Increased airway responsiveness to acetaldehyde in asthmatic subjects with alcohol-induced bronchoconstriction

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ABSTRACT: Bronchial responsiveness to acetaldehyde, a main factor in alcohol-induced bronchoconstriction, and methacholine were compared between 10 subjects with alcohol-induced bronchoconstriction and 16 asthmatic subjects without alcohol sensitivity.

In the alcohol-sensitive group, the geometric mean (geometric SEM (GSEM)) of the provocative concentration of methacholine ($PC_{20,meth}$) and acetaldehyde ($PC_{20,acet}$) causing a 20% fall in forced expiratory volume in one second were 0.947 mg·mL^{-1} (GSEM 0.139) and 21.0 mg·mL^{-1} (GSEM 0.112), respectively, which were not significantly different from those in the nonalcohol-sensitive group, which were 0.634 mg·mL^{-1} (GSEM 0.115) and 31.7 mg·mL^{-1} (GSEM 0.077), respectively. The ratio of airway responsiveness to acetaldehyde relative to methacholine (log $PC_{20,acet}/PC_{20,meth}$) was 1.345± 0.093 (mean±SEM) in the alcohol-sensitive group, which was significantly different from the value of 1.699±0.059 in the nonalcohol-sensitive group (p=0.0025). A significant correlation was observed between $PC_{20,meth}$ and $PC_{20,acet}$ in both the alcohol-sensitive group (r=0.742, p=0.0115) and nonsensitive group (r=0.882, p<0.0001).

In conclusion, the airways of asthmatic subjects with alcohol-induced bronchoconstriction have a selective hyperresponsiveness to acetaldehyde.
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Acetaldehyde, a rapidly generated metabolite of ethanol, is thought to be a main factor of alcohol-induced bronchoconstriction. In alcohol-induced bronchoconstriction, which occurs in response to intrinsic histamine released by acetaldehyde and is a condition that occurs as the result of a genetic alteration in the metabolism of alcohol, findings indicate that: 1) blood acetaldehyde and histamine concentrations increase during alcohol-induced bronchoconstriction [1]; 2) inhaled acetaldehyde provokes bronchoconstriction indirectly *via* histamine release in guinea-pigs [2] and asthmatic subjects [3]; 3) the activity of aldehyde dehydrogenase (ALDH), which oxidizes acetaldehyde to acetate, is a major factor in determining exacerbations of asthma after alcohol intake [4]; 4) among asthmatic patients in Western societies, who usually do not develop bronchoconstriction in response to alcohol, severe asthmatic attacks follow alcohol intake in those who are chronically alcoholic and taking the ALDH inhibitor disulfiram as a component of alcohol-abstinence therapy [5]; and 5) terfenadine, a selective histamine H$_1$-antagonist, completely inhibits alcohol-induced bronchoconstriction [6].

Although it has been demonstrated by the authors that inhaled acetaldehyde provokes bronchoconstriction in both asthmatic subjects with and without alcohol sensitivity [3, 7], it is unknown whether the airway response to acetaldehyde is augmented in subjects with alcohol-induced bronchoconstriction. It is therefore hypothesized that there is an increased airway responsiveness to acetaldehyde in asthmatic patients with alcohol-induced bronchoconstriction. In order to test this hypothesis, bronchial responsiveness to acetaldehyde and methacholine was compared in subjects with alcohol-induced bronchoconstriction and in a control group of asthmatic subjects without alcohol sensitivity.

Subjects and methods

Subjects

Ten alcohol-sensitive subjects (seven male, three female, aged 20–63 yrs), and 16 nonalcohol-sensitive asthmatic subjects (10 male, six female, aged 23–65 yrs), of whom nine were nonatopic and 17 were atopic, were studied (table 1). Each subject satisfied the American Thoracic Society definition of asthma [8]. Alcohol-induced bronchoconstriction was confirmed by a >20% decrease in peak expiratory flow (PEF) after drinking all forms of alcoholic beverages such as beer, whisky, brandy, wine and Japanese sake. Atopy was defined by the presence of a positive serum specific immunoglobulin (Ig)E to at least two common aeroallergens: grass pollen, tree pollen, cat dander, dog hair, *Dermatophagoides pteronyssinus* and *D. farinae*. Each subject had not taken antihistamines, sodium cromoglycate, inhaled corticosteroids or oral corticosteroids for ≥1 month prior to the study, and no subject had experienced an upper respiratory tract infection

