

Effects of pranlukast on aspirin-induced bronchoconstriction: differences in chemical mediators between aspirin-intolerant and tolerant asthmatic patients

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Background: Aspirin inhibits cyclooxygenase activity and modifies production of the arachidonate cascade in aspirin-induced asthma. The aim of the present study was to examine the effects of leukotriene (LT) receptor antagonist on aspirin challenge on eosinophil activity and chemical mediators released into the airway of asthmatic patients.

Methods: Aspirin oral provocation test was performed in aspirin-intolerant asthmatic patients (AIA; N = 7) and aspirin-tolerant asthmatic patients (ATA; N = 7). In AIA, LT receptor antagonist (pranlukast) was administered orally 2 hours before the test, and its inhibitory effects on sputum LTC₄+C₄, eosinophil cationic protein (ECP), eosinophil count, urinary LTE₄/creatinine (Cr), 11-dehydrothromboxane (11-dhTX) B₂/Cr, serum LTC₄+D₄, ECP, and peripheral blood eosinophil count were compared with the findings in ATA subjects.

Results: In AIA, aspirin induced an immediate reaction associated with increased urinary LTE₄/Cr and sputum ECP and a fall in urinary 11-dhTXB₂/Cr. Pranlukast inhibited the bronchial reaction and an increase in sputum ECP after threshold dosed of ASA, but failed to change aspirin-induced LT production in sputum and urine. In ATA, aspirin challenge was only associated with a fall in urinary 11-dhTXB₂.

Conclusions: Our results indicated that aspirin-induced asthma is associated with overproduction of LT with a shift to the 5-lipoxygenase series of the arachidonate cascade and that leukotriene receptor antagonist are useful for AIA through inhibition of production of LT and eosinophilic inflammation in the airway.

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INTRODUCTION

Aspirin-induced asthma constitutes approximately 10% of adult asthma cases.¹ The pathogenesis of aspirin intolerance is not yet clear.² All nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the production of prostaglandin or thromboxane A₂ and induce intolerance reactions in asthmatic patients sensitive to the drug.³ Recent studies have demonstrated that leukotrienes (LT) play an important role in airway

narrowing and other signs in aspirin-intolerant asthmatic patients (AIA), based on the findings that urinary LTE₄ was 2- to 10-fold higher in AIA than in aspirin-tolerant asthmatic patients (ATA).⁴⁻⁶ Several leukotriene modifiers inhibit the asthma response in oral or inhaled bronchial provocation tests (BPTs) with aspirin or NSAIDs⁷⁻¹⁰ and improve respiratory function by expanding the airway in AIA.¹¹

However, there are only a few studies that have examined the effects of LT modifiers on chemical mediators, eosinophils, eosinophil cationic protein (ECP), LT, and thromboxane A₂, on aspirin oral provocation. In the present study, we compared the changes in chemical mediators in the sputum,

urine, and blood in aspirin BPTs of AIA subjects treated with or without leukotriene receptor antagonist (LTRA; pranlukast) with those of ATA subjects.

METHODS

Subjects

After obtaining informed consent, seven inpatients with AIA and seven inpatients with ATA were enrolled in the present study (Table 1). The study was approved by the ethics committee of Nagasaki University Hospital. All patients were nonsmokers and had mild or moderate asthma based on the criteria of the updated National Heart, Lung, and Blood Institute Expert Panel II on Asthma.¹² All patients were in a stable condition and had been free of symptoms of respiratory infection for at least 6 weeks before enrollment in the study. Subjects were instructed to withhold inhaled adrenergic agents for 24 hours, aminophylline for 24 hours, antihistamines for 48 hours, and inhaled steroids for 48 hours before aspirin challenge. None were treated with LTRA.

