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Risk of Acute Bronchospasm and Bronchial Hyperreactivity from Inhaled Acid Aerosol in Healthy Subjects: Randomized, Double-blind Controlled Trial

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Aim. To determine the risk of developing acute bronchial spasm and bronchial hyperreactivity in healthy subjects after inhaling hydrochloric acid aerosol of different pH, since various characteristics of inhaled aerosol, particularly its acidity, contribute to the reduction in lung function in patients with a pre-existing disease.

Methods. Randomized, double-blind, placebo-controlled trial included 79 healthy volunteers. After submitting a written consent, they were randomized in 4 groups with approximately equal number of participants. Each group was exposed to acid aerosol of different pH. The respiratory lung function expressed by forced expiratory volume (FEV_1), forced expiratory flow at 50% (FEF_{50}), 75% (FEF_{25}), and between 25% and 75% of vital capacity ($FEF_{25/75}$), and non-specific bronchial reactivity were measured after inhalation of hydrochloric acid aerosol of pH 7.0, pH 5.0, pH 3.0, and pH 2.0 and compared with baseline values. Twelve subjects, who reached the threshold doses in both bronchial challenges, were tested again after receiving a systemic β-blockade with a single oral dose of propranolol.

Results. Significant bronchospasm developed after the inhalation of hydrochloric acid aerosol of pH 5.0, 3.0, and 2.0. After the inhalation of aerosols of pH 5.0 and pH 2.0, all parameters of respiratory lung function (FEV_1, FEF_{50}, FEF_{25}, and $FEF_{25/75}$) significantly decreased. After the inhalation of aerosol of pH 3.0, all parameters significantly decreased except for FEF_{25}, which showed no significant difference (1.84 ± 0.46 before vs 1.58 ± 0.49 after inhalation; $p = 0.07$). The inhalation of hydrochloric acid aerosol had no significant effect on the level of non-specific bronchial reactivity irrespective of its pH. Eight out of 12 subjects tested again after pretreatment with propranolol and with no significant change in the heart rate showed no change in non-specific bronchial reactivity in comparison with the 4 subjects who reacted with a significant decrease in the heart rate ($> 10\%$), as well as in non-specific bronchial reactivity ($p = 0.023$).

Conclusion. Inhalation of acid aerosol in healthy subjects induced a bronchial spasm, but had no effect on non-specific bronchial reactivity except in subjects under systemic adrenergic inhibition.

Key words: *aerosols; bronchial hyperreactivity; bronchial spasm; histamine; hydrochloric acid; lung; propranolol; respiratory function tests*

Environmental factors – chemical, physical, and biological – can provoke chronic inflammatory disorder of the respiratory tract characterized by various degree of airflow obstruction (1-5). The lungs, like most other tissues, do not handle acid well. Lowering of airway pH into the asthmatic range (pH 5.2 ± 0.2) produces bronchial spasm, impairs ciliary motility, increases mucus viscosity, damages the epithelium, and causes eosinophils to release bronchoconstrictors and proinflammatory substances. Acid pH range can also stimulate C fibers, which aggravate bronchoconstriction by sensory neuropeptide release, which in turn aggravates tissue acidosis (6).

Previous studies evaluated the importance of titrable acidity, osmolarity, inhaled droplet size, and specific chemical composition of aerosol in inducing bronchoconstriction in asthmatic patients (7-11). The decrease in airway pH may have additional consequences on asthma (8,12). Therapies directed at normalizing airway pH early in the course of an acute exacerbation of asthma could help prevent the cascade of events leading to airflow obstruction (6).

We postulated that the inhalation of acid aerosol of different pH could induce changes in airway patency and reactivity in a dose dependent manner even in healthy subjects. The aim of our study was to establish the risk of bronchoconstriction and changes in non-specific bronchial reactivity after exogenous airway acidification and to determine the possible role of adrenergic nervous system in these reactions in healthy subjects.

