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Occupational asthma caused by himic anhydride[1]

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ROSENMAN KD, BERNSTEIN DI, O'LEARY K, GALLAGHER JS, D'SOUZA L, BERNSTEIN IL. Occupational asthma caused by himic anhydride. *Scand J Work Environ Health* 13 (1987) 150—154. Acid anhydride compounds are reactive chemicals that have been previously associated with immunoglobulin E (IgE) mediated occupational asthma. Twenty workers with exposure to himic anhydride powder used for the manufacture of a synthetic flame retardant were questioned about respiratory symptoms. The study was initiated after one individual from the plant developed asthma. A test for serum-specific IgE to human serum albumin conjugates of himic anhydride, phthalic anhydride, hexahydrophthalic anhydride and trimellitic anhydride was performed for seven workers with respiratory symptoms associated with himic anhydride exposure. Three of the seven symptomatic workers who reported wheezing at work exhibited elevated specific IgE to two or more acid anhydride-human serum albumin conjugates. Radioallergosorbent inhibition studies performed with sera containing high levels of himic anhydride-human serum albumin specific IgE from a symptomatic worker demonstrated cross-allergenicity between himic anhydride-human serum albumin and hexahydrophthalic anhydride-human serum albumin allergenic determinants. This study demonstrated that himic anhydride can elicit IgE-mediated sensitization in the workplace.

Key terms: acid anhydride, cross allergenicity, flame retardants.

The acid anhydrides are reactive chemicals used widely in the chemical industry as plasticizers and for manufacturing epoxy resins. Immunoglobulin E (IgE) mediated sensitization and asthma have previously been described for workers exposed to four different acid anhydride compounds. They are phthalic anhydride, trimellitic anhydride, hexahydrophthalic anhydride, and tetrachlorophthalic anhydride (figure 1) (3, 4, 5, 8). Immediate hypersensitivity elicited by these agents has been demonstrated by both positive skin tests and elevated serum-specific IgE to human serum albumin conjugates of phthalic anhydride, trimellitic anhydride, hexahydrophthalic anhydride, and tetrachlorophthalic anhydride. Trimellitic anhydride exposure also results in a late-onset asthmatic response accompanied by fever and myalgias ("trimellitic anhydride flu") and has been associated with large elevations of IgG antibodies specific for trimellitic anhydride-human serum albumin (9).

Himic anhydride (3,6 endomethylene-Δ^4-tetrahydrophthalic anhydride) is a structurally related acid anhydride compound used in the United States to produce brominated fire retardants. This investigation describes clinical and immunologic findings in a group of workers exposed to himic anhydride. Laboratory studies examining allergenic determinants and cross-reactivity between himic anhydride and other acid anhydride conjugates are also presented.

Subjects and methods

Study population

The person who was the index case was seen at the New Jersey Occupational and Environmental Disease Clinic. After he was evaluated, an industrial hygiene

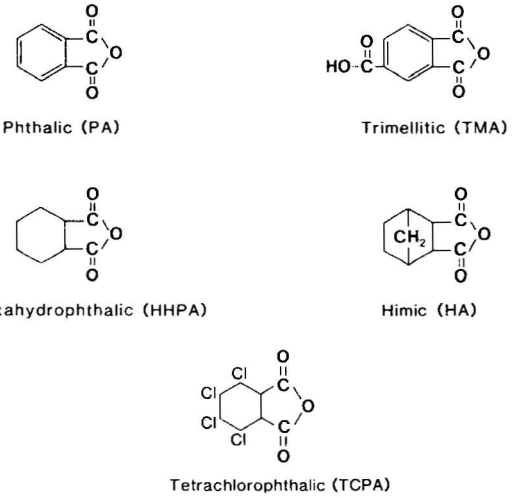

Figure 1. Chemical structures of the phthalyl and trimellityl anhydrides.

[1] Preliminary results were presented at the American Academy of Allergy and Immunology Meeting in Hollywood, Florida, 19—23 March 1983.

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walkthrough was conducted at the chemical plant where the person worked, and a follow-up questionnaire was administered by a physician to 19 other workers. Including the person representing the index case, 20 (13 chemical operators, 3 maintenance mechanics, 3 supervisors and 1 warehouse man) of 32 workers potentially exposed to himic anhydride were interviewed. Fifteen workers were interviewed because they were available on the first work shift of the day on which the interviews were scheduled. Four of the individuals volunteered for interviews during their off-work hours. Because of a lack of further cooperation from the management, skin testing could not be performed and only 7 of 20 workers were available for blood tests.