Protocol

Each of the seven asthmatic patients completed two BPTs. BPT1 was a single-blind aspirin oral challenge without pretreatment with pranlukast. The baseline FEV₁ was measured with a spirometer (Superspiro, DISCOM-21FX; Chest M.I. Co, Tokyo, Japan) and was confirmed to be >80% of the predicted value (baseline FEV₁). Clinical examination confirmed a lack of dyspnea or wheezing and rhonchi even on forced expirations. Aspirin challenge com-

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menced at 10 mg. In cases where the fall in FEV₁ was <15% of the baseline 10, 30, 60, 90, and 120 minutes after oral challenge, BPT was repeated using the next higher dose of aspirin. The doses were 20, 50, 100, 250, and 500 mg. In cases where the fall in FEV₁ exceeded 20% of baseline, BPT was considered positive, and the test was terminated, and the dose termed as the threshold dose of aspirin. FEV₁ was measured every hour until 12 hours after the last aspirin challenge. All challenge tests commenced at 9:00 AM. The subject was classified as AIA when the threshold was 250 mg or less, or as ATA when there was no reaction even after taking 500 mg of aspirin. In patients diagnosed as AIA, the same regimen was repeated for pranlukast after a 7-day washout period. Based on blood levels and the half-lives of pranlukast and aspirin, 7-day intervals were considered necessary and adequate for washout. Pranlukast (Ono Pharmaceutical Co, Osaka, Japan) was administered at a dose of 225 mg at the same time with aspirin at a dose one rank less than the threshold, and it was confirmed that FEV₁ did not fall by ≥15%. Then, the aspirin threshold was measured. Induced sputum samples for LTs, ECP and eosinophil number, urine samples for LTE4, 11-dhTXB2 and creatinine (Cr), and blood samples

for LTs, ECP, and eosinophil counts were obtained at 1 hour before the test, at the time when FEV₁ fell by 20% on BPT1, and concomitantly on BPT2 in AIA and 30 minutes after administration of 500 mg of aspirin in ATA.

Baseline Lung Function Tests

FEV₁ was measured with a spirometer (Superspiro, DISCOM-21FX; Chest M.I. Co). Findings were expressed as the percentage of the predicted normal, based on relevant reference standards.¹³

Sputum Induction and Processing

When appropriate sputum for analysis could not be obtained during any phase, sputum induction was performed using as following method. Sputum was induced using the method described by Pin et al¹⁴ by inhalation of hypertonic saline. The subject was instructed to gargle using tap water, then 3.6% saline at room temperature nebulized via an ultrasonic nebulizer (NE-U12; OMRON Co, Tokyo, Japan) was inhaled. Subjects were instructed to cough deeply after 3- and 5-minute intervals. Gargling between and before each induced cough was encouraged to minimize salivary contamination. The initial sample from the first cough was discarded. Expectoration or induced sputum was collected into a 50-mL

polypropylene tube kept at 4° C and processed within 2 hours. Spirometric tests were repeated after sputum induction. If FEV₁ decreased below 15% of the postsalbutamol value, the subject was required to stay in the laboratory until FEV₁ returned to the baseline value.

The volume of the sample was recorded, and the sample was diluted with 2 mL of Hanks' balanced salt solution containing 1% dithiothreitol (Sigma Chemicals, Poole, United Kingdom) and gently vortexed at room temperature. When uniform in consistency, samples were further diluted with Hanks' balanced salt solution and again briefly vortexed. They were then centrifuged at 400 *g* for 15 minutes at 4° C, the supernatant was decanted, and the cell pellet was resuspended. An adequate specimen was defined as one in which the number of squamous epithelial cells was <30% of inflammatory cells. Slides were then stained with May-Grunwald-Giemsa for differential cell counts, performed by an observer blinded to the clinical characteristics of the subjects. Two or more slides were used for counting and at least 300 inflammatory cells were counted in each slide. The supernatant fluid was kept at -80° C for subsequent assay of ECP. To measure LTC4 and D4 levels, the supernatant were

Table 1. Clinical Features of Aspirin-Intolerant and Aspirin-Tolerant Asthmatic Patients

	Age (Years)	Gender	Baseline FEV ₁ (% predicted)	Type (A/NA)	Severity	Aspirin threshold (mg)
AIA						
Patient 1	51	F	83.4	NA	Mild	20
Patient 2	50	F	98.2	NA	Mild	50
Patient 3	25	F	87.3	A	Mild	100
Patient 4	31	F	92.0	NA	Mild	250
Patient 5	48	F	94.5	NA	Mild	100
Patient 6	25	F	82.4	NA	Moderate	250
Patient 7	48	F	90.8	NA	Moderate	20
ATA						
Patient 1	33	M	92.9	A	Mild	
Patient 2	46	M	88.1	NA	Mild	
Patient 3	27	M	80.9	A	Mild	
Patient 4	24	M	102.1	A	Moderate	
Patient 5	35	F	98.3	A	Mild	
Patient 6	33	F	90.1	A	Mild	
Patient 7	53	M	82.3	NA	Moderate	

M indicates male; F, female; A, atopy; NA, nonatopy.

immediately 4-fold diluted with an LT extract (ethyl acetate:methanol solution at 2:1) and cryopreserved at -80°C .

