

Cromolyn sodium prevents bronchoconstriction and urinary LTE₄ excretion in aspirin-induced asthma

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Background: Inhalation of cromolyn sodium protects against sulpyrine-induced bronchoconstriction and prevents urinary leukotriene E₄ (u-LTE₄) excretion in aspirin-induced asthma.

Objective: This study was designed to investigate the protective effect of cromolyn sodium on airway responsiveness to the sulpyrine provocation test, and to investigate whether this protective activity is associated with a reduction in aspirin-induced urinary excretion of LTE₄, a marker of the cysteinyl leukotriene overproduction that participates in the pathogenesis of aspirin-induced asthma.

Methods: We evaluated the effects of pretreatment with cromolyn sodium on bronchoconstriction precipitated by inhalation of sulpyrine in ten adult patients with mild aspirin-induced asthma. Those who were in stable clinical condition and were hyperresponsive to sulpyrine provocation test were allocated to this study. Urinary leukotriene E₄ was measured using combined reverse phase high performance liquid chromatography (rp-HPLC)/enzyme immunoassay.

Results: Inhaled cromolyn sodium protects against aspirin-induced attacks of asthma through mechanisms not related to the bronchodilator property, but related to the improvement of the bronchial hypersensitivity, almost completely in all patients ($P < .001$). By contrast, after cromolyn sodium the maximum level of u-LTE₄ was significantly lower than control ($P < .05$).

Conclusion: Our results suggest for the first time that inhaled cromolyn sodium is one of the most useful inhibitors of aspirin-induced bronchoconstriction, probably acting by inhibiting the release of cysteinyl leukotrienes, and possibly other chemical mediators, by bronchial inflammatory cells.

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INTRODUCTION

Acetylsalicylic acid and other nonsteroidal anti-inflammatory agents with anti-cyclooxygenase activity induce severe, sometimes life-threatening

asthmatic attacks in about 10% of adults with asthma.^{1,2} Although aspirin-induced asthma has been recognized as a clinical entity since the beginning of this century, the exact pathologic mechanism for the production of this syndrome still remains obscure. Current evidence indicates that cyclooxygenase inhibition and increased production of sulfidopeptide leukotrienes play important roles in these obstructive reactions.

Cromolyn sodium is one of the most useful nonsteroidal therapeutic drugs for the treatment of bronchial asthma on infiltration of inflammatory cells.³ In vitro, cromolyn sodium inhibits histamine release from mast cells,⁴ the activation of inflammatory cells in-

cluding neutrophils, eosinophils and monocytes,⁵ antibody-dependent granulocyte-mediated cytotoxicity,⁶ and inhibits IgE production.⁷ In vivo, treatment of asthmatic patients with cromolyn sodium decreased eosinophils and house dust mite-specific IgE in bronchoalveolar lavage.⁸ These properties indicate that cromolyn sodium can modulate immune responses both in vitro and in vivo. Further, cromolyn sodium is also effective in nonatopic asthma, such as exercise-induced asthma⁹ and aspirin-induced asthma.^{3,10-12} The inhibitory action of cromolyn sodium on aspirin-induced bronchoconstriction is well documented, but its mechanism is less well defined. The aim of the present study is to confirm previous observations on the protective activity of inhaled cromolyn sodium against aspirin-induced asthma and to investigate the mechanism of protective effect of cromolyn sodium. In addition, this study was designed to examine whether this protective activity is associated with a reduction in urinary-aspirin-induced (u-LTE₄), a marker of the cysteinyl leukotriene overproduction that participates in the pathogenesis of aspirin-induced asthma.

METHODS

Subjects

We studied ten adult patients with clinical histories of aspirin-induced asthma. Sulpyrine and lysine aspirin and/or tolmetin sodium provocation tests were positive in all of them. All patients were diagnosed with mild or moderate asthma according to both Japanese Allergology Society's criteria and American Thoracic Society's criteria.¹³ The patients

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were in stable condition and had been free of symptoms of respiratory infections for at least 6 weeks (Table 1). Two patients required regular oral steroid medications (both less than 5 mg/day). Cromolyn sodium (20 mg/2 mL (1A), Fujisawa, Osaka, Japan) was stopped 2 weeks before this study. Other treatments were all withheld for at least 12 hours before the challenge test, except for slow release theophylline, which was withheld at least 24 hours in advance.¹⁴ The study was conducted according to the ethical standards of our medical center, which require informed consent from each patients.

