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Review Article

Investigating the Mechanisms of Alcohol-induced Asthma

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ABSTRACT

Introduction: The purpose of the present study was to determine the mechanisms of alcohol-induced asthma.

Methods: Oral ethanol provocation test was performed in Japanese asthmatics to measure pulmonary function, blood ethanol, acetaldehyde and histamine. Acetaldehyde dehydrogenase 2 (ALDH2) genotype was determined by polymerase chain reaction (PCR) and ethanol patch test. Human bronchi and mast cells were stimulated with acetaldehyde in vitro. Pure ethanol was orally administered to ALDH2-deficient mice to determine the plasma concentrations of ethanol, acetaldehyde, histamine, and enhanced pause (Penh) values. Mite allergen-sensitized mice were inoculated with intranasal acetaldehyde.

Results: Approximately half asthmatic subjects developed bronchoconstriction with concomitant increases in blood acetaldehyde and histamine, which was associated with genetically reduced ALDH2 activities. In vitro acetaldehyde stimulation induces bronchoconstriction and degranulation of human mast cells. ALDH2-deficient mice caused bronchoconstriction following oral ethanol provocation. Acetaldehyde also induced granulocytes macrophage colony stimulating factor (GM-CSF) production and nuclear factor (NF)-κB activation in human bronchi and deteriorated mite allergen-sensitized inflammation in a murine model of asthma.

Conclusions: Acetaldehyde has potential effects on human airway by two distinct mechanisms. As a metabolite of alcohol, its elevation following alcohol consumption induces airway mast cells to release histamine, which results in exacerbation of asthma in susceptible populations. As an air pollutant contained in products such as cigarette smoke, its inhalation potentially exacerbates airway inflammation.

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KEYWORDS: alcohol-induced asthma, acetaldehyde, acetaldehyde dehydrogenase (ALDH) 2, histamine, mast cell

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Introduction

Approximately half of the Japanese population is estimated to experience exacerbation of asthma after consumption of alcoholic drinks; however, the underlying mechanisms are yet to be determined. Acetaldehyde, a metabolite of alcohol, seems to play a critical role in alcohol-induced asthma in Japanese asthmatics, which associates with genetically controlled enzymatic activity of acetaldehyde dehydrogenase 2 (ALDH2), a primary catabolic enzyme of acetaldehyde. Additionally, acetaldehyde is not only a metabolite of alcohol but also is present in many products such as cigarette smoke. Acetaldehyde is thus considered as an air pollutant, which causes airway injury.[1] Thus, acetaldehyde potentially causes two distinct pathologic conditions in the airway, via primary mediator involved in alcohol-induced asthma in a subset of asthmatics and via an air pollutant to exacerbate airway inflammation. Here we show the mechanisms of alcohol-induced asthma utilizing human asthmatics, isolated human bronchi and ALDH2-deficient mice. We also show the effects of acetaldehyde on the airway inflammation in isolated human bronchi and in a murine model of asthma.

Methods

Oral ethanol provocation test in asthmatic subjects

Pure ethanol was dissolved in 5% glucose solution to yield a 10% ethanol solution. Asthmatic and healthy subjects drank 300 ml of this solution in 5-10 minutes. Determinations of pulmonary function, blood ethanol, acetaldehyde and histamine were performed before and 15, 30, 60, and 120 minutes after ethanol challenge. Patients showing a 20% or greater fall in forced expiratory volume in 1 second ($FEV_{1.0}$) following oral ethanol provocation were considered as responders.[2]

Pretreatment with a histamine H1 receptor antagonist

A 1 week wash-out period was allowed for positive responders to the oral ethanol provocation test. During the following week, the responder was given 2 mg of azelastine hydrochloride, a histamine H1 receptor antagonist, and the second oral ethanol provocation test was performed at the day after the last dose of azelastine.[3]

Determination of ALDH2 genotype by PCR and ethanol patch test

To determine *ALDH2* genotype, blood samples from asthmatics and healthy subjects were subjected for polymerase chain reaction (PCR) analysis.[4] PCR results were stratified into type NN (normal homozygote) or NM (mutant heterozygote) or MM (mutant homozygote). Ethanol patch test was also performed on the inner surface of the upper arm of the same subjects. A patch area that showed erythema of 15 mm represented a positive result for reduced ALDH2 activity.[5]

