Table 1. Clinical features of the patients divided into non-atopic and atopic group

	Sex	Age	FH or PH	Fever (day)	Wheeze	IgE (IU/ml)	RAST	Hospitalization period* (day)
Non-atopic 1	M	1 yr	–	1	–	111.8	HD 0, DF 0	9
Non-atopic 2	M	1 yr	–	4	–	15.7	HD 0, DF 0	13
Non-atopic 3	F	3 month	–	6	+	118.7	HD 0, DF 0	11
Non-atopic 4	F	1 month	–	1	+	0.5	HD 0, DF 0	10
Non-atopic 5	M	3 month	–	2	+	2.1	HD 0, DF 0	6
Non-atopic 6	M	1 yr	–	0	+	19.8	HD 0, DF 0	7
Atopic 1	F	10 month	BA	0	+	57.5	HD 0, DF 0	17
Atopic 2	F	4 yr	BA	1	+	42	HD 0, DF 0	13
Atopic 3	M	1 yr	BA	1	+	200	HD 5, DF 4	6
Atopic 4	M	4 yr	BA	5	+	819.3	HD 3, DF 3	19
Atopic 5	F	4 yr	BA/AD	1	+	6488.4	HD 6, DF 6	9
Atopic 6	M	2 yr	BA	2	+	4.2	HD 0, DF 0	17
Atopic 7	F	1 month	FH	1	+	2.2	HD 0, DF 0	15
Atopic 8	F	1 month	FH	1	+	0.5	HD 0, DF 0	13

Sex: m, male; f, female; FH, family history within one generation of allergic diseases; PH, past history; BA, bronchial asthma; AD, atopic dermatitis; fever, period of fever over 38°C; RAST, CAP RAST scores; HD, house dust; DF, *Dermatophagoides farinae*.
*p-value = 0.079.

patients (n = 14), the IFN-gamma concentration from PHA-stimulated PBMCs between the acute and convalescent phase is shown in Fig. 1a. The concentrations of IFN-gamma in all RSV-infected patients (n = 14) did not show remarkable change between the acute and convalescent phase (Table 2). RSV infected patients were divided into two groups, a non-atopic group (n = 6) and an atopic group (n = 8). In the non-atopic and atopic group, the IFN-gamma concentration from PHA-stimulated PBMCs is shown in Fig. 1b. There was no difference in IFN-gamma production by PHA-stimulated PBMCs in either group. In both groups, the IFN-gamma concentration from IL-12-stimulated PBMCs is shown in Fig. 1c. In the atopic group, the IFN-gamma concentration from IL-12-stimulated PBMCs was significantly lower in the acute phase than that in the convalescent phase ($p = 0.024$). Similarly, in the atopic group the IFN-gamma concentration from IL-18-stimulated PBMCs was significantly lower in the acute phase than that in the convalescent phase ($p = 0.0018$) (Fig. 1d). The change of IFN-gamma production by IL-18-stimulated PBMCs was more significant ($p = 0.0018$) than that by IL-12-stimulated PBMCs ($p = 0.024$). In addition, in the acute phase the IFN-gamma concentrations by IL-12- or IL-18-stimulated PBMCs in the atopic group were significantly lower than those in the non-atopic group ($p = 0.013$ or $p = 0.009$, respectively), while in the convalescent phase the IFN-gamma concentrations by IL-18-stimulated PBMCs in the atopic group were significantly higher than those in the non-atopic group ($p = 0.028$).

The concentrations of IL-4 in all RSV-infected patients (n = 14) between the acute and convalescent phase did not show any remarkable change (Fig. 2). Furthermore, no difference of IL-4 production between the non-atopic and atopic group was detected.

Discussion

In the present study, we have shown that in the atopic group in the acute phase the IFN-gamma concentrations by IL-12- or IL-18-stimulated PBMCs were significantly lower than those in the non-atopic group. Furthermore, the IFN-gamma concentrations by IL-18-stimulated PBMCs in the atopic group were significantly higher than those in the non-atopic group in the convalescent phase. However, no difference of IL-4 production between the non-atopic and atopic group was detected.