Subjects and Methods

Subjects

There were 79 healthy volunteers (3 women and 76 men) enrolled in the study. The subjects were recruited in the military out-patient department during their periodical medical examination in February 2001 at the Institute for Medical Research and Occupational Health. The mean (± standard deviation) age of the subjects was 32 ± 6 years. Inclusion criteria were willingness to participate in the trial and comply to the study protocol, age over 18 years, ability to make a reproducible lung function measurement, and lung function in the normal range. Exclusion criteria were any acute or chronic respiratory, cardiovascular, renal, hematological, neurological, or psychiatric disorders; use of medications that can influence bronchial reactivity measurements, such as β-adrenergic antagonists, calcium channel blockers, theophylline, amiodarone, ampicillin, anticoagulant therapy, barbiturates, benzodiazepines, quinidine, cimetidine, hydralazine, oral contraceptives, disopyramide, morphine, propafenone, and rifampicin; and infection of the upper respiratory tract within 6 weeks prior to trial.

Out of 83 subjects assessed for eligibility, 4 were excluded, which left 79 subjects to be included in the study. All subjects signed written consent after being fully informed about the study and the experimental protocol. Ethical Committees of Zagreb University School of Medicine and Institute for Medical Research and Occupational Health approved the study protocol.

Clinical Trial Protocol

A double-blind, placebo-controlled trial was performed at the Department for Occupational and Environmental Health, Institute for Medical Research and Occupational Health, Zagreb, Croatia, during March and April 2001. The protocol consisted of history taking, physical examination, baseline spirometry, randomization, non-specific bronchial reactivity measured by histamine challenge, histamine challenge after hydrochloric acid aerosol inhalation of pH 7.0, 5.0, 3.0, or 2.0, and histamine challenge after systemic adrenergic blockade with propranolol.

Methods

Spirometry. On the initial study day, the subjects underwent baseline spirometry. All spirometric tests were performed on a computerized pneumotachograph (Flowscreen, Jaeger GmbH, Würzburg, Germany). We used the registration of flow-volume curves which are the registration of flow as function of changes in pulmonary volumes.

Five parameters were measured and used for further analysis: forced expiratory volume in the 1st second (FEV_1), forced vital capacity (FVC), forced expiratory flow on 50% (FEF_{50}), 75%, (FEF_{25}), and between 25% and 75% of FVC ($FEF_{25/75}$). All spirometric tests were done according to the guidelines of the American Thoracic Society (ATS) (13). All values were cross-referenced to predicted values in terms of sex, age, height, and weight to test if they were in the normal range (14).

Randomization. After fulfilling all inclusion criteria, the subjects were allocated into four groups according to randomized sequence. For the need of randomization, we used STATISTICA for Windows, Kernel release 5.5 A software package (StaSoft Inc., Tulsa, OK, USA) to generate randomized numbers. Pre-generated numbers were allocated to each of the participants in closed envelopes by a nurse when they were approached for eligibility. Allocation sequence was revealed just before the first intervention (inhalation of acid aerosol).

Histamine challenge. On the same day after spirometry test was performed, the subjects were tested with a histamine challenge test to determine the level of their non-specific bronchial reactivity, according to the procedure described by Chai et al (15). The subjects inhaled doubling concentrations of histamine diphosphate saline solutions (Sigma Chemical Company, St. Louis, MO, USA) every 3 minutes, from a DeVilbiss nebulizer (model 646, DeVilbiss Health Care Co., Somerset, PA, USA) controlled by a dosimeter (KoKo dosimeter, Ferraris Respiratory, Louisville, CO, USA). Output of the nebulizer was calibrated to 11.9 ± 3.7 μL per inhalation. Subjects inhaled 5 inhalations at a pace of 15/minute for each concentration. Starting concentration of histamine diphosphate was 0.125 mg/mL and maximum used was 64 mg/mL. Histamine responsiveness was measured by FEV_1, FEF_{50}, FEF_{25}, $FEF_{25/75}$ after each inhaled dose. Non-specific bronchial reactivity was expressed as a slope of dose-response curves. Slope was calculated as a natural logarithm of percentage of decline of FEV_1 after last histamine dose divided by a cumulative dose of applied histamine (16).