In vitro acetaldehyde stimulation to human bronchi and airway mast cells

Human bronchi were prepared from the resected lung tissues of patients with lung cancer and were suspended in a Magnus bath containing Krebs-Henseleit solution. The upper surface of the specimen was suspended by an isometric transducer to record the contractile tension by a pen recorder. The suspended bronchi were stimulated with acetaldehyde and the contractile responses were recorded. 30 minutes after stimulation, buffer samples were collected and were subjected for determination of histamine by radioimmunoassay (RIA). Additionally, airway mast cells were isolated from lung tissue by means of immunomagnetic methods. These mast cells were directly stimulated with acetaldehyde and the concentrations of histamine in the cultured medium were determined by RIA.[6]

Animal model

ALDH2-deficient mice were bred using embryonic stem cells that were derived from C57BL/6 mice. The resulting mice were backcrossed into the BALB/c mice background. Exon 1 of *ALDH2* was replaced with the *Neo* cassette. Pure ethanol was orally administered to ALDH2-deficient and wild-type mice, and the plasma concentrations of ethanol, acetaldehyde, and histamine, in addition to enhanced pause (Penh) values, were determined and compared between the 2 groups.[7]

Effects of acetaldehyde on airway inflammation

Human bronchi were cultured in the presence of acetaldehyde for 24 hours and the concentrations of proinflammatory cytokines were determined. These tissues were also immunohistochemically stained for nuclear factor (NF)-κBp65.[8]

Intranasal inoculation of acetaldehyde to murine model of allergic asthma

Female Balb/c mice were intraperitoneally sensitized with mite allergen followed by intranasal (i.n.) mite allergen challenge to develop allergic airway inflammation. Thereafter, these mice were inoculated i.n. with low concentration (3%, 50 μl/mouse) of acetaldehyde. Hematoxylin and eosin-stained pulmonary pathology were

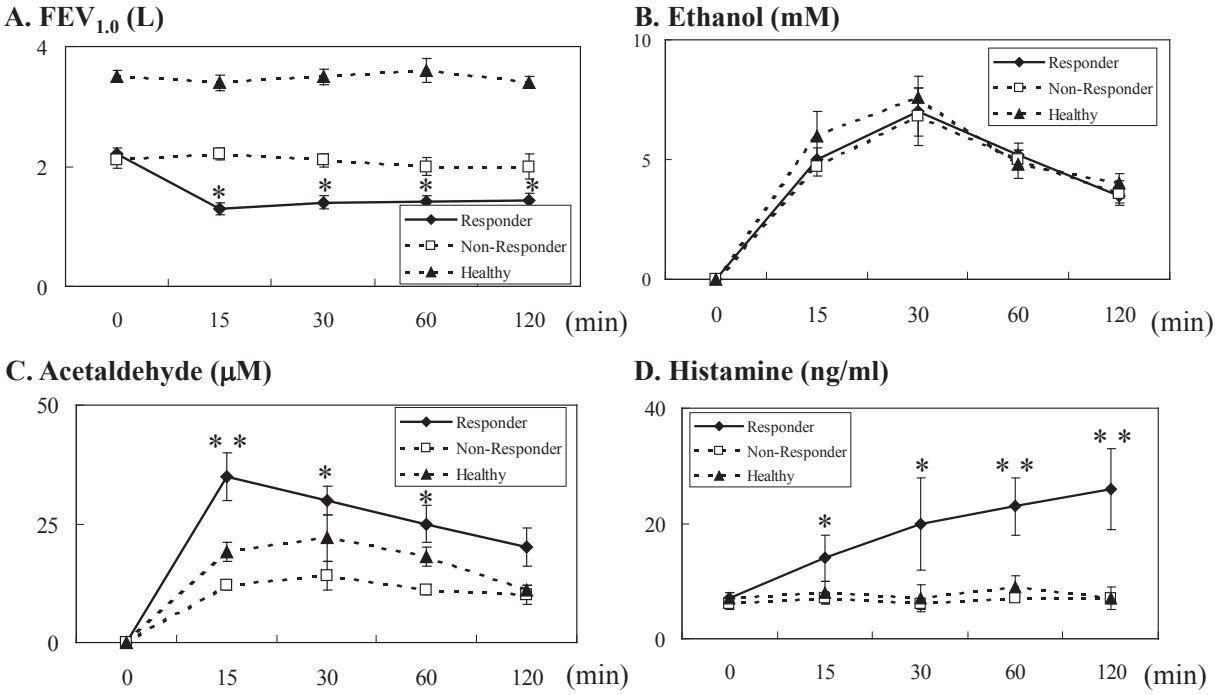

Fig. 1 Serial changes in (A) $FEV_{1.0}$, (B) serum ethanol, (C) acetaldehyde, and (D) histamine following oral ethanol provocation test. Bars represent mean (n = 21 for responders, n = 25 for non-responders, and n = 40 for healthy subjects) ± SEM. *p < 0.05 and **p < 0.01 vs non-responders.