We tried to determine whether lymphocytes in children with atopy or a family history of allergic diseases show a different immune response to RSV compared with non-atopic children. Our results suggest that the atopic disease or some genetic background of atopy might be one of the factors which prolongs RSV infection because of a reduced Th1 reaction to RSV, although we could not find any significant differences in the clinical course between the non-atopic group and atopic group.

Our results showed that reduced IFN-gamma expression was observed specifically in the atopic group in the acute phase. As low IFN-gamma expression in RSV is observed specifically in the

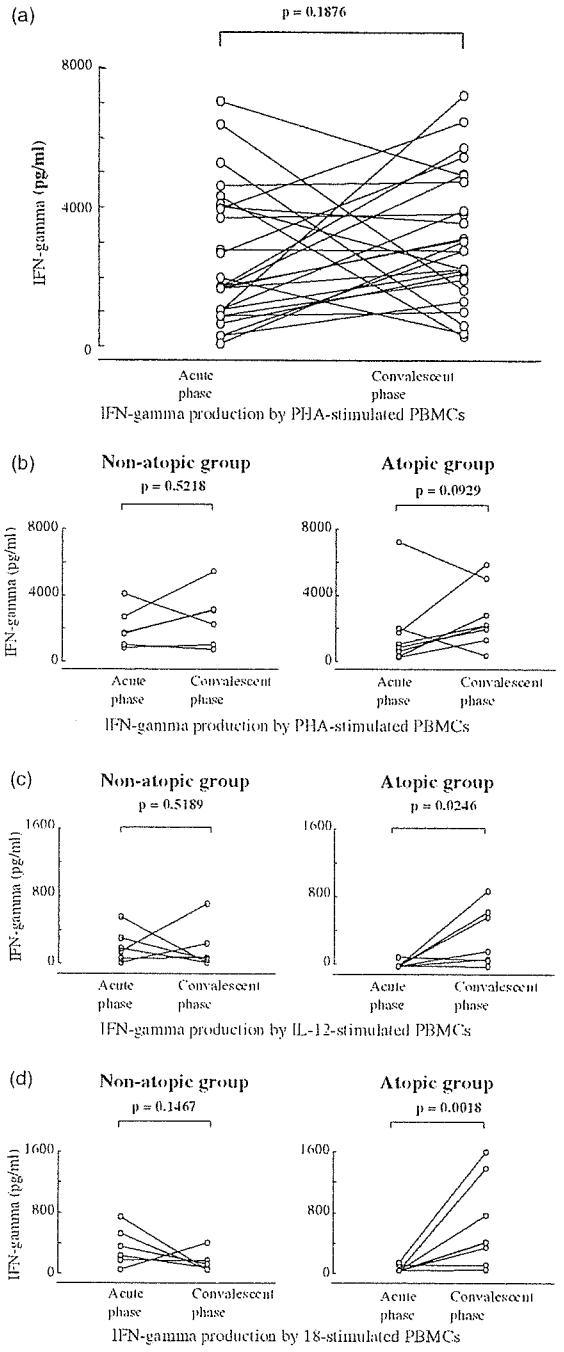

Fig. 1. (a) In all patients (n = 14), interferon (IFN)-gamma concentration from phytohemaglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) between the acute and convalescent phase. (b) In the non-atopic group (n = 6) and atopic group (n = 8), IFN-gamma concentration from PHA-stimulated PBMCs between the acute and convalescent phase. (c) In the non-atopic and atopic group, IFN-gamma concentration from IL-12-stimulated PBMCs between the acute and convalescent phase. (d) In the non-atopic and atopic group, IFN-gamma concentration from IL-18-stimulated PBMCs between the acute and convalescent phase.

atopic group, it may not be due to an inhibitory effect of RSV, which is capable of suppressing both non-specific and RSV-specific lymphocyte proliferation (12). In addition, there was no significant difference about ages between non-atopic and atopic group (Table 1). Reduced IFN-gamma production was observed only in atopic group. Therefore we assumed that a weak IFN-gamma response to RSV may not result from the immaturity of an infant's immune system.

One factor controlling the level of cytokine expression is genotype. Gentile et al. reported that the IFN-gamma genotype is related to the severity of a lower respiratory illness, the duration of intensive care unit stay and the frequency of otitis media (13). In Japanese children the association of IFN-gamma polymorphism with atopic asthma has been reported. It would be intriguing to investigate IFN-gamma polymorphism in our atopic group.

