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Letter to the Editor

Increased airway hyperresponsiveness to adenosine in patients with aspirin intolerant asthma

Dear Editor,

Airway hyperresponsiveness in bronchial asthma is commonly evaluated by inhalation of methacholine (Mch) or histamine. Asthma patients also show bronchoconstriction upon adenosine inhalation.[1] Inhaled adenosine 5′-monophosphate (AMP) is converted immediately to adenosine by 5′-nucleotidase in the mucosa of the respiratory tract, which activates mast cells (MCs).[2] Direct bronchial provocation with AMP causes immediate increases in the concentrations of prostaglandin D_2, histamine, and tryptase in the bronchoalveolar lavage fluid of asthma patients compared with healthy volunteers.[3] Premedication with the MC-stabilizing drugs cromolyn and nedocromil has been shown to reduce AMP-induced bronchoconstriction by 9.6- and 22.2-fold, respectively.[4]

Based on the reports mentioned above, we postulated that AMP-induced bronchoconstriction is an indirect effect of the histamine and prostaglandins generated by MC degranulation.[5] This type of bronchoconstriction by AMP may occur *via* a different mechanism compared with bronchoconstriction induced by Mch (which stimulates airway smooth muscle directly).

Aspirin-intolerant asthma (AIA) is a type of refractory bronchial asthma. Even under stable conditions, the number of MCs in the airway mucosa of AIA patients is ≈2.5-fold greater than that in non-AIA patients. Furthermore, levels of a marker of MC activation, urinary 9α,11β-prostaglandin $F_2\alpha$, are significantly higher in AIA patients.[6] Based on these findings, we hypothesized that MCs would show greater activation in AIA than in non-AIA, and that AIA patients would be more hypersensitive to AMP compared with non-AIA patients. To test these hypotheses, we compared the results of inhalation challenge tests using AMP and Mch conducted in patients with bronchial asthma diagnosed with AIA or non-AIA.

The Ethics Committee of Fujita Health University (Toyoake, Aichi, Japan) approved our study protocol. Study participants were 30 patients treated for bronchial asthma at an outpatient clinic in our hospital. Patients who reported an infection of the upper respiratory tract or attack of bronchial asthma within 4 weeks before the examination were excluded from the study. Among these 30 patients, 12 were diagnosed with AIA (4 males and 8 females; mean age, 42.6 years) and 18 with non-AIA (5; 13; 42.1 years). Characteristics, laboratory findings, and therapy of studied patients are shown in Table 1.

The diagnosis of AIA was confirmed by: inhalation provocation tests using tolmetin or sulpyrine; oral aspirin provocation test with a history of asthma attacks induced by nonsteroidal anti-inflammatory drugs (NSAIDs).[7] Patients with a negative result of the inhalation challenge test using sulpyrine/tolmetin, or oral aspirin provocation test with no history of NSAID intolerance, were classified as “non-AIA”.

For the bronchial provocation test, AMP chloride and Mch (Sigma–Aldrich; Saint Louis, MO, USA) were dissolved in physiologic (0.9%) saline in gradually increasing concentrations. Each dilution was inhaled using a nebulizer, and was interrupted if the forced expiratory volume in 1 s (FEV_1) decreased by 20% from its baseline value. A concentration of Mch and AMP that achieved a fall in FEV_1 of 20% (PC_{20}) was defined as the index for airway hyperresponsiveness.

Statistical analyses were undertaken using JMP v8.0 (SAS Institute, Cary, NC, USA). The Mann–Whitney *U*-test was used to determine differences in lung function, sex, age, immunoglobulin (Ig)E value, Mch and AMP thresholds (PC_{20}-AMP and PC_{20}-Mch, respectively) for airway hyperresponsiveness, and the ratio of PC_{20}-AMP/PC_{20}-Mch between AIA and non-AIA. We used ANCOVA to compare the regression coefficient between logPC_{20}-AMP and logPC_{20}-Mch in AIA and non-AIA. $p < 0.05$ was considered significant.

Table 1
Patient demographics, respiratory function, laboratory findings, and treatment in this study.

	AIA (n = 12)	Non-AIA (n = 18)	
Age (mean ± SD)	42.6 ± 17.0	42.1 ± 14.9	n.s.
Male	4	5	n.s.
Atopy (+/−)	6/3	12/6	n.s.
Severity (mild/moderate)	5/7	7/10	n.s.
FVC (ml, mean ± SD)	3006 ± 586	3085 ± 722	n.s.
FEV1.0 (ml, mean ± SD)	2430 ± 537	2365 ± 602	n.s.
FEV1.0% (%, mean ± SD)	81.4 ± 9.2	77.2 ± 6.8	n.s.
%FEV1.0 (%, mean ± SD)	90.3 ± 14.3	85.5 ± 13.3	n.s.
WBC (/μl, mean ± SD)	6300 ± 1500	6900 ± 2200	n.s.
Eosinophil (%, mean ± SD)	9.2 ± 6.3	6.5 ± 2.9	n.s.
IgE (U/ml, mean ± SD)	285.5 ± 272.4	421.3 ± 404.9	n.s.
Therapy			n.s.
ICS (μg, mean ± SD)	420 ± 261	360 ± 214	n.s.
LABA (n)	6	5	n.s.
Theophylline (n)	9	13	n.s.
LTRA (n)	10	14	n.s.

n.s., not significant; ICS, inhaled corticosteroid; LABA, long acting beta agonist; LTRA, leukotriene receptor antagonist; SD, standard deviation; WBC, white blood cells; FEV, forced expiratory volume; AIA, aspirin-intolerant asthma.