compared between 4 groups of mice; control, acetaldehyde inoculated only, mite allergen sensitized only, and mite allergen sensitized plus acetaldehyde inoculated.[9]

Results and Discussion

Mechanism of alcohol-induced asthma in vivo

55% of asthmatic subjects were regarded as responders by oral ethanol provocation test. The remaining asthmatics and healthy subjects did not show a significant fall in their pulmonary function and were regarded as non-responders (Fig. 1A). Blood acetaldehyde and histamine, but not ethanol, were significantly higher in responders than in non-responders (Fig. 1B, C, D). These results indicated that increase in blood acetaldehyde, but not ethanol, induces increased blood histamine levels, which cause bronchoconstriction in a subset, approximately half of Japanese asthmatics. To further confirm this mechanism, responders were pretreated with azelastine hydrochloride, a histamine H1 receptor antagonist, and were subjected for second oral ethanol provocation test. Pretreatment of azelastine completely inhibited ethanol-induced bornchoconstriction, suggesting a critical role of histamine in this phenomenon.

Genetic mechanism of alcohol-induced asthma

Physiological responses to alcohol differ among races. Mongoloid populations often show facial flushing, palpitation and nausea after small amount of alcohol consumption. In contrast European white populations generally drink large amount of alcohol. This difference in alcohol metabolism is based on differences in ALDH2 enzymatic activity among races. The gene encoding ALDH2 is located in the long arm of chromosome 12. ALDH2 enzyme becomes inactive when the amino residue 487 (glutamic acid) is replaced with lysine as a result of point mutation of the 12th exon. ALDH2 is a tertramer, and all 4 subunits must be normal for the enzyme to retain its activity. In the type MM (mutant homozygote) *ALDH2* gene, all 4 tetramers are absent, and thus no ALDH2 activity is present. In the type NM (mutant heterozygote) *ALDH2* gene, there are only a few normal tetramers, resulting in a low ALDH2 activity.[10] Types NN, NM, and MM make up to 56.4%, 39.4%, and 4.2% of the Japanese populations, respectively. In contrast, type NN is present in almost of all European white populations.[11] PCR analysis for *ALDH2* genotypes was performed to determine why only a subset of subjects developed increased blood acetaldehyde levels following oral ethanol provocation.

Table 1 The distribution of *ALDH2* genotype determined by PCR in asthmatic and healthy subjects.

ALDH2 genotype:	NN	NM	MM
Asthmatic	23 (50.0)	20 (43.5)	3 (6.5)
Healthy	22 (55.0)	16 (40.0)	2 (5.0)

Results are shown as n (%) of NN (normal homozygote), NM (mutant heterozygote), and MM (mutant homozygote).

Table 2 Relationship between *ALDH2* genotype determined by PCR and results of oral ethanol provocation test in asthmatic subjects.

ALDH2 genotype:	NN	NM	MM
Positive	4 (17.4)	14 (70.0)	3 (100)
Negative	19 (82.6)	6 (30.0)	0 (0)

Results are shown as n (%) of NN (normal homozygote), NM (mutant heterozygote), and MM (mutant homozygote).

Table 3 Relationship between *ALDH2* genotype determined by PCR and results of ethanol patch test in asthmatic subjects.

ALDH2 genotype:	NN	NM	MM
Positive	4 (5.9)	56 (82.4)	8 (11.8)
Negative	80 (100)	0 (0)	0 (0)

Results are shown as n (%) of NN (normal homozygote), NM (mutant heterozygote), and MM (mutant homozygote).

