

Tacrolimus reduces urinary excretion of leukotriene E₄ and inhibits aspirin-induced asthma to threshold dose of aspirin

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Background: The exact mechanism of aspirin-induced asthma is not clear. It has been postulated that precipitation of asthma attacks by aspirin is linked to inhibition of COX activity and massive release of cysteinyl leukotriene into the airway.

Tacrolimus, a macrolide-derived immunosuppressant, is used for immunosuppression in organ transplantation and also for allergic diseases such as atopic dermatitis.

Objective: We evaluated the effects of tacrolimus in aspirin-induced asthma by using a double-blind, crossover study design.

Methods: Twelve patients with aspirin-induced asthma (male:female, 3:9; mean age $\pm$ SD, 36.7 $\pm$ 7.2 years) received either tacrolimus (0.1 mg/kg) or placebo 2 hours before the threshold dose of oral aspirin.

Results: In the placebo arm, oral aspirin significantly decreased FEV₁ concomitant with significant increases in sputum eosinophilic cationic protein and urinary leukotriene E₄ levels. Tacrolimus significantly inhibited bronchoconstriction and abrogated aspirin-induced increase in both sputum eosinophilic cationic protein and urinary leukotriene E₄ levels.

Conclusion: The current study suggested that tacrolimus inhibited bronchoconstriction to a threshold dose of aspirin by inhibition of cysteinyl leukotriene excretion. (J Allergy Clin Immunol 2004;114:1278-81.)

Key words: Aspirin-induced asthma, tacrolimus, leukotriene eosinophilic cationic protein, 11-dehydrothromboxane B₂

A subset of patients with asthma is susceptible to disease exacerbation on ingestion of acetylsalicylic acid (ASA; aspirin) and other nonsteroidal anti-inflammatory drugs (NSAIDs). Although the exact mechanism of this distinct clinical syndrome, aspirin-induced asthma (AIA), has not been determined, it has been proposed that overproduction of cysteinyl leukotrienes (cysLTs), together with removal by ASA/NSAIDs of the brake

Abbreviations used

AIA: Aspirin-induced asthma

ASA: Acetylsalicylic acid

cysLT: Cysteinyl leukotriene

11-dhTXB₂: 11-Dehydrothromboxane B₂

ECP: Eosinophilic cationic protein

LTE₄: Leukotriene E₄

MRP: Multidrug resistance-associated protein

NSAID: Nonsteroidal anti-inflammatory drug

imposed by the bronchodilator prostaglandin E₂, may cause an asthma attack in patients with AIA.^{1,2}

Bronchial asthma is characterized by chronic airway inflammation, in which several cytokines and chemical mediators are involved.³⁻⁵ Corticosteroids inhibit the production of many inflammatory cytokines and recently were considered the first-line therapy for asthma.³ However, asthma is not always completely controlled even by high doses of inhaled corticosteroids, and corticosteroids cannot inhibit the release of chemical mediators.⁶ In contrast, tacrolimus, a macrolide-derived immunosuppressant, inhibits the release of chemical mediators, including histamine and cysLTs.⁷ Tacrolimus inhibits the production of cysLTs from eosinophils,⁸ neutrophils,⁹ and passively sensitized human lung tissues.⁷ Furthermore, tacrolimus has been used to prevent rejection in organ transplantation.^{10,11} Recently, tacrolimus was also used for some immune-mediated diseases, including atopic dermatitis^{8,12} and myasthenia gravis.¹³

On the basis of the above pharmacologic properties of tacrolimus, eg, inhibitory effects on cysLTs, we hypothesized that tacrolimus can inhibit aspirin-induced bronchoconstriction in AIA. Our results indicate that tacrolimus inhibits aspirin-induced bronchoconstriction via inhibition of cysLT excretion.