Several lines of evidence have shown the features of cytokine production in RSV infection. Pala et al. reported that enhanced IL-4 responses in children with a history of RSV bronchiolitis in infancy (14). Association of cytokine responses with disease severity in infants with RSV infection was reported (15, 16). van Benten et al., showed that RSV-induced bronchiolitis but not upper respiratory tract infection was accompanied by an increased nasal IL-18 response (17). Joshi et al. showed that RSV is associated with lower IFN-gamma production in young babies compared with upper respiratory tract infections (18). These results suggest that RSV infection changes the cytokine production and the RSV-induced cytokine production might be partly affected by the time and tissues of taking the samples.

The change of IFN-gamma production by IL-18-stimulated PBMCs was more significant ($p = 0.0018$) than that by IL-12-stimulated PBMCs ($p = 0.024$). This result suggests that in RSV infection the IL-18 receptor signal cascade might be specifically inhibited. Our previous reports showed that some atopic diseases are caused by impairment of IL-18Rα chain 950 del CAG, which downregulates IgE production (19). We have not yet determined what mechanism induces the predominant expression of the IL-18Rα chain cDNA in some atopic patients. Viral infection, such as RSV, and environmental factors might be candidate modulators of IL-18Rα chain transcript expression (20).

In conclusion, the suppression of IFN-gamma in the acute phase of RSV infection was observed only in the atopic group. These results suggest

Table 2. Interferon (IFN)-gamma and interleukin (IL)-4 production induced by phytohemaglutinin (PHA), IL-12 or IL-18 stimulation

	PHA acute	PHA convalescent	IL-12 acute	Il-12 convalescent	IL-18 acute	IL-18 convalescent	IL-4 acute	Il-4 convalescent
Non-atopic 1	2669.9	5445.4	177.1	15.6	198.1	37.1	70.73	48.07
Non-atopic 2	860.7	1027.1	15.6	244.6	15.6	362.3	39.1	46.3
Non-atopic 3	1001.2	3063.4	144.8	707.2	132	135.8	9.93	41.61
Non-atopic 4	1726.7	3115	547.9	15.6	308	80.4	36.68	10.63
Non-atopic 5	1685.3	2260	301.6	51.3	701.9	15.6	18.68	10.12
Non-atopic 6	4097	722.3	62.8	74.6	476.4	15.6	34.48	41.91
Mean ± SD	2006 ± 1209	2605 ± 1714	208 ± 193	184 ± 269	305 ± 249	107 ± 132	34 ± 20	33 ± 17
Atopic 1	1724.5	5697.3	15.6	523.9	15.6	34.7	34.57	24.38
Atopic 2	653.6	2122.5	15.6	92.7	15.6	372.6	18.83	87.38
Atopic 3	294.1	1315.7	15.6	15.6	15.6	307.2	17.1	60.81
Atopic 4	1990.4	410.2	116.2	81.3	91	92.7	43.27	105.67
Atopic 5	1063.6	2193.8	15.6	15.6	15.6	698.3	78.34	105.7
Atopic 6	317.9	2778.9	15.6	172.1	15.6	364.9	67.97	38.45
Atopic 7	7053.8	4916.7	23	794.7	119	1484.3	32.29	41.54
Atopic 8	869.9	1945.7	15.6	579.2	15.6	1286.2	33.23	10.59
Mean ± SD	1745 ± 2229	2672 ± 1781	29 ± 35	284 ± 302	37 ± 42	580 ± 538	40 ± 21	59 ± 36

Acute, acute phase; convalescent, convalescent phase.
PHA, IL-12, IL-18 = PHA, IL-12 or IL-18-induced IFN-gamma production (pg/ml); IL-4 = PHA-induced IL-4 production (pg/ml).

