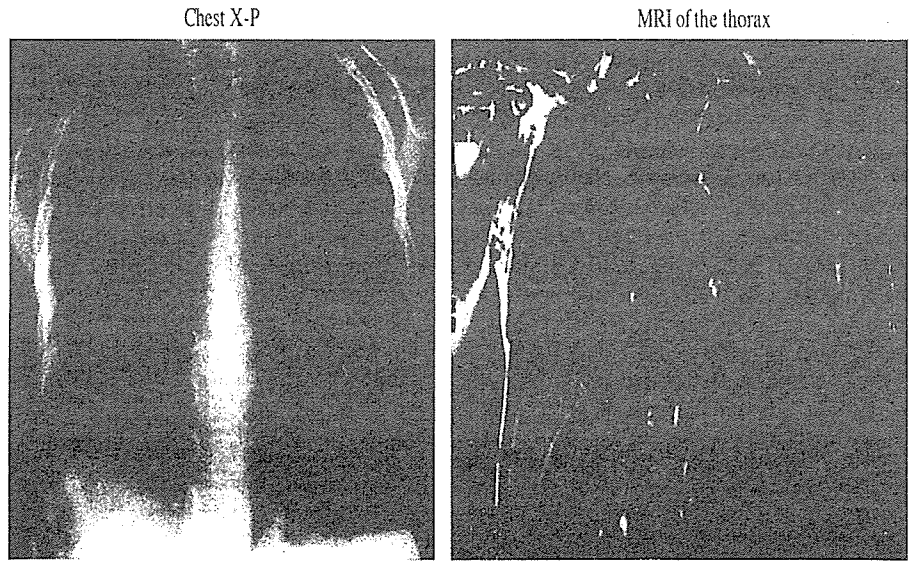

FIGURE 2. Chest x-ray and MRI of the thorax showing a coin lesion in the right hemithorax at the time of relapse.

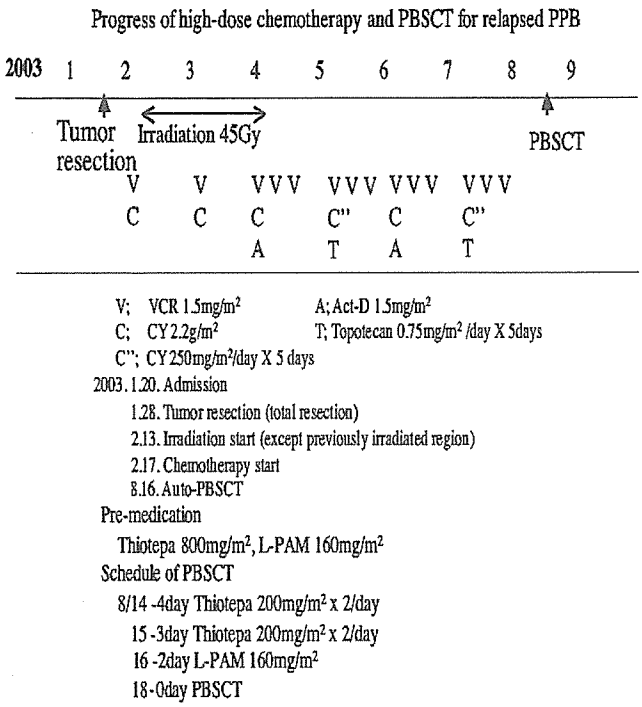

FIGURE 3. Progress of high-dose chemotherapy and PBSCT for relapsed PPB.

modeled after sarcoma treatment (eg, vincristine, actinomycin, and cyclophosphamide), the role of radiotherapy is more difficult to assess. Often, patients with great tumor bulk or with residual disease received radiotherapy with doses ranging from 4 to 55 Gy.[5,12] In a meta-analysis of 50 patients, reported by Priest and colleagues,[5] 16 children received radiation therapy, the majority of whom had purely solid tumors. Survival rates did not significantly differ among those treated with or without radiotherapy, although these data must be interpreted with caution.

Complete surgical excision is crucial; lobectomy or even pneumonectomy should be considered to achieve surgical radicality with free margins to prevent local recurrence.[7] Chemotherapy with agents such as vincristine, actinomycin D, doxorubicin, ifosfamide, etoposide, epirubicin, and cisplatin has been used in many patients. These drugs are also included in protocols for childhood soft tissue sarcomas. The use of intracavity cisplatin was reported in 2 cases.[13]

de Castro et al[10] administrated high-dose chemotherapy using melphalan, etoposide, and carboplatin, followed by autologous hematopoietic stem cell transplantation in a 5-year-old girl patient. She relapsed 4 months later and died about 9 months after the completion of high-dose therapy. They concluded that the role of high-dose chemotherapy and autologous hematopoietic stem cell transplantation was likely to be limited in PPB.

In our case, high-dose chemotherapy and PBSCT was performed when PPB had relapsed. Local recurrence developed in 1 of 7 type I PPBs (14%) and in 18 of 43 type II and type III PPBs (46%).[5] In relapsed cases prognosis is poor. A single case dose not prove the value of stem cell transplantation for treatment of PPB. Although the patient could have been cured with second surgery alone or by the surgery and subsequent chemotherapy, in relapsed PPB, high-dose chemotherapy and PBSCT should be considered.

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Allergology International. 2006;55:317-320

ORIGINAL ARTICLE

Pharmacokinetics of Beclomethasone Dipropionate in an Hydrofluoroalkane-134a Propellant System in Japanese Children with Bronchial Asthma

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ABSTRACT

Background: Hydrofluoroalkane-134a (HFA) has been shown to be a safe replacement for chlorofluorocarbons (CFCs) as a pharmaceutical propellant, with the advantage that it has no ozone-depleting potential. This is the first report of the pharmacokinetics of beclomethasone dipropionate (BDP) delivered from a pressurized solution formulation using an HFA propellant system (HFA-BDP) in Japanese children with bronchial asthma.

Methods: Plasma concentrations of beclomethasone 17-monopropionate (17-BMP), a major metabolite of BDP, following an inhaled dose of HFA-BDP (200 μg as four inhalations from 50 μg/actuation) in five Japanese children with bronchial asthma were quantified and analyzed by a non-compartmental analysis to obtain pharmacokinetic parameters.

Results: The area under the concentration-time curve from time zero to the last quantifiable time (AUC_{0-t}) was 1659 ± 850 pg · h/mL (arithmetic mean ± standard deviation (SD)), the maximum concentration observed (C_{max}) was 825 ± 453 pg/mL and the apparent elimination half-life ($t_{1/2}$) was 2.1 ± 0.7 hours. The time to reach C_{max} (T_{max}) was 0.5 hours in all patients. No special relationship was observed between these parameters and age or body weight. These parameters were compared with the previously reported parameters of American children with bronchial asthma. The Japanese/American ratio of the geometric means of each parameter was 1.36 for AUC_{0-t}, 1.04 for C_{max} and 1.4 for $t_{1/2}$. The median of T_{max} was 0.5 hours in American patients as well as Japanese patients.

Conclusions: The pharmacokinetics of HFA-BDP in Japanese children with bronchial asthma are reported for the first time and a similarity to those in American children is suggested.

KEY WORDS

beclomethasone dipropionate, children with bronchial asthma, hydrofluoroalkane-134a, Japanese, pharmacokinetics

INTRODUCTION

Hydrofluoroalkane-134a (HFA) has been shown to be a safe replacement for chlorofluorocarbons (CFCs) as a pharmaceutical propellant, with the advantage that it has no ozone-depleting potential.[1] Metered dose inhalers (MDIs) of beclomethasone dipropionate (BDP), using CFCs as propellant system in a press-and-breathe (P&B) inhalation device (CFC-BDP), have been used for many years in patients with bronchial asthma, but the Montreal Protocol represents international agreement to cease CFCs production and use. HFA is a suitable replacement propellant for CFCs in MDIs, as it does not deplete stratospheric ozone. The BDP solution product using HFA as propellant system (HFA-BDP) has a smaller particle size

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Received 1 December 2005. Accepted for publication 24 March 2006.