METHODS

Subjects

Twelve patients with AIA, admitted to the Second Department of Internal Medicine, Nagasaki University School of Medicine, were enrolled in the current study. Table I summarizes patient character-

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istics. Patients were 3 males and 9 females with an average age of 36.7 years. Bronchial asthma was diagnosed according to the Global Initiative for Asthma guidelines.¹⁴ Atopy was defined by a positive skin prick test using 10 common aeroallergens (Torii, Tokyo, Japan) and/or IgE (RAST), and 7 patients were atopic. All patients had a history of asthma attacks occurring within 2 hours after the intake of ASA/NSAID medication, orally or as suppositories. AIA was also diagnosed by oral aspirin provocation test as described below. All patients were in a stable condition and had been free of symptoms of respiratory infection for at least 8 weeks before enrollment in the current study. None of the patients had other diseases that could influence the immune system, and none was on oral steroids or cysLT modifiers. None took aminophylline or antihistamines within 48 hours before the study. Before the aspirin provocation test, subjects were instructed to withhold inhaled corticosteroids for 48 hours and inhaled adrenergic agents for 24 hours. The current study was approved by the ethics committee of Nagasaki University Hospital, and written informed consent was obtained from each patient.

Oral aspirin provocation test

Oral aspirin provocation test was performed by using the method described in our previous report.¹⁵ Clinical examination confirmed the lack of dyspnea, wheezing, or rhonchi even on forced expirations on the day of the test. The baseline FEV₁ was measured before the test with a spirometer (Superspiro, DISCOM-21FX; Chest MI Co, Tokyo, Japan) and was confirmed to be >80% of the predicted value. After oral provocation test of aspirin (5, 10, 20, 50, 100, 250, 500 mg), FEV₁ was measured at 15, 30, 60, 90, and 120 minutes after the oral consumption of aspirin. In patients who showed <15% fall in FEV₁ from baseline even at 120 minutes after the oral challenge, the oral challenge test was repeated by using the next higher dose of aspirin. In patients who showed >20% fall in FEV₁ from baseline or >15% fall associated with worsening of asthma-related symptoms, the test was considered positive, ie, the patient was considered to have AIA, and the dose of aspirin was considered the threshold dose.

Study design

The study design was a randomized, placebo-controlled, double-blind, crossover study. After the initial aspirin oral provocation test, all eligible patients (n = 12) entered a 1-week washout period. Thereafter, patients were randomly assigned to receive either placebo (n = 6) or tacrolimus (Prograf; Fujisawa Co, Osaka, Japan) at 0.1 mg/kg body weight (n = 6) 2 hours before the second aspirin oral provocation test using the threshold dose of aspirin. After the second aspirin oral provocation test, all patients entered another 1-week washout period, and then the third aspirin oral provocation was performed in a crossover manner. Table I shows the dose of tacrolimus administered to each patient. Samples of peripheral blood, sputum, and urine were obtained from all patients before the test and when the patient showed a positive reaction. When the patient did not show any positive reaction to the threshold dose of aspirin by tacrolimus, samples were obtained at the same time of the attack of the first provocation test.

Sputum processing

All subjects were instructed to gargle by using tap water before sputum collection to reduce the possible effect of contamination. Subjects who could not cough up sputum received nebulized saline via an ultrasonic nebulizer (NE-U12; OMRON Co, Tokyo, Japan) to induce sputum production. Subjects were instructed to cough deeply after intervals of 3 to 5 minutes, and sputum samples were obtained from each patient. Sputum samples were processed as described in our previous reports,^{15,16} and the slides were stained with May-Grunwald-Giemsa for differential cell counts. The sample was

TABLE I. Patient characteristics

Number	Age (y)	Sex	Atopy	Step	Threshold dose of aspirin (mg)	Provocation dose of tacrolimus (mg)
1	31	F	Yes	3	250	5.5
2	48	F	Yes	3	100	6.0
3	25	F	Yes	2	250	5.0
4	48	F	Yes	3	20	4.5
5	37	F	Yes	4	20	6.5
6	31	F	No	3	100	5.0
7	32	M	Yes	3	100	6.0
8	35	F	No	2	250	5.0
9	32	M	Yes	2	100	8.0
10	44	F	No	3	50	6.0
11	39	F	No	3	100	6.0
12	38	M	No	2	250	6.5

diluted with 2 mL Hank's balanced salt solution containing 1% dithiothreitol (Sigma Chemicals, Poole, United Kingdom) and gently vortexed at room temperature. Samples were then centrifuged at 400g for 15 minutes at 4°C, and the supernatant was kept at -80°C for subsequent eosinophil cationic protein (ECP) assays.