Results demonstrated that significant difference did not occur in the frequency of *ALDH2* genotypes between asthmatics and healthy subjects (Table 1). The percentage of responders to oral ethanol provocation test was higher among those with inactive *ALDH2* genotypes (NM and MM) than those with the normal active types (NN) (Table 2). Results of ethanol patch testing correlated well with *ALDH2* genotype determined by PCR (Table 3) and further confirmed that reduced ALDH2 activity based on *ALDH2* genotype differences was found in significantly higher in responders. Taken together, we propose the following mechanism of alcohol-induced asthma. Orally administered ethanol is decomposed by alcohol dehydrogenase to yield acetaldehyde, which is then degraded by ALDH2 to yield acetic acid. In approximately half of Japanese subjects, the enzymatic activities of ALDH2 are genetically reduced and thus blood acetaldehyde levels are markedly elevated following alcohol consumption. Acetaldehyde induces histamine release, resulting in facial hot flushes and bronchoconstriction, i.e. alcohol-induced asthma. In contrast, ALDH2 activity is normal in almost all of European Caucasian white; thus, they develop neither facial hot flushes nor alcohol-induced asthma since blood acetaldehyde does not markedly increase after alcohol consumption.

Mechanism of alcohol-induced asthma in vitro

Although it is likely that mast cells play a critical role in acetaldehyde-induced bronchoconstriction via production of histamine in human asthma as abovementioned, there had been few studies to evaluate the direct effects of acetaldehyde on mast cells.[12, 13] Thus, human resected bronchi and isolated mast cells were stimulated with acetaldehyde *in vitro*. Acetaldehyde increased airway muscle tone, which was associated with a significant increase in the release of histamine (medium stimulation 26.3 ± 4.8 M/g vs acetaldehyde stimulation 98.7 ± 8.9 M/g; $p<0.05$). Acetaldehyde also directly induced a significant histamine release from isolated human airway mast cells. Taken together, these *in vitro* experiments had repeatedly shown and confirmed the *in vivo* experiments that acetaldehyde directly stimulates mast cells to release histamine, which causes airway smooth muscle constriction.

Animal model

We established an ALDH2-deficient mouse line to compare responses between wild-type and ALDH2-deficient mice receiving orally administered ethanol. The results

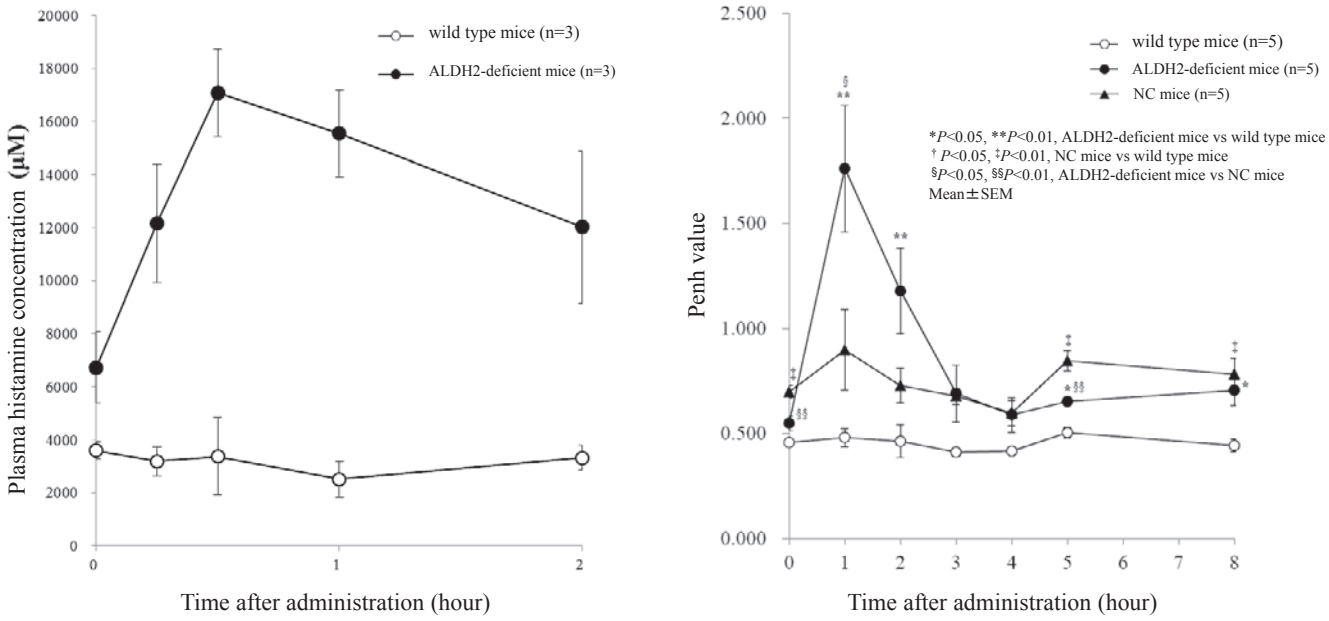

Fig. 2 Plasma histamine concentrations (left) and Penh (right) after oral ethanol challenge.