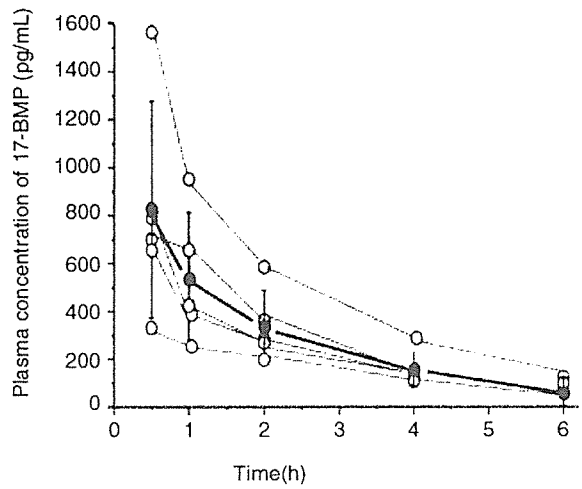

Fig. 1 Individual (○) and mean (●) plasma concentration-time profiles of beclomethasone 17-monopropionate (17-BMP) in Japanese children with bronchial asthma following administration of 200 μg of beclomethasone dipropionate (BDP). Each error bar represents the standard deviation.

distribution (by mass) than the CFC-BDP suspension product, which results in improved intrapulmonary deposition and airway availability of HFA-BDP when compared with CFC-BDP.[2] The HFA-BDP has an equivalent effect to the twice dose of CFC-BDP[3] and has been approved and used in adult patients in numerous countries. As CFC-BDP has also been essential in the treatment of children with bronchial asthma, the indications and usage of HFA-BDP for children need to be established urgently. Although the pharmacokinetics of HFA-BDP in American children with bronchial asthma have already been reported,[4,5] they have not yet been reported in Japanese children. The objective of this clinical trial was to clarify the pharmacokinetics of HFA-BDP in Japanese children with bronchial asthma.

METHODS

Five Japanese patients with stable mild asthma, between the ages of 6 years and 15 years (3 men and 2 women), were enrolled in this open-label study. Written informed consent was obtained from all patients and their parents or legal guardians in accordance with the Declaration of Helsinki. The independent medical ethics review committee of Gifu University approved the protocol. Each patient received 200 μg BDP per period, as four inhalations of 50 μg actuation P & B. Time zero for each dose was defined as the time when the inhaler was first actuated. Blood samples were collected before administration, and at 0.5, 1, 2, 4, 6, 9, 12 and 24 hours after administration. Immediately after collection, the tube was inverted and the blood was cooled on ice and centrifuged at 2000 rpm for 10 minutes at 7℃, and the isolated plasma was pippeted into two tubes (0.5 mL per tube). In each tube, 15 μL of acetic acid was added and mixed. Mixed samples were frozen immediately in a dry ice/methanol bath and stored frozen at under −70℃ until analysis. As almost all the BDP-derived material in plasma is reported to be the active metabolite, beclomethasone 17-monopropionate (17-BMP),[4] the plasma 17-BMP concentration was determined using an LC MS MS method with a lower limit of quantitation of 75 pg/mL developed by Harrison *et al.*[5] with a few modifications. The pharmacokinetic parameters, area under concentration-time curve from time zero to the last quantifiable time ($AUC_{0\text{-}t}$), maximum concentration observed (C_{max}), time to reach C_{max} (T_{max}) and apparent elimination half-life ($t_{1/2}$) were calculated by a non-compartmental analysis of each plasma concentration-time profile using WinNonlin ver.4.0 (Pharsight Corporation). The effects of age (6 to 14 years) and body weight (22.8 to 85.0 kg) on $AUC_{0\text{-}t}$, C_{max} and $t_{1/2}$ were analyzed by linear regression analysis of log-transformed parameters against age or body weight.

The parameters of Japanese children obtained in this study were compared with the previously reported parameters of American children with bronchial asthma provided by 3M Pharmaceuticals (USA).[6] For log-transformed parameters of $AUC_{0\text{-}t}$, C_{max} and $t_{1/2}$, means and their 90% confidence intervals were calculated for the differences between Japanese and American parameters and back-transformed to geometric means and their 90% confidence intervals for the ratios of Japanese parameters to American parameters. The log-transformed means of parameters were also compared by Student's *t*-test, considering p values of less than 0.05 to indicate statistically significant differences.

RESULTS AND DISCUSSION

PHARMACOKINETICS OF HFA-BDP IN JAPANESE CHILDREN WITH BRONCHIAL ASTHMA

The individual plasma concentration profiles of 17-BMP in Japanese children with bronchial asthma and their mean profile are shown in Figure 1. Pharmacokinetic parameters are shown in Table 1. The arithmetic mean ± standard deviation (SD) of $AUC_{0\text{-}t}$ was 1659 ± 850 pg · h/mL, C_{max} was 825 ± 453 pg/mL and $t_{1/2}$ was 2.1 ± 0.7 hours. T_{max} was 0.5 hours in all patients. The coefficient of variability of $AUC_{0\text{-}t}$ and C_{max} (51.2% and 54.9%, respectively) was larger than that of $t_{1/2}$ (31.4%), which indicated a higher variability of the rate and extent of absorption than the elimination rate, possibly because of variability in the patients' inhalation techniques. No significant effect of age (6 to 14 years) or body weight (22.8 to 85.0 kg) on pharmacokinetic parameters was observed (Table 2, p = 0.145). This result suggests that no dose adjustment

Table 1 Pharmacokinetic parameters of beclomethasone 17-monopropionate (17-BMP) in Japanese children with bronchial asthma following administration of 200 μg beclomethasone dipropionate (BDP)

Subject	Sex	Age (years)	Body weight (kg)	$AUC_{0\text{-}t}$ (pg · h/mL)	C_{max} (pg/mL)	T_{max} (h)	$t_{1/2}$ (h)
1	Male	6	25.4	3062	1565	0.5	1.7
2	Female	9	36.3	1462	703	0.5	2.1
3	Female	14	85.0	1524	828	0.5	2.8
4	Male	9	22.8	1507	699	0.5	1.2
5	Male	11	27.4	741	331	0.5	2.6
Mean		10	39.4	1659	825	0.5	2.1
SD		NC	NC	850	453	NC	0.7
CV (%)		NC	NC	51.2	54.9	NC	31.4
Geometric mean		NC	NC	1501	732	NC	2.0

$AUC_{0\text{-}t}$, area under concentration-time curve; C_{max}, maximum concentration observed; T_{max}, time reach to C_{max}; $t_{1/2}$, apparent elimination half-life.

SD, standard deviation; CV, coefficient of variability.

NC, not calculated.

Table 2 Effects of age and body weight on pharmacokinetic parameters of beclomethasone 17-monopropionate (17-BMP) in Japanese children with bronchial asthma

	$AUC_{0\text{-}t}$	C_{max}	$t_{1/2}$
Age	0.216	0.288	0.145
Body weight	0.833	0.951	0.203

Each value represents the *p*-value from regression analysis of each parameter against age or body weight of 5 patients in this study.

$AUC_{0\text{-}t}$, area under concentration-time curve; C_{max}, maximum concentration observed; $t_{1/2}$, apparent elimination half-life.

based on age or body weight in the range of this study is needed, though much more data are required to derive a conclusion.

COMPARISON OF HFA-BDP PHARMACOKINETICS BETWEEN JAPANESE AND AMERICAN CHILDREN WITH BRONCHIAL ASTHMA

The plasma concentration profiles of 17-BMP in Japanese and American children with bronchial asthma following a 200 μg BDP dose using the HFA-propellant system are shown in Figure 2. Pharmacokinetic parameters and their ratios (Japanese / American) are shown in Table 3. The differences of log-transformed mean 17-BMP parameters ($AUC_{0\text{-}t}$, C_{max} and $t_{1/2}$) between Japanese and Americans were not significant ($p = 0.13$). The median of T_{max} in American children was the same as that in Japanese children. The estimated Japanese / American $AUC_{0\text{-}t}$ ratio was 1.36 with a 90% confidence interval of 0.870 to 2.13. The estimated Japanese / American C_{max} ratio was 1.04 with a 90% confidence interval of 0.671 to 1.62. The estimated Japanese / American $t_{1/2}$ ratio was 1.4 with a 90% confidence interval of 0.96 to 1.9. All confidence intervals contained 1, suggesting the possibility of the equivalence of the parameters between Japanese and Americans. The wide confidence intervals obtained may be due to variability in patients' inhalation techniques, and an insufficient limit of quantitation, which may affect the last quantifiable time-point and the apparent elimination half-life.