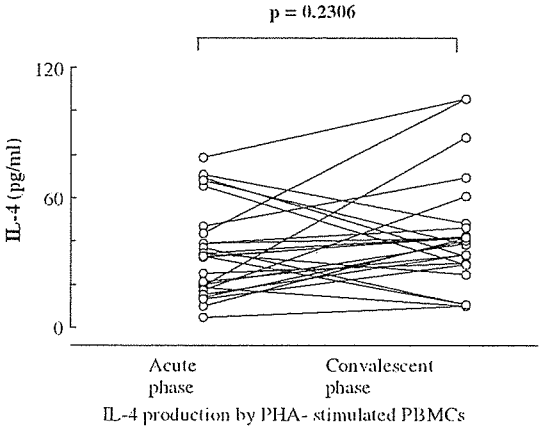

Fig. 2. In all patients (n = 14), IL-4 concentration from phytohemaglutinin-stimulated peripheral blood mononuclear cells between the acute and convalescent phase.

that the significant difference between the non-atopic and atopic group in regard to the immune response to RSV might be caused in part by genetic factors.

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Inosiplex Affects the Spectra of Proton Magnetic Resonance Spectroscopy in Subacute Sclerosing Panencephalitis

ABSTRACT

In vivo magnetic resonance techniques such as magnetic resonance imaging (MRI) and magnetic resonance spectroscopy have been some of the most useful tools for evaluation of neurologic diseases. In subacute sclerosing panencephalitis, magnetic resonance spectroscopy can be an additional tool for evaluation of disease progression or the efficacy of the treatment, such as interferon or inosiplex, compared with MRI. Inosiplex is one of the effective drugs for subacute sclerosing panencephalitis, but our in vivo and in vitro magnetic resonance spectroscopic study indicated that inosiplex affects the spectra, suggesting a possible failure of neurologic evaluation in a patient with subacute sclerosing panencephalitis treated with inosiplex. (*J Child Neurol* 2006;21:177–178; DOI 10.2310/7010.2006.00048).

We previously reported a cerebral magnetic resonance spectroscopic study of a 13-year-old girl with subacute sclerosing panencephalitis.[1] The spectrum clearly demonstrated the early subtle changes in subacute sclerosing panencephalitis, indicating the usefulness of magnetic resonance spectroscopy in evaluation of disease progression or therapeutic efficacy; however, there was an unidentified peak at 2.9 ppm. A further study using a drug solution and the patient's urine indicated that the resonance should be from the methyl of 2-hydroxypropyldimethylammonium. Moreover, the results also cautioned us about the probable contamination of inosiplex resonances on those of the brain itself.

Magnetic resonance spectroscopic examinations were performed using a conventional whole-body system (Signa Horizon 1.5 Tesla, General Electric). Localization of a 20 × 20 × 20 mm region of interest in her right frontal lobe was achieved using short echo time (30 milliseconds) point-resolved spectroscopic sequences. All of the spectra taken from the brain

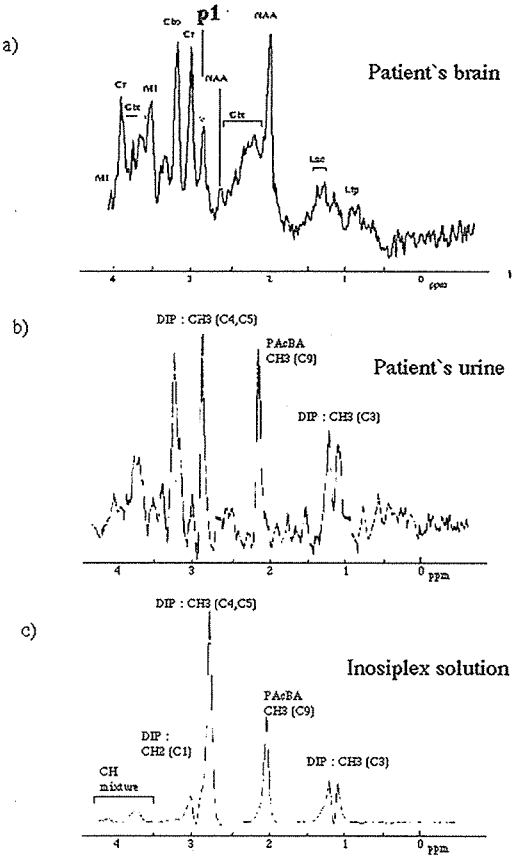

Figure 1. Magnetic resonance spectroscopy. *A,* The spectrum obtained from the region of interest of the left frontal lobe. *B,* The spectrum obtained from the region of interest of the patient's urine. *C,* The spectrum obtained from the region of interest of the inosiplex solution. Cr = creatine and phosphocreatine; Cho = choline; DIP = 2-hydroxypropyldimethylammonium; Glx = glutamate and glutamine; Lac = lactate; Lip = lipids; NAA = *N*-acetylaspartate; MI = *myo*-inositol; p1 = additional peak; PAcBA = *p*-acetamidobenzoate.

showed a decrease in *N*-acetylaspartate resonance, an increase in *myo*-inositol and choline resonances, and the presence of a lactate signal (Figure 1A).[1] An additional peak was observed at the resonances corresponding to γ-aminobutyric acid (GABA; 2.9–3.0 ppm, resonance p1 in Figure 1A).

