Acetaldehyde at a Low Concentration Synergistically Exacerbates Allergic Airway Inflammation as an Endocrine-Disrupting Chemical and as a Volatile Organic Compound

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Key Words
Acetaldehyde  ·  Allergic airway inflammation  ·  Asthma bronchiale  ·  Endocrine-disrupting chemical(s)  ·  Volatile organic compound(s)

Abstract
Background: Acetaldehyde is an endocrine-disrupting chemical (EDC) and a volatile organic compound (VOC). It is also a carcinogen and teratogen that causes bronchoconstriction in a subset of asthmatics. However, the mechanism through which acetaldehyde acts as an EDC/VOC causing allergic airway inflammation remains unknown. Objectives: To determine the effects of a low concentration of acetaldehyde, which itself did not trigger airway inflammation, on extant allergic airway inflammation in a murine model of allergic asthma. Methods: We compared airway hyperresponsiveness (AHR), lung pathology, serum IgE and airway concentrations of cytokines among four groups of BALB/c mice [control, Dermatophagoides farinae (Df) allergen-sensitized (AS), intranasally acetaldehyde-injected (ALD) and AS-ALD mice]. Results: Physiological and histological differences were not evident between ALD and control mice. AS mice developed AHR and allergic airway inflammation characterized by goblet cell hyperplasia and eosinophilic infiltration. Both AHR and airway eosinophilia were significantly enhanced in AS-ALD compared with AS mice. Serum total and Df-specific IgE were significantly increased in both AS and AS-ALD mice compared with control and ALD mice, but comparable between AS and AS-ALD mice. Mite allergen sensitization significantly increased interleukin-5 and granulocyte macrophage colony-stimulating factor, and decreased interferon-γ levels in the airways; injecting acetaldehyde into airways with allergic inflammation significantly increased the levels of these inflammatory cytokines. Conclusions: Exposure to acetaldehyde can enhance allergic airway inflammation in asthma.

Introduction

Endocrine-disrupting chemicals (EDCs) confer health risks such as toxicity, carcinogenicity, mutagenicity, immunotoxicity and neurotoxicity in humans [1–5]. A relationship between EDCs and allergic diseases has been established [6–10], but the exact mechanism underlying this relationship remains obscure. Volatile EDCs are generally referred to as volatile organic compounds (VOCs). Exposure to VOCs such as formaldehyde or acetaldehyde can cause sick building syndrome or bronchial asthma [6–10].
Bronchial asthma is characterized by chronic airway inflammation and airway hyperresponsiveness (AHR) [11–15]. Among the various inflammatory cells, type 2 T-helper lymphocytes (Th2), which produce Th2 cytokines that regulate allergic airway inflammation, are typically located in the airways of asthma patients [12]. In particular, the Th2 cytokine interleukin (IL)-5 promotes the maturation and activation of eosinophils [11–15]. The type 1 T-helper lymphocyte (Th1) cytokine interferon (IFN)-γ inhibits the biological effects of Th2 cytokines. Th2 immunity is dominant over Th1 immunity in asthma [11, 12]. The most common trigger of acute exacerbation of asthma among both children and adults is viral respiratory tract infection [16]. Although the precise underlying mechanism of virus-induced asthma exacerbation remains unknown, viral infection probably exacerbates Th2-dominant allergic airway inflammation [11, 16].

Several other factors can exacerbate asthma. We previously reported that alcohol consumption exacerbates asthma in about half of the Japanese patients with asthma [17–19]. Acetaldehyde, a metabolite of alcohol, plays a critical role in this alcohol-induced bronchoconstriction via stimulation of mast cells/basophils to produce histamine [20]. In addition to being an alcohol metabolite, acetaldehyde is also a VOC that has been linked to sick building syndrome and asthma [6–10], and it might have various adverse effects on humans [1–5]. In fact, acetaldehyde in cigarette smoke inhibits ciliary motility via phosphokinase C-dependent mechanisms [21]. Taken together, these findings indicate that acetaldehyde affects airway inflammation as a VOC. Nonetheless, little is known about interactions between acetaldehyde and allergic airway inflammation. The present study investigates the effects of acetaldehyde as an EDC/VOC on extracellular allergic airway inflammation induced by mite allergens in a novel murine model of asthma.

**Materials and Methods**

**Acetaldehyde Concentration**

The concentration of acetaldehyde used herein was determined by preliminary experiments based on published findings [22, 23]. Various concentrations of acetaldehyde were injected intranasally into mice daily for 1 week as described below and then lung specimens were histologically evaluated. We concluded that 50 μg of acetaldehyde does not directly damage murine airways since this dose neither caused tissue damage nor inflammatory changes in this model. This concentration was lower than that used in a previous study of humans [23].