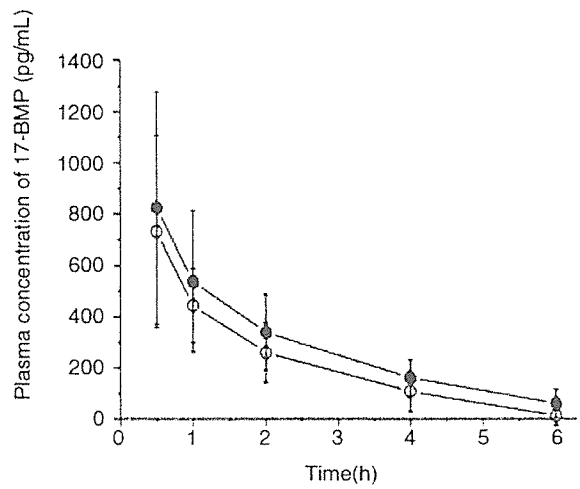

Fig. 2 Japanese (●) and American (○) plasma concentration-time profiles of beclomethasone 17-monopropionate (17-BMP) following administration of 200 μg beclomethasone dipropionate (BDP). Each point represents the mean ± standard deviation of 5 Japanese or 18 American children with bronchial asthma.

In conclusion, the pharmacokinetics of HFA-BDP

Table 3 Comparison of plasma pharmacokinetics of beclomethasone 17-monopropionate (17-BMP) between Japanese and American children with bronchial asthma following administration of 200 μg beclomethasone dipropionate (BDP)

Country		AUC_{0-t} (pg · h/mL)	C_{max} (pg/mL)	T_{max} (h)	$t_{1/2}$ (h)
Japanese	Geometric mean 90% confidence interval	1501 (1013-2224)	732 (497-1079)	0.5 (median) (0.5-0.5) (min-max)	2.0 (1.5-2.6)
American	Geometric mean 90% confidence interval	1102 (891-1365)	703 (570-867)	0.5 (median) (0.08-1.0) (min-max)	1.5 (1.2-1.8)
Japanese/ American ratio	Geometric mean 90% confidence interval	1.36 (0.870-2.13)	1.04 (0.671-1.62)	1.0 (median ratio)	1.4 (0.96-1.9)

AUC_{0-t}, area under concentration-time curve; C_{max}, maximum concentration observed; T_{max}, time reach to C_{max}; $t_{1/2}$, apparent elimination half-life.

in Japanese children with bronchial asthma were investigated and the following parameters (arithmetic mean ± SD or median for T_{max}) were obtained; AUC_{0-t} (1659 ± 850 pg · h/mL), C_{max} (825 ± 453 pg/mL), $t_{1/2}$ (2.1 ± 0.7 hours) and T_{max} (0.5 hours). These parameters were similar to those of American children with bronchial asthma.

In this study, the pharmacokinetic properties of HFA-BDP did not show much difference between the two countries. Therefore, propellant systems using CFCs should be replaced by systems using HFA as soon as possible in countries where such products have already been launched, from the standpoint of protection of the ozone layer. In addition, the introduction of HFA products, e.g. HFA-BDP, is strongly recommended in countries where such products are not clinically available.

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Allergology International. 2006;55:287-293

ORIGINAL ARTICLE

A Randomized Open-Label Comparative Study of Montelukast versus Theophylline Added to Inhaled Corticosteroid in Asthmatic Children

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ABSTRACT

Background: Inhaled corticosteroids (ICSs) are widely used in combination with other classes of drugs for treatment of childhood asthma. The efficacy and the safety of montelukast added to low-dose ICS therapy were compared with those of sustained-release theophylline added to low-dose ICS therapy in asthmatic children in the present study.

Methods: Following the 2-week run-in period, 6-to 14-year old patients receiving treatment with ICSs were randomized to treatment for 4 weeks with either montelukast 5 mg once daily or sustained release theophylline 5–8 mg/kg (dry syrup) or 100–200 mg (tablet) twice daily. Patients also received a fixed dose of ICS throughout the run-in and treatment periods. The primary efficacy endpoint was the change from baseline in peak expiratory flow (PEF) at Week 2.

Results: A significant increase in morning PEF was observed in the add-on montelukast group as compared with the add-on theophylline group at Week 2 (change from baseline of 22.8 L/min *vs.* 8.7 L/min; $p = 0.041$ for between-group difference) and at Week 4 (31.0 L/min *vs.* 9.8 L/min; $p = 0.012$). A significant increase in evening PEF was observed in the add-on montelukast group as compared with the add-on theophylline group at Week 4 (24.7 L/min *vs.* 8.7 L/min; $p = 0.027$). There were no significant differences between the treatment groups in incidences of clinical and laboratory adverse experiences.

Conclusions: The results indicate that montelukast added to low-dose ICS is an effective and safe option for the treatment of asthma in children.

KEY WORDS

childhood asthma, inhaled corticosteroid, montelukast, peak expiratory flow, sustained-release theophylline

INTRODUCTION

Bronchial asthma is a chronic inflammatory disease characterized by airway hyper-responsiveness and episodic respiratory symptoms, such as breathlessness, wheezing, chest tightness and coughing.[1,2] Numerous cell types, including eosinophils, T cells, mast cells, basophils, and neutrophils, play a role in triggering airway inflammation.[3] Cysteinyl leukotrienes (CysLTs) and other mediators released by such inflammatory cells have been shown to play a critical role as determinants of pathological conditions in bronchial asthma.[4-6] Montelukast is a selective CysLT$_1$ receptor antagonist that reduces asthmatic inflammation and airway resistance and prevents bronchoconstriction.[7-10]

Inhaled corticosteroids (ICSs) are used as medication for early intervention and long-term management of childhood asthma. ICSs are effective because they directly reach the airway and intensively inhibit airway inflammation.[11-13] However, when the amount of drug deposited in the respiratory tract increases with

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Received 15 November 2005. Accepted for publication 9 March 2006.

use of higher dose, risks of adverse drug reactions also increase.[11,14] Therefore, some reports have recommended combination of ICS with other classes of drugs than ICS monotherapy with increased doses.[15-17] Such combined therapy for long-term asthma management has been shown to be more effective in controlling mild to severe persistent asthma in children. Candidates for concomitant drugs include CysLT1 receptor antagonists, long-acting inhaled β2-agonists, and sustained-release theophylline. However, there have been few comparative studies done on these types of drugs when combined with low-dose ICS in children with asthma. In this study, the efficacy and safety of oral administration of montelukast was compared to those of sustained-release theophylline in asthmatic children in the treatment with ICS.

METHODS

PATIENTS

Eighty-four children, male: 51 (60.7%), female: 33 (39.3%), aged 6–14 years, with unstable asthma symptoms despite low dose ICS therapy were enrolled in the study. Patients had mild to severe persistent asthma according to the Japanese Pediatric Guidelines[11] and mild to moderate persistent asthma as defined by the GINA guidelines.[18] Before the 2-week run-in period, patients were confirmed to have airway reversibility and reproducible peak expiratory flow (PEF) measurement. During the 2-week run-in period, patients were confirmed to have symptoms (recurrent coughing, or mild or moderate asthma attacks). The following patients were excluded from the study: patients on continuous therapy with oral or injectable corticosteroids; patients who had used oral antiallergic drugs within the 2 weeks prior to the run-in period; patients who used a long-acting corticosteroid within the 1 year prior to the run-in period; and patients with complications that could affect the evaluation of efficacy, such as bronchiectasis. Patients with a history of serious adverse drug reaction to theophylline or other xanthine derivatives and patients who had previously used montelukast were also excluded from the study.

Parents or guardians gave written consent prior to the start of the study. The study was approved by the institutional review board of each participating site.

STUDY DESIGN

This study was done as a multi-center, randomized, open-label study conducted between June 2003 and August 2004. Twenty-four sites around Japan participated, involving a total of 61 affiliated specialists in pediatric asthma treatment. Following a 2-week run-in period, patients were randomized to treatment for 4 weeks with either montelukast 5 mg chewable tablet administered once daily at bedtime or sustained release theophylline 5–8 mg/kg (dry syrup) or 100–200 mg (tablet) twice daily (Fig. 1). Patients also received a fixed dose of inhaled corticosteroid in the run-in and treatment periods (CFC-beclomethasone dipropionate 100–400 μg/day, or fluticasone propionate 100–200 μg/day). The central random allocation of the study drug was performed using the minimization method involving study centers and body weight as factors. Laboratory tests (hematology, blood chemistry, urinalysis) were performed at the beginning and the completion of treatment. Pulmonary function tests (FEV_1 and FVC) were performed at the time of laboratory tests whenever possible.