Resonance of CH2 (C4) in GABA has a triplet splitting pattern at 2.9 ppm, and resonance detection of GABA on in vivo magnetic resonance spectroscopy usually requires specifically edited pulse sequences.[2] The resonance shape and the intensity observed in our patient suggested a contribution of the resonances from the drugs, especially inosiplex (4.8 g/day, oral administration). Inosiplex is a 1:3 complex of inosine and *p*-acetamidobenzoate. Reported resonance assignments of the chemicals are shown in Table 1. The chemical shift value of the observed unknown resonance from the brain is very similar to that of CH3 (C4, C5) in 2-hydroxypropyldimethylammonium, which has a high singlet peak resonance at 2.9 ppm originating from 18 protons per molecule.

Next, spectra were obtained from the patient's urine in a 50 mL tube and inosiplex solution in a 50 mL tube using the same machine and conditions. The spectra from urine clearly showed the presence of a resonance complex similar to that of the inosiplex solution, confirming the intensive excretion of the unchanged inosiplex compound and its metabolites (Figure 1B and C).[3,4] 2-Hydroxypropyldimethylammonium has no detectable binding to the serum proteins and distributes into the brain at a high concentration.[3,4] According to the experimental data in animals, [4] we can assume that 2-hydroxypropyldimethylammonium should be present at around several hundred micromolar concentrations in the brain of a patient

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.

Zenichiro Kato, MD, PhD,
Department of Pediatrics, Graduate School of Medicine
Center for Emerging Infectious Diseases (CEID)
Center for Advanced Drug Research (CADR)
Gifu University
Takahiko Asano, MD, PhD,
Department of Radiology
Graduate School of Medicine
Gifu University
Naomi Kondo, MD, PhD,
Department of Pediatrics, Graduate School of Medicine
Center for Emerging Infectious Diseases (CEID)
Center for Advanced Drug Research (CADR)
Gifu University
Gifu, Japan

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Address correspondence to Zenichiro Kato, Department of Pediatrics, Graduate School of Medicine, Gifu University, Yanagido 1-1, Gifu 501-1194, Japan. Tel: +81 (58) 230 6386; fax: +81 (58) 230 6387; e-mail: zen-k@cc.gifu-u.ac.jp.

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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

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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

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From the Department of Pediatrics, Graduate School of Medicine, Gifu University, 1-1 Yanagido, Gifu 501-1194, Japan.
Reprints: Hideo Kaneko, Department of Pediatrics, Graduate School of Medicine, Gifu University, 1-1 Yanagido, Gifu 501-1194, Japan (e-mail: hideo@cc.gifu-u.ac.jp).