Measurement of LTs Concentrations in Sputum, Urine, and Serum

The concentrations of LTC₄, LTD₄, and LTE₄ were determined by radioimmunoassay (RIA), which had a low detection limit of 20 pg/mL (leukotriene C₄ [³H] assay system, TRK 905; Amersham, Arlington Heights, IL; peptidyl-leukotriene [³H] RIA kit, NEK-043; NEN, Boston, MA).

Measurement of Urinary 11-dhTXB₂

Urinary samples were collected in a specially designed tube (with EDTA-2Na, indomethacin, and trasylol) and immediately centrifuged. The supernatant was cryopreserved at -80°C for later determination of 11-dhTXB₂. The concentration of 11-dhTXB₂ in the urine was determined by RIA (thromboxane B₂ [¹²⁵I] RIA kit; NEN). The low detection limit was 35 pg/mL.

Measurement of Sputum and Serum ECP

Blood samples for measurement of ECP were collected in specially designed tubes (Vacutainer blood collection tube; Becton Dickinson, Mountain View, CA), left at room temperature for 1 hour and centrifuged at 1,300 *g* for 10 minutes. The concentrations of ECP in the serum and sputum were determined by RIA with a lower limit of detection of 2.0 $\mu\text{g/L}$ (Pharmacia ECP RIA; Pharmacia, Piscataway, NJ).

Data Analysis

Values were expressed as means $\pm$ standard error of the mean. Changes in FEV₁ on BPT1 and BPT2 and in the concentrations of chemical mediators were analyzed using a two-tailed Student's *t* test with the level of significance (*P* value) set at 0.05.

RESULTS

Bronchoprovocation Results

The threshold value in AIA ranged from 20 to 250 mg (Table 1). A fall of $>20\%$ in FEV₁ was observed 15 to 30 minutes after oral administration of the

threshold dose (Fig 1). FEV₁ was measured hourly up to 12 hours after the test, but no patient in this study developed a late asthmatic response. Treatment with pranlukast improved aspirin-induced fall in FEV₁ compared with the falls in FEV₁ on BPT1 ($P < 0.01$). In contrast, FEV₁ in ATA was not affected by oral administration of 500 mg of aspirin (Fig 1, right panel).

LTC₄+D₄ and ECP Concentrations and Eosinophil Count in the Sputum

LTC₄+D₄:LTC₄+D₄ concentration was significantly higher in the sputum of AIA than ATA at baseline (217 ± 85 vs 83 ± 25 pg/mL; $P < 0.05$; Fig 2). Sputum LTC₄+D₄ levels in AIA rose significantly during aspirin challenge and pranlukast failed to alter this effect (without pranlukast, 228 ± 140 to $1,750 \pm 233$ pg/mL; $P < 0.01$, with pranlukast 217 ± 85 to $1,510 \pm 210$ pg/mL). There was no significant difference between the levels attained in the presence or absence of pranlukast. In contrast, sputum LTC₄+D₄ levels did not change in ATA during aspirin challenge.

Eosinophil Count

There was no significant difference between AIA and ATA with respect to the eosinophil count in the sputum before aspirin challenge (Fig 2). In addition, there was virtually no change in sputum eosinophil counts in both groups after aspirin challenge.