Table 1. – Subject characteristics

Subject No.	Age yrs	Sex	Atopy	FVC % pred	FEV1 % pred	FEV1/FVC %	Treatment
Alcohol-sensitive asthmatic subjects							
1	26	M	+	115.1	104.1	83.5	Sa
2	28	M	-	109.0	88.3	75.7	Sa, Th
3	31	M	+	112.3	83.5	69.7	Sa, Th
4	42	M	+	114.3	103.9	76.3	Sa
5	36	F	-	94.1	93.8	88.9	Sa
6	57	M	+	94.3	83.3	72.9	Sa, Th
7	20	M	+	109.5	81.0	69.0	Sa, Th
8	26	M	+	102.6	88.0	77.6	Sa
9	26	F	+	107.7	89.6	82.4	Sa
10	63	F	-	103.3	103.2	79.4	Sa, Th
Mean	35.5			106.2	91.9	77.5	
SEM	4.5			2.4	2.8	2.0	
Alcohol-tolerant asthmatic subjects							
1	24	M	+	113.7	87.0	71.5	Sa
2	27	M	+	124.3	89.4	66.5	Sa, Th
3	45	M	+	114.5	91.2	70.3	Sa, Th
4	65	M	-	103.0	101.6	75.6	Sa, Th
5	39	F	+	109.4	105.7	79.8	Sa
6	23	F	-	91.5	65.5	59.1	Sa, Th
7	57	F	-	86.3	74.4	74.0	Th
8	37	M	+	108.2	96.1	78.3	Sa
9	52	M	-	83.5	78.4	80.1	Sa
10	33	F	+	120.9	100.7	76.6	Sa, Th
11	47	F	-	97.4	106.8	77.5	Sa, Th
12	26	M	+	129.9	121.1	85.6	Sa
13	32	M	+	108.5	92.2	71.4	Sa, Th
14	29	M	+	117.7	98.1	76.8	Sa, Th
15	23	F	+	106.0	95.7	90.3	Sa
16	52	M	-	90.3	67.9	80.5	Sa, Th
Mean	37.6			106.6	92.0	74.6	
SEM	3.2			3.4	3.7	2.0	

FVC: forced vital capacity; FEV1: forced expiratory volume in one second; M: male; F: female; Sa: salbutamol *via* metered-dose inhaler; Th: orally administered theophylline.

in the preceding month or during the study. Permitted medication, which remained unchanged during the study, included inhaled β_2-agonists and oral theophylline. This study was carried out when their symptoms were mild and stable. All medication was stopped at 13:00 h on the previous day to allow a washout time of at least 24 h. The bronchial responsiveness to inhaled acetaldehyde or methacholine was then measured at 13:00 h on the test day. Informed consent was obtained from all subjects. This study was approved by the ethics committee of the authors university hospital.

Bronchial responsiveness to inhaled acetaldehyde or methacholine

Bronchoprovocation to acetaldehyde or methacholine was performed on two occasions 2 weeks apart, in a double-blind, randomized, crossover fashion. Acetaldehyde and methacholine were dissolved in physiological saline solution to make concentrations of 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40 and 80 mg·mL^{-1}. Saline and each solution were inhaled from a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA, USA) operated by compressed air at 5 L·min^{-1}. The nebulizer output was 0.14 mL·min^{-1}. Saline was inhaled first for 2 min and forced expiratory volume in one second (FEV1) was measured. If

the change in FEV1 from the baseline value was ≤10%, inhalation of acetaldehyde or methacholine was started, and if the saline solution caused a change in FEV1 >10%, the test was stopped or postponed. Acetaldehyde or methacholine was inhaled for 2 min by tidal mouth breathing and followed immediately by measurements of FEV1. Increasing concentrations of acetaldehyde or methacholine were inhaled until a fall of ≥20% in FEV1 occurred. FEV1 was measured three times and the highest FEV1 value of three attempts was recorded each time.

