

Short communication

Occupational asthma and rhinitis due to glutaraldehyde: changes in nasal lavage fluid after specific inhalatory challenge test

Background: Glutaraldehyde (GA) is a known respiratory sensitizers, and some studies have reported occupational asthma in exposed workers. Specific changes in nasal lavage fluid (NLF) induced by high-molecular-weight allergen provocation in sensitized subjects were described previously. The purpose of this study was to evaluate the changes in cytogram, protein content, eosinophil cationic protein (ECP), and mast-cell tryptase concentrations in NLF after GA inhalation challenge in patients with a positive history of GA-induced asthma and late or dual asthmatic response due to exposure to low-level GA.

Methods: A single-blind, placebo-controlled study was performed on 11 health workers with occupational asthma and rhinitis due to GA. The control groups comprised 10 atopic subjects with perennial asthma and rhinitis and 10 healthy ones. A “nasal pool” technique was used to evaluate the examined parameters in nasal washings before and 30 min, 4 h, and 24 h after the inhalatory provocation with GA and placebo.

Results: There was a significant increase in eosinophil number and percentage, and albumin, ECP, and tryptase concentrations in NLF from patients with occupational asthma and rhinitis when compared to controls.

Conclusions: The results indicate the immunologic mechanism of GA-induced asthma and the applicability of the “nasal pool” technique as the diagnostic procedure in GA-induced airway allergy.

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Glutaraldehyde (GA) is a biocide commonly used for high-level disinfection and cold sterilization. In recent years, GA has emerged as the main cause of occupational asthma among health-care workers (1).

Diagnosis of GA-induced asthma is based mainly on a provocation test performed at the workplace with monitoring of PEFR followed by fully controlled provocation challenges in the laboratory (2). However, the isolated spirometric changes induced by GA seem to be inadequate for diagnosing occupational asthma – the specific smell of GA enables the patient to recognize the agent (3). Moreover, the identification of anti-GA specific IgE in the serum is of insufficient diagnostic value (4). Thus, stronger evidence for the diagnosis is necessary – such as an objective estimation of the provocation test results. GA-induced asthma is often accompanied by rhinitis (5). The nose is the part of the respiratory system that is most easily accessible for morphologic and pathophysiologic evaluations (6).

The aim of the present study was to assess the applicability of the “nasal pool” technique for the diagnosis of GA-induced occupational airway allergy, as well as to evaluate the cellular response and the changes in protein

level, eosinophil cationic protein (ECP), and mast-cell tryptase concentration in nasal lavage fluid (NLF) after exposure to low-level GA.

Material and methods

Subjects

Three groups of nonsmoking subjects were enrolled for the study. Group A comprised 11 health-care workers – eight nurses, one laboratory technician, one radiographer, and one ward attendant (mean age: 48.3 ± 7.8 years, seniority 26 ± 7.6 , occupationally exposed to GA for 6 ± 3.2 years) with occupational asthma due to GA. All the subjects reported a history of asthmatic symptoms in relation to GA exposure in the workplace and showed a 20% or more decrease in FEV₁ during a controlled specific inhalatory challenge test with GA, compared to the values obtained after saline challenge. Individual data of patients with GA-induced asthma are shown in Table 1.

Group B consisted of 10 atopic patients (mean age 39.5 ± 7.7 years) with perennial asthma and rhinitis. Group C consisted of 10 healthy individuals (mean age 33.5 ± 12.5 years). Both the control groups had negative results of inhalatory challenge with GA.

The study participants had not received any systemic or local medication, except inhaled, short-acting β_2 -agonists, for 14 days before the study. None of the controls (groups B and C) had been occupationally exposed to GA in the past. The subjects could not be

subjected to GA exposure for at least 14 days before the onset of the project. All the patients were hospitalized in a GA-free environment during the course of the study.

Study protocol

The study was designed as a biphasic, crossover, single-blind trial. At phase I, the subjects were challenged with 0.9% saline as placebo. At least 7 days later, the allergen challenge with 2% GA was performed. The test was done by painting the solution onto a 2 m² piece of cardboard in a challenge chamber for 15 min (2).