Industrial hygiene

Himic anhydride is the starting material in a synthetic chemical reaction to produce a flame retardant product, ethylene-(5,6-dibromonorbornene-2,3-dicarboximide). The product is produced in a batch process which requires the manual debagging of approximately 50 (25 kg/bag) bags for each kettle charge. The first step in the production process involves the dumping of the himic anhydride into a tank containing toluene and acetic acid to make a slurry. The slurry is then transferred to a reaction vessel and reacted with 1,2 ethylenediamine to form an intermediate product, ethylene-bis (5-norbornene, 2,3-dicarboximide). The solvent and water are removed from the intermediate product, which is then reacted with bromine to form the final product. The highest potential exposure to himic anhydride occurs during the debagging operation. At the time of our investigation this operation was performed in a local exhaust hood equipped with a plexiglass door. After the bags were slit by the operator, the material was dropped into the tank through a grill opening in the hood. The operator then bent into the hood to break up larger chunks of himic anhydride powder with a stick. The empty himic anhydride bags were folded and placed into a plastic-lined drum.

During the debagging operation the chemical operator wore a 3M-8725 respirator, safety glasses, a hard hat, rubber boots, and a disposable spun-bonded olefin suit with hood and booties. After the debagging operation was completed, the operator showered for 60 s before removing the disposable coveralls and leaving the charging area. After removing the coveralls, the operator was required to take a body shower and put on a clean work uniform.

Exposure to himic anhydride during the debagging operation had been evaluated by the company. Although details of this sampling and results were not provided, it was reported that the operator's exposure was below detectable limits (< 0.5 mg/m^3).

The company did not restrict the number of operators that debagged or unloaded himic anhydride. Thus approximately 30 employees were potentially exposed to himic anhydride. The described process was new. Prior to the institution of this system, himic anhydride was debagged without an exhaust hood in a non-enclosed area with workers having the option of wearing respirators. No other protective equipment was provided. The debagging operation takes place on one floor and the solvent and water removal operation on the floor below. The floor was made of open steel grating. No other anhydride was used in the building. In another production building at the plant phthalic anhydride was used in a similar chemical process.

Immunologic studies

The acid anhydride compounds himic anhydride, phthalic anhydride, hexahydrophthalic anhydride, and trimellitic anhydride were conjugated to human serum albumin and lysine protein carriers (4). The chemical characterization of the acid anhydride protein conjugates was determined after acid hydrolysis with analytical methods previously described (6, 7).

A modified radioallergosorbent test was used to measure specific IgE against human serum albumin conjugates of phthalic anhydride, hexahydrophthalic anhydride, himic anhydride, and trimellitic anhydride (4). Twenty milligrams per milliliter of conjugated proteins were coupled to methylcellulose discs activated with cyanogen bromide dissolved in acetonitrile. After washing with phosphate buffered saline, the test serum was incubated for 3 h at room temperature. Subsequently, the discs were washed with phosphate buffered saline and incubated with 50 000 counts per minute of ^{125}I-radiolabeled horse anti-human IgE for 16 h. After a final washing with phosphate buffered saline, the counts per minute of ^{125}I-anti-IgE bound were measured, and specific IgE was expressed as the percentage of binding or counts per minute of ^{125}I-anti-IgE bound divided by the total counts per minute added to each tube multiplied by 100. Binding of greater than two standard deviations above the mean binding of the negative control group was considered to be significant. The negative control samples of serum were obtained from unexposed research laboratory personnel.

Radioallergosorbent inhibition studies were performed on test samples of serum from the himic anhydride sensitized worker (number 1) selected on the basis of high specific IgE binding to himic anhydride-human serum albumin (2). An aliquot of 0.05 ml of various molar concentrations of human serum albumin and lysine conjugates of phthalyl ligands were incubated at 37° for 2 h with 0.05 ml of the test serum. Subsequently, the mixture was incubated with coupled discs of himic anhydride-human serum albumin for 2 h, followed by an addition of ^{125}I-anti-IgE. The percentage of reaginic inhibition by an individual

reagent was calculated according to the following formula:

$$\left[1 - \frac{\text{counts per minute } ^{125}\text{I-anti-IgE bound by preabsorbed sample}}{\text{counts per minute } ^{125}\text{I-anti-IgE bound by nonabsorbed samples}}\right] \times 100.$$

