

Effects of suplatast tosilate on allergic eosinophilic airway inflammation in patients with mild asthma

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Background: Bronchial asthma is a chronic inflammatory disease characterized mainly by infiltration of the airway mucosa by various inflammatory cells, notably eosinophils. T_H2-type cytokines are suggested to be deeply involved in the pathogenesis of asthma.

Objective: We sought to determine the suppressive effects of suplatast tosilate, an inhibitor of T_H2-type cytokines, on eosinophilic inflammation of the bronchial mucosa in patients with mild asthma.

Methods: Airway hyperresponsiveness tests, pulmonary function tests, eosinophil measurements in induced sputum, and bronchial mucosa biopsies were performed before and after treatment with suplatast tosilate for 6 weeks in 15 patients with mild asthma and in 13 control patients with mild asthma not receiving suplatast tosilate. This study was performed as a case-controlled open study.

Results: In the treatment group a significant improvement in the provocation concentration of histamine was observed ($P < .05$). Improvements in peak expiratory flow ($P < .01$) and in symptom score ($P < .05$) were also noted in the suplatast tosilate-treated group. Moreover, the average number of infiltrating eosinophils and EG2⁺ cells significantly decreased (both $P < .05$), as did the ratios of eosinophils and EG2⁺ cells in sputum (both $P < .01$). The average number of CD4⁺ and CD25⁺ T lymphocytes also decreased (both $P < .05$).

Conclusion: Suplatast tosilate appears to inhibit allergic airway inflammation mediated by T_H2-type cytokine and to improve clinical symptoms in patients with mild asthma. (J Allergy Clin Immunol 2003;111:958-66.)

Key words: Airway hyperresponsiveness, airway inflammation, airway remodeling, bronchial asthma, EG2⁺ cells, eosinophils, induced sputum, suplatast tosilate, T_H2-type cytokines

Bronchial asthma is a chronic disease characterized mainly by localized luminal stenosis and allergic inflammation of the airway. Even in mild cases, bronchial asthma

Abbreviations used

AHR: Airway hyperresponsiveness

PEF: Peak expiratory flow

ma might be aggravated by various factors, including allergenic stimulation, infection, and fatigue. Moreover, asthmatic symptoms might become severe after several years of sporadic attacks. Various inflammatory cells, notably eosinophils, are involved in the airway inflammatory process.^{1,2} Recent studies indicate that the number of activated eosinophils is increased in the bronchial mucosa, even in the earliest stage of bronchial asthma.^{3,4}

T_H2-type cytokines are suggested to play a major role in the pathogenesis of allergic inflammation⁵; IL-4, for example, is involved in IgE production and vascular cell adhesion molecule 1 intensity in endothelial cells, and IL-5 is known to activate eosinophils, inducing them to infiltrate the bronchial mucosa.^{6,7}

Suplatast tosilate (IPD; Taiho Pharmaceutical Co, Ltd, Tokyo, Japan) has been shown to inhibit the production of IL-4 by human T_H cells⁸ and to selectively inhibit the production of IL-4 and IL-5 in the mouse T_H2 clone D10G4.1.⁹ The Japanese Guidelines for the Diagnosis and Management of Asthma¹⁰ include T_H2-type cytokine inhibitors in the list of drugs that can be used in the treatment of asthma. Suplatast tosilate was administered to patients with mild asthma to investigate the effects on human airway inflammation and asthma symptoms (step 1 or 2).¹¹ The degree of airway inflammation was evaluated through biopsies of the bronchial mucosa and airway hyperresponsiveness (AHR) tests. The ratio of eosinophils in induced sputum was also analyzed. Peak expiratory flow (PEF) levels and asthmatic symptoms were monitored during the study period. The results obtained in the treatment group were compared with those of the control group, which was given no anti-inflammatory drugs, and compared between groups.

METHODS

Subjects

Twenty-eight subjects were recruited from outpatients with asthma of step 1 or 2¹¹ visiting Doai Memorial Hospital who had never received steroid therapy. They were randomly allocated to the suplatast tosilate group or the control group.

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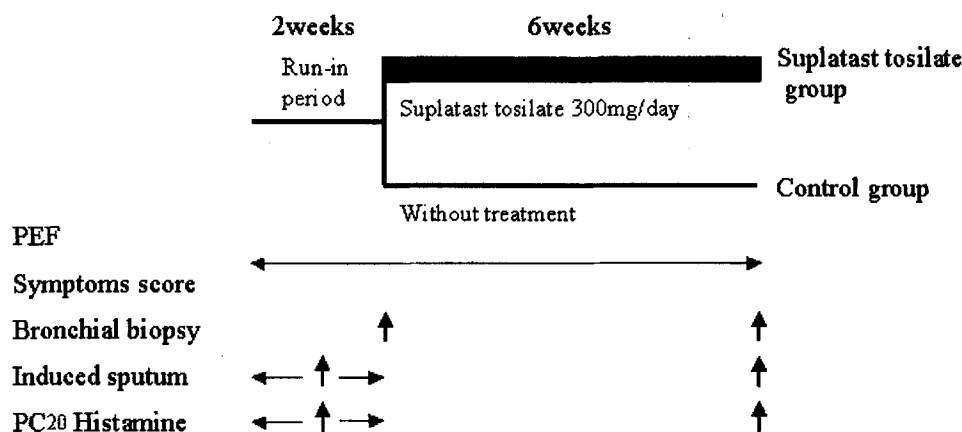


FIG 1. Study protocol. After an initial 2-week run-in period, the treatment group was administered suplatast tosilate for 6 weeks. Bronchial mucosal biopsy was performed before and after treatment.