The Regional Bioethics Committee approved the study protocol, and all the participants gave their written consent prior to the study.

The estimation of GA levels in the air

The concentration of GA during exposure was estimated with a Hewlett-Packard gas chromatographer with a flame-ionization detector (FID) and HP-17 capillary column (30 m × 0.54 mm × 2.00 µm) (7).

Skin prick tests and IgE evaluation

Skin prick tests (SPT) were performed on the volar part of the forearm with a standard battery of common allergens including tree and grass pollen, molds, feathers, and house-dust mite (Allergopharma, Germany). The negative control was allergen diluent, and the positive one was histamine dihydrochloride solution at 1 mg/ml. All the tested sites were examined after 15 min: a wheal of 3 mm greater than negative control was considered positive.

Total serum IgE was evaluated by the Uni-CAP System (Uppsala, Pharmacia, Sweden).

Nasal lavage and challenge procedure

All the procedures were performed as in the "nasal pool" method (8). Nasal washings were collected immediately before the provocation and 30 min, 4 h, and 24 h afterward; the washings processing has been described in detail elsewhere (9).

Symptom score

The number of sneezes and the degree of mucosal edema, rhinorrhea, and itching were evaluated. The total symptom score (SS) ranged from 0 to 8 and represented the sum of the scores for sneezing (0 sneezes: 0 points; 1–4 sneezes: 1 point; >4 sneezes: 2 points), rhinorrhea (none: 0 points; mild: 1 point; abundant: 2 points), mucosal edema (none: 0 points; mild: 1 point; nasal block: 2 points), and itching (none: 0 points; itching of the nose or throat: 1 point; itching of the nose and throat: 2 points). Positive clinical challenge was defined as a total score of more than 3 points (10).

Mediator levels

Nasal ECP and tryptase concentrations were measured by radioimmunoassay (RIA kits, Pharmacia, Sweden) according to the manufacturer's protocol. The samples for these assays were collected before and 30 min, 4 h, and 24 h after the provocation test.

Pulmonary function

Bronchial response was measured by serial monitoring of forced expiratory volume in 1 s (FEV₁) and peak expiratory flow rate (PEFR) with a spirometer (Vicat 2A, Mijnhart, The Netherlands) – before and 5 min, 30 min, 1 h, 2 h, 4 h, 6 h, and 24 h after the provocation. All the subjects were instructed

beforehand how to use the peak flow meter for hourly PEF measurements. Histamine challenge was performed according to Cockcroft (11).

Statistics

The data were analyzed by a parametric two-way ANOVA and Tukey's test for multiple comparisons. The differences were regarded as significant at $P < 0.05$.

Results

The estimation of GA levels in the air

The mean concentration of GA in the air during the challenge tests was 0.32 ± 0.08 mg/m³; i.e., below the occupational exposure standards (12, 13).

Symptom score, and cellular and biochemical findings

Symptom score, and cellular and biochemical findings in NLF induced by GA and placebo in GA-sensitive subjects, and by GA in atopic and healthy controls participating in the study can be seen in Table 2.

Symptom score

The allergen challenge induced the symptoms of rhinitis in all subjects from group A. The symptoms were severe in that group (score 6.73 ± 0.79). The reaction to GA in subjects from groups B and C was slight (scores 2.0 ± 0.82 and 1.3 ± 0.82). Statistical analysis revealed significant differences between groups at 30 min, 4 h, and 24 h after the provocation – the score after challenge with GA in group A was significantly higher than that after challenge with placebo in the same group and than after GA in groups B and C. Within-group comparisons also revealed significant differences in groups A, B, and C. In group A, the score after GA challenge was significantly higher at 30 min, 4 h, and 24 h than before the challenge. In groups B and C, GA also induced a significant increase in clinical score at 30 min, but this increase did not persist until 24 h.