Data analysis

The provocative concentration of acetaldehyde (PC20, acet) and methacholine (PC20,meth) causing a 20% fall in FEV1 were determined by linear interpolation from the log dose/response curve. PC20,meth and PC20,acet values were logarithmically transformed for analysis and reported as the geometric mean (geometric SEM (GSEM)). A 20% fall in FEV1 was not obtained in one of 16 subjects without alcohol-induced bronchoconstriction at the highest concentration of acetaldehyde administered, and, therefore, a minimal estimate was obtained by calculating the cumulative PC20,acet on the next doubling concentration beyond the highest administered. The responsiveness of the airways to acetaldehyde relative to methacholine was
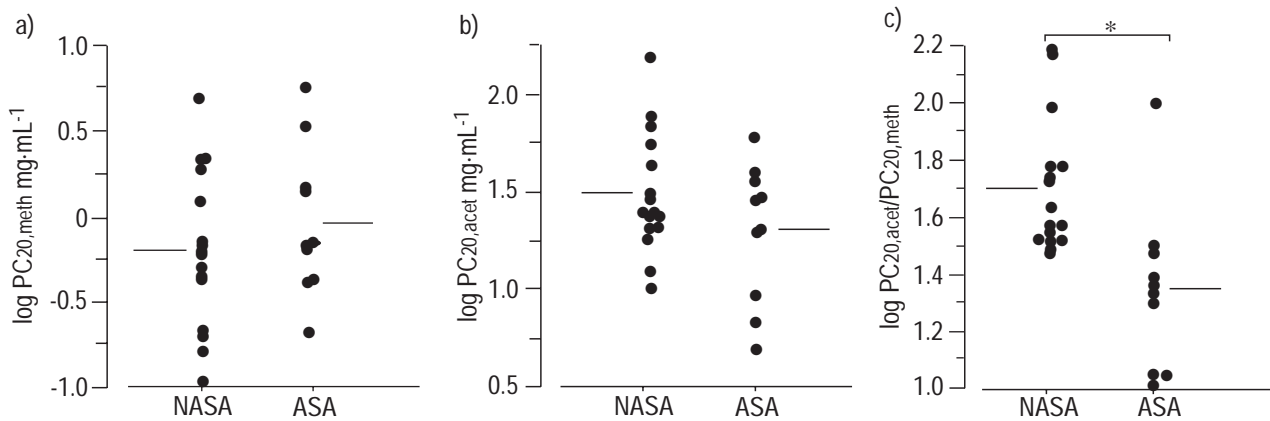
Fig. 1. – Bronchial responsiveness to methacholine (a) and acetaldehyde (b), and relative response of acetaldehyde to methacholine (c) in alcohol-sensitive asthmatic (ASA) and nonalcohohol-sensitive asthmatic (NASA) subjects. Bars indicate geometric mean. $PC_{20,meth}$, $PC_{20,acet}$: provocative concentration of methacholine and acetaldehyde causing a 20% fall in forced expiratory volume in one second, respectively. *: p=0.0025.

calculated as log ($PC_{20,acet}$/$PC_{20,meth}$) and reported as the mean±SEM. Variation between the unpaired values was statistically analysed using the unpaired Student′s t-test. Correlations were obtained using Pearson′s correlation co-efficient. Significance was based on a 95% confidence level (p<0.05).

Results

The geometric means of $PC_{20,meth}$ and $PC_{20,acet}$ in the alcohol-sensitive group were 0.947 mg·mL^{-1} (GSEM 0.139) and 21.0 mg·mL^{-1} (GSEM 0.112), respectively, which were not significantly different from those in the nonalcohol-sensitive group, which were 0.634 mg·mL^{-1} (GSEM 0.115) (p=0.35) and 31.7 mg·mL^{-1} (GSEM 0.077) (p=0.18), respectively (fig. 1). The responsiveness of the airways to acet-aldehyde relative to methacholine was 1.345±0.093 in the

alcohol-sensitive group, which was significantly dif-ferent from the nonalcohol-sensitive group (1.699±0.059) (p=0.0025) (fig. 1). A significant correlation was observ-ed between $PC_{20,meth}$ and $PC_{20,acet}$ in both the alcohol-sensitive group (r=0.742, p=0.0115) and nonsensitive group (r=0.882, p<0.0001) (fig. 2). There was no signi-ficant difference between the responsiveness of the air-ways to acetaldehyde compared to methacholine between atopic and nonatopic subjects in the nonalcohol-sensitive group.

Discussion

In this study, the bronchial responsiveness to acetalde-hyde and methacholine in asthmatic subjects with and without alcohol-induced bronchoconstriction was investi-gated. The results showed that bronchial responsiveness to acetaldehyde relative to methacholine was augmented in asthmatic subjects with alcohol-induced bronchoconstric-tion when compared to asthmatic subjects who were tolerant to alcohol, and that a positive correlation between $PC_{20,meth}$ and $PC_{20,acet}$ was statistically significant in both the alcohol-sensitive group and nonsensitive group. Since the authors [3] have already reported that inhalation of acetaldehyde by the same method causes no significant bronchoconstriction in healthy nonasthmatic subjects, no control subject without asthma participated in this study.