Acid aerosol inhalation. On the following day, each group of subjects inhaled aerosol of different pH. The solutions of hydrochloric acid (HCl) were prepared by using 0.9% NaCl and 0.2 mol/L KCl, 0.2 mol/L HCl, 0.1 mol/L NaOH, 0.1 mol/L K_2HPO_4, and 0.1 mol/L Na_2HPO_4. The prepared solutions were standardized to the designated pH by use of Iskra MA 5730 pH-meter (Iskra, Kranj, Slovenia) in Clinical Toxicological Chemistry Unit, and labeled as X, Y, Z, or Q to secure double blinding for the pH of solution, ie, thus neither the investigator nor subjects were aware of the pH of the solution used. Unblinding was available after the statistical analysis of all results was performed. Groups X, Y, Z, and Q inhaled HCl aerosol of pH 5.0, pH 3.0, pH 7.0, and pH 2.0, respectively. DeVilbiss 646 nebulizer was used for each inhalation, which lasted 3 minutes. After the inhalation of HCl aerosol, subjects were again challenged with histamine according to the same protocol as the previous day.

Twelve subjects who reached the threshold doses of histamine diphosphate (fall of $FEV_1 \geq 20\%$ in relation to baseline) on both study days continued the study. In these subjects, we provoked a systemic blockade of β-adrenergic nervous system with a single oral dose (80 mg) of propranolol. This allowed us better insight into the role and importance of adrenergic nervous system in the regulation of airway diameter. The propranolol effect, ie, the degree of β-blockade, was evaluated by measuring the heart rate (> 10% decrease from baseline) by palpating the pulse of the radial artery. Five of these 12 subjects were from group Y (pH 3.0) and 7 were from group Q (pH 2.0). Ninety minutes after the oral dose of propranolol, inhalation of HCl aerosol and histamine challenge were repeated according to the same protocol used on a previous day.

Statistical Analysis

Comparisons between repeated measurements of lung function parameters and non-specific bronchial reactivity were performed with paired t-test. To test for normality of distribution, before using t-test, Kolmogorov-Smirnov test was applied. For comparisons between groups, the one-way and repeated measures analyses of variance were used, with *post hoc* comparison of means. Before performing analysis of variance we tested for the homogeneity of variances. Comparisons of qualitative variables among groups were performed with Pearson chi-square test. The level of statistical significance was set at $p < 0.05$. We used STATISTICA for Windows software package.

Results

The flow of participants through the study is shown in Figure 1.

There were no significant differences between the groups in their age ($p = 0.33$; analysis of variance), sex ($p = 0.13$; Pearson chi-square test), and smoking habits ($p = 0.73$; Pearson chi-square test) (Table 1). Analysis of variance revealed no significant differences between the groups in the baseline values of their lung function measures (FVC, FEV_1, FEF_{50}, FEF_{25}, and $FEF_{25/75}$; Table 2).

Bronchoconstriction after Acid Aerosol Inhalation

We compared the mean values of lung function parameters measured before and after the inhalation of acid aerosol (Table 3). In the group inhaling HCl aerosol of pH 7.0, only the values of $FEF_{25/75}$ were significantly lower from baseline values after the inhalation (paired t-test, $p = 0.02$). In the group inhaling HCl aerosol of pH 5.0, the values of all lung parameters were significantly lower than the baseline values after