ECP

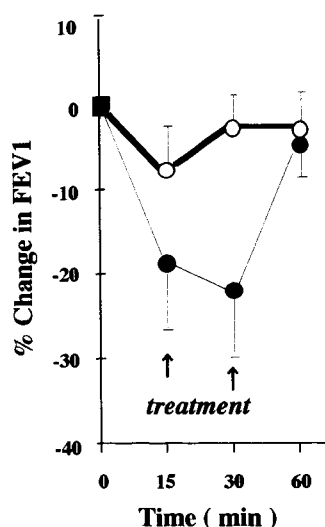
There was no difference between AIA and ATA with respect to sputum ECP levels before aspirin challenge. However, in AIA, sputum ECP increased significantly by aspirin challenge without pretreatment with pranlukast (111 ± 22 to 303 ± 55 $\mu\text{g/L}$; $P < 0.05$). This rise was abrogated by pretreatment with pranlukast (87 ± 43 to 100 ± 51 $\mu\text{g/L}$). In ATA, aspirin oral challenge did not modify ECP levels in the sputum (Fig 2).

Changes in Urinary LTE₄, 11-dhTXB₂

LTE₄

Changes in urinary LTE₄ were similar to those noted in the sputum LTC₄+D₄. Urinary LTE₄ at baseline was significantly higher in AIA than in ATA

Aspirin intolerant asthmatics (n=7)



Aspirin tolerant asthmatics (n=7)

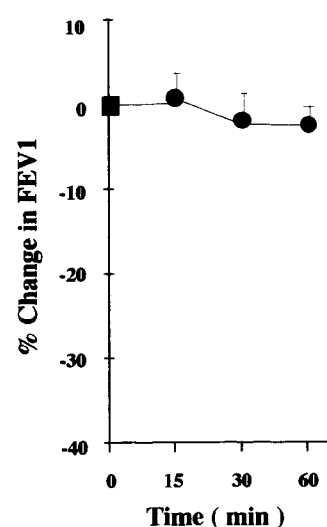


Figure 1. Aspirin provocation test with or without pranlukast in AIA and without pranlukast in ATA. ● indicates provocation without pranlukast; ○, provocation with pranlukast. Data are means $\pm$ standard deviation (SD) of seven subjects in each group.

LTC4+D4 in sputum (pg/ml)

% of eosinophils in sputum (%)

ECP in sputum (μ g/l)

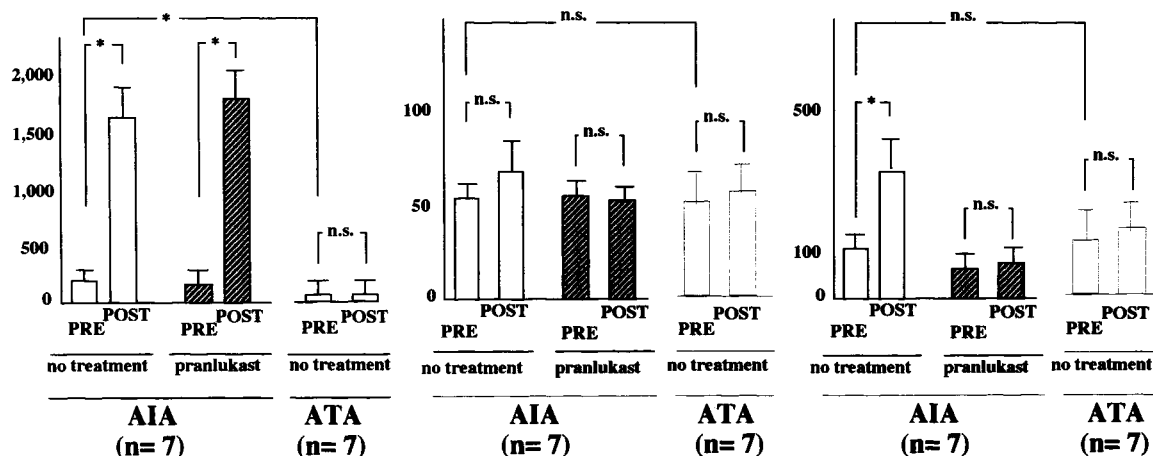


Figure 2. Changes in sputum LTs, ECP, and percent of eosinophils in sputum after aspirin oral challenge in AIA and ATA subjects. Data are means $\pm$ SD. * $P < 0.05$.

(340 ± 211 vs 238 ± 126 pg/mg Cr; $P < 0.05$; Fig 3). Aspirin challenge significantly increased urinary LTE4 in AIA (277 ± 167 to 586 ± 378 pg/mg Cr; $P < .05$). This increase was not suppressed by pretreatment with pranlukast (340 ± 211 to 552 ± 379 pg/mg Cr; $P < 0.05$).