**Animals and Immunization Protocol**

An animal model of mite allergen-sensitized asthma was generated [24, 25]. Four groups (n = 8 per group) of 4- to 6-week-old female BALB/c mice (Charles River Japan, Yohokama, Japan) were housed at the Laboratory Animal Center for Biochemical Research, Nagasaki University School of Medicine. All mice were immunized twice intraperitoneally on days 1 and 14 with 0.5 mg of Dermatophagoides farinae (Df, American house dust mite, LG-5339; Cosmo Bio, Tokyo, Japan) per mouse precipitated in aluminum hydroxide. These mice were then challenged intranasally with 50 μl of phosphate-buffered saline (PBS) [control group and acetaldehyde inoculated group (ALD)] or with 50 μg of Df allergen/50 μl [allergen-sensitized group (AS) and AS-ALD group] on days 14, 16 and 18 [25]. The ALD and AS-ALD groups were each intranasally injected with 50 μg of acetaldehyde (Sigma, St. Louis, Mo., USA) from days 14–20. We determined AHR on day 20 in unrestrained mice using whole-body plethysmography. All mice were sacrificed by dislocation of the cervical vertebrae on day 21 and peripheral blood was collected. Bronchoalveolar lavage fluid (BALF) was obtained from half of the mice in each group using 0.5 ml of ice-cold PBS. Lung tissue samples were obtained from the other half of each group of mice for pathological evaluation. The procedures were reviewed and approved by the Nagasaki University School of Medicine Committee on Animal Research. All experiments were repeated at least three times.

**Determination of AHR**

We measured airway responsiveness to inhaled methacholine (MCh; Sigma) in unrestrained mice using whole-body plethysmography (PULMOS-I; M.I.P.S., Osaka, Japan) to determine AHR in this model. AHR is expressed as calculated specific airway resistance (sRaw), which closely correlates with pulmonary resistance measured using conventional two-chamber plethysmography in ventilated animals. The four murine groups were exposed to PBS for 5 min and subsequently to increasing concentrations (6, 12, 25 and 50 mg/ml) of MCh in PBS using an ultrasonic nebulizer (NE-U17; Omron, Kyoto, Japan). We then calculated sRaw values from recordings taken for 3 min after each dose.

**Pathological Evaluation of Pulmonary Inflammation**

Lung sections from each group were stained with hematoxylin and eosin and evaluated (magnification ×400) at least three times by three observers in a blinded fashion [25]. The number of eosinophils and the total number of nuclei in three randomly selected airway samples were determined. Eosinophils were reported as percentage of the total cells in the airways.

**Determination of Serum IgE Levels**

Serum concentrations of total IgE and Df-specific IgE were measured in duplicate using enzyme-linked immunosorbent assays (ELISA). Total serum IgE concentrations were determined using a rat anti-mouse IgE antibody (Ab; PharMingen, San Diego, Calif., USA) and biotin-conjugated rat anti-mouse IgE mAb (PharMingen) [20]. Other 96-well ELISA plates were coated overnight at 4°C with 5 μg/ml of Df extract to measure Df-specific IgE. Serum samples (1:10) were incubated for 2 h at room temperature in the Df-coated plates before incubation with biotin-
conjugated rat anti-mouse IgE mAb. The optical density at 405 nm (OD$_{405}$) was determined using an automatic ELISA plate reader. The total serum IgE level (expressed as µg/ml) was determined using a mouse IgE standard (PharMingen). The $D_f$-specific serum IgE levels are expressed as OD$_{405}$.

Analysis of BALF
We evaluated BALF samples using a hemocytometer and light microscopy. Each BALF sample was centrifuged for 10 min at 400 g at 4°C, and cytokines were analyzed in the supernatants. The cell pellets were resuspended in 1 ml of PBS. The total number of cells in the BALF was counted using a hemocytometer, and cells on Cytospin slides were fixed and visualized by May-Giemsa staining. Three observers performed differential counts of 200 cells. Absolute cell numbers were calculated as the product of the total and differential cell counts, and the absolute number of eosinophils in the BALF was calculated. The concentrations of IFN-γ, IL-5 and granulocyte macrophage colony-stimulating factor (GM-CSF) in the BALF supernatants were determined by ELISA (Quantikine; R&D Systems, Minneapolis, Minn., USA), as described by the manufacturer.

Statistical Analysis
Results are expressed as means ± SEM. Data were evaluated using repeated-measure ANOVA with a Bonferroni multiple comparison test. A value of $p < 0.05$ was considered significant.

Results

Acetaldehyde Enhanced AHR in a Murine Model of Asthma
We measured airway responsiveness to inhaled MCh (fig. 1). The sRaw value did not significantly increase in response to PBS inhalation in any group, but significantly increased in the AS and AS-ALD groups compared with the control group after inhaling 25 and 50 mg/ml of MCh, indicating AHR in this animal model of bronchial asthma. Acetaldehyde at this concentration did not result in AHR. sRaw significantly increased in the AS-ALD compared with the AS group after inhaling 50 mg/ml of MCh. These results suggest that acetaldehyde at this concentration enhanced AHR.