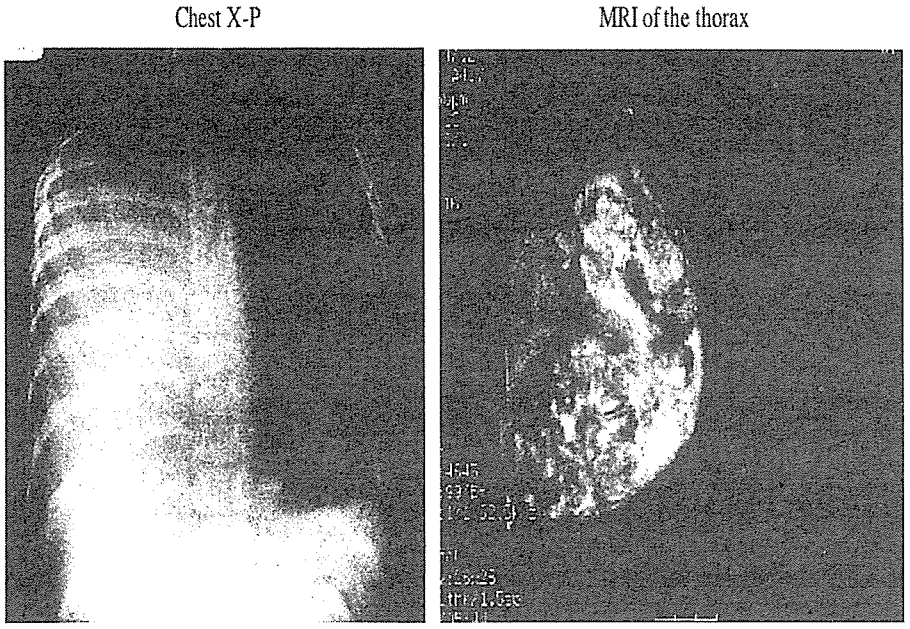

FIGURE 1. Chest x-ray and MRI of the thorax showing a tumor mass in the right hemithorax.

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

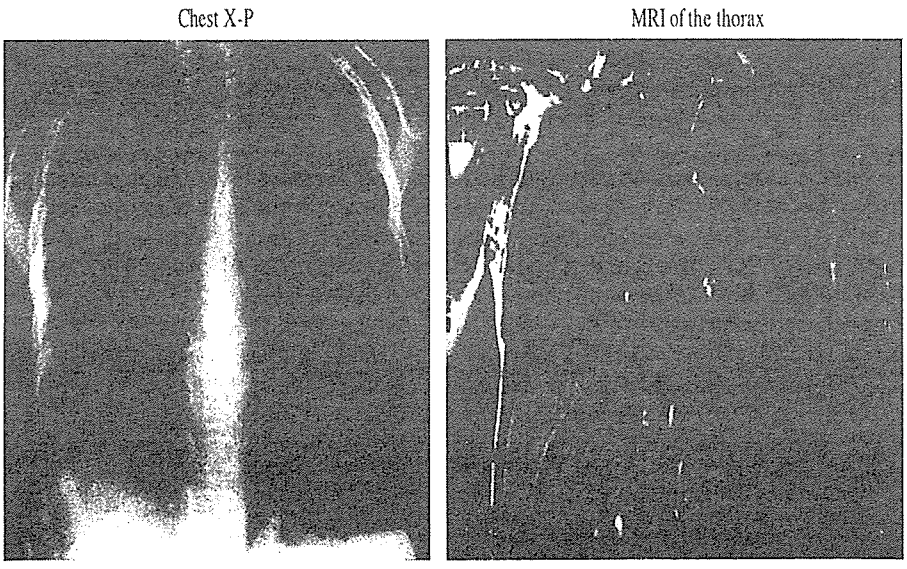

FIGURE 2. Chest x-ray and MRI of the thorax showing a coin lesion in the right hemithorax at the time of relapse.

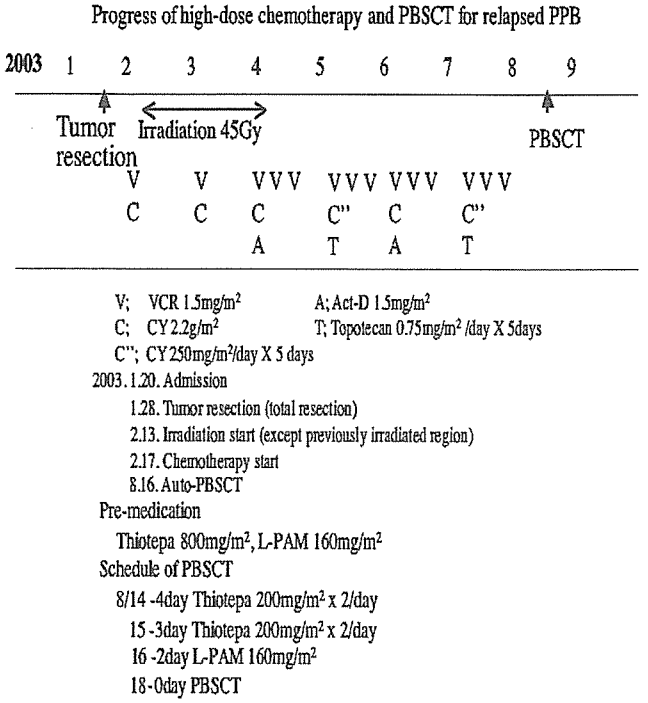

FIGURE 3. Progress of high-dose chemotherapy and PBSCT for relapsed PPB.