Study Design

Bronchial challenge with sulpyrine was performed according to the standard method of Japanese Allergology Society as previously described.¹⁵ All patients were given this provocation test twice after pretreatment with cromolyn sodium or 2 mL of saline as placebo. The sulpyrine provocation test with inhaled placebo was performed as the preliminary test. At least seven days after the preliminary test, the patients were given the other challenges.¹⁶ Each study day began with inhalation of nebulized saline to rule out nonspecific obstructive reactions; FEV₁ was measured 5 minutes before, and 5, 10, and 15 minutes after completion of inhalation. If FEV₁ did not fall more than 10%, the subject entered the proper study. All patients underwent a sulpyrine bronchial provocation test on two occasions, separated by at

least seven days. The first sulpyrine provocation test was preceded by inhalation of 2 mL of saline as placebo, the second sulpyrine provocation test was preceded by inhalation of cromolyn sodium. Five minutes after the inhalation, spirometry was performed. Then the provocation test with inhaled sulpyrine was begun, and the mean provocative dose of sulpyrine that caused a 20% fall in FEV₁ (PD₂₀) was determined. Strictly speaking, PD₂₀ is used when concentrations are described, it is impossible to measure the value of PD₂₀ utilized in our usual clinical protocol of sulpyrine inhalation test. As we usually perform sulpyrine inhalation tests to diagnose aspirin-induced asthma in accordance with standard protocol of our medical center, this representation is for the sake of convenience. This expression can be interpreted in a general sense. Spirometry was performed and recorded at 5, 10, 15, and 30 minutes after ending any inhalation. The provocation test was terminated by the administration of an inhaled bronchodilator if FEV₁ fell by more than 35% from baseline. The higher of the first two satisfactory recordings was taken for analysis.

Urine was collected before challenge, at 2-hour intervals, and 4-hour intervals thereafter and stored at -20 °C until assay.

Sulpyrine Provocation Test

This test was performed using a jet nebulizer driven by compressed air (Nihon Shoji Co., Ltd, Osaka, Japan).

Fresh pulverized sulpyrine (Astra Japan, Inc, Osaka, Japan) was dissolved each day in saline to produce the concentrations of reagents. The sulpyrine provocation test was performed at the following concentrations: 10, 20, 100, 250, 500, and 1250 mg/mL except for one subject. These concentrations are in accordance with our daily protocol of sulpyrine in inhalation test to diagnose aspirin-induced asthma. As one subject was too sensitive to challenge at these doses of sulpyrine, the sulpyrine provocation test was performed at 4 and 10 mg/mL. Subjects were asked to inhale the sulpyrine solution from 10 or 4 mg/mL for three minutes. If FEV₁, measured at the time points as mentioned above after the inhalation, did not drop by more than 10% of the initial values, a higher concentration of sulpyrine was given. The provocation was stopped when FEV₁ fell to ≥20% from the baseline or when the maximum dose of sulpyrine was reached. Dose-responses for sulpyrine were constructed and used for calculation of PD₂₀. One patient failed to produce a 20% decrease in FEV₁ after inhalation of the highest sulpyrine dose. For statistical evaluation, their PD₂₀ values were set as equal to the highest cumulative dose of sulpyrine given.

These tests were performed on a flow-integrating computerized pneumotachograph (Discom-21FX II, Chest M.I. Co, Ltd, Tokyo, Japan).

Table 1. Patient Characteristics at Time of Entry into the Study*

Patient No†	Sex	Age, yr	Atopy	FEV ₁ , % pred	FVC, % pred	Eosino, %	Medication
1	M	36	HD, Gr	66	98	8	T, B
2	M	32	Cd	60	96	4	IS, T, B
3	M	20	HD, C, Gr	77	120	7	IS, B
4	M	45	none	54	92	5	T, B
5	F	26	Cd	61	104	4	OS, B
6	F	48	none	67	94	5	T, B
7	F	40	none	68	84	4	T, B
8	F	42	none	52	96	6	OS, T, B
9	F	54	HD, Cd	74	121	10	T, B
10	F	56	none	51	79	11	T, B

* HD, house dust; C, cat; Gr; grass; Cd, candida; T, theophylline; B, Inhaled β_2 -agonist on demand; IS, inhaled steroid; and OS, oral steroid.

† Patient numbers do not indicate the sequence of entry into the study.