Measurement of eosinophilic cationic protein, cysLTs, and 11-dehydrothromboxane B₂

Sputum levels of ECP, urinary LTE₄, and urinary 11-dehydrothromboxane B₂ (11-dhTXB₂) were determined by using radioimmunoassay (Pharmacia ECP RIA, Pharmacia, Uppsala, Sweden; peptidyl-leukotriene RIA kit, NEK-043 NEN, Life Science Products, Lansing, Mich; and thromboxane B RIA kit, DuPont, NEN, Boston, Mass).^{15,16} The collected urine samples were immediately frozen and stored at -80°C for the assay according to the instructions manual of the kit. Each sample was measured 3 times, and the average value was reported. The lower detection limits of ECP, cysLTs, and 11-dhTXB₂ were 2.0 µg/mL, 10 pg/mL, and 3.0 pg/mL, respectively. The concentration of urinary creatinine was also determined for correction reference.

Measurement of tacrolimus concentration in peripheral blood

Peripheral blood was obtained from each patient 4 hours after the oral administration of tacrolimus. The concentration of tacrolimus was measured by an enzyme immunoassay (Tacrolimus-II Dainapack; Abbott Japan, Tokyo, Japan).

Statistical analysis

Data were expressed as means ± SDs. Changes in FEV₁ and concentrations of chemical mediators were analyzed by using a 2-tailed Student *t* test with the level of significance (*P* value) set at .05.

RESULTS

Tacrolimus inhibits oral aspirin-induced bronchoconstriction

All subjects showed significant (*P* < .01) reduction of FEV₁ (mean, 22.4%) after placebo treatment. This fall in FEV₁ was completely inhibited in all subjects by admin-

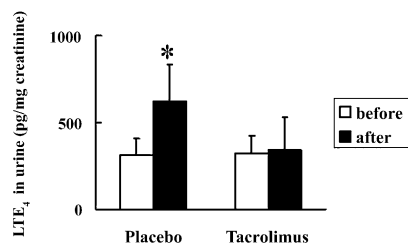


FIG 1. Changes in urinary LTE_4 before and after oral aspirin provocation test in patients pretreated with placebo or tacrolimus. Data are means $\pm$ SDs. * $P < .01$ vs. before the aspirin challenge test.

istration of tacrolimus. In the placebo arm, all subjects developed wheezing, as well as intense rhinorrhea, nasal congestion, congestion of the conjunctiva, and periorbital edema. In the tacrolimus arm, neither nasal nor ocular reactions occurred.

Tacrolimus inhibits oral aspirin-induced rise in sputum ECP level

There was no significant change in eosinophil count in the sputum between baseline and postaspirin challenge. There was no difference in serum ECP between baseline and postaspirin challenge, irrespective of treatment with or without tacrolimus (data not shown). In the placebo arm, oral aspirin provocation resulted in a significant increase in sputum ECP (baseline, $79.2 \pm 11.3 \mu\text{g/mL}$; postaspirin challenge, $157.8 \pm 34.2 \mu\text{g/mL}$; $P < .01$). This difference disappeared in the tacrolimus arm (baseline, $75.8 \pm 11.5 \mu\text{g/mL}$; postaspirin challenge, $76.1 \pm 14.3 \mu\text{g/mL}$; $P > .1$).

Tacrolimus inhibits urinary LTE_4 excretion after oral aspirin provocation

Aspirin oral provocation significantly ($P < .01$) increased urinary LTE_4 in the placebo arm. Tacrolimus significantly ($P < .01$) inhibited the aspirin-induced rise in urinary LTE_4 (Fig 1). On the other hand, aspirin oral provocation significantly ($P < .01$) reduced urinary 11-dhTXB₂ based on its inhibition of COX activity. Neither placebo nor tacrolimus significantly altered the urinary 11-dhTXB₂ excretion level (Fig 2). These results suggest that tacrolimus inhibits cysLTs excretion without affecting COX activity.

