

# Hydrofluoroalkane-Beclomethasone Dipropionate Effectively Improves Airway Eosinophilic Inflammation Including the Distal Airways of Patients with Mild to Moderate Persistent Asthma as Compared with Fluticasone Propionate in a Randomized Open Double-Cross Study

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## ABSTRACT

**Background:** To evaluate whether hydrofluoroalkane-beclomethasone dipropionate (HFA-BDP) controls eosinophilic inflammation, including that in the distal airways, more effectively than fluticasone propionate (FP) Diskus®.

**Methods:** Fifty patients with well-controlled mild to moderate persistent asthma using FP for more than 6 months were randomly assigned to FP and HFA-BDP groups, and the treatment regimens of the two groups were switched twice between FP and HFA-BDP in a double cross-over manner at 3-month intervals after 2-week washout periods. Evidence of eosinophilic inflammation in blood and induced sputum samples was assessed, together with pulmonary function testing and an Asthma-related Quality of Life Questionnaire (AQLQ) survey after each treatment period.

**Results:** The peripheral blood differential eosinophil count and sputum levels of eosinophil cationic protein (ECP) showed reciprocal changes during the study periods in both groups. The blood differential eosinophil count was significantly lower during the HFA-BDP than during the FP treatment period in both the FP ( $p = 0.004$ ) and the HFA-BDP ( $p = 0.020$ ) group. The late-phase induced sputum ECP level was significantly decreased during the HFA-BDP treatment period in both the FP ( $p = 0.016$ ) and the HFA-BDP group ( $p = 0.023$ ). The significant elevation of surfactant protein D values in the late-phase sputum observed in both groups indicated that late-phase sputum was obtained mainly from proximal peripheral airways. Both symptom and activity limitation domains of the AQLQ in the HFA-BDP group significantly increased after switching from FP to HFA-BDP. There were no significant changes in pulmonary function indices in either group at any time during the study.

**Conclusions:** HFA-BDP improved residual eosinophilic inflammation in asthmatic airways, including distal airways, more effectively than FP.

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**KEY WORDS**

asthma, Asthma-related Quality of Life Questionnaire (AQLQ), eosinophil cationic protein (ECP), fluticasone propionate (FP) Diskus<sup>®</sup>, hydrofluoroalkane-beclomethasone dipropionate (HFA-BDP)

**INTRODUCTION**

Eosinophilic inflammation in bronchial asthma is now known to frequently involve even the small airways and alveoli, as well as more distal bronchioles.<sup>1,2</sup> It is, therefore, important in asthma therapy to choose drugs with adequate delivery to the distal airways, in order to optimize the control of asthma and prevent progression of airway remodeling. However, the following three critical clinical questions must first be addressed. First, can we obtain effective control of asthmatic inflammation in both central and peripheral airways (<2 mm diameter) using inhaled corticosteroids, the gold standard for asthma therapy? Second, using the same pharmacologically effective doses of various types of inhaled corticosteroid (ICS), can we expect an equal degree of anti-inflammatory effects in the peripheral airways? Third, can ordinary clinical examinations such as pulmonary function tests directly describe the status of inflammation in the peripheral airways and be utilized for evaluating the therapeutic efficacies of drugs against peripheral inflammation?

To answer the above questions, we previously performed two clinical studies. In the first, we confirmed that 85 patients with stable mild to moderate persistent asthma who had used the dry powder-type inhaled steroid (DPI) for at least 6 months consistently maintained good asthma control, based on routine clinical examinations, such as pulmonary function tests.<sup>3</sup> Nevertheless, increased numbers of eosinophils were detected in 30–40% of late-phase induced sputum samples from these patients, and this was accompanied by significantly increased sputum eosinophil cationic protein (ECP) levels.<sup>3</sup> Most of the residual eosinophilic inflammation was ameliorated by switching treatment from the DPI to hydrofluoroalkane-134a beclomethasone dipropionate (HFA-BDP) extra-fine aerosol therapy for 4 weeks.<sup>3</sup> In our other study, during a one-year observation period after changing from DPI to HFA-BDP, we found virtually no increases in either eosinophil counts or the ECP levels in late-phase induced sputum.<sup>4</sup> These results raised three possibilities for approaching the residual inflammation in the distal airways. First, even in patients on regular long-term treatment with DPIs in whom stable daily control has apparently been maintained, the possibility of residual eosinophilic inflammation existing in some of the distal airways must be suspected. Second, routine examinations, such as pulmonary function tests, are of limited value for directly evaluating changes in the distal airways, and

other methods must be developed. Third, optimal delivery to peripheral airways is obtained with HFA-BDP, the aerosolized ultra-fine particles of which are 1.1  $\mu\text{m}$  in diameter on average.<sup>5,6</sup> This ICS may be a good candidate for treating residual eosinophilic inflammation in the distal airways. Accordingly, we decided to revisit the above questions using a stringent study design. The aim of this study was to determine whether HFA-BDP is more effective for controlling eosinophilic inflammation in asthmatic airways, including that in distal airways, than the FP Diskus<sup>®</sup>.