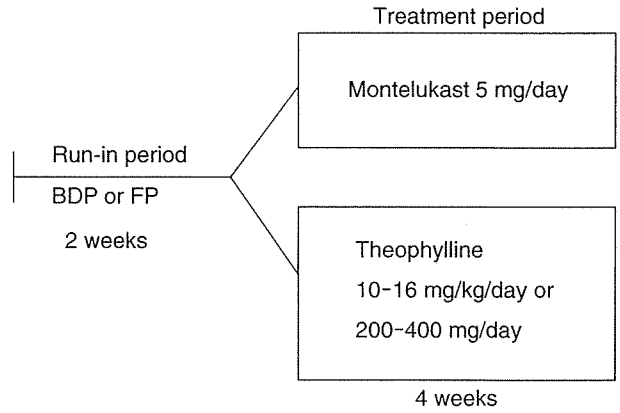

Fig. 1 Study design. BDP = beclomethasone dipropionate; FP = fluticasone propionate.

EVALUATION OF EFFICACY AND SAFETY

The primary efficacy endpoint was the change from baseline in PEF at Week 2. PEF was measured daily with a Mini-Wright PEF meter (Clement Clark International; Harlow, UK) three times upon awakening and three times at bedtime, and the maximum value at each time was recorded. Patients kept a daily asthma diary from the beginning of the run-in period to the completion of treatment, and daily recorded asthma-related symptoms (asthma attacks, coughing, daily activities, nighttime sleep), morning and evening PEF values, treatment compliance with study medication, and use of other concomitant drugs such as inhaled β2-agonist.

Clinical and laboratory adverse experiences were recorded during the study. Patients also assessed tolerability at the completion of the 4-week treatment period (or at discontinuation).

STATISTICAL ANALYSIS

The per-protocol set (PPS) was defined as the primary efficacy analysis population. The analyses were also performed in the full analysis set (FAS) to examine the stability of the study results. Summary statistics of the observed values and the changes from baseline (defined as the mean over the 2-week run-in period) as well as their 95% confidence intervals were

Table 1 Patient Demographics and Other Baseline Characteristics (Per-Protocol Set)

Treatment groups	Montelukast	Theophylline
Number of subjects	39 N (%)	36 N (%)
Gender		
Male	21 (53.8)	23 (63.9)
Female	18 (46.2)	13 (36.1)
Age		
6－9 yrs	21 (53.8)	26 (72.2)
10－14 yrs	18 (46.2)	10 (27.8)
Mean ± SD	9.4 ± 2.4	8.8 ± 2.2
Body weight		
< 30 kg	22 (56.4)	22 (61.1)
≥ 30 kg	17 (43.6)	14 (38.9)
Mean ± SD	34.0 ± 14.3	28.7 ± 7.8
Asthma severity		
Mild persistent	24 (61.5)	18 (50.0)
Moderate persistent	12 (30.8)	16 (44.4)
Severe persistent	3 (7.7)	2 (5.6)
Duration of asthma		
Mean ± SD	5.3 ± 3.4	5.6 ± 3.7
Dose of inhaled corticosteroid †		
< 200 μg/day	11 (28.2)	10 (27.8)
≥ 200 to 300 μg/day	13 (33.3)	18 (50.0)
≥ 300 μg/day	15 (38.5)	8 (22.2)
Mean ± SD	261.6 ± 102.3	235.9 ± 86.5
Eosinophils		
< 6%	12 (30.8)	17 (47.2)
≥ 6%	27 (69.2)	19 (52.8)

† Equivalent to dose of beclomethasone dipropionate

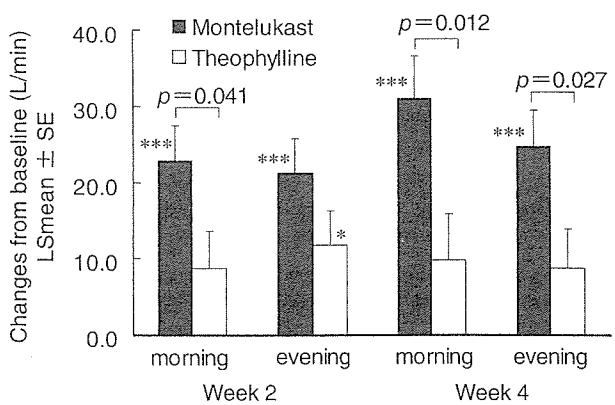

Fig. 2 Comparison of the changes from baseline in morning and evening PEF between montelukast and theophylline. *** $p < 0.001$ and * $p < 0.05$ compared with baseline.

computed at each time point and for each treatment group. Statistical analyses were performed for PEF at Week 2 (defined as the mean over treatment between Week 1 and 2) and Week 4 (defined as mean over treatment between Week 3 and 4). If there were no data for analysis at Week 4, then the value at Week 2 was extrapolated, using the Last Observation Carried Forward method. Comparisons of the change from baseline between treatment groups were performed using an analysis-of-covariance model involving treatment as a factor and baseline value as a covariate. Within-group comparisons of the values at each time point with baseline were also performed using Student's *t*-test for the least squares mean (hereinafter LSmean) of change.

For those patients included in the analysis of safety, the numbers and percentages of patients reporting adverse experiences were summarized by treatment groups.

RESULTS

Of 84 randomized patients, 79 patients completed the study, while 5 patients withdrew. The reasons for withdrawal were: occurrence of adverse experience in 3 patients, use of prohibited concomitant drug in 1

Table 2 Summary statistics for PEF (morning, evening), numbers of mild asthma attacks and Inhaled β_2-agonist use

Item	Group	N	Baseline	Week 2	Week 4	Week 4 (LOCF)
Morning PEF	M	39	264.7 ± 12.1	287.4 ± 11.7	295.6 ± 12.0	295.6 ± 12.0
(L/min)	T	36	261.3 ± 11.6	270.2 ± 12.1	273.1 ± 12.0	269.3 ± 11.9
Evening PEF	M	39	278.2 ± 12.3	299.2 ± 11.9	302.5 ± 12.0	302.5 ± 12.0
(L/min)	T	36	270.4 ± 11.5	282.5 ± 11.6	278.4 ± 12.0	279.2 ± 11.4
Mild Asthma Attacks	M	39	0.89 ± 0.17	0.28 ± 0.12	0.27 ± 0.11	0.27 ± 0.11
(times/week)	T	36	1.02 ± 0.25	0.56 ± 0.32	0.58 ± 0.24	0.56 ± 0.22
Inhaled β_2-Agonist Use	M	26	5.93 ± 1.42	4.37 ± 1.29	4.15 ± 1.16	4.15 ± 1.16
(times/week)	T	20	5.68 ± 1.76	4.73 ± 1.94	3.50 ± 1.61	4.58 ± 1.91

M: Montelukast, T: Theophylline, Mean ± SE

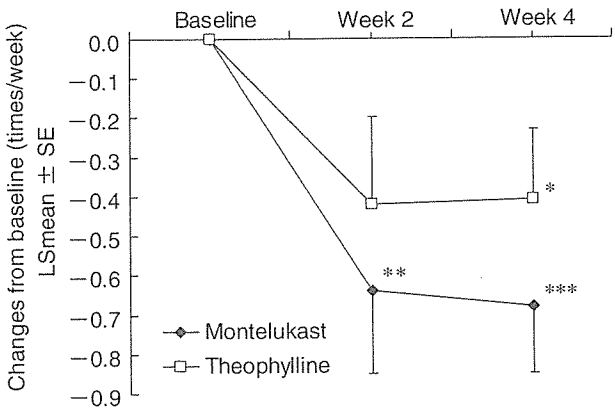

Fig. 3 Comparison of the changes from baseline in mild asthma attacks between montelukast and theophylline. *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ compared with baseline.

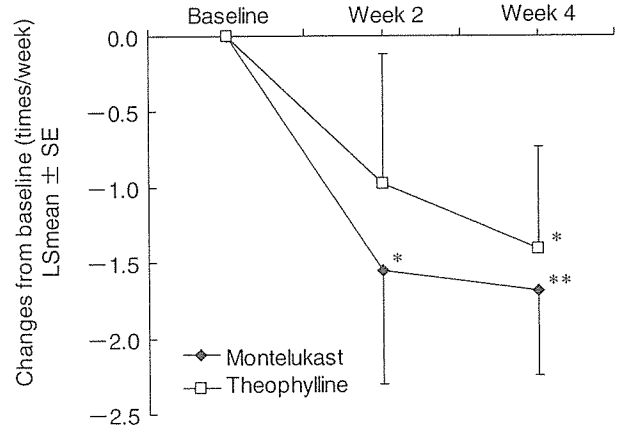

Fig. 4 Comparison of the changes from baseline in inhaled β_2-agonist use between montelukast and theophylline. ** $p < 0.01$ and * $p < 0.05$ compared with baseline.

patient, and deviation of study visit schedule in 1 patient. Seventy-five patients with data on PEF at Week 2 (primary endpoint) were eligible for efficacy analysis. Nine patients were excluded from the efficacy analysis set, and the reasons for exclusion were: use of prohibited concomitant drug in 3 patients, violation of study procedure in 3 patients, change in living environment in 1 patient, insufficient study period and insufficient frequency of PEF measurements in 1 patient, and noncompliance with drug administration and insufficient frequency of PEF measurements in 1 patient.