11-dhTXB2

Urinary 11-dhTXB2 concentrations at baseline in AIA and ATA were not significantly different. Aspirin challenge resulted in a decrease in urinary

11-dhTXB2 in AIA, and such fall was not influenced by pretreatment with pranlukast (Fig 3). The decrease in urinary 11-dhTXB2 was significantly greater in ATA ($1,024 \pm 377$ pg/mg to 292 ± 130 pg/mg creatinine) than AIA (882 ± 222 pg/mg to 468 ± 179 pg/mg creatinine; $P < 0.01$). Additional analysis suggested that this difference was dependent on the pharmacodynamic of aspirin; the amount of aspirin in ATA (500 mg) was higher than in AIA (20 to 250 mg).

Changes in Serum LTs, ECP, and Eosinophil Count

Aspirin challenge did not influence serum LTC4+D4 and ECP concentrations and eosinophil count in AIA and in pranlukast-pretreated or untreated ATA (Fig 4).

DISCUSSION

The main findings of the present study were: 1) sputum LTs and urinary LTE4 concentrations were greater in AIA than in ATA during asthma stable state, 2) in AIA, aspirin oral challenge increased the concentrations of LTs and ECP in the sputum and LTE4 in the urine and induced asthma attack, and 3) in AIA, pretreatment with pranlukast abrogated aspirin-induced increase in sputum ECP but did not affect sputum LTs and urinary LTE4. These findings indicate that overproduction of LT and eosinophilic inflammation of the airway are involved in the onset of AIA.

With regard to the differences between AIA and ATA with respect to aspirin-induced FEV₁ changes and chemical mediators, previous studies have shown that urinary LTE4 is 2- to 10-fold greater in AIA than in ATA during stable asthma state.⁴⁻⁶ The present finding showed that urinary LTE4 in AIA was approximately 1.4-

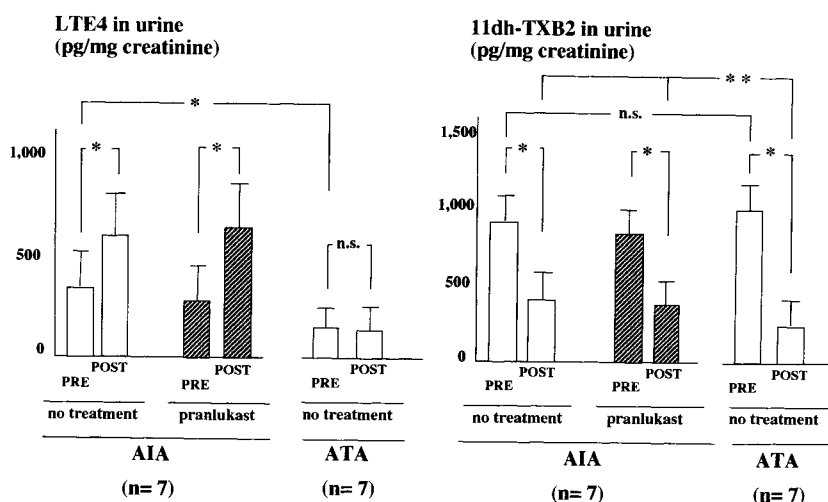


Figure 3. Changes in urinary LTE4 and 11-dhTXB2 after aspirin oral challenge in AIA and ATA subjects. Data are means $\pm$ SD. * $P < 0.05$; ** $P < 0.01$.