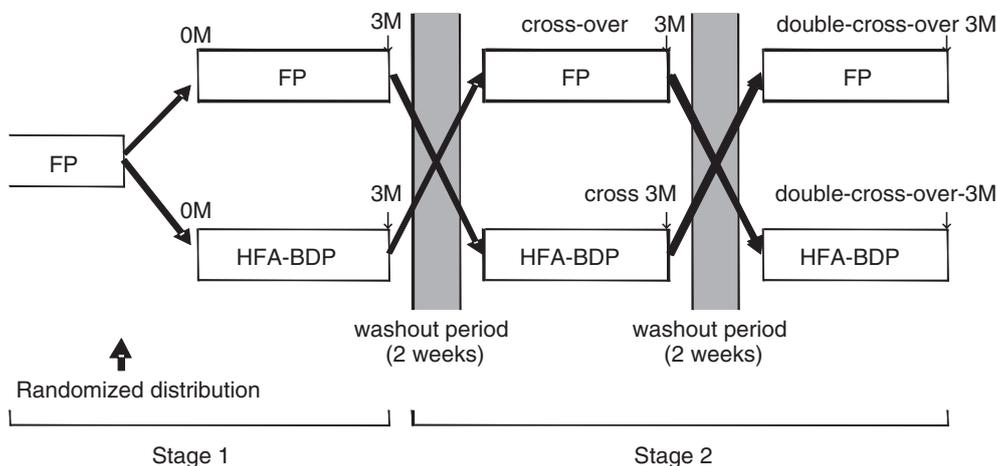
**METHODS****SUBJECTS**

The study was approved by the ethics committees of Showa University (IRB approval No. 326) and Tohno-Kousei hospital (IRB approval No. 8), and all patients gave written informed consent. The study subjects were 50 patients with mild to moderate persistent asthma (classified according to the Global Initiative on Asthma (GINA) guidelines)<sup>7</sup> stably controlled with the FP Diskus<sup>®</sup>, with good compliance and adherence to treatment for more than 6 months. We excluded patients from the study who met the following exclusion criteria: 1) daily oral steroid use, 2) underlying chronic obstructive pulmonary disease (COPD) or other chronic respiratory diseases, 3) severe hepatic or renal disease, heart failure, hematologic diseases or other grave complications, 4) taking medication for chronic respiratory infectious diseases, and 5) history of poor drug compliance. Before enrollment in the study, the clinical significance and purpose of the study, and the possible disadvantages of participation associated with switching inhaled corticosteroids twice in a double cross-over manner, were explained in detail to each patient. After obtaining written informed consent from each participant, they were randomly divided into two groups: the “FP Diskus<sup>®</sup> continuation” group (hereinafter, FP Diskus<sup>®</sup> group) and the “switch to HFA-BDP” group (hereinafter, HFA-BDP group). Patient characteristics are presented in Table 1. The dosage of HFA-BDP in the HFA-BDP group was set to be equivalent to that of the FP used previously. Only three types of medications, namely, long-acting beta<sub>2</sub>-agonists, leukotriene receptor antagonists and/or theophylline, used before the study, were allowed as concurrent drugs during the study, with the doses of these drugs being kept constant throughout the study periods. This study was carried out in accordance with the principles embodied in the Helsinki Declaration of 1995 (as revised in Edinburgh 2000).

**Table 1** Patient characteristics

	FP group	HFA-BDP group	Statistical analysis
Number of patients	25	24	
Gender (male/female)	12/13	17/7	$p = 0.148$
Mean age (years)	61.0±17.4	67.9±13.1	$p = 0.124$
Duration of asthma (months)	42.8±45.0	49.1±40.9	$p = 0.612$
Atopic type/non-atopic type	7/18	4/20	$p = 0.496$
Smoker/ex-smoker/non-smoker	0/7/18	1/10/13	$p = 0.314$
Other concurrently administered medications (LABA/LTRA/theophylline)	7/12/18	9/11/19	$p = 0.875$

Abbreviations: FP, Fluticasone Propionate Diskus®; HFA-BDP, hydrofluoroalkane-beclomethasone dipropionate; LABA, long-acting beta2-adrenergic agonists; LTRA, leukotriene receptor antagonist.