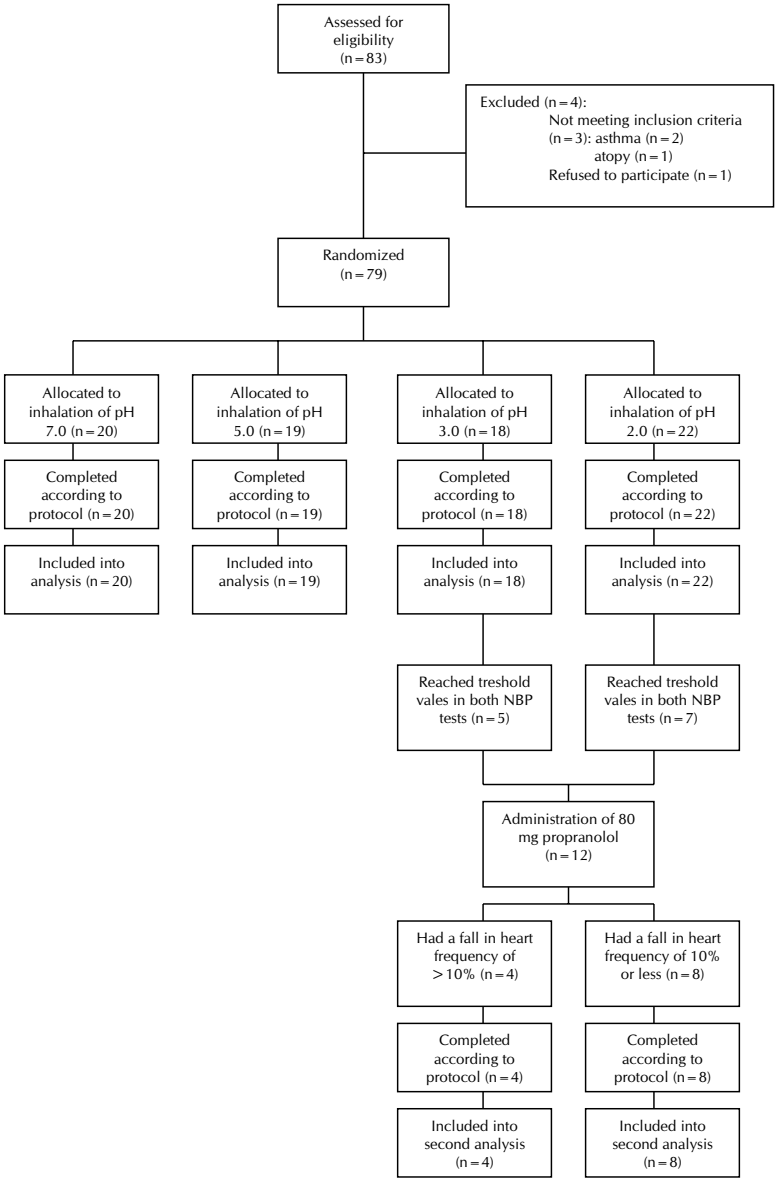

Figure 1. Flow of participants through the study.

the inhalation (Table 3). In the group inhaling HCl aerosol of pH 3.0, all lung function parameters were significantly lower after the inhalation than before, except for FEF_{25} (1.84 ± 0.46 vs 1.58 ± 0.49, $p = 0.07$; Table 3). In the group inhaling HCl aerosol of pH 2.0, all values of lung function parameters were significantly lower after the inhalation. Between-group comparison (repeated measures analysis of variance) showed no significant difference except for FEF_{50} that showed the greatest decrease with the inhalation of HCl aerosol of pH 2.0 ($p = 0.019$).

Non-specific Bronchial Reactivity after Exposure to Acid Aerosol

Comparison between baseline values of non-specific bronchial reactivity and non-specific bronchial reactivity after the inhalation of HCl aerosols of pH 7.0, 5.0, 3.0, and 2.0 showed no significant differences in response ($p = 0.85$, repeated measures analysis of variance, Table 4).

Non-specific Bronchial Reactivity after Exposure to Acid Aerosol under Systemic β Blockade

After taking oral dose of propranolol for β-adrenergic blockade, 4 (responders) out of 12 subjects reacted with significant decrease in the heart rate (>10%). The remaining 8 (non-responders) subjects showed no significant change in their heart rate (Fig. 2). Non-specific bronchial reactivity after the exposure to HCl aerosol of pH 2.0 and pH 3.0 was significantly increased only in subjects who reacted with a significant decrease in heart rate ($p = 0.012$; repeated measures analysis of variance).