Eighty-three patients were eligible for analysis of safety; 1 patient in the theophylline group was excluded because of delayed performance of the patient's laboratory tests.

The dose of inhaled corticosteroid (mean ± SD, on the beclomethasone dipropionate equivalence basis) in the 75 eligible patients for efficacy analysis was 261.6 ± 102.3 μg/day in the montelukast group and 235.9 ± 86.5 μg/day in the theophylline group; there was no significant difference between the two groups. There were also no significant differences between the two treatment groups with respect to other baseline characteristics (including sex, age, body weight, and severity grade of asthma) (Table 1).

PEF IMPROVEMENT

The LSmean change from the baseline in morning PEF at Week 2 was 22.8 L/min in the montelukast group ($p < 0.001$: within group comparison from baseline), and 8.7 L/min in the theophylline group ($p = 0.078$: within group comparison from baseline), demonstrating a significant improvement in the montelukast group compared with the theophylline group ($p = 0.041$: between group comparison). At Week 4, the change from baseline in morning PEF was 31.0 L/min in the montelukast group ($p < 0.001$), and 9.8 L/min in the theophylline group ($p = 0.107$), demonstrating a significant improvement in the montelukast group compared with the theophylline group ($p = 0.012$) (Fig. 2, Table 2).

The LSmean change in evening PEF at Week 2 from baseline was 21.3 L/min in the montelukast group ($p < 0.001$) and 11.7 L/min in the theophylline group ($p = 0.013$). The difference between the groups

in the change from baseline was not significant (p = 0.137). At Week 4, the change from baseline in evening PEF was 24.7 L/min in the montelukast group ($p < 0.001$) and 8.7 L/min in the theophylline group (p = 0.096), indicating a significant improvement in the montelukast group compared with the theophylline group (p = 0.027) (Fig. 2, Table 2).

MILD ASTHMA ATTACKS

A mild asthma attack was defined as an episode of mild wheezing occasionally associated with mild intercostal or tracheosternal retractions. The LSmean change from the baseline in the number of mild asthma attacks (including wheezing) at Week 2 was −0.64 times/week in the montelukast group (p = 0.004 for difference from baseline) and −0.42 times/week in the theophylline group (p = 0.061 for difference from baseline). The change at Week 4 was −0.68 times/week in the montelukast group ($p < 0.001$) and −0.41 times/week in the theophylline group (p = 0.024). No significant differences between the groups were observed in the changes at Week 2 and Week 4 (Fig. 3, Table 2).

INHALED β_2-AGONIST USE

The LSmean change from baseline in the number of inhaled β_2-agonist use at Week 2 was −1.55 times/week in the montelukast group (p = 0.046) and −0.98 times/week in the theophylline group (p = 0.261). The change at Week 4 was −1.69 times/week in the montelukast group (p = 0.005) and −1.41 times/week in the theophylline group (p = 0.044). No significant differences between the groups were observed in the changes at Week 2 and Week 4 (Fig. 4, Table 2).

PERIPHERAL BLOOD EOSINOPHILS

Eosinophil levels were not significantly affected by either treatment with add-on montelukast or theophylline and no significant difference was observed between the two treatments (data not shown).

SUBGROUP ANALYSIS BY BODY WEIGHT IN THE MONTELUKAST GROUP

Study subjects on montelukast were stratified into subgroups by body weight (<30 kg and ≥30 kg), and differences in PEF and in safety were assessed. The changes from baseline values in morning and evening PEF were similar between the subgroups at Week 2 and Week 4; there were also no significant differences between the two subgroups in safety assessments (data not shown).

SAFETY ASSESSMENT

There were no clinically meaningful differences between the treatment groups in the incidence of clinical or laboratory adverse experiences. Two drug-related clinical adverse experiences were seen but they were mild and transient: 1 patient (2.4%) in the montelukast group developed headache and 1 patient (2.4%) in the theophylline group had queasiness. Two serious clinical adverse events, status asthmaticus and asthma aggravation, were reported in 1 patient in each treatment group; however, these were not judged to be drug-related. Two patients (4.8%) in the montelukast group developed drug-related laboratory adverse experiences: 1 patient had increased total protein (baseline: 6.8 g/dL, Week 4: 8.7 g/dL, normal range value: 6.3–7.9 g/dL); 1 patient had increased total bilirubin (baseline: 0.9 mg/dL, Week 4: 1.7 mg/dL, normal range value: 0.1–1.0 mg/dL) and positive urobilinogen urine (baseline: ±, Week 4: +, normal range value: ±). Drug-related serious laboratory adverse experiences were not reported. No drug-related adverse experiences were clinically significant.

DISCUSSION

Theophylline is a widely used medication for the treatment of asthma, mostly because of its ease of use, low cost and good anti-inflammatory effects;[19] thus, it was selected for a positive control, as an add-on agent to ICS in this study. In this study, the mean theophylline dosage was 9.8 mg/kg/day (4.7–15.7 mg/kg/day). Sugimoto *et al.* reported that the mean serum theophylline concentration was 8.8–13.1 μg/ml when 7-to 10-year old asthmatic children were given theophylline at a dose of 16 mg/kg/day in the steady state.[20] In addition, Nakashima *et al.* reported that the mean serum theophylline concentration was 5.5–7.3 μg/ml when healthy adult male subjects were administered 400 mg/day (approximately 6.1 mg/kg/day) in the steady state.[21] The ranges of serum theophylline concentration in the present study were assumed to be between the values of the above two studies.[20,21] In this study, the investigators determined whether or not to perform serum concentration measurement for patients mainly consisting of those whose asthma symptoms were not improved. As a result, the serum theophylline concentration was measured in three patients: 1.3 and 3.1 μg/ml (this patient was measured twice at a dose of 8.2 mg/kg/day), under the detection limit of 2.0 μg/ml (10.4 mg/kg/day), and 6.5 μg/ml (12.0 mg/kg/day), respectively. When used as complementary therapy in patients not optimally controlled by low-to-high dose ICS, montelukast has shown to improve the control of asthma and reduce exacerbations, and to be a good alternative to increasing a dose of ICS or given an additional long-acting β_2-agonist.[22,23]

This study shows that montelukast plus ICS demonstrated significant improvement in morning and evening PEF at week 2 and 4 compared to the baseline results with ICS alone. Theophylline plus ICS demonstrated significant improvement in evening PEF at Week 2, compared to the baseline value. Children administered concomitant montelukast and ICS demonstrated a significantly greater improvement in

morning PEF at Week 2 and morning and evening PEF at Week 4 in comparison with concomitant treatment of theophylline and ICS. The improvement in PEF observed with add-on montelukast in the early stage within 2 weeks of the therapy is consistent with the results of a study in adult patients with bronchial asthma, who reported significant improvement in morning PEF from its baseline after 1–3 days of therapy with add-on montelukast.[22]

To investigate the influence of severity and duration of disease, subgroup analyses by severity (mild *vs.* moderate and severe) and duration of disease (<5 years *vs.* ≥5 years) were performed. In all the subgroups, montelukast showed significant improvement from baseline at Week 2 in the morning PEF, whereas theophylline did not (data not shown). These findings indicate that the addition of montelukast to the therapy resulted in improvement in PEF as early as Week 2, independent of the severity and duration of disease.

Diurnal variation in PEF is an useful indicator for evaluation of asthma, which is possibly related to airway hyper-responsiveness.[24] The exploratory data analysis demonstrated that the mean diurnal variation in PEF decreased in the montelukast group from the baseline value of 9.3 ± 5.2% to 7.2 ± 4.2% at Week 2 ($p = 0.005$), to 6.1 ± 3.6% at Week 4 ($p < 0.001$), however it was unchanged in the theophylline group (baseline: 8.8 ± 7.3%, Week 2: 9.0 ± 9.0, $p = 0.794$, Week 4: 7.3 ± 5.0, $p = 0.077$). The result suggested that the addition of montelukast to ICS provided more improvement for diurnal variation in PEF than theophylline.