Study design

This case-controlled open study was carried out from January through June 1996. The protocol (Fig 1) was reviewed and approved by the Institutional Review Board of Doai Memorial Hospital. Before the study, each subject was fully informed of the purpose and procedures of the study, and written informed consent was obtained from all subjects. After an initial 2-week run-in period, the treatment group was administered 100 mg of suplatast tosilate 3 times daily for 6 weeks. PEF was measured twice daily in the morning and evening, and the patients were asked to keep an asthma diary. The symptoms score was calculated from those records. An AHR test, induced sputum collection, and a bronchial biopsy were performed at the beginning and end of the study. The biopsy specimens were stained by the methods described below to determine changes in the numbers of infiltrating eosinophils, EG2⁺ cells, and CD3⁺, CD4⁺, CD8⁺, and CD25⁺ cells. The induced sputum was examined for the percentage of eosinophils.

Airway hyperresponsiveness

AHR was evaluated according to the standard method established by the Japan Cooperative Research Group on Inhalation Test Standardization.¹² Antiasthmatic agents were withdrawn 12 hours before the test. Solutions of histamine dihydrochloride serially diluted 1:2 with saline were used as inhalants. FEV₁ measured before the test was taken as the baseline value. Using a no. 646 DeVilbiss nebulizer (DeVilbiss Health Care Inc, Somerset, Pa), the patient inhaled saline solution with 5 L/min compressed air for 2 minutes while breathing normally. When it was confirmed that FEV₁ did not decrease by 10% or greater, the patient inhaled histamine solutions, starting with the lowest concentration of 39 µg/mL. FEV₁ was measured immediately after the inhalation, and the concentration of the histamine solution was repeatedly doubled (20,000 µg/mL maximum) until the FEV₁ immediately after the inhalation decreased by 20% or greater from the baseline value. The concentration of histamine solution that caused FEV₁ to decrease by 20% (PC₂₀ histamine) was calculated and analyzed as an index of AHR.

Bronchial biopsy

Atropine sulfate and pentazocine were administered intramuscularly as pretreatment at doses of 0.25 mg and 7.5 mg, respectively, followed by inhalation of 3 mL of 2% xylocaine and 0.3 mL of nebulized salbutamol sulfate (equivalent to 1.5 mg of salbutamol) and

endotracheal instillation of 4% xylocaine by using the Jackson-Rees spray. Patients inhaled 100 µg of salbutamol sulfate in 2 puffs through a metered-dose inhaler to prevent an asthma attack. A fiberoptic bronchoscope (Olympus Optical Co, Ltd, Tokyo, Japan) was inserted into the bronchus, surface anesthesia was induced with 2% xylocaine, and specimens were obtained with biopsy forceps. Both in the treatment group and the control group, bronchial biopsy was performed before and after the study period of 6 weeks, collecting from the right bronchi before treatment and from the left bronchi after treatment.

Immunohistochemistry. The biopsy specimens were immersed in embedding medium (Tissue Mount; Chiba Medical, Saitama, Japan) and stored at -80°C until stained. The specimens were cut into serial thick sections (6 µm) with a cryostat and attached to glass slides for hematoxylin and eosin staining and immunohistochemistry. For immunohistochemistry, the sections were fixed in acetone for 10 minutes at -20°C. The slides were first incubated with each primary mouse mAb and then incubated again with the peroxidase-labeled goat anti-mouse IgG antibody. The enzyme activity was visualized with diaminobenzidine or new fuchsin, and finally, slides were counterstained briefly with methyl green. As the primary antibody, eosinophil cationic protein clone EG2 (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden) was used for activated eosinophils, and CD3 (Leu-4; Becton Dickinson Immunocytometry Systems, San Jose, Calif), CD4 (Leu-3a; Becton Dickinson Immunocytometry Systems), CD8 (anti-human Leu-2a; Becton Dickinson Immunocytometry Systems), and DCO-CD25 and ACT1 (DAKO A/S, Glostrup, Denmark) were used for T lymphocytes.

Counting of cells in each sample. The number of infiltrating eosinophils in the bronchial mucosa (lamina propria) was determined on hematoxylin and eosin-stained slides. The area was measured with National Institutes of Health image software, and the number of eosinophils in that field was counted with a light microscope at a magnification of 600×. We calculated the number of infiltrated cells per square millimeter of field. Other infiltrated cells stained with EG2 or stained with lymphocytic cell surface antigen, CD3, CD4, CD8, and CD25 were counted in the same area of serial sections. To determine the number of these lymphocytic antigen-positive cells, we counted the number of cells in 10 high-magnification fields (400×) in the lamina propria and expressed the resulting figure per square millimeter. Infiltrated cells were counted by 2 examiners independently of each other and blinded to patient background and study design.