Analysis of u-LTE₄

Urinary LTE₄ was measured using compound rp-HPLC (Class LC-10 system: Shimadzu, Kyoto, JAPAN)/enzyme immunoassay as previously described.¹⁷ ³H-LeukotrieneE₄ (168.4 Ci/mmol.; NEN, DuPont, Dreierich, Germany) was used as internal standard; anti-LTE₄ antibody (Cayman Chem, Ann Arbor, MI) showed a cross reactivity of 10% to 25% versus LTC₄-LTD₄ and <0.01% versus LTB₄. Creatinine concentration was measured using a standard assay kit (Sigma, St. Louis, MO) and the results, corrected according to the observed recovery, were expressed as the amount of LTE₄ per mg of creatinine.

Statistical Analysis

Statistical evaluation was performed using a StatView J-4.02 for Macintosh (Abacus Concepts, Inc, Berkeley, CA).

All PD₂₀ values were logarithmically transformed before analysis, and summary statistics were expressed as mean ± standard deviation (SD). Student's paired t test was used to compare mean differences. Wilcoxon signed-rank test were used for comparison of the two treatment groups at the same time point regarding FEV₁ and u-LTE₄ values. A P value less than or equal to .05 (two-sided) was considered significant.

RESULTS

None of the patients had a severe obstructive reaction to sulpyrine, and all could be followed for longer than 120 minutes. Bronchial responses to inhaled cromolyn sodium are presented in Table 2. Baseline FEV₁ and FVC were not significantly affected by inhalation of either cromolyn sodium or

Table 2. Bronchial Responses in Baseline FEV₁ and FVC Affected by Inhalation of Placebo and Cromolyn Sodium

DSCG* (-)	DSCG (+)	
	Before	After
FVC, L	3.25 ± 0.82	3.11 ± 0.93
FEV ₁ , L	2.08 ± 0.62	2.07 ± 0.85

* DSCG = cromolyn sodium.

placebo. The results showed that we have not confirmed a bronchodilator effect with cromolyn sodium in this study. After pretreatment with cromolyn sodium or placebo, challenge with sulpyrine caused an obstructive reaction in all patients. The data of PD₂₀ in all patients are displayed in Table 3. After inhalation of cromolyn sodium, PD₂₀ values were obviously improved in all patients. Furthermore, all PD₂₀ values transformed logarithmically were elevated significantly (Table 3, Fig 1). Mean log PD₂₀ was 2.08 ± 0.45 (SD) after cromolyn sodium versus 1.22 ± 0.47 before cromolyn sodium (*P* < .001). The results indicated that inhalation of cromolyn sodium protects against sulpyrine-induced bronchoconstriction almost completely.

Data for preplacebo and postplacebo PD₂₀ are not significantly different on the two days (placebo and cromolyn sodium) (data not shown). Mean baseline u-LTE₄ was 902 ± 242 picogram (pg)/mg creatinine before cromolyn sodium versus 924 ± 258 pg/mg creatinine on the placebo day (not significant). After placebo, challenge with sulpyrine caused a significant increase in u-LTE₄, reaching a maximum value of 2296 ± 528 pg/mg creatinine. (Fig 2, *P* < .05 compared with baseline). By contrast, after cromolyn sodium, the maximum level of u-LTE₄ was 788 ± 252 pg/mg creatinine, which was significantly lower than placebo (*P* < .05)

Table 3. Individual Data for PD₂₀ Sulpyrine.

Patient No.	PD ₂₀ -Sulpirine, mg/mL	
	Placebo	DSCG
1	10	100
2	10	100
3	10	250
4	20	100
5	20	100
6	20	100
7	250	1250
8	10	100
9	20	100
10	4	20

DSCG (+): cromolyn sodium and DSCG (-): control.

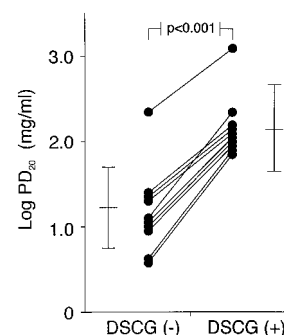


Figure 1. Individual results of PD₂₀ sulpyrine at baseline period and after cromolyn sodium. DSCG(+), cromolyn sodium and DSCG(-), control. Both results are shown in mean with the bar indicating SD. All PD₂₀ values were logarithmically transformed before analysis. Summary statistics were expressed as mean ± SD.

but not significantly different from baseline. Although the total dose of sulpyrine was higher on the cromolyn sodium day, u-LTE₄ is significantly lower than the placebo day. No significant correlation was observed between the maximum fall in FEV₁ or the concentration of sulpyrine inhaled and the corresponding maximum increasing in u-LTE₄ after placebo, or between the protection afforded by cromolyn sodium on FEV₁ decrease and u-LTE₄ excretion.