**Fig. 1** Study protocols.

## STUDY PROTOCOL AND METHODS

The study protocol was divided into two stages (Fig. 1). During the 1st stage, we used the same design as in our previous study.<sup>3</sup> The peripheral blood differential eosinophil count and serum cortisol, as well as the ECP levels in induced sputum samples, were examined before and three months after the start of the study. Sputum was induced by having the subject inhale 10% hypertonic saline for 15 minutes, and all induced sputum samples were sequentially collected over 30 minutes. We regarded the first induced sputum samples during 15 minutes of hypertonic saline inhalation as early-phase samples, while those finally collected after the inhalation period were taken as late-phase samples. After saliva removal, the samples were diluted five-fold with saline, stirred on a Vortex mixer for one minute, and centrifuged at 4,000 G for 30 minutes at 4°C. The supernatant was collected, mixed with a 1 mg/mL solution of protamine sulfate in 0.01 N hydrochloric acid at a ratio of 9 : 1, and stored frozen at -70°C. The sputum ECP level was measured using an ECP FEIA kit (Pharmacia & Upjohn Diagnostics AS, Uppsala, Sweden). The sputum

surfactant protein (SP)-D level was measured using an SPD ELISA kit (Yamasa Co. Ltd. Chiba, Japan). Various pulmonary function indices were measured using a pulmonary function test apparatus (SP-750, Fukuda Electronics, Tokyo, Japan). We also administered the health-related Quality of Life (QOL) questionnaire developed by Juniper (Asthma-related Quality of Life Questionnaire; AQLQ)<sup>8</sup> to all participants before and at three months after the enrollment. Appropriate permission to use the questionnaire was obtained prior to starting the study.

Before starting the 2nd stage of the parallel-group and double cross-over study, there was a two-week washout period (Fig. 1). After the first cross-over period of three months and another washout period of two weeks, the ICS was again switched to the original ICS which had been assigned at the time of enrollment in the study and the same parameters as described above for the first stage were examined. Each parameter was examined at 3 months after each switch, and other medications except for the ICS were continued to be used during each washout period.

**Table 2** Changes in (A) percentages of plasma and sputum eosinophils and sputum ECP, (B) AQLQ scores and (C) pulmonary function during the first 3 months (first stage) of the study

## (A) Percentages of plasma and sputum eosinophil and sputum ECP

FP group	Before	3 months later	Statistical significance (p value)	HFA-BDP group	Before	3 months later	Statistical significance (p value)	Statistical differences between the two groups (p value)
Plasma eosinophils (%) (n = 25)	3.8±2.7	3.6±3.0	0.526	Plasma eosinophils (%) (n = 24)	3.6±2.4	2.5±2.3	0.028 ( * )	0.039 ( * )
Serum cortisol (µg/dL) (n = 25)	13.9±5.8	15.7±7.1	0.088	Serum cortisol (µg/dL) (n = 24)	13.3±6.0	15.2±13.6	0.541	0.254
Sputum eosinophils (%) (Early phase) (n = 13)	9.7±8.8	11.0±6.2	0.679	Sputum eosinophils (%) (Early phase) (n = 13)	10.7±6.1	7.3±3.6	0.091	0.573
Sputum eosinophils (%) (Late phase) (n = 13)	12.8±11.7	11.6±6.6	0.626	Sputum eosinophils (%) (Late phase) (n = 13)	12.3±7.0	6.2±2.5	0.007 ( ** )	0.028 ( * )
Sputum ECP (Early phase) (n = 13)	632.5±1024.5	874.2±336.1	0.626	Sputum ECP (Early phase) (n = 13)	306.2±426.8	246.7±305.8	0.644	0.715
Sputum ECP (Late phase) (n = 13)	1740.7±2261.3	1737.0±4659.3	0.997	Sputum ECP (Late phase) (n = 13)	1569.5±2278.0	156.3±149.7	0.044 ( * )	0.282

## (B) AQLQ scores (median (25 percentile – 75 percentile))

FP group (n = 25)	Before	3 months later	Statistical significance (p value)	HFA-BDP group (n = 24)	Before	3 months later	Statistical significance (p value)	Statistical differences between the two groups (p value)
Symptoms	6.42 (5.90 – 6.83)	6.42 (5.58 – 6.92)	0.715	Symptoms	5.96 (5.33 – 6.67)	6.42 (5.46 – 6.67)	0.033 ( * )	0.883
Activity limitation	5.63 (5.25 – 6.28)	5.88 (5.41 – 6.50)	0.232	Activity limitation	5.38 (4.69 – 5.88)	5.50 (4.94 – 6.50)	0.036 ( * )	0.282
Emotional function	6.40 (5.60 – 6.80)	6.40 (5.60 – 6.80)	0.712	Emotional function	5.60 (5.20 – 6.70)	6.10 (5.30 – 6.60)	0.127	0.901
Exposure to environmental stimuli	6.25 (5.75 – 6.75)	6.25 (5.75 – 6.75)	0.968	Exposure to environmental stimuli	5.75 (5.25 – 6.88)	6.00 (5.13 – 6.75)	0.307	0.608