TABLE I. Demographic, clinical, and immunohistologic characteristics of individuals recruited in this study

Subject no.	Sex	Age (y)	Duration of disease	Type	IgE (IU/mL)	PC ₂₀ histamine (μg/mL)		FEV ₁ % (predicted)	
						Before	After	Before	After
Suplatast tosilate treatment group									
1	F	37	34	A	105	1070	2430	75.4	77.4
2	M	61	8	A	980	220	540	63.2	67.4
3	F	40	5	A	45	3000	3150	102.1	97.9
4	M	31	24	A	106	375	2400	77.6	76.2
5	M	44	5	A	1119	200	1470	115.0	119.2
6	F	47	22	A	23	565	305	54.9	69.0
7	M	52	20	A	291	78	113	88.1	58.3
8	M	54	10	A	170	2550	6000	96.0	84.3
9	F	48	6	A	200	312	310	105.6	97.2
10	F	38	10	A	2177	850	290	94.2	97.5
11	M	68	26	A	439	860	480	63.4	64.5
12	M	35	12	A	13	88	630	71.0	62.8
13	M	55	29	A	239	47	54	63.5	62.9
14	F	44	10	A	18	480	1750	113.2	93.4
15	F	21	7	A	32	5100	10,300	116.9	108.3
Median ± SD		44.0 ± 12.0	10.0 ± 9.6	–	170.0 ± 598.5	480 ± 1424	630 ± 2793*	88.1 ± 21.1	77.4 ± 18.8
Control group									
1	F	37	8	A	1210	5000	1740	104.1	97.5
2	M	49	5	A	48	152	88	80.0	54.4
3	M	57	23	A	530	205	350	86.0	87.0
4	M	53	11	A	30	850	2550	80.8	87.7
5	F	40	9	A	23	78	64	95.4	74.4
6	M	64	25	A	289	680	860	74.0	86.5
7	M	32	11	A	2150	855	1200	108.3	102.9
8	M	65	16	A	11,112	215	143	63.1	47.8
9	F	22	6	A	178	2900	1300	118.6	110.7
10	M	60	22	A	1177	39	39	104.0	95.7
11	F	49	22	A	199	78	111	63.3	72.4
12	M	62	21	NA	4	86	50	64.2	56.7
13	F	53	19	A	377	9500	9000	89.7	81.8
Median ± SD		53.0 ± 13.2	16.0 ± 7.1	–	289.0 ± 3006.0	215 ± 2783	350 ± 2433	86.0 ± 18.3	86.5 ± 19.3

A, Atopic; TH, theophylline; β₂, oral β₂ stimulant; iβ₂, inhaled β₂ stimulant; NA, nonatopic.

*P < .05 between the 2 values.

†P < .01 between the treatment and control groups.

Induced sputum

Expectoration was induced according to the method of Pin et al,¹³ whereby the patient inhaled hypertonic saline solution (5% maximum) with a nebulizer. The collected sputum was gently homogenized in a 10-mL disposable syringe, and 10-fold volumes of 10 mmol/L dithiothreitol (Boehringer Mannheim Co, Ltd, Mannheim, Germany) dissolved in Dulbecco-PBS (Gibco Laboratories Life Technologies Inc, Grand island, NY) were added. The cell-suspended solution was mixed and deprived of its viscosity by emptying and filling the syringe several times. It was filtered through gauze and a 40-μm rating nylon mesh (Nippon Rikagaku Kikai Co, Ltd, Tokyo, Japan) and then washed twice with Dulbecco-PBS, spun by means of centrifugation (280g for 10 minutes at 4°C), and finally suspended in Dulbecco-PBS containing 0.1% BSA.¹⁴ The suspension was smeared on a slide and stained with May-Giemsa stain. For immunostaining, poly-L-lysine-coated slides (Muto Pure Chemicals Co, Ltd, Tokyo, Japan) were used, and the suspension was smeared on them. The slides were stored at -80°C until stained. Cell fractionation was calculated after counting more than 400 cells, excluding epithelial components. The ratio of

eosinophil count to the total lower airway cell count in each sputum sample was also calculated.

Statistics

Data were analyzed by using statistics software (Stat View-J v5.0; Abacus Concepts, Inc, Berkeley, Calif). All data were expressed as the median ± SD. Data obtained at the beginning and end of the study were assessed by using the Wilcoxon signed-rank test, and differences between the treatment and control groups were assessed for statistical significance by using the Mann-Whitney *U* test. PEF data were analyzed by using the Kruskal-Wallis test with repeated measures. The difference was considered significant when *P* values were less than .05.