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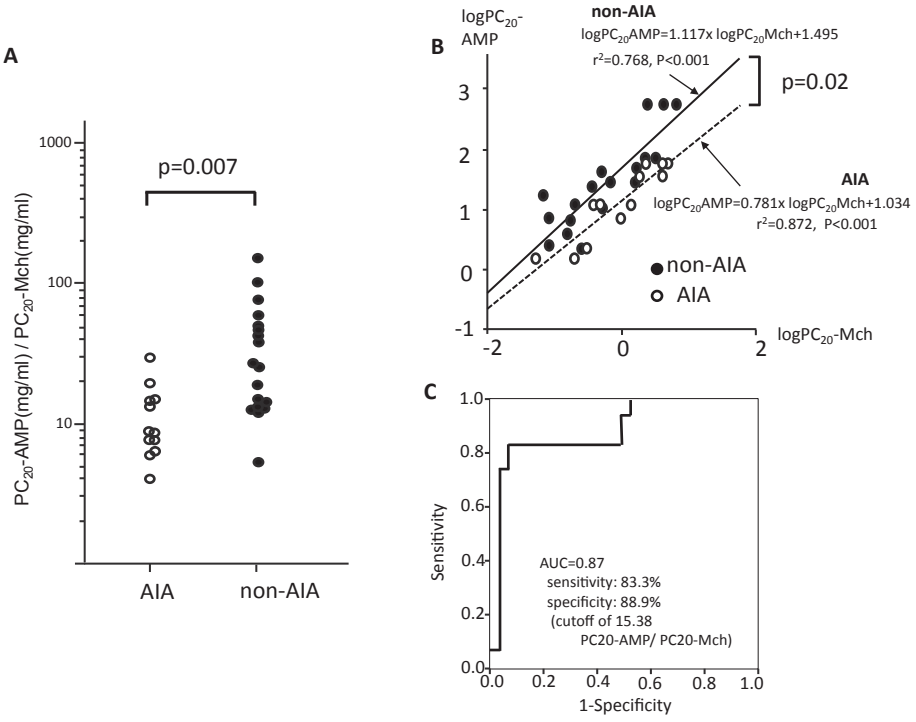

Fig. 1. A. The ratio of PC20-AMP/PC20-Mch in AIA patients was significantly lower than in non-AIA patients (Mann–Whitney *U*-test, $p = 0.007$). **B.** A significant positive correlation was observed between logPC20-AMP and logPC20-Mch in both AIA and non-AIA patients ($p < 0.001$). Comparative analysis of the two regression slopes showed that individual PC20-AMP/PC20-Mch was significantly lower in AIA than in non-AIA ($p = 0.02$). **C.** Receiver Operating Characteristic (ROC) analysis showing the ratio of PC20-AMP/PC20-Mch is a good marker for discriminating between AIA and non-AIA (ROC AUC: 0.87). The highest specificity (88.9%) and sensitivity (83.3%) were gained at a cutoff of 15.38 for PC20-AMP/PC20-Mch.

Between AIA and non-AIA, there were no significant differences with regard to clinical background and lung function (Table 1). There were no significant differences in PC_{20}-AMP and PC_{20}-Mch between the two groups (data not shown). However, the ratio of PC_{20}-AMP/PC_{20}-Mch was significantly lower than that observed in non-AIA patients (Mann–Whitney *U*-test, $p = 0.007$) (Fig. 1A). A significant positive correlation was observed between logPC_{20}-AMP and logPC_{20}-Mch in AIA and non-AIA patients ($r = 0.781$ and 1.117, respectively, $p < 0.001$) (Fig. 1B). Comparative analyses of the two regression slopes showed that individual PC_{20}-AMP/PC_{20}-Mch was significantly lower in AIA than in non-AIA ($p = 0.02$) (Fig. 1B). These results suggested that sensitivity to AMP was significantly higher in AIA patients compared with non-AIA patients. The ratio of PC_{20}-AMP/PC_{20}-Mch was a good marker to discriminate between AIA and non-AIA with an area under the receiver-operating-characteristics curve (ROC AUC) of 0.87. Analyses of ROC curves showed that the highest specificity (88.9%) and sensitivity (83.3%) were gained at a cutoff of 15.38 for PC_{20}-AMP/PC_{20}-Mch (Fig. 1C).

Recently, it has been recognized that airway MCs have a major role in AIA pathophysiology.[8] Adenosine induces airway hyperresponsiveness through activation of adenosine receptors or P2Y12 receptors (receptor for adenosine diphosphate and leukotriene E4) on MCs.[9] Genetic polymorphism of the gene for the adenosine receptor has been reported to be associated with AIA.[10] Taking these findings into consideration, it appears that adenosine-induced bronchoconstriction in AIA is high specifically because of increased activation of MCs in the airways of AIA patients.

AIA patients exhibit more severe asthmatic symptoms than non-AIA patients,[8] so the diagnosis and appropriate treatment of AIA patients is important. A NSAID-challenge test is essential for the diagnosis of AIA, but is associated with problems such as sensitivity, specificity, and risks.[7] Thus, several trials to investigate better diagnostic methods for AIA have been reported. These trials included *in vitro* diagnostic methods such as arachidonic-acid derivatives in peripheral blood polymorphonuclear leukocytes or cluster of differentiation (CD)11b expression on the eosinophil surface.[7]

Our study suggests that AIA patients may be more sensitive to AMP-induced bronchoconstriction than non-AIA patients, and that bronchial hypersensitivity measured by Mch inhalation was similar in AIA and non-AIA patients. We speculate that the AMP provocation test may reflect MC activation in the airways of AIA patients. Differential hyperresponsiveness of airways to inhalation of AMP and Mch might be a useful marker to distinguish AIA from non-AIA.

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Conflict of interest

The authors have no conflict of interest to declare.

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