## (C) Pulmonary function during the first 3 months (first stage) of the study

FP group (n = 25)	Before	3 months later	Statistical significance (p value)	HFA-BDP group (n = 24)	Before	3 months later	Statistical significance (p value)	Statistical differences between the two groups (p value)
%VC	98.8±13.0	99.8±12.5	0.454	%VC	96.4±18.9	96.7±18.5	0.877	0.569
%FVC	93.6±14.4	96.4±13.3	0.203	%FVC	91.0±20.3	90.1±17.5	0.693	0.407
%FEV1.0	95.7±29.6	96.8±30.5	0.504	%FEV1.0	94.3±26.7	94.2±25.8	0.974	0.612
FEV1.0%-G	75.8±13.3	74.4±13.1	0.296	FEV1.0%-G	69.2±13.0	70.2±13.8	0.481	0.868
MEFR%	76.1±28.5	76.5±27.0	0.917	MEFR%	73.3±36.8	71.0±38.7	0.766	0.816
PEF%	69.6±15.7	73.6±20.7	0.195	PEF%	67.4±27.7	71.5±24.5	0.247	0.080
FEF50 (L/sec)	2.37±1.37	2.18±1.34	0.185	FEF50 (L/sec)	2.12±1.64	1.97±1.37	0.179	0.600
FEF75 (L/sec)	0.80±0.49	0.74±0.50	0.418	FEF75 (L/sec)	0.58±0.40	0.63±0.47	0.340	0.921

**Table 3** Comparison of early phase and late phase sputum findings at the start of the study

Phase	FP group (N = 13)		HFA-BDP group (N = 13)	
	Early-phase	Late-phase	Early-phase	Late-phase
Sputum induction rate	63.60%		73.70%	
ECP	632.5 ± 1024.3	1740.7 ± 2261.3 ( <i>p</i> = 0.086)	306.2 ± 426.8	1569.5 ± 2278.0 ( <i>p</i> = 0.078)
SPD	19.7 ± 24.2	42.1 ± 42.9 ( <i>p</i> = 0.044)	27.3 ± 29.6	46.3 ± 23.3 ( <i>p</i> = 0.042)
Eo%	9.7 ± 8.8	12.8 ± 11.7 ( <i>p</i> = 0.319)	10.7 ± 6.1	12.3 ± 7.0 ( <i>p</i> = 0.548)

Sputum induction rate: rates which induced sputum were obtained in the two phases.

### STATISTICAL ANALYSIS

The significance level was set at 5%. The peripheral blood differential eosinophil count, serum cortisol level and the values of pulmonary function indices, are presented as means ± SD, while sputum ECP levels and the AQLQ scores are presented as means ± SE and median values (25<sup>th</sup>–75<sup>th</sup> percentile), respectively. The patient characteristics in the two ICS groups shown in Figure 1 were analyzed using an unpaired *t* test and Fisher's exact probability test. Statistical analyses of the time-course of changes in the peripheral blood differential eosinophil count, the induced sputum ECP, pulmonary function values, and the AQLQ scores were analyzed by the Kruskal-Wallis method and the Wilcoxon signed-rank test with Bonferroni's correction. Statistical comparisons between the two groups were made by the Friedman test. Statistical analyses were carried out using JMP, version 5.0.1a (SAS Institute Inc., Cary, NC, USA).

### RESULTS

Soon after the start of the study, one elderly patient in the HFA-BDP group dropped out due to the onset of dementia.

#### RESULTS FROM THE FIRST STAGE OF THE STUDY

The changes in various parameters during the 3-month period of the first stage of the study are shown in Table 2 (A)–(C). The peripheral blood differential eosinophil count in the HFA-BDP group was significantly decreased as compared with that in the FP group (Table 2 (A)). The sputum eosinophil percentages and ECP values in the late-phase samples were also significantly decreased in the HFA-BDP group. The scores for both symptom and activity limitation domains of the AQLQ were also significantly improved over the three months of the first stage (Table 2 (B)). On the other hand, there were no significant changes in the various pulmonary function indices (Table 2 (C)). Table 3 shows the comparison between early- and late-phase sputum samples. The sputum SP-D levels in the late-phase sputum samples

were significantly higher than those in the early-phase sputum samples in both groups. The sputum eosinophil percentages and ECP values were also significantly elevated in the late-phase samples at the start of the study (Table 3).

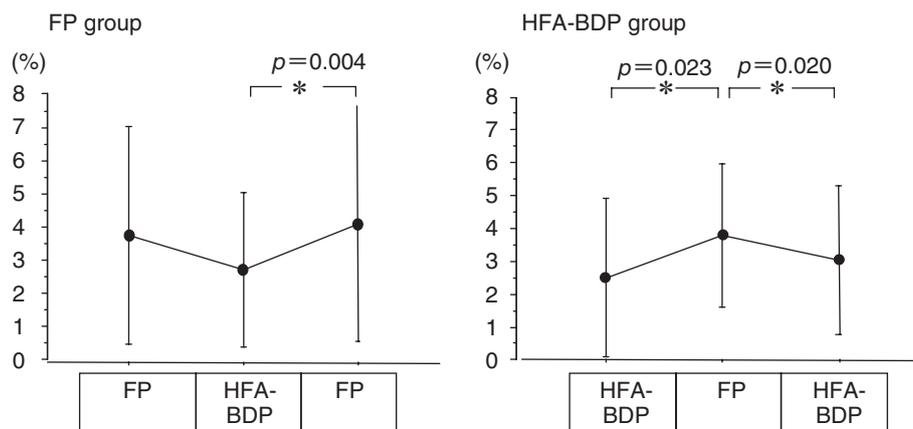
#### RESULTS FROM THE SECOND STAGE OF THE STUDY