RESULTS

The background data of the patients are shown in Table I. All but one patient in the control group had atopic asthma. There were no statistically significant differences in age, duration of illness, serum level of IgE,

Eosinophils (cells/mm ²)		CD3 (cells/mm ²)		CD4 (cells/mm ²)		CD8 (cells/mm ²)		CD25 (cells/mm ²)		Combined drugs
Before	After	Before	After	Before	After	Before	After	Before	After	
150	33	102.8	128.8	79.6	50.7	47.8	55.0	18.8	4.3	TH, β_2 , I β_2
680	80	55.0	169.4	40.7	62.5	63.7	93.5	11.6	4.3	TH, β_2
33	28	81.3	181.4	75.1	81.3	71.9	71.9	3.1	6.3	TH
100	90	176.6	179.5	78.2	62.5	39.1	81.1	0.0	0.0	TH, β_2 , I β_2
900	111	81.1	149.1	65.7	68.1	65.2	115.8	9.4	3.1	I β_2
—	—	—	—	—	—	—	—	—	—	TH, β_2 , I β_2
160	200	140.4	137.5	79.6	56.5	52.2	69.5	7.2	2.9	TH, β_2 , I β_2
566	45	141.9	71.0	57.9	17.4	47.8	47.8	7.2	7.2	TH, β_2 , I β_2
—	—	—	—	—	—	—	—	—	—	TH, I β_2
—	—	—	—	—	—	—	—	—	—	TH, β_2 , I β_2
533	133	149.1	111.4	52.1	33.3	23.2	50.7	1.5	0.0	TH, β_2 , I β_2
922	328	154.9	120.1	65.2	50.7	71.9	71.9	17.4	1.5	TH, β_2 , I β_2
112	50	154.9	199.8	98.5	86.9	82.5	189.6	2.9	0.0	TH, β_2 , I β_2
—	—	—	—	—	—	—	—	—	—	TH
15	20	141.9	121.6	137.6	79.6	68.1	75.3	13.0	7.2	I β_2
160.0 ± 379	80.0 ± 92.3*	88.1 ± 21.1	137.5 ± 37.6	75.1 ± 25.8	62.5 ± 20.8*	63.7 ± 17.2	71.9 ± 40.1*	7.2 ± 6.3	3.1 ± 2.7*	—
33	30	81.3	122.0	75.1	112.6	71.9	62.5	3.1	15.6	TH
900	809	81.1	46.9	65.7	31.3	65.2	18.8	9.4	9.4	I β_2
160	16	140.4	53.2	79.6	31.3	52.2	18.8	7.2	0.0	TH, β_2 , I β_2
475	566	46.9	141.9	31.3	57.9	18.8	47.8	6.3	7.2	TH, β_2 , I β_2
44	44	79.1	71.9	43.4	37.5	40.3	31.3	4.7	0.0	TH, β_2 , I β_2
75	533	68.8	149.1	43.8	52.1	15.6	23.2	25.1	1.5	TH, β_2 , I β_2
162	922	28.1	154.9	12.5	65.2	18.8	71.9	6.3	17.4	TH, β_2 , I β_2
480	893	198.3	90.7	78.2	56.3	76.7	31.3	17.4	15.6	TH, β_2 , I β_2
15	23	141.9	37.5	137.6	28.1	68.1	15.6	13.0	6.3	I β_2
130	66	124.5	84.4	50.7	40.7	69.5	25.0	14.5	3.1	TH, β_2 , I β_2
1171	400	165.7	43.8	56.3	28.1	59.4	28.1	17.4	21.9	TH, β_2 , I β_2
180	283	46.9	122.0	21.8	56.3	12.5	59.4	0.0	6.3	β_2 , I β_2
200	187	100.0	34.4	37.5	21.9	31.2	9.4	6.2	6.3	TH
162.0 ± 309.6	283.0 ± 346.1	86.0 ± 18.3	84.4 ± 44.7	50.7 ± 32.2	40.7 ± 24.0	52.2 ± 24.1	28.1 ± 19.8†	7.2 ± 7.0	6.3 ± 7.0	—

Asthma, rhinitis,
other respiratory
diseases

PC₂₀ histamine, or FEV₁ percent predicted between the treatment and control groups. There was no significant difference in infiltrating cells between the control and treatment groups, except for CD8⁺ T lymphocytes, after supalast tosilate administration ($P < .05$).

Improvement in PEF, symptom score, and AHR

In the treatment group PEF gradually increased, showing a significant increase from baseline values in the fifth week (Fig 2, A). At the end of the study, significant increases in both morning and evening PEF values were observed (morning, from 391.4 to 416.9 L/min; evening, from 403.2 to 424.7 L/min; $P < .01$). The symptom scores also improved, showing a significant decrease after 6 weeks of treatment (from 5.6 to 2.6, $P < .05$; Fig 2, B). In the control group PEF showed no significant changes (morning, from 384.8 to 382.4 L/min; evening, from 397.4

to 397.3 L/min), and the symptom scores did not decrease (from 5.0 to 6.4). There were no significant changes in the peripheral blood eosinophil counts, serum levels of IgE, or IgE RAST results in either group (data not shown).

AHR, expressed as PC₂₀ histamine, significantly improved in the treatment group (from 474.4 to 843.4 μ g/mL, $P < .05$; Fig 3). In the control group, on the other hand, there were no significant changes in PC₂₀ histamine values (from 411.6 to 378.5 μ g/mL).