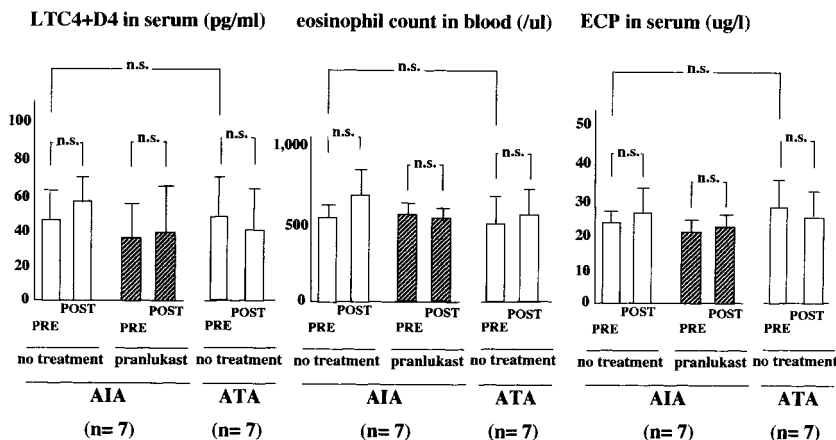


Figure 4. The changes in serum LTs, ECP, and eosinophil counts after aspirin oral challenge in AIA and ATA subjects. Data are means $\pm$ SD.

fold that in ATA subjects, which was less than that reported in the above studies. In addition, sputum LTs, which reflect the condition of the airway, were 2.6-fold greater in AIA than in urinary LTE4, compared with ATA subjects. This supports the notion that there is greater overproduction of LT in AIA than in ATA subjects.

In the oral provocation test using aspirin or NSAID in AIA, a dose of aspirin of 30 to 150 mg (mean: 60 mg) often induces an asthma attack.³ In the present study, a dose ranging from 20 to 250 mg (mean: 113 mg) was required to induce asthma attacks. Careful analysis of the response of our subjects showed the lack of correlation between aspirin threshold and severity of asthma, eg, in subject 1 with AIA, 20 mg of aspirin induced bronchoconstriction, although she was known to have mild asthma; however, 250 mg of aspirin induced bronchoconstriction in subject 6, a moderate asthmatic with AIA.

Previous studies have shown that during aspirin challenge, AIA subjects exhibit eosinophil or EG2-positive eosinophil infiltration of the airway and a rise in urinary LTE4.^{4-6,15,16} These findings suggest that in AIA, aspirin or NSAIDs cause airway narrowing by increasing the production of LTs and activation of eosinophils. In the present study, induced sputum LTs and urinary LTE4 increased 7.0- and 1.6-fold, respectively, and sputum ECP in-

creased 2.7-fold without a local increase in eosinophil number in the airway. However, in ATA subjects, sputum eosinophil number, ECP, and LTs did not increase even with a 500-mg aspirin challenge. Therefore, these results suggest that the pathogenesis of AIA involves an increase in LTs and an activation of eosinophil in the airways (although recruitment of circulatory eosinophils may occur later) as demonstrated in our results by the elevation of ECP. At the same time, major basic protein and/or eosinophil peroxidase may be produced by activated eosinophils, and it is possible that these mediators induce airway narrowing.¹⁷

Several studies have examined the effects of the aspirin challenge test on LT modifiers in AIA subjects. With regard to the effects of LTRA, previous studies examined the effect of pranlukast on dypyrone inhalation challenge⁷ and on sulpyrine inhalation challenge,¹⁸ the effect of SK&F 104353 on aspirin oral challenge⁶ and the effect of MK-0679 on lysine-aspirin inhalation challenge.¹⁹ With regard to LT synthesis inhibitor, previous studies examined the effects of zileuton and ZD2138, a 5-lipoxygenase inhibitor, on aspirin oral challenge.^{8,9} In all studies, each treatment effectively lowered FEV₁ during the provocation test. With regard to changes in urinary LTE4, LTRA cannot inhibit aspirin-induced increase in

urinary LTE4,^{11,18} whereas LT synthesis inhibitors inhibit aspirin-induced increase in urinary LTE4.^{8,9} However, there are no studies that have investigated the changes in LT or ECP in the airways. Urinary LTE4 may reflect LT production in the airway, but to clarify the pathogenesis of asthma in AIA subjects, it will be necessary to use sputum, bronchoalveolar lavage fluid, or biopsy specimens from the airway. With regard to the methods for provocation, inhalation provocation tests were used in approximately one half of the above studies, although clinically, aspirin oral challenge is more useful for a definite diagnosis.² Therefore, our studies were performed using aspirin oral challenge and collection of induced sputum from the airway.

In this study, we reported that pranlukast inhibited the activation of eosinophils and airway narrowing during aspirin oral provocations in AIA subjects. These results suggest that pranlukast is effective for AIA and that its effect is probably attributable to an inhibition of mechanisms involved in airway inflammation.

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