Discussion

Our results suggest that even in healthy subjects there is a dose-response effect of exogenous acid aerosol on airway patency, with the greatest effect

Table 1. Characteristics of 79 healthy subjects in four tested groups

Characteristics	pH of inhaled aerosol			
	pH 7.0 (n = 20)	pH 5.0 (n = 19)	pH 3.0 (n = 18)	pH 2.0 (n = 22)
Sex (F/M)*	1/19	0/19	2/16	0/22
Age (years)†	32.5 ± 6.0	32.2 ± 5.6	33.4 ± 5.6	30.4 ± 3.7
Smokers/non-smokers‡	14/6	13/6	13/5	12/10

*F/M = female/male.
†Mean ± standard deviation, no significant differences (p = 0.330, one-way ANOVA).
‡ No significant differences (p = 0.730, chi-square test).

Table 4. Non-specific bronchial reactivity (NBR, mean ± standard deviation) before and after the exposure to acid aerosol in four tested groups (n = 79)

Non-specific bronchial reactivity*	pH of inhaled aerosol			
	pH 7.0 (n = 20)	pH 5.0 (n = 19)	pH 3.0 (n = 18)	pH 2.0 (n = 22)
Before exposure	-1.23 ± 0.88	-1.34 ± 1.86	-1.96 ± 1.68	-1.82 ± 1.76
After exposure	-1.27 ± 0.81	-1.41 ± 1.82	-1.76 ± 1.87	-1.61 ± 1.35

*Repeated measures analysis of variance, p = 0.85.

Table 2. Baseline values of FVC, FEV_1, FEF_{50}, FEF_{25}, and $FEF_{27/75}$ expressed as the percentage (mean ± SD) of predicted values (values were cross-referenced to predicted values in terms of sex, age, height, and weight, ref. 14) in four tested groups

Lung function parameter	Groups according to pH of inhaled aerosol				
	pH 7.0 (n = 20)	pH 5.0 (n = 19)	pH 3.0 (n = 18)	pH 2.0 (n = 22)	p*
FVC	108.8 ± 13.3	106.8 ± 9.6	101.1 ± 7.6	109.3 ± 12.6	0.102
FEV_1	106.5 ± 11.2	104.8 ± 10.8	99.2 ± 9.9	108.3 ± 11.8	0.071
FEF_{50}	96.5 ± 13.3	94.7 ± 20.0	96.3 ± 19.5	102.3 ± 24.6	0.615
FEF_{25}	80.8 ± 17.8	82.6 ± 29.7	72.8 ± 18.9	91.9 ± 25.1	0.090
$FEF_{25/75}$	92.2 ± 14.0	92.6 ± 22.1	86.7 ± 22.5	99.6 ± 25.0	0.303

*Analysis of variance.

Table 3. Lung function parameters (mean ± standard deviation) after exposure to acid aerosol in four tested groups according to the pH of the inhaled aerosol*