Effect on airway inflammation

Bronchial mucosa biopsies were performed at the beginning and end of the study. In the supalast tosilate group paired bronchial mucosa biopsies could be performed in 11 of 15 subjects. The reasons biopsies could not be performed in 4 subjects were as follows. One subject agreed to all tests except bronchial mucosa biopsy. One subject experienced mild bronchospasm during

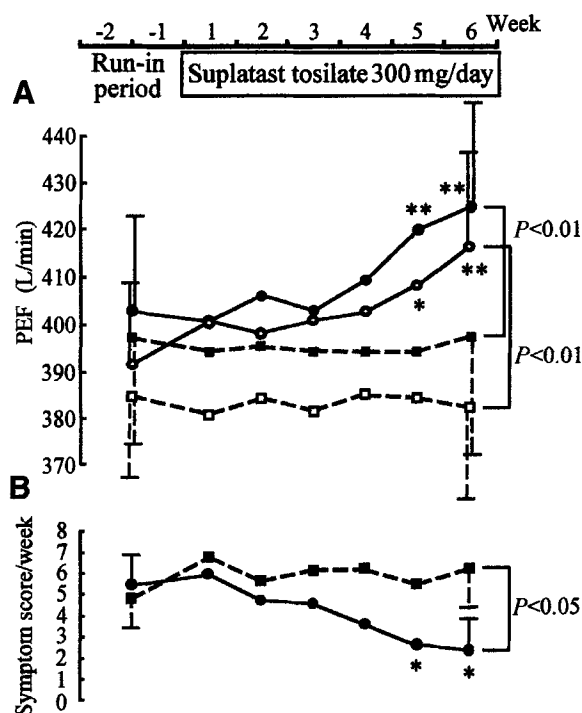


FIG 2. A, Changes in PEF observed in the suplatast tosilate group (circles) and in the control group (squares). Open symbols, Morning PEF; filled symbols, evening PEF. B, Symptom score per week in the suplatast tosilate group (filled circles) and in the control group (filled squares). * $P < .05$ and † $P < .01$ compared with baseline values.

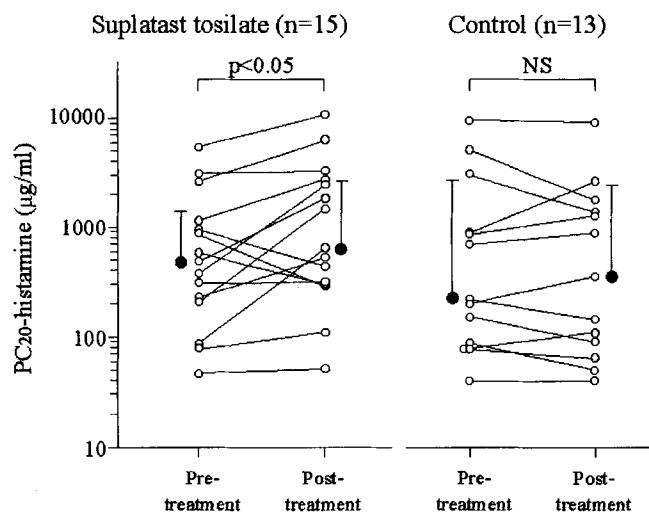


FIG 3. Changes in AHR expressed as PC₂₀ histamine in 15 patients treated with suplatast tosilate and 13 control subjects.

bronchoscopy before the administration of suplatast tosilate, and biopsy could not be conducted. Thus it was impossible to obtain the preadministration and postadministration specimens. The other 2 subjects underwent bronchoscopy before the administration but refused bronchoscopy after the treatment period. In the control group paired biopsy could be performed both before and after administration in all 13 subjects. However, one

patient refused to continue taking part in this clinical study during the suplatast tosilate administration period.

The pretreatment and posttreatment findings of the bronchial mucosa obtained from each of the patients are shown in Fig 4. The diffuse infiltration of eosinophils (Fig 4, A, left) and EG2⁺ cells (Fig 4, B, left) observed before treatment was notably inhibited after treatment (Fig 4, right).