Cellular findings

The provocation with GA resulted in an increase in the total number of leukocytes in nasal washings from all the groups at all time points. The differences between the groups were statistically significant at 4 and 24 h after the provocation – the total leukocyte count in group A after GA challenge was significantly higher than after placebo challenge in the same groups, and than after GA challenge in groups B and C. Significant differences induced by GA in the total leukocyte count were observed in group A between 0 and 30 min, 4 h, and 24 h.

Table 1. Demographic data, and clinical and laboratory findings of patients with occupational asthma and rhinitis due to GA ($n=11$)

Patient Initials	Job	Age (years)	Seniority (years)	Duration of exposure to GA (years)	Results of SPT with common allergens	Total IgE level (ku/l)	Asthmatic response during SIPT with GA	PC _{20H} Pre-post-SIPT with GA
M.S.	Theater nurse	40	20	6	—	45	Dual	4.2–1.0
G.B.	Nurse	52	34	3	—	60.5	Late	1.08–0.67
M.T.	RTG technician	62	42	10	+	10.7	Late	1.9–0.35
A.K.	Laboratory technician	56	28	6	—	27	Late	0.83—not done
E.P.	Nurse at endoscopy unit	51	21	3	—	24.9	Late	5.71–0.67
B.M.	Nurse	38	18	2	—	11.8	Late	16–3.2
T.B.	Ward attendant	53	24	6	—	17.5	Dual	6.4–0.26
H.M.	Nurse	48	30	10	—	40.1	Dual	4.8–0.15
D.K.	Nurse	50	30	10	—	136	Late	5.16–1.11
A.R.	Nurse at USG unit	38	18	2	+	89	Late	14.5–1.1
I.O.	Theater nurse	43	21	8	—	31.1	Late	5.6–3.3

SPT: skin prick test; SIPT: specific inhalatory provocation test; GA: glutaraldehyde; PC_{20H} – provocation concentration of histamine producing 20% fall in FEV₁.

The neutrophil proportion changed significantly after GA challenge in groups A and B – at 4 and 24 h, it was higher than before the challenge.

An analysis of the total eosinophil count revealed significant differences only in group A after GA provocation. The values were higher at 30 min, 4 h, and 24 h than before the challenge. Moreover, the groups differed significantly at 24 h. In group A, the eosinophil count after GA challenge was higher than in the other groups and than after placebo provocation in the same group.

An analysis of the proportion of eosinophils revealed significant differences between the groups at 30 min, 4 h, and 24 h after GA challenge – in group A, the percentage was higher after GA provocation than in the B and C groups, and than after placebo challenge in group A. Within-group comparisons revealed significant differences only in group A after GA provocation. The eosinophil percentage in group A was higher at 4 and 24 h than before the challenge and than at 30 min afterward (Fig. 1).

An analysis of the total basophil count revealed

significant differences between the groups at 4 and 24 h after provocation. At these time points, the basophil count in group A after GA challenge was significantly higher than after placebo administration in this group and than in groups B and C. Within-group analysis revealed significant differences only in group A after provocation with GA. The basophil count was significantly higher at 4 h than before the challenge and at 24 h than before, and at 30 min and 4 h after the provocation.

An analysis of the proportion of basophils revealed significant differences between the groups at 30 min, 4 h, and 24 h after GA challenge – at 30 min afterward, the percentage was higher in group A than after placebo challenge, and than in group C; at 4 and 24 h, the basophil proportion in group A was significantly higher than after placebo challenge in the same group, and than after GA challenge in groups B and C. Within-group comparisons revealed significant differences only in group A after GA provocation. The basophil percentage in that group was higher at 30 min, 4 h, and 24 h after than before the challenge (Fig. 1).