Lung function parameters		pH of inhaled aerosol										
		pH 7.0 (n = 20)			pH 5.0 (n = 19)			pH 3.0 (n = 18)			pH 2.0 (n = 22)		
		baseline/ after inhalation†	% change from baseline (95% CI)	p‡	baseline/ after inhalation	% change from baseline (95% CI)	p†	baseline/ after inhalation	% change from baseline (95% CI)	p†	baseline/ after inhalation	% change from baseline (95% CI)	p†
FEV_1 (L)	b	4.36 ± 0.58	1.06	0.06	4.47 ± 0.50	1.50	0.02	4.07 ± 0.54	2.75	0.036	4.68 ± 0.63	2.44	0.003
	a	4.31 ± 0.61	(0.01-2.10)		4.40 ± 0.52	(0.31-2.68)		3.95 ± 0.52	(0.17-5.33)		4.56 ± 0.60	(0.89-4.00)	
FEF_{50} (L/s)	b	4.88 ± 0.81	2.26	0.21	4.98 ± 1.19	5.72	0.003	4.94 ± 1.05	5.99	0.004	5.50 ± 1.53	6.51	0.001
	a	4.78 ± 0.94	(-1.21-5.73)		4.70 ± 1.19	(2.36-9.08)		4.65 ± 1.07	(2.37-9.60)		5.12 ± 1.40	(2.75-10.27)	
FEF_{25} (L/s)	b	1.80 ± 0.38	3.75	0.25	1.92 ± 0.53	11.37	< 0.001	1.84 ± 0.46	14.26	0.07	2.28 ± 0.81	6.73	0.02
	a	1.73 ± 0.44	(-3.07-10.57)		1.71 ± 0.52	(7.74-15.00)		1.58 ± 0.49	(0.21-22.33)		2.08 ± 0.68	(0.87-12.58)	
$FEF_{25/75}$ (L/s)	b	4.14 ± 0.73	4.09	0.02	4.24 ± 0.98	7.19	< 0.001	4.10 ± 0.84	8.93	0.014	4.78 ± 1.42	6.54	< 0.001
	a	3.98 ± 0.78	(0.93-7.25)		3.93 ± 0.95	(3.77-10.60)		3.74 ± 0.96	(2.01-15.83)		4.44 ± 1.23	(3.16-9.94)	

*Abbreviations: FEV_1 – forced expiratory volume in the 1st second; FEF_{50} – forced expiratory flow on 50% vital capacity; FEF_{25} – forced expiratory flow on 75% vital capacity; and $FEF_{27/75}$ – forced expiratory flow between 25% and 75% vital capacity, 95% CI – 95% confidence interval.
†Paired t-test.
‡b – baseline; a – after inhalation.

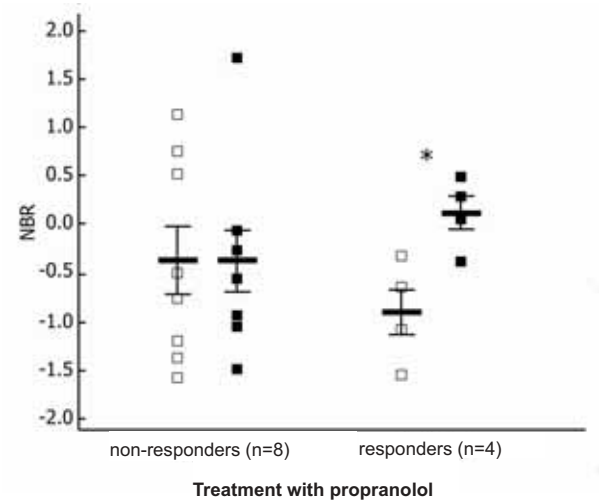

Figure 2. Non-specific bronchial reactivity (NBR) after the exposure to acid aerosol (open squares), and after the exposure to acid aerosol with β-adrenergic antagonist pre-treatment (closed squares) in non-responders and responders (> 10% decrease in heart rate, to a single dose of 80 mg of orally administered propranolol. Results are presented as mean ± standard error of mean (SEM). Asterisk, p = 0.012; repeated measures analysis of variance.

achieved at pH 3.0 (and plateau afterwards) for most of the measured parameters. The FEF_{50} showed to be the most sensitive parameter for its evaluation. Non-specific bronchial reactivity did not change after acid aerosol inhalation, suggesting that airway defense mechanisms were activated. This assumption is corroborated with the significant non-specific bronchial reactivity change after acid aerosol inhalation in the group of 4 responders who had significant adrenergic blockade after pretreatment with propranolol.