DISCUSSION

Although the mechanism of aspirin-induced asthma remains unproved, it is a widely accepted hypothesis that bronchial overproduction of leukotrienes and inhibition of prostaglandin synthesis are involved in the pathogenesis of aspirin-induced asthma.^{3,18-20} The inhibitory effect of acetylsalicylic acid on cyclooxygenase increases formation of bronchoconstrictor leukotrienes via shunting of unmetabolized arachidonic acid into metabolism by the 5-lipoxygenase. The severity and rapidity of bronchospasm induced by cyclooxygenase-inhibiting drugs in aspirin-induced asthma is directly related to the dose and to the potency of the drug to inhibit the cyclooxygenase. Knapp et al²¹ showed that excretion of LTE₄ was increased during aspirin-induced asthma episodes, but the degree

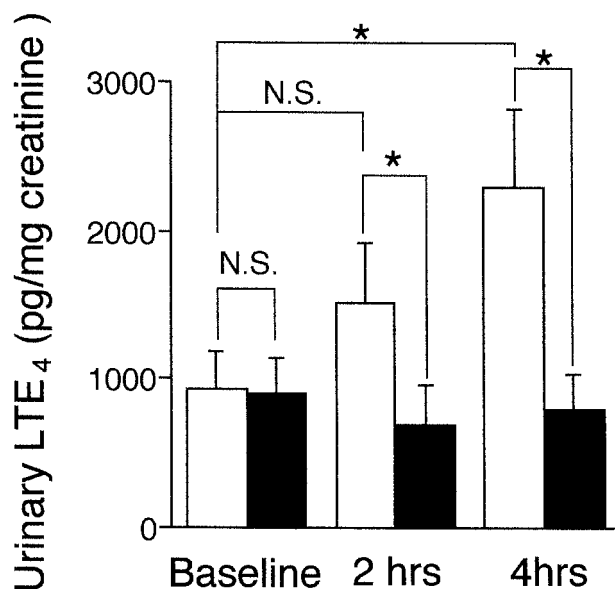


Figure 2. Mean urinary LTE_4 before and during the observation period after placebo (white bars) or cromolyn sodium (black bars). Asterisks indicate statistically significant difference: * $P < .05$. NS: not significant.

of increase for individual patients did not correlate with the degree of bronchospasm or inhibition of platelet thromboxane B_2 formation. The results indicate that although the endogenous synthesis of potent bronchoconstrictor leukotrienes increases during aspirin-induced bronchospasm, it is unlikely that a direct shunting of unmetabolized arachidonate into leukotriene synthesis represents the mechanism of aspirin-induced asthma.

Our results indicate that pretreatment with cromolyn sodium effectively attenuates aspirin-precipitated bronchoconstriction as previously described.^{3,10-12} Although some previous reports indicated cromolyn sodium may have a bronchodilating effect,²² we have not confirmed the bronchodilator effect with cromolyn sodium in this study (Table 2). We demonstrated that inhaled cromolyn sodium protects against bronchoconstriction of aspirin-induced asthma through mechanisms not related to bronchodilating properties, but related to improvement in the hypersensitivity that is evident in almost all patients. By contrast, after cromolyn sodium, the maximum level of u-LTE_4 was significantly lower than

the control. Data for preplacebo and postplacebo PD_{20} on placebo day and preplacebo PD_{20} on cromolyn sodium day are not significantly different (data not shown), but postcromolyn sodium PD_{20} was higher on the cromolyn sodium day (Table 3). These findings confirm that cromolyn sodium is highly effective in preventing aspirin-induced asthma and suggest that this effect is mediated by inhibition of sulfidopeptide leukotriene production. The observation that cromolyn sodium also abolishes sulpyrine-induced excretion of u-LTE_4 , a relatively stable metabolite of cystinyl leukotrienes, strongly suggests that the protective activity is due to inhibition of release of chemical mediators from bronchial inflammatory cells. There is considerable evidence from pharmacologic studies²³ and studies on excretion of u-LTE_4 ²⁴⁻²⁶ and bronchoalveolar lavage²⁷ that the main inflammatory mediators associated with aspirin-induced bronchoconstriction are cysteinyl leukotrienes that probably originate from eosinophils and mast cells *in vivo*.²⁸