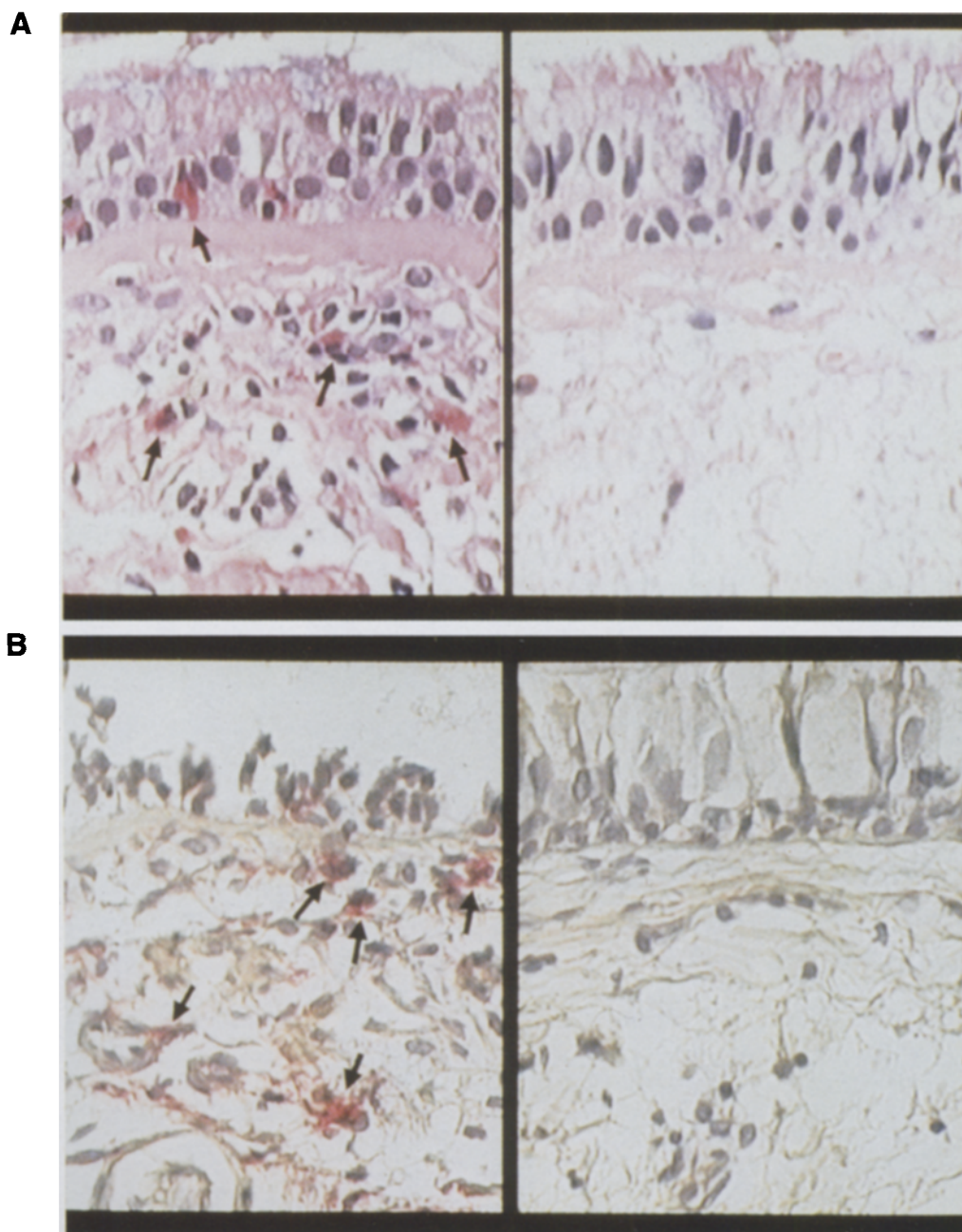


FIG 4. Histologic findings of airway mucosa before and after treatment with suplastast tosilate: **A**, eosinophils stained with hematoxylin and eosin; **B**, EG2⁺ cells stained with antibody eosinophil cationic protein clone EG2 (*left*, before treatment; *right*, after treatment; original magnification 400 $\times$). Arrows indicate eosinophils (Fig 4, A) and EG2⁺ cells (Fig 4, B).

In the suplastast group the mean number of eosinophils infiltrating the bronchial mucosa was 160 ± 349 cells/mm² before treatment. After treatment, this number significantly decreased to 80 ± 92 cells/mm² ($P < .05$). The number of EG2⁺ cells in this group also significantly decreased from 125 ± 328 cells/mm² before treatment to 44 ± 84 cells/mm² after treatment ($P < .05$). In the control group, on the other hand, the mean number of eosinophils and the number of EG2⁺ cells did not change significantly (Fig 5).

Marked inhibition of eosinophils by suplastast tosilate was also observed in induced sputum (Fig 6). The ratio of eosinophils significantly decreased in the suplastast tosilate group, from $6.3\% \pm 8.8\%$ before treatment to $1.4\% \pm 6.3\%$ after treatment. In the control group the ratio of eosinophils remained unchanged (from $2.4\% \pm 8.7\%$ to $0.5\% \pm 9.9\%$, Fig 7).