Many technical details limit the comparability of different studies dealing with this problem. The results of previous studies demonstrated that titrable acidity, specific chemical composition, droplet size, and osmolarity as well as pH must be considered when evaluating the airway response to acid aerosol (7-10). The results obtained by Fine et al (7) suggested that large particles of acid aerosols produced more intensive bronchoconstriction than did smaller ones. The same study also suggested that the bronchoconstrictor potency of acid aerosols, expressed as a change in specific airway resistance (SRaw), is related to their total available hydrogen ion concentration (titrable acidity) and not merely to their free hydrogen ion concentration. The relationship between the tit-

rable acidity and bronchoconstriction potency is in accordance with the notion that acid aerosols cause bronchoconstriction, at least partly, by altering airway surface pH.

The studies with acid aerosol inhalation were mostly performed in asthmatic patients. Koenig et al (17) exposed subjects with extrinsic asthma and exercise-induced bronchial spasm to physical exercise and aerosol droplet of sodium chloride (NaCl) or sulfuric acid (H_2SO_4) and found that exposure to the H_2SO_4 aerosol produced larger reductions in $Vmax_{75}$ as well as significant changes in $Vmax_{50}$ FEV_1 and total respiratory resistance (RT). Similar results were reported by Linn et al (18). The results reported by Utell et al (10) indicated that asthmatics demonstrated bronchoconstriction after exposure to acid sulfate aerosol and dose-response relationship to inhaled sulfates. In asthmatic patients it is not only exogenous acid aerosol that may aggravate asthmatic symptoms, but also a significant acidity produced endogenously in airways due to inflammatory process. Hunt et al (19) found that water derived from lower airways was acidified in patients with acute asthma. The water pH was sufficient to cause both NO evolution from endogenous NO_2^- and the necrosis of eosinophils. Furthermore, pH normalized during glucocorticoid treatment (19). That observation suggested that airway pH was an important determinant of expired NO concentration and airway inflammation, and that it might have a role in pathophysiology of acute asthma (8). It has been shown that both adolescent and adult asthmatic subjects are much more sensitive to effects of inhaled sulfur dioxide (SO_2, a common constituent of ambient acid aerosols) than predicted from the studies among healthy adults (4,5). From these results and results of our study, it seems that physiological defense mechanisms are significantly deprived in asthmatics and that this is possibly connected with the function of adrenergic nervous system.

Blockade of the β-adrenergic receptors probably excluded the dilating effect of adrenergic nerves on airways, which is why acidity could induce not only bronchial spasm but also the change in non-specific bronchial reactivity. These results imply that the autonomic nervous system plays an important role in regulating airway diameter and that its dysfunction is likely to contribute to the pathogenesis of airway diseases (20,21). Matsumoto et al (22) suggested that bronchoconstriction was regulated by the prejunctional modulation of the cholinergic system via α- and β-adrenoreceptors. Our results are in accordance with those reported previously (7,10,16), ie, that inhalation of acid aerosol leads to significant changes in pulmonary function. However, the direct comparison among studies is not possible because there were differences in clinical characteristics of subjects, measured parameters of pulmonary function, used aerosols, and experimental protocols.

There are several limitations to our study. One of them is a relatively small study sample. We would need larger groups of subjects (>400 participants) to have 80% statistical power to indicate the significant change in non-specific bronchial reactivity after acid aerosol inhalation at $\alpha = 0.05$. Also, the 3-minute inhalation of acid aerosol is a relatively short time, but due to ethical considerations it was considered as an optimum quantity for the reflection of acute responses. The comparison of responders and non-responders to single oral dose of propranolol included a small number of participants in our study, but in a group of responders showed significant effects on non-specific bronchial reactivity. Since our trial included only healthy subjects (mostly men), our conclusions are limited only to that population and our results can not be compared to those in other studies on asthmatic patients. However, we believe that this study is a good starting point to evaluate this matter further.

To the best of our knowledge, this is the first trial investigating all these parameters in a series among the same group of subjects.

We conclude that inhalation of acid aerosol in healthy subjects can, to a certain extent, induce a bronchial spasm, has no effect on non-specific bronchial reactivity, and can increase non-specific bronchial reactivity in subjects with lower tone of adrenergic part of autonomic nervous system.

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