The mechanism of cromolyn sodium on aspirin-induced asthma still remains uncertain. The protective activity of

cromolyn sodium is not limited to aspirin-induced asthma but has also been shown to be extremely effective in non-aspirin-sensitive patients against many other kind of stimuli, as mentioned above, and effectively protects against the early and late asthmatic reactions to allergen.²⁹ The late asthmatic reaction is not associated with increased excretion of u-LTE_4 ³⁰ and is not affected by 5-lipoxygenase inhibitors,³¹ which effectively protect against aspirin-induced asthma.^{23,25} This indicates that the effect of cromolyn sodium *in vivo* is not limited to the inhibition of leukotriene release, but also affects the release of other inflammatory inhibitors. The mechanism of cromolyn sodium on aspirin-induced asthma still remains uncertain. Previous reports showed that cromolyn sodium inhibits eosinophil function,^{32,33} activation of neutrophil following anti-IgE anaphylaxis,³⁴ and mast cell secretion.³⁵ Hoshino et al³⁶ suggested that cromolyn sodium inhibits the expression of adhesion molecules as an anti-inflammatory action, and decreases the number of inflammatory cells in the airways of asthmatic patients. In addition, Kay et al³⁷ indicated that cromolyn sodium directly inhibits the secretory properties of inflammatory cells and that in turn might have important implications in modulating mechanisms contributing to the inflammatory component of asthma and allergic disease. These studies indicate that cromolyn sodium directly inhibits the secretory properties of inflammatory cells and that in turn might have important implications in modulating mechanisms contributing to the inflammatory component of asthma and allergic disease. It may also help to explain why compounds with considerably greater mast cell stabilizing properties than cromolyn sodium have been so disappointing when they are evaluated clinically.

On the other hand, several previous reports suggested that cromolyn sodium inhibits bronchial hypersensitivity through tachykinin antagonist properties.³⁸⁻⁴¹ Tachykinins, in particular neurokinin A, neurokinin B, and substance

P, produce a number of airway effects that may contribute to asthma and other allergic diseases, such as bronchial eosinophilia,⁴² smooth muscle hyperproliferation,⁴³ and inducing the production of inflammatory metabolites on alveolar macrophages.⁴⁴ Tachykinins also have been identified in the respiratory tract and implicated in mediating neurogenic inflammation of the airways. Airway neurogenic inflammation is caused by neuropeptides released from airway sensory nerves, and may be involved in the pathogenesis of asthma.^{45,46} In this study, our results obtained through cromolyn sodium treatment of patients with aspirin-induced asthma are unique in that the inhalation of cromolyn sodium protects against sulpyrine-induced bronchoconstriction and prevents u-LTE₄ excretion at statistically significant levels, the results resembling the effect of inhaled PGE₂ reported by Sestini et al.⁴⁷ The results of this study suggest that the protective activity against sulpyrine-induced bronchoconstriction depends upon interaction between cromolyn sodium and the PGE₂ system in aspirin-induced asthma. Some previous studies report that tachykinin modifies arachidonic acid metabolism, induces a significant increase in PGD₂, PGE₂, and thromboxane B₂ synthesis.^{44,48} On the contrary, PGF_{2α} causes the release of tachykinin;⁴⁹ furthermore bradykinin and tachykinin cause indirect bronchoconstriction by liberation of leukotrienes.⁵⁰ These findings and our results suggested that the efficacy of cromolyn sodium in aspirin-induced asthma may be due to inhibition of the production of leukotrienes through tachykinin antagonist properties. The question of whether neurogenic inflammation is involved in aspirin-induced asthma remains unanswered. To determine the exact relationship among neurogenic inflammation that is involved in tachykinin, arachidonic acid metabolism, and pathogenesis of aspirin-induced asthma, additional studies will be required.

In addition, Stevenson et al reported that during oral challenges with acetylsalicylic acid bronchoconstriction occurred routinely despite pretreatment with 40 mg of cromolyn sodium.⁵¹ In-

halation of sulpyrine is a relatively weak stimulus to mast cell discharge and LT formation when compared with oral acetylsalicylic acid challenges. The difference between inhalation challenge with sulpyrine and oral challenge with full therapeutic doses (650 mg) of acetylsalicylic acid should be made clear; our data do not indicate that cromolyn would afford protection against a therapeutic dose of aspirin in a patient with a history of aspirin-induced asthma.

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