A reduction in mild asthma attacks and in β_2-agonist use is indicative of improvement in asthma control. Add-on montelukast further reduced the frequency of mild asthma attacks (compared to baseline values) throughout the study, while add-on theophylline was more effective only at Week 4. Also, inhaled β_2-agonist use during Week 2 or Week 4 (compared to baseline use) was significantly reduced with add-on montelukast, but not with add-on theophylline. These results suggest that montelukast added to ICS can decrease asthma-related symptoms more than theophylline added to ICS in asthmatic children. Therefore, it is concluded that montelukast is more effective than theophylline as add-on therapy to low dose ICS in improving pulmonary measures and asthma-related symptoms in asthmatic children.

Peripheral blood eosinophil levels serve as an indicator of airway inflammation.[25] Montelukast is known to decrease peripheral blood eosinophil levels.[26] However, eosinophil levels did not show any significant change from the baseline value in both treatment groups in this study. It is thought that the number of patients might not be sufficient to demonstrate significant change.

Montelukast showed additional improvement in PEF to ICS alone because it is believed to have different mechanisms of action from those of ICS in suppressing airway inflammation. It is known that despite treatment with corticosteroids, airway inflammation persists in asthmatic patients.[27] While ICSs affect many inflammatory pathways in asthma, they have little impact on CysLTs.[28] The results from several large-scale clinical studies provide support for this view of a dual pathway of airway inflammation.[22,23,29]

Montelukast is indicated with one dose of 5 mg for 6-to 14-year old patients, in whom body weight ranged widely. Therefore, in this study the influence of body weight was investigated. The efficacy and safety results from stratifying patients into subgroups (<30 kg and ≥30 kg) confirmed the appropriateness of the use of one dose for pediatric patients in that age range. The recent study, which was a multicenter, randomized, double-blind trial for 6-to 14-year old patients with mild asthma, revealed that the efficacy and safety did not differ greatly regardless of body weight when 5 mg montelukast was administrated.[30]

During four weeks of treatment in children with asthma on ICS therapy, both montelukast and theophylline showed a favorable safety profile. In addition, the MOSAIC study,[31] which was a 12-month, multicenter, randomized, double-blind trial for 6-to 14-year old patients with mild asthma, showed that montelukast was generally well tolerated for the treatment period (12-months), clinical and laboratory drug-related adverse experience represented 4.4% and 0.5% in the montelukast group, respectively.

In summary, this study suggests that when combined with ICS therapy, montelukast is an effective and safe option for long-term management of childhood asthma. Furthermore, taking into account the mode of administration, dose management and convenience of handling, montelukast may be considered superior to sustained-release theophylline as add-on therapy to ICS in asthmatic children.

ACKNOWLEDGEMENTS

The authors thank the following investigators for their participation in the study; Kenichi Tokuyama, Hiroyuki Mochizuki, Hirokazu Arakawa, Hiroyuki Inui, Taeru Kitabayashi, Takanori Imai, Ryoko Ito, Tokuko Mukouyama, Kuniteru Aoki, Shigeharu Kyo, Ichiro Miyata, Morimitsu Tomikawa, Atsushi Shibata, Kenichi Akashi, Kosaburo Shichijo, Tsutomu Matsumoto, Akira Akasawa, Hidetoshi Kawahara, Taro Kamiya, Tetsuji Ishii, Yukihiro Ohya, Tomoko Suda, Hiroko Watanabe, Masami Narita, Ichiro Nomura, Kazuko Nakagawa, Ryosuke Inoue, Akira Hoshioka, Toshikazu Tsubaki, Akiko Yamaide, Hideo Kaneko, Toshiyuki Fukao, Takahide Teramoto, Eiko Matsui, Osamu Fukutomi, Takehiko Miyagawa, Masanori Yamada, Satoko Suzuki, Hiroatsu Agata, Komei Ito, Masashi Morishita, Takao Fujisawa, Kosei Iguchi, Jun Atsuta, Jiro Takei, Yasuo Arai, Michiko Haida, Kyoko Shiraishi, Yasuko Tanaka, Etsuko Tagaya,

Yoshio Sakamoto, Kojiro Otsuka, Yasumasa Nomoto, Tetsuya Ozeki, Itaru Terashima, Akira Ozaki, Toshikazu Nagakura.

We express our thanks to Banyu Pharmaceutical Co., Ltd., Tokyo, Japan for their kind support of this study.

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気管支喘息領域におけるオーダーメイド治療と遺伝子多型

Tailor-made Medicine and Gene Polymorphisms in Bronchial Asthma

はじめに

アレルギー疾患は，遺伝的要因と環境要因が絡み合って発症する．アレルギー発症に関する種々のアンケートや問診から家族集積性が認められることから，アレルギー疾患の発症には何らかの遺伝的要因がかかわっていると考えられる．さらに，アレルギーの病態の多様性からアレルギーに関連する遺伝子は多彩であると考えられる．本稿では，その多彩なアレルギー関連遺伝子のうち，特に薬剤反応性と関連のある遺伝子を中心に自験例を含めて，気管支喘息領域におけるオーダーメイド治療と遺伝子多型を概説する．

薬理遺伝学とオーダーメイド治療

現在，安定期の気管支喘息の長期管理薬としては，吸入ステロイド薬を中心に抗アレルギー薬，β_2アドレナリン受容体刺激薬（特に長時間作用性），徐放性テオフィリン薬などさまざまな選択肢が存在する．いずれの薬剤も有効性は確立しているが，吸入ステロイド薬ですらその有効性は70～90%の患者にとどまるといわれている．今後は，個々の気管支喘息患者にとって最善の治療法の組み合わせを見い出していくこと，つまりオーダーメイド化が大切であると考えられる．そして，その薬剤反応性の個体差を規定する遺伝的要因を検討する分野を薬理遺伝学とよぶ．

気管支喘息領域の薬理遺伝学につき，β_2アドレナリン受容体刺激薬，抗アレルギー薬としてロイコトリエン受容体拮抗薬，Th2サイトカイン抑制薬を中心に述べる．

β_2アドレナリン受容体刺激薬

β_2アドレナリン受容体刺激薬は喘息発作時のリリーバー（短時間作用性吸入薬）あるいは安定期のコントローラー（長時間作用性吸入薬，経口薬，貼付薬）として用いられている．そして，β_2アドレナリン受容体刺激薬の急性気管支拡張効果とβ_2アドレナリン受容体多型との関連性がいくつか報告されている[1)2)]．β_2アドレナリン受容体はカテコラミンの作用を調節するG蛋白結合型受容体で，イントロンのない遺伝子（ADRB2）として5q31-32にコードされている．単一多型と薬剤感受性としては，β_2アドレナリン受容体刺激薬の急性気管支拡張効果とArg16Gly多型との関連を検討し，Gly16型受容体をもつ場合，単回のβ_2アドレナリン受容体刺激薬負荷時の気管支拡張反応が弱いという結果が報告されている[2)]．Arg16Gly多型はβ_2アドレナリン受容体刺激薬との結合能や，G蛋白とのカップリングに直接影響していないにもかかわらず，それらの違いがみられる理由として，内因性カテコラミンあるいは以前に使用していたβ_2アドレナリン受容体刺激薬によるdown regulationの可能性が考えられている．ただし，これらは後方視的研究であることや，ハプロタイプに関する検討不足などが指摘されている．そこで，2000年には複数の多型の組み合わせで規定されるハプロタイプと表現型の間に相関がみられることが新たに報告された[3)]．プロモーター領域とコード領域に存在する13ヵ所の多型の組み合わせを12種類のハプロタイプに分類し，ハプロタイプとmRNA，受容体の発現レベルとの相関が示され，さらにハプロタイプによって薬剤反応性に違いがあることが示されている．単一の多型ではなく複数の多型の組み合わせ，すなわちハプロタイプによる変異が個人の疾患感受性や薬剤感受性を決める因子となることが示唆された．

ロイコトリエン受容体拮抗薬

ロイコトリエン受容体拮抗薬は，単剤で気管支拡張効果と抗炎症効果の両者を有し，気道リモデリングも改善させる可能性

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Introduction

Allergic diseases are caused by complex interactions of genetic and environmental factors. Since various questionnaires and medical interviews on the onset of allergic diseases suggest familial accumulation, it is believed that genetic factors are involved in the development of allergic diseases. Furthermore, the highly various pathology of allergic diseases suggests that multiple genes are involved in the allergy. In this paper, among various allergy-associated genes, genes associated with responses to drugs are discussed, including our own experience, in order to outline tailor-made medicine and gene polymorphisms in bronchial asthma.