The peripheral blood differential eosinophil count in the FP group was significantly lower during the HFA-BDP-assigned period than during the FP-assigned period (Fig. 2). In contrast, in the HFA-BDP group, the peripheral blood differential eosinophil count was significantly elevated during the FP-assigned period. The two graphs in Figure 2 show the reciprocal changes in these two groups. The AQLQ scores for both groups are shown in Figure 3. In the HFA-BDP group, the scores in both symptom and activity limitation domains significantly increased after the switch (second cross-over) from FP to HFA-BDP. Figure 4 shows the sputum ECP levels over the double cross-over study period in the two groups; the sputum ECP levels also showed reciprocal changes in the two groups. In particular, the ECP level in the late phase sputum samples in the HFA-BDP group significantly increased after the FP-assigned period as compared with that after the other HFA-BDP-assigned period (*p* = 0.012 and *p* = 0.023) (Fig. 4). There were no significant changes between the two groups in the various pulmonary function indices over the second stage of the study (Table 4).

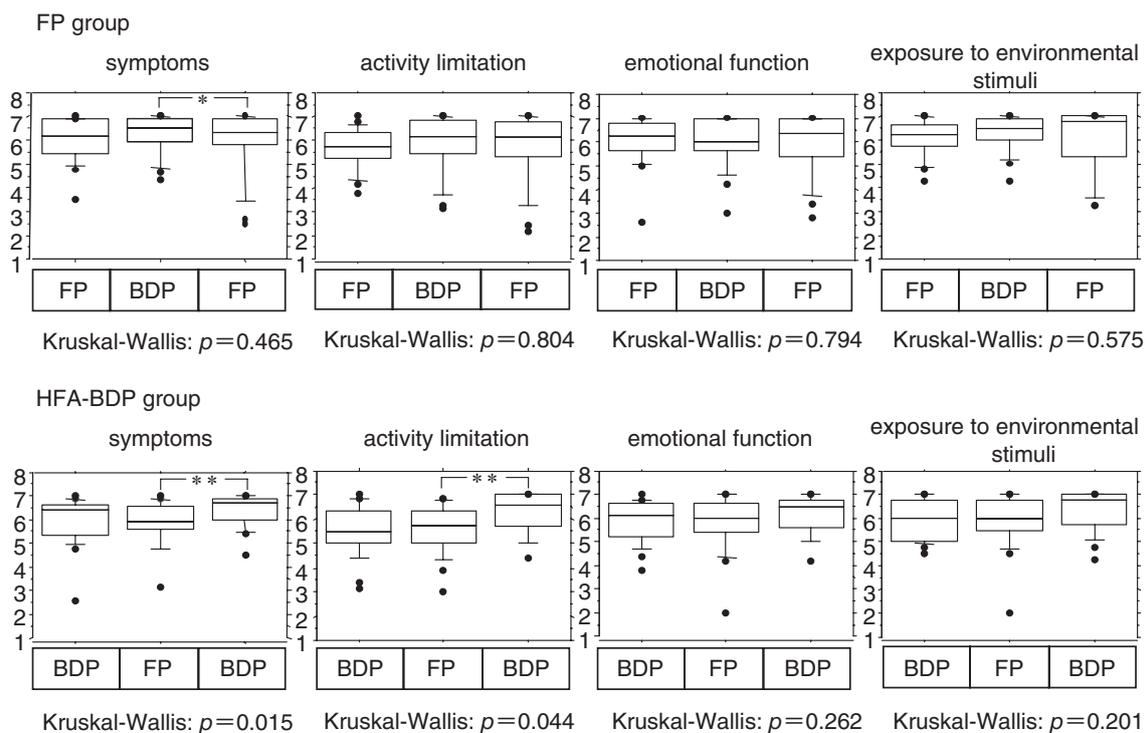
### DISCUSSION

The results of this randomized double cross-over study provide strong evidence that HFA-BDP is more effective at controlling eosinophilic inflammation in asthmatic airways, including that in the distal airways, than the FP Diskus®.