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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ORIGINAL ARTICLE

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

Takahide Teramoto[1], Toshiyuki Fukao[1], Yoshiko Tomita[2], Yoshiaki Terauchi[2], Kaori Hosoi[1], Eiko Matsui[1], Minako Aoki[1], Naomi Kondo[1] and Haruki Mikawa[3]

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

[1]Department of Pediatrics, Graduate School of Medicine, Gifu University, Gifu, [2]Pharmacokinetics Research Laboratories, Dainippon Sumitomo Pharmaceutical Co., Ltd. and [3]Kansaidenryoku Hospital, Osaka, Japan.
Correspondence: Takahide Teramoto, Department of Pediatrics, Graduate School of Medicine, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan.
Email: t-tera@cc.gifu-u.ac.jp
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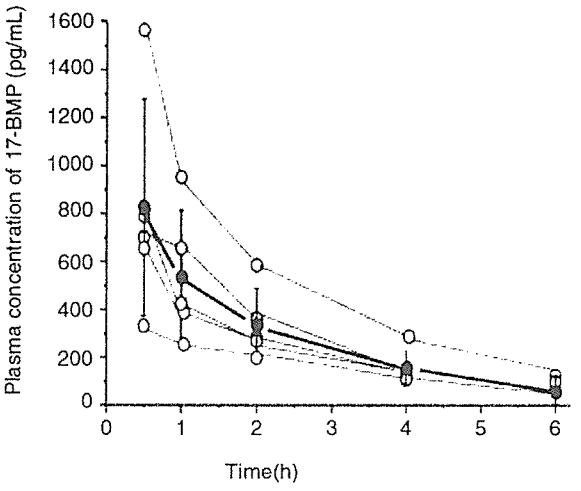

Fig. 1 Individual (○) and mean (●) plasma concentration-time profiles of beclomethasone 17-monopropionate (17-BMP) in Japanese children with bronchial asthma following administration of 200 μg of beclomethasone dipropionate (BDP). Each error bar represents the standard deviation.

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

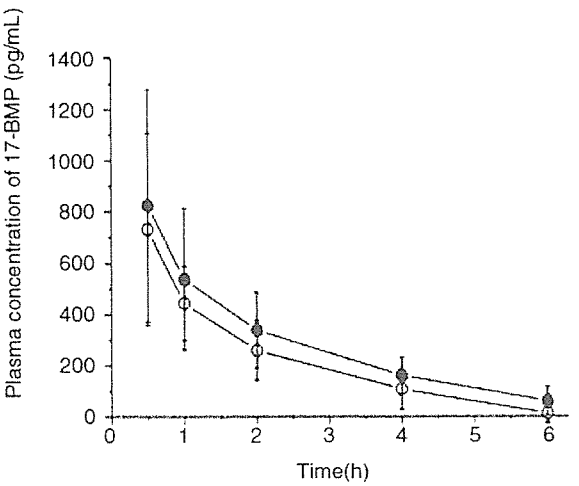

Fig. 2 Japanese (●) and American (○) plasma concentration-time profiles of beclomethasone 17-monopropionate (17-BMP) following administration of 200 μg beclomethasone dipropionate (BDP). Each point represents the mean ± standard deviation of 5 Japanese or 18 American children with bronchial asthma.

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 timepoint and the apparent elimination half-life.

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

Naomi Kondo[1], Toshio Katsunuma[2], Yasuhei Odajima[3] and Akihiro Morikawa[4]

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

[1]Department of Pediatrics, Graduate School of Medicine, Gifu University, Gifu, [2]Department of Pediatrics, The Jikei University School of Medicine, Tokyo, [3]Department of Pediatrics, Saitama Medical School, Saitama and [4]Department of Pediatrics and Developmental Medicine, Gunma University Graduate School, Gunma, Japan.
Correspondence: Dr Toshio Katsunuma, Department of Pediatrics, The Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo 105-8461, Japan.
Email: tkatsunuma@jikei.ac.jp
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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 $CysLT_1$ receptor antagonists, long-acting inhaled β_2-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.