Pharmacogenetics and tailor-made medicine

There are various choices of drugs for long-term management of stable bronchial asthma such as inhaled steroid, β_2 adrenargic receptor agonist (especially long-acting type), and theophylline. Although the efficacy has been proven for all these drugs, the effective rate is 70-90% even for inhaled steroid. For improved future treatment, it is important to determine the best treatment combination for each patient through tailor-made medicine. The investigation of genetic factors that determine the individual differences in response to drugs is called pharmacogenetics.

In this paper, the pharmacogenetics of bronchial asthma mainly regarding the β_2 adrenargic receptor agonist, anti-allergy drugs (leukotriene receptor antagonist and Th2 cytokine suppressor) are discussed.

β_2 adrenargic receptor agonist

The β_2 adrenargic receptor agonist is used as a reliever (short-acting inhaled formula) for asthma attacks or as a controller (long-acting inhaled formula, oral formula, plaster formula) for maintenance of a stable state. There are several reports on the association between the bronchodilating effect of the β_2 adrenargic receptor agonist and polymorphisms of the β_2 adrenargic receptor[1)2)]. The β_2 adrenargic receptor is a G-protein coupled receptor that regulates the action of catecholamine and its gene (ADRB2), which does not have an intron, resides in 5q31-32. The association between the acute bronchodilating effect of the β_2 adrenargic receptor agonist and Arg16Gly polymorphism has been investigated and it has been reported that the bronchodilating effect of a single administration of β_2 adrenargic receptor agonist is weaker in patients with Gly16 allele[2)]. Since Arg16Gly polymorphism does not directly affect binding with the β_2 adrenargic receptor agonist or coupling with the G-protein, the cause of difference in drug response is believed to be the down regulation by intrinsic catecholamine or the previously used β_2 adrenargic receptor agonist. However, these studies are retrospective and the haplotypes have not been fully considered. In 2000, associations between haplotypes (determined by multiple polymorphisms) and phenotypes were reported[3)]. Based on 13 polymorphisms in the promoter and coding regions, 12 haplotypes were categorized, and the association between the haplotypes and the mRNA and protein levels of the receptor was demonstrated, furthermore, it was shown that the drug response differed depending of the haplotypes. It was suggested that the combination of multiple polymorphisms (not an individual polymorphism), namely haplotypes might determine the individual disease susceptibility or drug susceptibility.

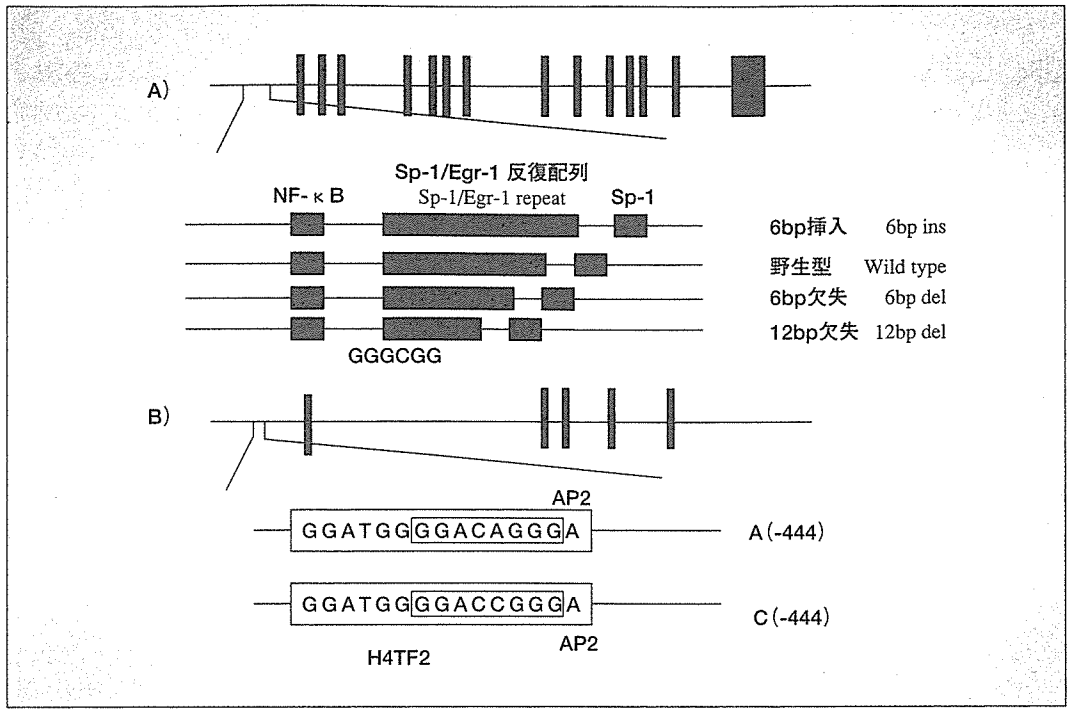

図1 5-リポキシゲナーゼ遺伝子およびロイコトリエンC4合成酸素遺伝子における多型
Fig. 1 Polymorphisms in the 5-lipoxygenase gene and the leukotriene C4 synthase gene
A) 5-リポキシゲナーゼ遺伝子とプロモーター領域のマイクロサテライト型多型：5-リポキシゲナーゼ遺伝子の5′上流領域には転写因子Sp-1結合モチーフであるGGGCGG配列が5回連続で繰り返しており，この領域は同時に転写因子Egr-1の結合モチーフ(GCGGGGGCG)の繰り返し領域にもなっている．繰り返し配列が1回挿入されるタイプ，1ないし2回欠失するタイプが存在する．
B) ロイコトリエンC4合成酵素遺伝子とプロモーター多型：ロイコトリエンC4合成酵素の翻訳開始部位の444塩基上流のアデニンがシトシンに置換する多型が存在する．この多型により新たな転写因子H4TF2の結合モチーフが生じるがこの調節因子がロイコトリエンC4合成酵素遺伝子の転写を正に制御している可能性があると考えられている．
A) 5-lipoxygenase gene and microsatellite polymorphisms in the promoter region: in the 5′ upstream region of the 5-lipoxygenase gene, there are 5 repeating Sp-1 (transcription factor) binding motifs (GGGCGG) that are also Egr-1 (transcription factor) binding motifs (GCGGGGGCG). There is one insertion mutation or one or two deletion mutations of the repetitive sequence.
B) Leukotriene C4 synthase gene and promoter polymorphisms: there is an adenine-cytosine substitution at the 444 base upstream from the translation initiation site of leukotriene C4 synthase. This mutation creates the H4TF2 (transcription factor) binding motif and this regulatory factor may positively regulate the transcription of the leukotriene C4 synthase gene.

があることがわかっている．システィニルロイコトリエン(CysLTs：LTC4, LTD4, LTE4)は喘息気道局所において気管支平滑筋収縮，好酸球遊走，活性化，粘液分泌など喘息の病態に直結する多彩な生物活性を発揮する．その受容体は，CysLT1RとCysLT2Rの2種類が同定されており，CysLTsによる気道収縮作用，血管透過性亢進作用，粘液分泌亢進作用はCysLT1Rを介して発現される．

ロイコトリエン受容体拮抗薬は気管支喘息患者の60～70%に有効であると考えられている．ロイコトリエン関連薬剤の反応性を規定する候補遺伝子として5-リポキシゲナーゼ遺伝子，ロイコトリエンC4合成酵素遺伝子の多型が複数のグループにより報告されている．

1) 5-リポキシゲナーゼ遺伝子多型

5-リポキシゲナーゼ遺伝子のプロモーター領域には転写因子Sp-1結合モチーフ(GGGCGG)が5回繰り返しており，この領域は同時に転写因子Egr-1の結合モチーフ(GCGGGGGCG)の繰り返し領域にもなっている(**図1A**)．この繰り返し回数が異なる

Leukotriene receptor antagonist

The leukotriene receptor antagonist alone has both bronchodilating and anti-inflammatory effects and may be able to improve airway remodeling. In the asthmatic airway, cysteinyl leukotrienes (LTC4, LTD4, and LTE4) have various biological actions that are directly associated with the pathology of asthma such as constriction of bronchial smooth muscle, recruitment /activation of eosinophils, and mucous secretion. Two types of receptors, namely CysLT1R and CysLT2R have been identified and airway constriction, increased blood vessel permeability, and increased mucous secretion are mediated by CysLT1R.

The leukotriene receptor antagonist is thought to be effective in 60 to 70% of bronchial asthma patients. As candidate genes that determine the response to leukotriene related drugs, polymorphisms of 5-lipoxygenase gene and leukotriene C4 synthase gene have been reported by several groups.