The changes in the numbers of CD3⁺, CD4⁺, CD8⁺, and CD25⁺ cells in the bronchial mucosa were also analyzed (Table I). In the suplastast tosilate group CD4⁺ and

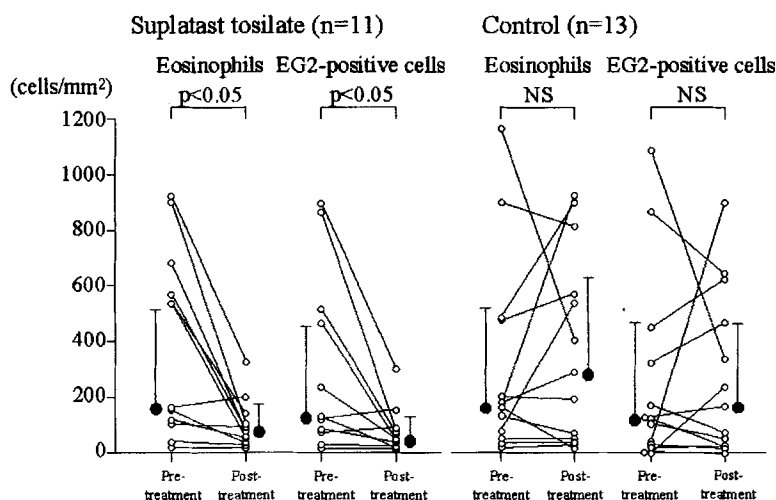


FIG 5. Changes in eosinophil (stained with hematoxylin and eosin) and EG2⁺ cell (stained with antibody eosinophil cationic protein clone EG2) counts in bronchial mucosa (11 patients treated with suplatast tosilate and 13 control subjects; original magnification 400 $\times$).

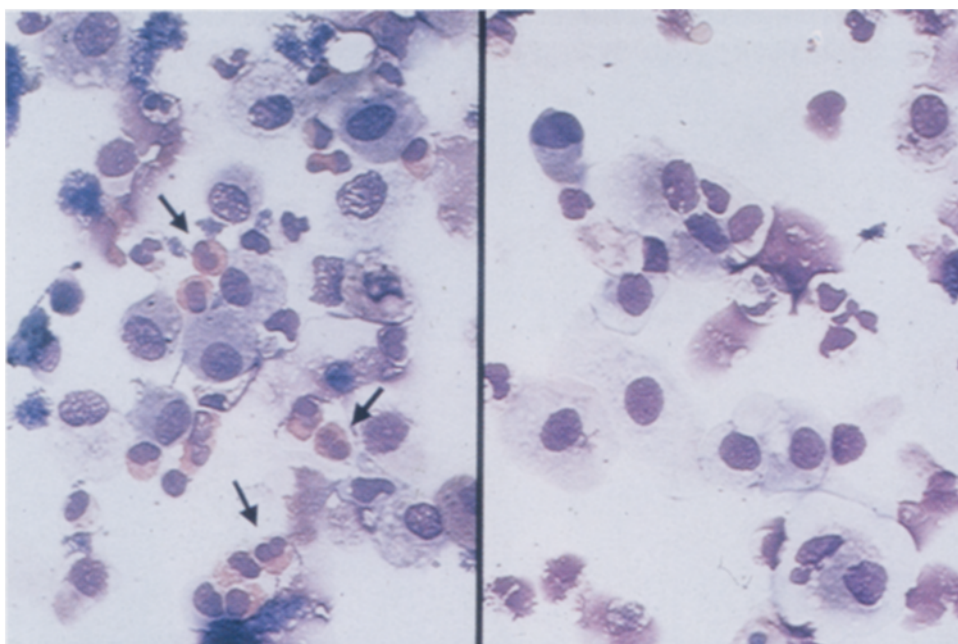


FIG 6. Eosinophils in the sputum before (left) and after (right) treatment with suplatast tosilate (May-Giemsa staining, original magnification 400 $\times$). Arrows indicate eosinophils.

CD25⁺ T lymphocytes decreased significantly, whereas CD8⁺ T lymphocytes showed a significant increase. There was no significant change in the number of any type of cell in the control group.

DISCUSSION

Anti-inflammatory drugs, such as beclomethasone dipropionate, disodium cromoglycate, and nedocromil, are used as drugs for the long-term management of chronic allergic inflammation in bronchial asthma. In the 1997 edition of the National Institutes of Health Guidelines,¹¹

oral leukotriene receptor antagonists won recognition as drugs that improve mild persistent asthma (step 2). As pointed out in the 1995 edition of the Japanese Guidelines, oral antiallergic agents, along with steroid inhalants and disodium cromoglycate, are considered beneficial for mild asthma. However, although the latest Japanese guidelines for asthma management¹⁰ described T_H2-type cytokine inhibitors as useful in the treatment of asthma, little clinical work has been done to evaluate their effects on airway inflammation. Hence we evaluated the efficacy of suplatast tosilate in airway inflammation, especially eosinophilic infiltration, in patients with mild asthma.