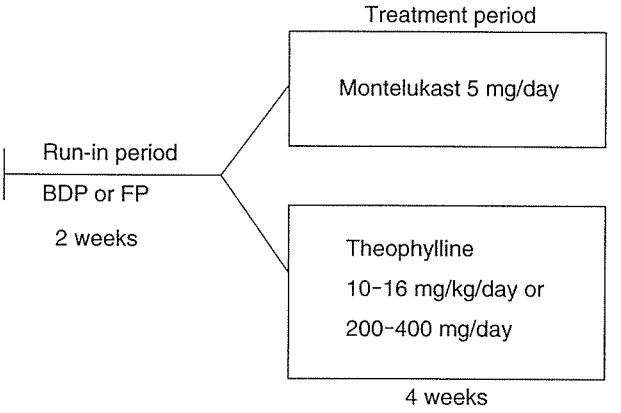

Fig. 1 Study design. BDP = beclomethasone dipropionate; FP = fluticasone propionate.

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 β_2-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

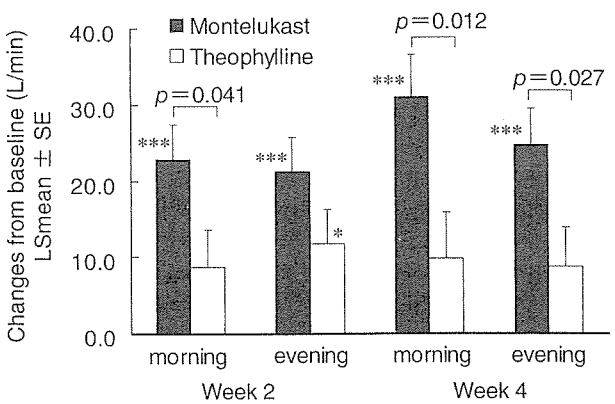

Fig. 2 Comparison of the changes from baseline in morning and evening PEF between montelukast and theophylline. *** $p < 0.001$ and * $p < 0.05$ compared with baseline.

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

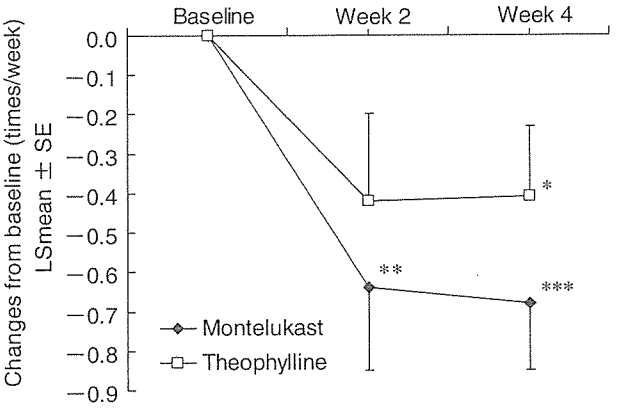

Fig. 3 Comparison of the changes from baseline in mild asthma attacks between montelukast and theophylline. *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ compared with baseline.

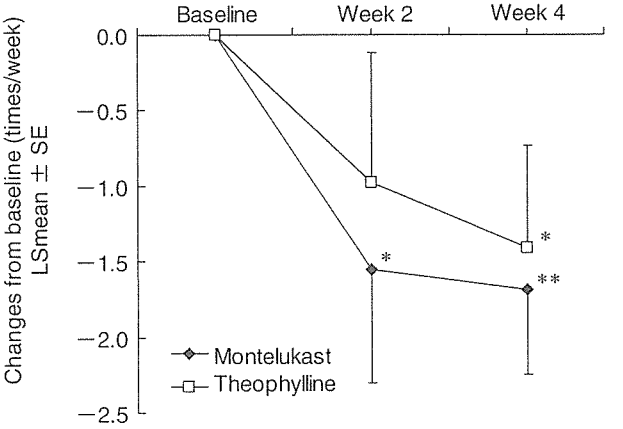

Fig. 4 Comparison of the changes from baseline in inhaled β_2-agonist use between montelukast and theophylline. ** $p < 0.01$ and * $p < 0.05$ compared with baseline.

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.

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