During the first stage of the study, we reconfirmed the results obtained in our previous study, as follows.<sup>3</sup> Despite good and stable control of asthma being maintained with continuous FP administration for at least 6 months, residual eosinophilic inflammation



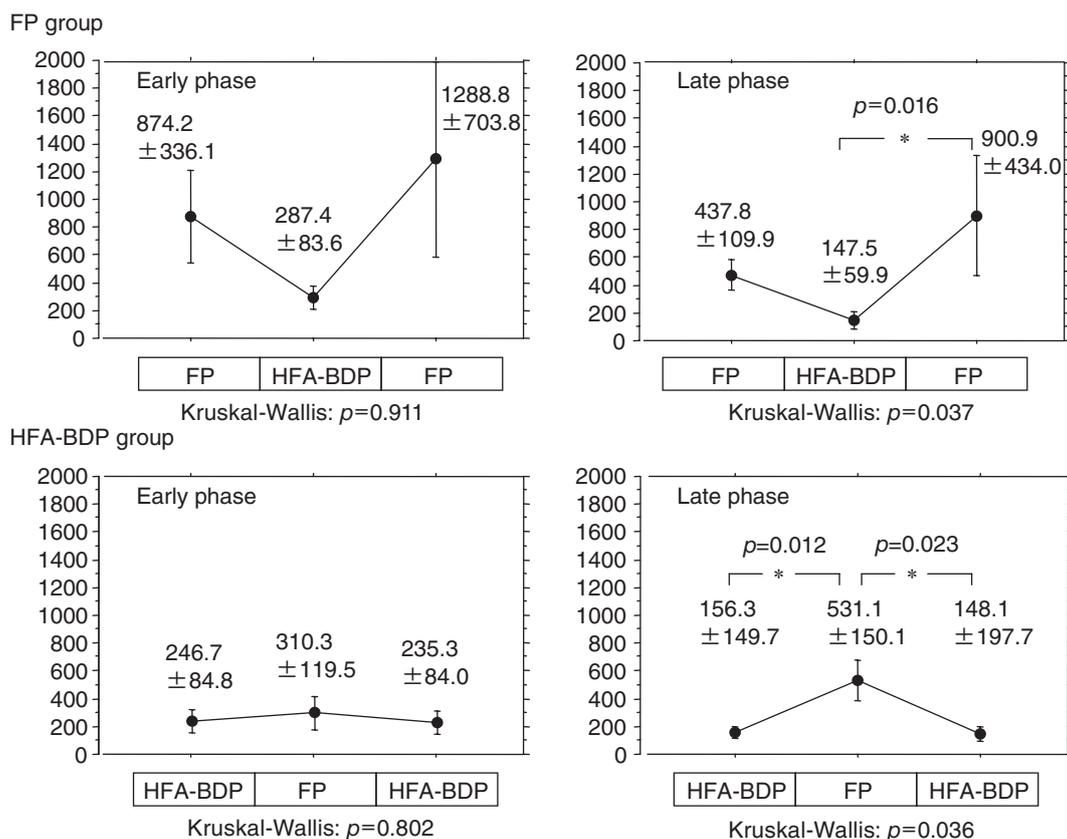
**Fig. 2** Changes in peripheral blood eosinophil percentages during the 2nd stage of the study. Statistical significance: \* $P/2 < 0.05$  and \*\* $P/2 < 0.01$  compared with the peripheral blood differential eosinophil count during the first cross-over stage.



**Fig. 3** AQLQ score changes in the FP and HFA-BDP groups during the 2nd stage of the study. Statistical significance: \* $P/2 < 0.05$  compared with the AQLQ scores during the first cross-over stage.

in the asthmatic airways, including distal airways, persisted (Table 2). Furthermore, significant improvement in this residual inflammation was observed in the HFA-BDP group. Both the peripheral blood differential eosinophil count and the scores in two AQLQ domains, namely, symptoms and activity limitation, also improved significantly in the HFA-BDP group during this stage, accompanied by significant improvements in the eosinophil percentages and

ECP levels in late-phase sputum samples (Table 2). Although dose-related suppression of serum and urine cortisol levels at higher doses has been reported with HFA-BDP,<sup>9</sup> we found no significant adrenal suppression at the conventional dose used in this study. The reason why HFA-BDP could reduce the peripheral blood eosinophil count with no adrenal suppression was unclear. However, a small dose of inhaled HFA-BDP may possibly go through the alveoli



**Fig. 4** Changes in sputum ECP levels during the 2nd stage of the study. Statistical significance: \*\*  $P/2 < 0.01$  compared with the sputum ECP levels during the first cross-over stage.

into the circulation, which may result in obtaining beneficial suppression of eosinophils in peripheral blood.