Low Acetaldehyde Concentration Worsened Airway Inflammation
Figure 2a–d shows representative pathological features of the four groups of mice. Airway inflammation was not significantly increased in ALD compared with control mice. Goblet cell metaplasia and cellular infiltrate with eosinophils were identified in AS mice. The mean number of infiltrating eosinophils per 10 perivascular areas was significantly increased in AS-ALD compared with AS mice (means ± SEM: 8.7 ± 12.1 vs. 22.1 ± 9.7, $p < 0.05$). Analysis of the cellular components of BALF revealed significantly more lymphocytes and eosinophils in the airways of AS than in control mice, and airway eosinophilia was significantly increased in AS-ALD compared with AS mice (fig. 2e).

Acetaldehyde Did Not Change Serum IgE Levels
Figure 3 shows serum total IgE and $D_f$-specific IgE levels. These values were significantly increased in AS and AS-ALD compared with control and ALD mice. Total IgE or $D_f$-specific IgE did not significantly differ between control and ALD mice.

Acetaldehyde Increased Inflammatory Cytokine Concentrations in BALF
Figure 4 shows IL-5, GM-CSF and IFN-γ levels in BALF. Like the pathological lung profile, the cytokine profile was not significantly altered in ALD mice compared with control mice, whereas IL-5 and GM-CSF were significantly increased, and IFN-γ was significantly decreased in AS compared with control mice. Levels of IL-5, GM-CSF and IFN-γ were significantly increased in AS-ALD compared with AS mice, and a low dose of acetaldehyde significantly increased inflammatory cytokine levels in AS mice.
Discussion

The major findings of the present study are as follows. Intranasal injection of a low concentration of acetaldehyde, which itself did not trigger airway inflammation, worsened AHR, significantly exacerbated extant allergic airway inflammation induced by mite allergens and increased the production of Th1 and Th2 cytokines. Acetaldehyde is commonly encountered in the environment. Cigarette smoke and vehicle exhaust emissions contain both acetaldehyde and formaldehyde [2, 6–8, 21], and these chemicals are also found in paints, plastic products and adhesives, for example [2, 7, 9]. In addition, some fruits naturally contain acetaldehyde [2], and acetalde-
cause of genetically lower levels or absent activity of aldehyde dehydrogenase 2, a primary enzyme involved in acetaldehyde metabolism [17–20]. We previously confirmed that an increased blood acetaldehyde concentration stimulates human mast cells in bronchi to release histamine, thus causing bronchoconstriction [17–20]. The present findings suggest that acetaldehyde has a proinflammatory effect in the pathophysiology of asthma in addition to a bronchoconstrictive effect.
The immunological effects of alcohol on infectious diseases have received increased attention [29]. In fact, alcoholism is considered a risk factor for infections such as pneumonia [30]. A growing body of evidence points to alcohol as an important modifier of mucociliary clearance, which is the first line of defense in the lungs [28]. Acetaldehyde activates phosphokinase C in airway cells and might be linked to the release of airway oxidants [21]. Aytacoglu et al. [31] reported that alcohol could cause lung damage. In contrast, little is known about interactions between alcohol and allergic inflammation. One mechanism through which inhaled acetaldehyde might enhance allergic airway inflammation is physical damage to the airway epithelium, which would enhance the penetration of mite allergens into the airways, resulting in an increased IgE response. However, this was not proven in the present study. Clarisse et al. [32] measured indoor air concentrations of aldehydes and found very low levels. The combustion of cigarettes remarkably increases airborne aldehyde concentrations [33]. Thus, smoking tobacco increases the amount of exposure to acetaldehyde compared with the low concentration generated in the present study, in which levels of mite allergen–specific IgE antibody were comparable between AS and AS-ALD mice. We previously disclosed that acetaldehyde, but not alcohol, stimulates GM-CSF production from the airway epithelium in vitro through the activation of nuclear factor (NF)-κB in lung tissue from patients with lung cancer [34]. The present study also demonstrated that a low concentration of acetaldehyde significantly increased airway production of GM-CSF induced by mite allergen in vivo. Since GM-CSF is a growth factor for dendritic cells that serve as the primary antigen-presenting cells in the airways, the present findings suggest that the maturation of dendritic cells by acetaldehyde-induced GM-CSF production enhances adaptive immunity and thus exacerbates allergic airway inflammation. Although the mechanism of acetaldehyde–increased GM-CSF production remains uncertain, the present findings indicate that acetaldehyde can exacerbate allergic airway inflammation via an EDC pathway.

In conclusion, acetaldehyde might be involved in the pathogenesis of asthma via two pathways. One is that increased blood levels of acetaldehyde, which may be due to genetically reduced or lack of aldehyde dehydrogenase 2 activity in some Asian populations, stimulate mast cells to release histamine after oral alcohol intake, and this causes bronchoconstriction. The other is that inhaled acetaldehyde acting as an EDC enhances allergic airway inflammation.

References