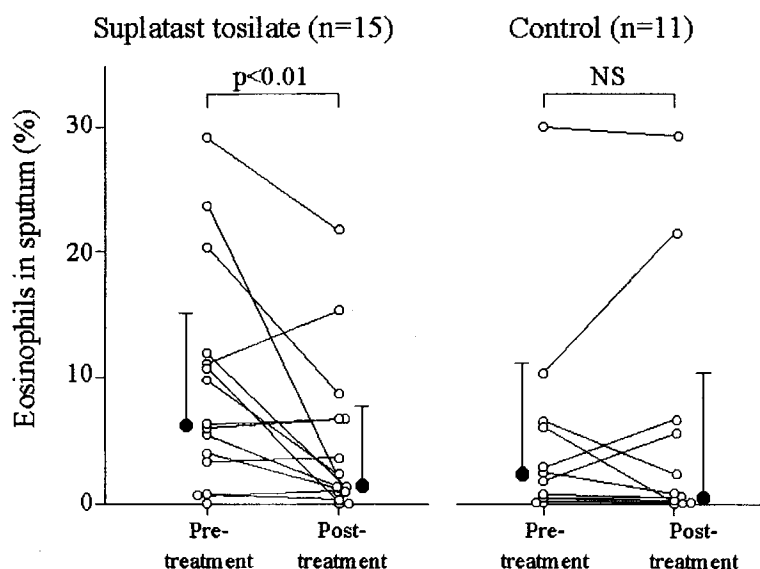


FIG 7. Changes in the percentage of eosinophils in sputum from 15 treated patients and 11 control subjects.

After beginning suplatast tosilate administration, PEF values and symptom scores showed significant improvement. In addition, AHR improved significantly, with significant decreases in the numbers of eosinophils and EG2⁺ cells in the bronchial mucosa and sputum. Suplatast tosilate has been reported to inhibit the production of cytokines by T_H2 cells, IgE production, and the expression of the low-affinity IgE receptor (CD23).⁸ In these ways suplatast tosilate seems to play a role in the inhibition of allergic inflammation.

Various factors combine to induce allergic inflammation, but it is the 2 major cell subsets, IgE mast cells and CD4⁺ lymphocytes, that recognize the triggering antigens. It is difficult to explain all the pathogenetic aspects of allergic inflammation by means of the IgE mast cell pathway alone. Rather, there are many reports suggesting that CD4⁺ T lymphocyte-mediated reactions are important in this respect.¹⁵ CD4⁺ T lymphocytes are classified as T_H1 (producing, for example, IL-2 and IFN- γ) and T_H2 (eg, IL-4, IL-5, and IL-3) according to the kind of cytokines they produce,¹⁶ and T_H2 is considered predominant in allergic diseases.¹⁷ When challenged by antigens, the number of CD4⁺ T lymphocytes expressing T_H2-type cytokine mRNA is reported to increase significantly in bronchoalveolar lavage fluid and airway mucosa, with a parallel increase in eosinophil count.¹⁸ Whether the CD4⁺ cell-eosinophil pathway comes into operation seems to depend on the biologic activity of T_H2-type cytokines.

Suplatast tosilate might inhibit the T_H2 cell-eosinophil pathway, considering that it inhibits the antigen-challenged production of IL-4 and IL-5 by murine and human T_H2-like clones.⁸ Our present study provides clinical evidence that, in addition to decreasing eosinophils and EG2⁺ cells to a notable extent, as previously reported,¹⁹ suplatast tosilate also inhibits the T_H2 cell-eosinophil pathway. It

has recently been reported that suplatast tosilate is effective against chronic eosinophilic pneumonia.^{20,21} Presumably, suplatast tosilate modifies this disease by means of the same mechanism as that found during allergic inflammation in asthma. Recent studies indicate that it inhibits AHR and late asthmatic response in sensitized animals.^{22,23} The results imply that suplatast tosilate inhibits a process other than mast cell degranulation, probably by inhibiting eosinophil-T cell interaction.

In the present study it was found that CD4⁺ and CD25⁺ T lymphocytes significantly decreased in number after treatment with suplatast tosilate, along with a similar decrease in the number of eosinophils. This result suggests that this compound might suppress eosinophil infiltration as a result of T-cell inhibition. In contrast, there was a significant increase in CD8⁺ T lymphocytes, suggesting the possibility that suplatast tosilate might inhibit CD4⁺ T lymphocytes by increasing CD8⁺ T lymphocytes. The exact mechanism of this T-cell interaction remains to be elucidated.

Recently, suplatast tosilate at a low concentration has been demonstrated to suppress eosinophil migration stimulated by IL-5.²⁴ Moreover, one of the authors has recently observed that suplatast tosilate induces apoptosis of eosinophils (K.O., manuscript in preparation). Although the mechanism is not yet fully elucidated, suplatast tosilate might reduce the number of eosinophils in the bronchial mucosa and in the sputum through such direct action of the agent on eosinophils.

Tamaoki et al²⁵ reported it was possible to reduce the dose of inhaled corticosteroid administered to steroid-dependent asthmatic patients if combined with suplatast tosilate. Although bronchial biopsy and sputum collection were not performed in their study, they reported decreased concentrations of serum eosinophil cationic protein in the suplatast group. Thus the steroid-sparing effects of

suplatast tosilate might be a result of T_H2 -type cytokine inhibition and subsequent eosinophil suppression.

Today, it is believed that to inhibit airway inflammation as early as possible, it is important to prevent mild asthma from becoming serious and refractory.²⁶ The results of our study indicate that suplatast tosilate holds promise as a drug that can be used to treat cases of mild asthma by inhibiting T_H2 cell-mediated inflammation. If administered with or instead of a steroid inhalant, suplatast tosilate might effectively attenuate asthma symptoms, and furthermore, it might prevent airway remodeling in mild asthma.

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