During the second stage, we directly compared the preventive effects on airway inflammation of two types of ICS in a randomized double cross-over manner. The results revealed inverse changes in the peripheral blood differential eosinophil count in both groups (Fig. 2), suggesting that HFA-BDP has greater efficacy against eosinophilic inflammation in asthmatic airways. A previous study demonstrated significantly greater reductions from baseline in mean peripheral blood differential eosinophil counts and serum ECP levels in patients treated with HFA-BDP than in those receiving HFA-FP.<sup>10</sup> Based on the finding of a higher rate of deposition in the lungs of radio-labeled HFA-BDP (53% ex-actuator) as compared with CFC-FP (12 to 13%) or CFC-BDP (4%),<sup>11</sup> it is suggested that many HFA-BDP extra-fine particles reach the peripheral airways, and some may even enter the circulation via alveoli. During the second stage, HFA-BDP significantly improved inflammation in the distal airways, as reflected mainly by the results of analyzing the late-phase sputum samples (Fig. 4). A randomized placebo-controlled study by

Hauber *et al.* showed that HFA-BDP reduced eosinophilic inflammation and T helper 2-type cytokine expression in both early- and late-phase sputum samples, whereas budesonide did so only in the early-phase samples.<sup>12</sup> This also suggested the superiority of HFA-BDP in terms of delivery into the distal airway. Herein, we obtained a significant decrease in the AQLQ score in the FP group when the ICS was changed from HFA-BDP to FP in the double cross-over study (Fig. 3). These results may indicate a correlation between improved QOL and reduced eosinophilic inflammation with the use of HFA-BDP. On the other hand, our results confirmed the lack of significant changes in pulmonary function test parameters during the second stage (Table 4). Other previous studies also failed to detect any significant differences in pulmonary function parameters, including daily peak flow rates, between patients receiving HFA-BDP versus FP at equivalent dose ratios.<sup>13-15</sup> These observations indicated that pulmonary function parameters may be of limited efficacy for recognizing inflammatory changes in the distal airways. However, despite the urgent need to devise simple and relatively non-invasive means of detecting inflammation in the peripheral airways, no such methods have as yet been

**Table 4** Changes in pulmonary function during the 2nd stage of the study

	FP Group				HFA-BDP Group				Statistical significance between both groups (p value)
	3M	Cross 3M	Double cross 3M	Statistical significance (p value)	3M	Cross 3M	Double cross 3M	Statistical significance (p value)	
%VC	99.8 ±12.2	99.5 ±12.5	99.5 ±11.5	0.148	96.7 ±18.5	98.6 ±18.8	97.2 ±18.1	0.537	0.289
%FVC	96.4 ±13.3	94.3 ±14.3	93.1 ±12.7	0.110	90.1 ±17.5	92.4 ±25.8	89.8 ±20.7	0.335	0.781
%FEV1.0	96.8 ±30.5	95.4 ±28.1	93.7 ±24.4	0.205	94.2 ±25.8	94.1 ±25.1	95.2 ±28.1	0.809	0.721
FEV1.0%-G	74.4 ±13.1	74.7 ±11.5	75.9 ±12.6	0.240	70.2 ±13.8	68.4 ±12.7	69.0 ±11.0	0.370	0.515
MEFR%	76.5 ±27.0	68.7 ±32.0	70.7 ±31.5	0.501	71.0 ±38.7	76.1 ±38.6	78.3 ±39.8	0.483	0.899
PEF%	73.6 ±20.7	73.5 ±20.2	71.6 ±19.0	0.502	71.5 ±24.5	69.5 ±23.8	69.5 ±26.4	0.653	0.446
FEF50 (L/sec)	2.18 ±1.34	1.99 ±1.09	2.2 ±1.18	0.509	1.97 ±1.37	1.93 ±1.36	1.85 ±1.39	0.431	0.551
FEF75 (L/sec)	0.74 ±0.50	0.75 ±0.53	0.77 ±0.54	0.849	0.62 ±0.47	0.53 ±0.41	0.55 ±0.41	0.708	0.364

established. A previous study by Fahy *et al.* found that eosinophil percentages and ECP levels in induced sputum samples from both patients with asthma and healthy subjects were greater than those in bronchoalveolar lavage fluids or bronchial washings, suggesting that analysis of induced sputum samples may be useful for evaluating the condition of peripheral airways.<sup>16</sup> Furthermore, Gershman *et al.* reported that sputum samples collected at least 12 minutes after the start of induction with 3% saline and collected every four minutes during a 20-minute induction contained significantly higher levels of surfactant protein A.<sup>17</sup> These findings suggest that analysis of late-phase induced sputum samples may more clearly reflect the conditions of distal airways. In this study, patients inhaled 10% hypertonic saline for 15 minutes, and the sputum specimens were sequentially collected over a period of 30 minutes. As a result, the sputum levels of SP-D, secreted by alveolar type II cells,<sup>18</sup> were significantly elevated in the late-phase induced sputum samples (Table 3), suggesting that these samples were mainly from the proximal peripheral airways. Although we cannot be certain that the method of sputum induction in this study obtains samples optimally reflecting the condition of the peripheral airways, it is reasonable to assume that the sputum collected more reliably reflects the condition of the distal airways than that obtained with other routine examinations. When counting the numbers of eosinophils, while we found some squamous cells in the early-phase sputum samples, marked elevation of macrophage numbers was detected in the late-phase ones. However, one of the greatest limitations of inha-

lational methods for sputum induction is that we cannot consistently obtain both early- and late-phase sputum samples of adequate quantity (induction rate, 60–70%) at the same time during the same induction procedure.