Inhaled acetaldehyde causes bronchoconstriction indir-ectly *via* endogenously released histamine in guinea-pigs [2] and asthmatic subjects [3]. Although the origin of the histamine released by acetaldehyde in asthmatic airways is not clear, acetaldehyde causes dose-dependent hista-mine release from the leukocytes of asthmatic subjects *in vitro* [1]. At present, the mechanism responsible for the increased acetaldehyde responsiveness of asthmatic air-ways in alcohol-sensitive subjects is unclear, but possi-bilities include the increased releasability of histamine releasers such as leukocytes and an inability to metabolize acetaldehyde. SHIMODA *et al.* [1] reported that leukocyte histamine release induced by acetaldehyde was not dif-ferent between alcohol-sensitive and nonalcohol-sensitive asthmatic patients, making it unlikely that the change in histamine releasability plays an important role in the
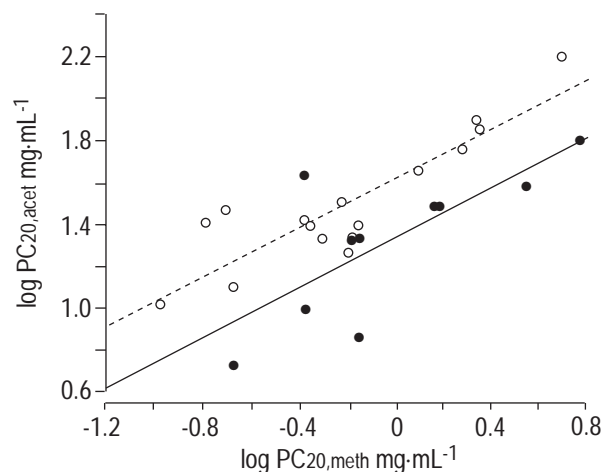

Fig. 2. – Relationship between bronchial responsiveness to metha-choline and acetaldehyde in alcohol-sensitive asthmatic (●) and non-alcohol-sensitive asthmatic (○) subjects. The regression lines for nonalcohol-sensitive asthmatics (- - - -) and alcohol-sensitive asthmatics (———) subjects are shown. $PC_{20,meth}$ and $PC_{20,acet}$: provocative concentration of methacholine and acetaldehyde causing a 20% fall in forced expiratory volume in one second, respectively. A significant correlation was observed between $PC_{20,meth}$ and $PC_{20,acet}$ in both the alcohol-sensitive group (r=0.742, p=0.0115) and nonalcohol-sensitive group (r=0.882, p<0.0001).

selective hyperresponsiveness to acetaldehyde in subjects with alcohol-induced bronchoconstriction.

Ethanol is oxidized to acetaldehyde, which in turn is oxidized to acetate, mainly by ALDH, which consists of two main isozymes with low and high Michaelis-Menten constant (K_m; the substrate concentration at which an enzyme-catalysed reaction proceeds at half its maximum velocity) for aldehyde [9–11]. Approximately 50% of Japanese people lack the low K_m enzyme (ALDH type 2 (ALDH2)), and show an elevation of serum acetaldehyde concentration due to their inability to metabolize acetaldehyde [12–14], which is a major factor in determining exacerbations of asthma after alcohol intake [4]. For example, it has been reported that a significant bronchoconstriction (>20% decrease in FEV1) was observed after oral ethanol challenge in three of 16 (19%) Japanese asthmatic patients with a normal homozygous ALDH2 genotype (normal ALDH2 activity), 10 of 14 (71%) Japanese asthmatics with a mutant heterozygous ALDH2 genotype (low ALDH2 activity), and two of two (100%) Japanese asthmatics with a mutant homozygous ALDH2 genotype (no ALDH2 activity) [4]. Since ALDH2 is reported to be expressed in human lungs [10, 15] and the genetic polymorphism at the ALDH2 gene locus occurs in Oriental populations, it is intriguing to predict that the pulmonary ethanol-metabolizing capabilities may vary in individuals with different genetic phenotypes. Although the lung ALDH2 activity was not examined in the present study, it is possible that the lung ALDH2 activity plays an important role in relative bronchial hyperresponsiveness to acetaldehyde in alcohol-sensitive asthmatics. However, further studies are required to draw a conclusion.

In summary, this study has demonstrated an increased airway responsiveness to acetaldehyde relative to methacholine in asthmatic subjects with alcohol-induced bronchoconstriction. It is believed that this is the first report suggesting bronchial hyperresponsiveness to acetaldehyde in alcohol-sensitive asthmatic subjects.

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