Table 2. Symptom score, and cellular and biochemical findings in nasal lavage fluid (NLF) induced by glutaraldehyde (GA) and placebo in GA-sensitive subjects, and by GA in atopic and healthy controls participating in study

	GA-sensitized subjects								Atopics				Healthy subjects			
	Placebo				GA											
	Before	30 min	4 h	24 h	Before	30 min	4 h	24 h	Before	30 min	4 h	24 h	Before	30 min	4 h	24 h
Score	0.45	1.36	0.64	0.36	0.45	7.55	7.09	3.27	0.70	2.00	1.30	0.40	0.00	1.30	0.10	0.10
Total cell count ($\times 10^3/\text{mm}^3$)																
Leukocytes	2.16	7.65	5.17	12.33	8.28	24.85	47.38	85.58	3.94	9.84	17.69	26.90	2.39	6.42	12.16	8.57
Eosinophils	0.12	0.18	0.44	0.11	0.73	2.86	8.76	18.58	0.00	0.07	0.16	0.80	0.00	0.00	0.00	0.10
Basophils	0.00	0.00	0.06	0.00	0.00	0.40	1.09	2.43	0.00	0.00	0.08	0.40	0.00	0.00	0.00	0.00
Cell percentage (%)																
Neutrophils	24.00	44.55	51.73	41.45	28.39	42.45	54.64	58.64	31.50	41.60	59.00	59.30	14.70	31.10	29.70	29.70
Eosinophils	1.45	1.36	1.27	1.36	5.91	8.00	11.18	12.55	0.30	0.30	0.70	1.30	0.40	0.00	0.40	0.00
Basophils	0.09	0.27	0.27	0.18	0.09	1.00	2.00	2.45	0.20	0.50	0.70	0.50	0.00	0.20	0.20	0.00
Permeability index (%)	12.98	7.74	6.76	7.94	10.84	13.41	20.52	25.85	13.24	10.09	8.35	10.06	9.93	9.12	10.17	7.49
ECP (μl)	2.05	3.37	2.55	2.43	2.63	21.59	34.25	59.28	1.90	3.71	2.26	2.91	1.90	1.90	1.90	2.03
Tryptase (μl)	<1	<1	<1	<1	<1	1.55	1.64	1.58	<1	<1	<1	<1	<1	<1	<1	<1