Tacrolimus has no adverse effects

Careful physical and blood examinations found no significant adverse effects in any subjects. The mean concentration of tacrolimus in peripheral blood was $6.8 \pm 0.9 \text{ ng/mL}$ and did not exceed 10 ng/mL in any of the subjects. Previous reports revealed the safety and efficacy of this concentration.¹⁷⁻¹⁹

DISCUSSION

The current study examined the effects of tacrolimus in AIA and found that a single pretreatment with tacrolimus

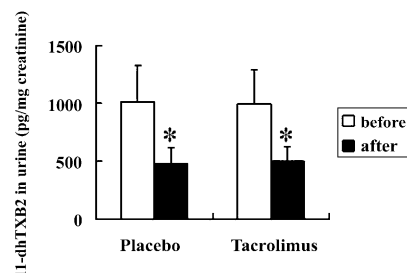


FIG 2. Changes in urinary 11-dhTXB₂ before and after oral aspirin provocation test in patients pretreated with placebo or tacrolimus. Data are means $\pm$ SDs. * $P < .01$ vs. before the aspirin challenge test.

at 0.1 mg/kg body weight (1) completely inhibited the fall in FEV₁ and clinical symptoms after oral provocation of the threshold dose of aspirin and (2) significantly inhibited aspirin-induced increase in sputum ECP and urinary LTE_4 levels. It should be emphasized that the current study used only the threshold dose of aspirin, suggesting that giving a full dose of ASA/NSAIDs after tacrolimus treatment might cause an asthma attack. In addition, subjects in the current study had relatively mild AIA, because we chose subjects who could stop controller medications. Thus, we cannot exclude the possibility that tacrolimus may not inhibit asthma attacks in patients with a more severe form of AIA.

The exact mechanism of AIA is still unclear. Although several other mediators are involved, cysLTs represent an important compound in the mechanism of AIA. It has been postulated that precipitation of asthma attacks by aspirin is linked to inhibition of COX activity associated with a massive release of cysLTs into the airway.²⁰ Overproduction of cysLTs occurs mainly on the surface of eosinophils and macrophages in the airway when the COX activity on the arachidonic acid cascade is suppressed by ASA/NSAIDs.²⁰ To date, leukotriene modifiers are available to inhibit cysLT activity *in vivo*, and several reports showed the usefulness of these drugs in the treatment of AIA.^{5,16} To our knowledge, the current study is the first to show that tacrolimus inhibits aspirin-induced bronchoconstriction in patients with AIA and that such an effect is associated with a reduction of aspirin-induced urinary LTE_4 excretion.

The oral provocation dose (0.1 mg/kg) of tacrolimus selected in our study was based on previous reports.¹⁷⁻¹⁹ In the current study, no patient developed any adverse effects by using this oral provocation dose.

The mechanism of inhibition of aspirin-induced bronchoconstriction by tacrolimus is still unknown. Long-term use of tacrolimus as an immunosuppressant shows anti-inflammatory/antiallergic effects via suppression of proinflammatory cytokines.²¹⁻²³ Tacrolimus reduces the expression of the high-affinity Fcε receptor on antigen-presenting epidermal dendritic cells.²⁴ These cells might be the targets of tacrolimus in allergic diseases. However, it is unlikely that tacrolimus inhibited airway inflammation as an immunosuppressant in the current study,

because it was orally administered only once and just 2 hours before the aspirin provocation. LTC₄ is released from leukotriene-synthesizing cells by an ATP-dependent export carrier and pump.²⁵⁻²⁷ Currently, this ATP-dependent export pump is known as multidrug resistance-associated protein (MRP). MRP plays a crucial role in multidrug resistance of malignant tumors.²⁸ Increased MRP levels in tumor cells cause multidrug resistance by decreasing the intracellular anticancer drug concentration.²⁸⁻³⁰ Interestingly, tacrolimus serves as a substrate for the pump³¹ and thus inhibits its transporter function.³¹⁻³³ Taken together, the aforementioned studies suggest that tacrolimus may inhibit aspirin-induced bronchoconstriction via inhibition of transporter function of MRP, which transports leukotriene from the cytoplasm to the extracellular space. Although the involvement of MRP in the inflammatory response has been reported,³⁴ no studies have evaluated the role of MRP in AIA. If patients with AIA show some abnormalities of MRP expression, the clinical strategy for AIA should be altered dramatically.

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