In conclusion, this randomized double cross-over study showed that HFA-BDP can control residual eosinophilic inflammation in asthmatic airways, including that in distal airways, more effectively than FP. Routine pulmonary function test parameters are of limited efficacy for recognizing inflammatory changes in the distal airways, while sputum induction with inhalation of 10% hypertonic saline for 15 minutes appears to be an easy, relatively non-invasive method allowing selective analysis of secretions from the distal airways.

## REFERENCES

1. Hamid Q, Song Y, Kotsimbos TC *et al.* Inflammation of small airways in asthma. *J Allergy Clin Immunol* 1997; **100**:44-51.
2. Martin RJ. Therapeutic significance of distal airway inflammation in asthma. *J Allergy Clin Immunol* 2002; **109**:S 447-60.
3. Ohbayashi H. [The small airway inflammation of asthmatic patients who have used dry powder type inhaled steroid for moderate-long term evaluated by induced sputum and the efficacy of HFA-BDP (QVAR) inhalation]. *Averugi [Jpn J Allergol]* 2005; **54**:24-35 (in Japanese).
4. Ohbayashi H. One-year evaluation of the preventative effect of Hydrofluoroalkane-beclomethasone dipropionate on eosinophilic inflammation of asthmatic peripheral airways. *Respiration* 2006; **73**:146-53.
5. Leach CL, Davidson PJ, Boudreau RJ. Improved airway

- targeting with the CFC-free HFA-beclomethasone metered-dose inhaler compared with CFC-beclomethasone. *Eur Respir J* 1998;**12**:1346-53.
6. Ederle K, Multicentre Study Group. Improved control of asthma symptoms with a reduced dose of HFA-BDP extrafine aerosol: an open-label, randomised study. *Eur Rev Med Pharmacol Sci* 2003;**7**:45-55.
  7. National Institutes of Health, National Heart, Lung, and Blood Institute, Global Initiative for Asthma. *Global Strategy for Asthma Management and Prevention*. 2002;102-14.
  8. Juniper EF, Buist AS, Cox FM, Ferrie PJ, King DR. Validation of a standardized version of the Asthma Quality of Life Questionnaire. *Chest* 1999;**115**:1265-70.
  9. Fowler SJ, Orr LC, Wilson AM, Sims EJ, Lipworth BJ. Dose-response for adrenal suppression with hydrofluoroalkane formulations of fluticasone propionate and beclomethasone dipropionate. *Br J Clin Pharmacol* 2001;**52**:93-5.
  10. Aubier M, Wettenger R, Gans SJ. Efficacy of HFA-beclomethasone dipropionate extra-fine aerosol (800 microg day<sup>-1</sup>) versus HFA-fluticasone propionate (1000 microg day<sup>-1</sup>) in patients with asthma. *Respir Med* 2001;**95**:212-20.
  11. Leach CL, Davidson PJ, Hasselquist BE, Boudreau RJ. Lung deposition of hydrofluoroalkane-134a beclomethasone is greater than that of chlorofluorocarbon fluticasone and chlorofluorocarbon beclomethasone: a cross-over study in healthy volunteers. *Chest* 2002;**122**:510-6.
  12. Hauber H, Taha R, Bergeron C, Miqounov V, Hamid Q, Olivenstein R. Effects of hydrofluoroalkane and dry powder-formulated corticosteroids on sputum inflammatory markers in asthmatic patients. *Can Respir J* 2006;**13**:73-8.
  13. Van Aalderen WM, Price D, De Baets FM, Price J. Beclomethasone dipropionate extra-fine aerosol versus fluticasone propionate in children with asthma. *Respir Med* 2007;**101**:1585-93.
  14. Lasserson TJ, Cates CK, Jones AB, Steele EH, White J. Fluticasone versus HFA-beclomethasone dipropionate for chronic asthma in adults and children. *Cochrane Database Syst Rev* 2006;**2**:CD005309.
  15. Fairfax A, Hall I, Spelman R. A randomized, double-blind comparison of beclomethasone dipropionate extra-fine aerosol and fluticasone propionate. *Ann Allergy Asthma Immunol* 2001;**86**:575-82.
  16. Fahy JV, Wong H, Liu J, Boushey HA. Comparison of samples collected by sputum induction and bronchoscopy from asthmatic and healthy subjects. *Am J Respir Crit Care Med* 1995;**152**:53-8.
  17. Gershman NH, Liu H, Wong HH, Liu JT, Fahy JV. Fractional analysis of sequential induced sputum samples during sputum induction: evidence that different lung compartments are sampled at different time points. *J Allergy Clin Immunol* 1999;**104**:322-8.
  18. Mason RJ. Biology of alveolar type II cells. *Respirology* 2006;**11**:S12-5.