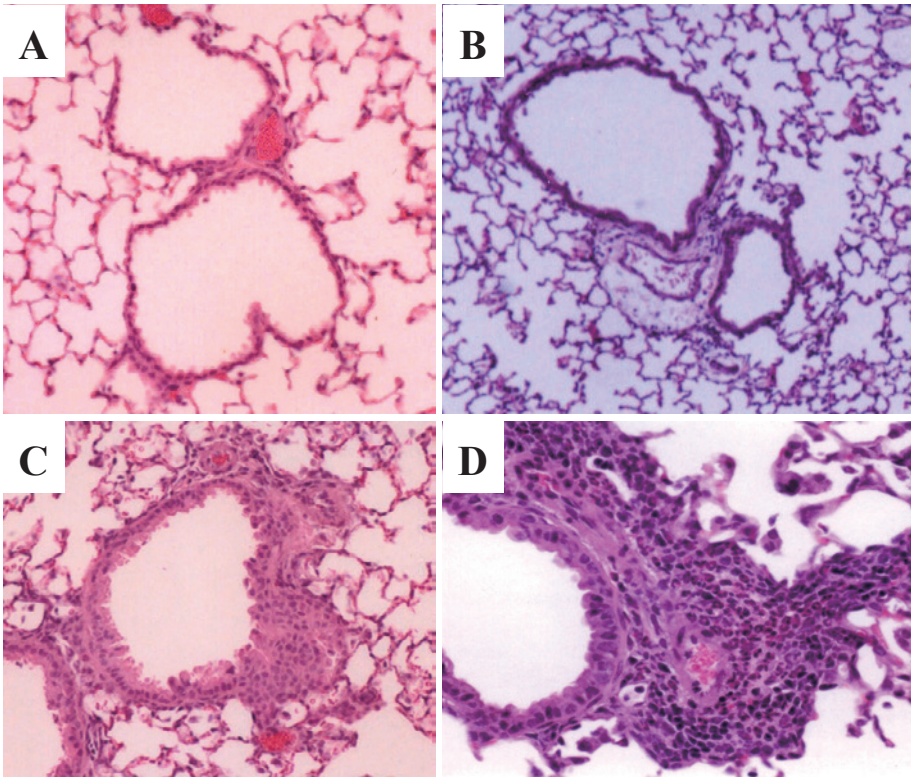

Fig. 3 Pulmonary pathology of (A) control, (B) acetaldehyde inoculated, (C) mite allergen sensitized, and (D) mite allergen sensitized and acetaldehyde inoculated BALB/c mice. Representative microphotographs of each (n = 4) group are shown.

showed that the plasma concentrations of acetaldehyde ($-p<0.0001$) and histamine ($-p<0.005$) were significantly higher, and the Penh values ($-p<0.01$) were significantly greater in the ALDH2-deficient mice, although plasma ethanol levels were not different (Fig. 2).

Effects of acetaldehyde on airway inflammation

Since acetaldehyde could be involved in airway inflammation as an air pollutant, we measured production of an

inflammatory cytokine, granulocyte macrophage colony stimulating factor (GM-CSF) and activation of NF-κB, a transcription factor, from acetaldehyde stimulated human bronchi. Acetaldehyde significantly increased GM-CSF production from human bronchi and nuclear translocation of NF-κB in airway epithelium. GM-CSF and NF-κB are critically involved in the development and maintenance of airway inflammation, environmental acetaldehyde might enhance allergic airway inflammation observed in human asthma. In fact, low concentration of acetaldehyde, which per se could not cause airway inflammation, significantly enhanced pre-existing mite allergen-induced allergic airway inflammation (Fig. 3). Although the underlying mechanism is yet to be determined, it is possible that acetaldehyde-induced GM-CSF could activate inflammatory cells including eosinophils, macrophages and dendritic cells.

Concluding remarks

Collectively, our experimental results indicate that acetaldehyde has potential effects on human airway by 2 distinct mechanisms. As a metabolite of alcohol, its elevation following alcohol consumption induces airway mast cells to release histamine, which results in exacerbation of asthma in susceptible populations. As an air pollutant contained in products such as cigarette smoke, its inhalation potentially exacerbates airway inflammation.

Conflicts of interest: None declared

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