1) 5-lipoxygenase gene polymorphisms

In the promoter region of the 5-lipoxygenase gene, there are 5 repeating Sp-1 (transcription factor) binding motifs (GGGCGG) that are also Egr-1 (transcription factor) binding motifs (GCGGGGGCG) (Fig. 1A). There are microsatellite polymorphisms with different numbers of repetitions. Deletion of the GGGCGG sequence results in decreased promoter activity due to decreased Egr-1 binding, and insertion of the sequence results in increased promoter activity[4)-6)]. In 1998, Drazen et al. reported that among mild asthma patients who received 5-lipoxygenase inhibitor (currently not used in Japan), 52% of the 104 patients with at least one wild type allele responded to the drug and none of the 10 patients with no wild type alleles fulfilled the criteria of "responder"[7)].

2) Leukotriene C4 synthase gene polymorphisms

The leukotriene C4 synthase gene resides in the long-arm of chromosome 5 and has an adenine-cytosine substitution polymorphism at 444 base upstream from the translation initiation site (A-444C). This substitution creates the binding motif for a transcription factor, H4TF2. Therefore, this mutation may positively regulate the transcription of leukotriene C4 synthase gene (Fig. 1B). The frequency of this gene polymorphism allele is relatively high through the races (27% in Caucasian and 20% in Japanese). It has been reported that the leukotriene receptor antagonist improves respiratory functions better in patients with the mutated allele compared to wild type[8)-10)].

We administered a leukotriene receptor antagonist for 4 weeks in 10 bronchial asthma patients (age between 6 and 15) and investigated the symptom score and urine LTE4. The symptom score showed a significant decrease after 2 weeks of administration. However, 3 out of 10 patients (30%) showed no improvement, which corresponded closely with previous studies. In the comparison between the group of patients that showed improvements (responders) and the group of patients that did not show improvements (non-responders), the urine LTE4 level was higher in responders compared to non-responders. Furthermore, the urine LTE4 level decreased after 4 weeks of administration of the oral leukotriene receptor antagonist (Fig. 2). It was suggested that the leukotriene receptor antagonist worked better in patients with increased CysLT production. Out of these 10 patients, 3 had leukotriene C4 synthase gene A-444C allele and they all were responders.

Th2 cytokine suppressor

It has been reported in the type-I allergic reaction experimental model that Th2 cytokine suppressor suppresses IgE antibody production, histamine release from mast cells, and degranulation from the mesentery.

We administered a Th2 cytokine inhibitor in childhood bronchial asthma patients for 8 weeks and investigated the cytokine production from peripheral blood mononuclear cells before and after the administration. Although the interleukin (IL)-4 level did not show a significant change after 8 weeks of Th2 cytokine inhibitor administration, IFN-γ and IL-12 showed increases after administration in the responder group (Fig. 3). The Pearson correlation coefficient was investigated for parameters, including the serum IgE, number of eosinophils, and IL-5, and only IFN-γ showed a significant difference. In the investigation of gene polymorphisms and drug responses, the above-mentioned leukotriene C4 synthase gene A-444C polymorphism and IL-13 gene R110Q polymorphism showed significant differences between responders and non-responders. Patients with wild-type alleles in leukotriene C4 synthase gene A-444C polymorphism and/or wild-type alleles in IL-13 gene R110Q polymorphism tended to be responders. This may be related to the reports that suggest that CysLT1R expression is modified by IFN-γ and IL-13[11)12)]. Since *in vitro* experiments showed that IFN-γ and IL-13 dose-dependently increased CysLT1R expression in airway smooth muscle cells, it is speculated that IFN-γ

マイクロサテライト多型が存在し，GGGCGG 配列の欠失変異では Egr-1の結合低下によりプロモーター活性が減少し，挿入変異では増加する[4)-6)]．1998年 Drazen らは，5-リポキシゲナーゼ阻害薬(2006年9月現在，日本では使用されていない)を投与された軽症喘息患者のうち，野生型遺伝子を少なくとも1つもつ104例では52%が responder であったのに対し，変異遺伝子しかもたない10例では，responder の基準にあてはまる症例がみられなかったと報告した[7)]．

2) ロイコトリエン C4合成酵素遺伝子多型

ロイコトリエン C4合成酵素遺伝子は5番染色体長腕に位置し，ロイコトリエン C4合成酵素の翻訳開始部位の444塩基上流のアデニンがシトシンに置換する多型が存在する(A-444C)．この多型により新たな転写因子 H4TF2の結合モチーフが生じる．そこで，この多型によりロイコトリエン C4合成酵素遺伝子の転写が正に制御される可能性があると考えられている(図1B)．この遺伝子多型のアリル頻度は，白色人種で27%，日本人で20%と人種に関係なく比較的高頻度に認められる．変異型は野生型に比してロイコトリエン受容体拮抗薬による呼吸機能の改善がよいとの報告がある[8)-10)]．

筆者らは，6歳から15歳の気管支喘息患者10名を対象にロイコトリエン受容体拮抗薬を4週間投与し，症状点数の変化および尿中 LTE4排泄量の変化について検討した．症状点数は投与2週間以降有意な低下を認めた．しかし，10症例の検討で3例(30%)は症状の改善が認められず従来の報告と一致していた．症状の改善を認めた群を responder 群とし，認めなかった群を non-responder 群とすると，responder 群では non-responder 群に比べて，投与前の尿中 LTE4排泄量が多かった．さらに，ロイコトリエン受容体拮抗薬内服4週後の LTE4排泄量は投与前に比べて減少していた(図2)．CysLT 産生を亢進する方向に酵素遺伝子が働く症例ほどロイコトリエン受容体拮抗薬が有効となる可能性が示唆される．遺伝子多型との関連では，対象となった10名中ロイコトリエン C4合成酵素遺伝子 A-444C 多型が3名に認められ，3名とも responder 群であった．

Th2サイトカイン抑制薬

Th2サイトカイン抑制薬はI型アレルギー反応の実験モデルにおいて IgE 抗体産生を抑制するとともに，肥満細胞からのヒスタミン遊離抑制および腸間膜からの脱顆粒を抑制することが報告されている．

小児気管支喘息患者を対象に Th2サイトカイン抑制薬を8週間投与し，投与前後の末梢血リンパ球からのサイトカイン産生量を検討した．Th2サイトカイン抑制薬8週間投与前後でインターロイキン(interleukin：IL)-4産生量は有意な変化はみられなかったが，IFN-γ，IL-12は responder 群で，産生量が投与により増加する傾向がみられた(図3)．その他，血清 IgE，好酸球数，IL-5などのパラメーターを用いて Pearson の相関係数をみたが，薬剤反応性との間に有意差を認めたものは IFN-γの変化量のみであった．遺伝子多型と薬剤反応性についての検討では，前出のロイコトリエン C4合成酵素遺伝子 A-444C 多型と IL-13遺伝子 R110Q 多型が responder 群，non-responder 群で有意差を認めた．ロイコトリエン C4合成酵素遺伝子 A-444C 多型および IL-13遺伝子 R110Q 多型の野生型は変異型に比して Th2サイトカイン抑制薬の responder が多いという結果であった．このことは，CysLT1R の発現が IFN-γ，IL-13によって変化するとの報告と関連している可能性がある[11)12)]．つまり，*in vitro* の実験で気道平滑筋細胞に IFN-γ，IL-13を添加すると時間，濃度依存性に CysLT1R の発現が増強するため，responder 群では，Th2サイトカイン抑制薬投与前の IFN-γ産生量が低く，CysLT1R の発現が低下しており，Th2サイトカイン抑制薬が効果的である可能性が推測される．

遺伝子診断キットとオーダーメイド治療

現在までに，気管支喘息を中心とするアレルギーの病因候補遺伝子として高親和性 IgE レセプター (FcεRI)β鎖遺伝子，IL-4レセプター (IL-4R)α鎖遺伝子，IL-4遺伝子，IL-13遺伝子などの遺伝子が候補にあげられてきた．筆者らはアレルギー反応系のうち，特に抑制系の中心である IFN-γ，IL-12に焦点をあて，シグナル伝達系の遺伝子について検索してきた．

このような成績を含めて，世界の報告をもとに，筆者らはアレルギーを遺伝子学的に新たに分類することを試みた．アレルゲンが侵入して各種のアレルギー症状が出現するまでの経路における遺伝子異常の報告を整理すると図4のようになる．分類は(1)アレルギー反応のうち抗原特異性を決定する部位として HLA クラスⅡ-ペプチド-T 細胞レセプターにおける抗原認識部位(2) IgE 産生亢進の機序における B 細胞内外に関する部位(3)メディ