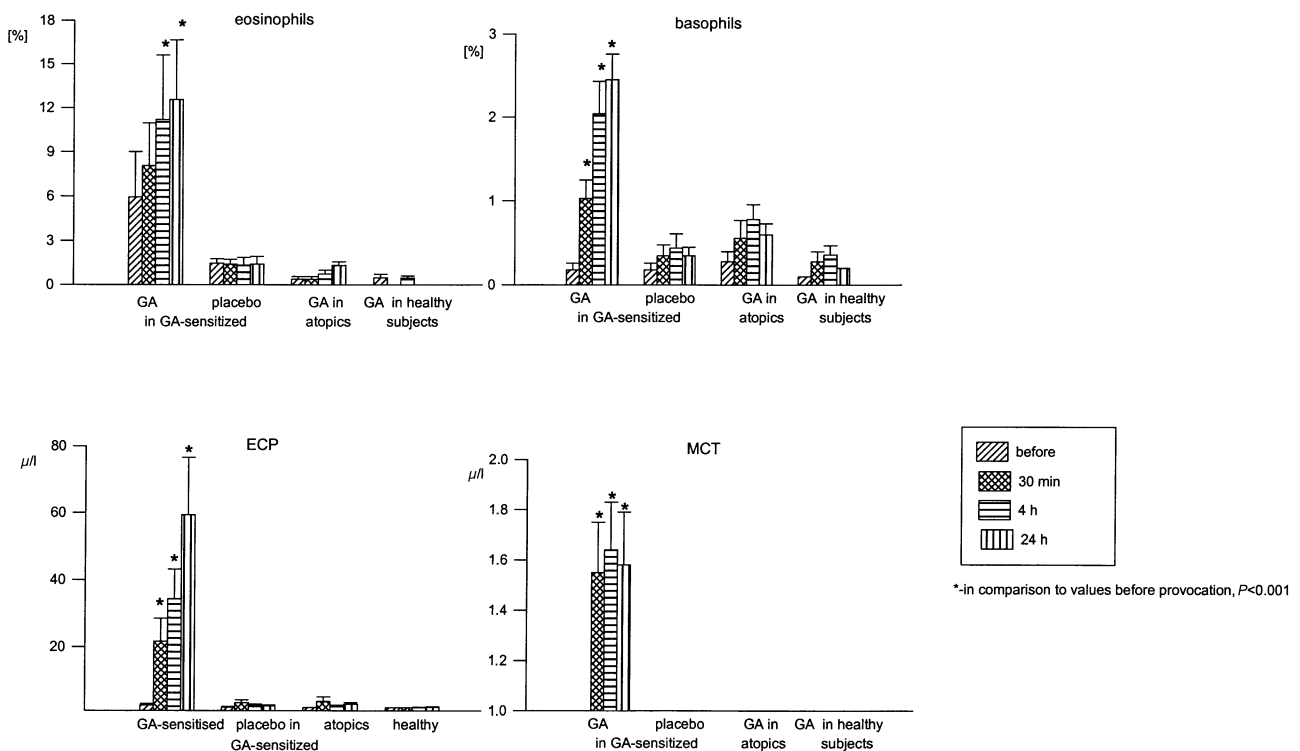


Figure 1. Changes in proportion of eosinophils and basophils, and in level of eosinophil cationic protein (ECP) and mast-cell tryptase (MCT) in nasal lavage fluid induced by glutaraldehyde (GA) and placebo in GA-sensitive subjects, and by GA in atopic and healthy controls participating in study. Data expressed as mean $\pm$ standard error.

Permeability index

Significant differences between the groups were observed at 4 and 24 h after GA challenge – in group A, the permeability index was significantly higher than after placebo administration and than in groups B and C after GA challenge. Moreover, only in group A could significant differences be observed between permeability indices at successive time points – at 4 and 24 h, the values were higher than before the challenge and than at 30 min afterward.

Mediator levels

The analysis of ECP concentration revealed significant differences between the groups at 4 and 24 h – the values obtained in group A after GA challenge were significantly higher than after placebo administration in the same group, and than after GA challenge in groups B and C. Within-group comparisons revealed significant differences only in group A after GA challenge, in ECP concentration at 30 min, 4 h, and 24 h, since the challenge was significantly higher than before the provocation (Fig. 1).

The analysis of tryptase levels revealed significant differences between the groups at all time points after the provocation: at 30 min, 4 h, and 24 h. At all these time points, tryptase concentration in group A after GA challenge was significantly higher than after placebo administration in the same group and than after GA

challenge in groups B and C. Statistically significant differences were also found in group A after GA challenge – the tryptase level was higher at 30 min, 4 h, and 24 h than before the provocation test (Fig. 1).

Discussion

Many agents in the workplace can cause rhinitis symptoms, as a result of specific immune reaction or as a direct irritant effect. Upper airway symptoms precede the development of asthma due to high-molecular-weight agents and usually start at the same time as asthmatic symptoms in subjects exposed to low-molecular-weight sensitizers. The magnitude of rhinitis is generally lower in subjects sensitized to low-molecular-weight compounds than in those allergic to high-molecular-weight agents (14). Mild or moderate rhinitis is usually of little importance for patients suffering from dyspnea and is not often reported spontaneously.

The methodology of nasal challenges with occupational agents, particularly low-molecular-weight agents that cannot be diluted in saline solutions, needs to be developed (15). Such a methodology would be helpful when an irritant or odorous substance is under investigation, when the subject firmly believes that it is the cause of the symptoms, or when a compensation claim is involved. Nasal challenge would also be very

useful for diagnostic purposes in subjects with rhinitis but no asthmatic symptoms to distinguish between the allergic and irritant effects of occupational agents (9, 16, 17). Moreover, nasal lavage examination can be an alternative to bronchoalveolar lavage in asthmatic patients with concomitant rhinitis (18).

While there is much clinical evidence of airway sensitization by GA in exposed subjects, there is very little immunologic evidence to support these observations (4, 19). GA seems to belong to the growing group of low-molecular-weight chemicals for which there is a poor correlation between specific IgE antibody and clinical symptoms. Specific IgE can be detected only in a small percentage of exposed workers who report work-related respiratory symptoms, although there is experimental evidence that GA has the potential to induce the quality of immune activation necessary for the production of IgE antibody and the development of respiratory sensitization (19). It is important to note that even in the absence of the production of specific IgE antibody, TH2-type cells and their cytokine products may well directly promote the development of allergic hypersensitivity reactions in the respiratory tract. Airways inflammation and asthma may be induced by the direct action of IL-5 (possibly acting with IL-4) on eosinophil accumulation and activation (20, 21).

The nasal challenge method, along with the estimation of cell number and the biochemical examination of the lavage fluid or secretion, allows one to distinguish between the allergic and irritant effects of various substances on the respiratory system (9, 16, 17, 22, 23). All subjects with occupational asthma who participated in the study had a late-phase asthmatic reaction after specific challenge with GA, and three of them revealed an additional early reaction starting within 30 min after exposure. It is unlikely that the effect of GA on the airways was solely irritant, as there was a latent interval after the first exposure, the induced reactions were predominantly late, and changes in responsiveness were seen after late reactions (2).

In the present study, some control subjects had rises in their nasal symptoms immediately after GA challenge, but the reappearance of nasal symptoms, particularly obstruction, and to a lesser extent itching, took place only in patients with occupational asthma. These findings make the nasal symptom score a useful parameter in the separation of responders from nonresponders.

The increase in the number of granulocytes and total protein level in NLF has been observed in the irritant reaction. It was, however, brief and did not affect the relative number of eosinophils and basophils or the relative concentration of albumin (16). In the present study, we observed, as typical of the allergic reaction, a prolonged increase in the total cell count and eosinophil number, a less pronounced but very characteristic increase in metachromatic cell

number, and an increase in the albumin/protein ratio for up to 24 h as well as in the symptom score value after specific challenge in patients with GA-induced occupational asthma. Although basophils and neutrophils also increased after challenge, the eosinophils showed the most significant and persistent rise as well as the most significant correlations with clinical findings. Another effect found after GA challenge in GA-sensitized patients was a prolonged increase in ECP level. The first increase in ECP concentration was observed 4 h after nasal challenge, in parallel with a significant increase in the eosinophil count and index permeability. Wang et al. (24) noticed that the combined measurement of the percentage of eosinophils together with the ECP concentration in nasal secretions seems to be very useful for monitoring and assessing the condition of chronic nasal inflammation in patients with allergic rhinitis. Other authors also observed a significantly higher ECP level in nasal fluid and eosinophil number and percentage in nasal brush or fluid 24 h after high- and low-molecular-weight allergen challenge in patients with occupational asthma (9, 16, 17, 23). We regarded the nasal challenge test as positive when the increase in eosinophils and in permeability index persisted for up to 24 h after the challenge.

The results concerning mast-cell tryptase levels in the NLF after the GA challenge were surprising. Tryptase is considered the most reliable marker in the monitoring of mast-cell activation, especially during the early phase of nasal allergic reaction (25). Prolonged elevated tryptase levels in NLF were observed only in patients with GA asthma. In the controls, we did not find any rise in tryptase level, and all measurements were below the detectability of the method. GA has the property of cross-linking of proteins, but the immunologic consequences of such action have not as yet been explained (4). Local metabolism of GA in the nasal mucosa may lead to formation of new proteins – and new allergens. A direct influence of GA on various protein receptors probably also takes place. We cannot explain the pathophysiologic background of this observation, but we suspect an immunologic rather than toxic mechanism, because elevated tryptase levels were not observed in the controls.

The results of the present study indicated the relevance of changes in NLF and spirometry due to specific inhalatory challenge. NLF examination allows us to identify patients with occupational asthma and rhinitis due to GA.

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