Results

Case report

The worker (number 1) representing the index case was a 28-year-old white male who had come to the clinic in June 1982 with complaints of nasal stuffiness, swelling of his face, rash, and difficulty in breathing while at work. He had worked at the present chemical company for three years. In his first year with the company he had worked in the production building where himic anhydride was used. One year after the start of his employment he was transferred to the himic anhydride debagging operation. Five minutes after starting this new job, he stated he began to sneeze, experienced a cold sweat, and then developed hives accompanied by swelling and redness. He was transferred out of the area, but three months later he was reassigned to the same site. At this time he described developing swelling of his face and hives while near the reaction kettle, and he was again transferred. One year later the himic anhydride debagging process was moved to his work area. He subsequently experienced persistent nasal stuffiness, scratchy throat, and wheezing at work. In the fall of 1981, he began to cough during and after work. By the winter of 1982 he had experienced three separate asthmatic attacks which required visits to a physician. He had never worked in the second building at the plant where phthalic anhydride was used. Sinus films showed mucosal thickening of the ethmoid and maxillary sinuses. His chest radiograph was normal. At the time he was seen in our clinic, he was being treated with terbutaline and prednisone. He had no personal or family history of allergies, hay fever, or asthma. He smoked one pack of cigarettes per day. He had previously worked for five years in a warehouse and on a paint thinner production line in another chemical plant.

At the time of his first visit, he had been on sick leave for two weeks. His physical examination was normal. Laboratory testing revealed normal pulmonary function indices including a forced vital capacity of 5.80 l (113 % of predicted); a forced expiratory volume in one second of 4.55 l (107 % of predicted); and a mean forced expiratory flow during the middle half of the forced vital capacity of 2.61 l (98 % of predicted). There were no differences between the pre- and postbronchodilator studies. His white count was 7 300 with 1 % eosinophils. Serum-specific IgE to conjugates of himic anhydride-human serum albumin, phthalic anhydride-human serum albumin, and hexahydrophthalic anhydride-human serum albumin, as determined by the radioallergosorbent test, were markedly positive. These results (table 1) exhibited 12.7, 20.9, and 19.5 % binding of radiolabeled anti-IgE for himic anhydride-human serum albumin, phthalic anhydride-human serum albumin and hexahydrophthalic anhydride-human serum albumin, respectively. Binding to a substrate of tetrachlorophthalic anhydride-human serum albumin was elevated (4.9 %), but it was not considered significant because it did not exceed two standard deviations above the mean (2.98 %) of the control group.

After the patient terminated his employment at the plant, his symptoms gradually resolved. He had one subsequent asthma attack when he went back to the plant to pick up his last pay check. One year after leaving work, he was asymptomatic without medication.

Results of the follow-up examinations

Of the other 19 workers interviewed, seven complained of a runny or stuffy nose, and three of wheezing.

Table 1. Summary of symptoms and immunologic results (radioallergosorbent test) from the worker of the index case and six other workers exposed to himic anhydride. (HA = himic anhydride, HSA = human serum albumin, PA = phthalic anhydride, HHPA = hexahydrophthalic anhydride, TMA = trimellitic anhydride)

Worker	Years worked	Symptoms	Percentage of ^{125}I-anti-IgE binding			
			HA-HSA	PA-HSA	HHPA-HSA	TMA-HSA
1[a]	3	Rhinorrhea, wheezing, hives	12.7	20.9	19.5	4.9
2	3	Rhinorrhea	1.0	.9	1.7	.9
3	4	Nasal stuffiness	1.6	1.0	1.9	1.2
4	3	Rhinorrhea	1.1	1.8	1.1	1.0
5	5	Rhinorrhea, wheezing	2.5	3.7	5.7	3.0
6	5	Rhinorrhea	2.0	2.7	1.9	1.1
7	3	Rhinorrhea, wheezing	6.1	4.3	4.0	2.5
Laboratory controls[b]		None	3.38 ± 1.69 (N = 9)	2.15 ± 1.01 (N = 30)	1.96 ± 0.87 (N = 14)	2.98 ± 1.1 (N = 12)

[a] Index case.
[b] The figures given are the means and standard deviations for the number of persons presented in parentheses.

Although there appeared to be an associative trend between symptomatic status and duration of employment, the statistical analysis of this possible relationship did not produce significant results (chi-square trend, P = 0.18).

Immunologic testing was conducted for the worker who was the index case and for six other employees (table 1). They all complained of upper respiratory symptoms. Three also complained of wheezing and shortness of breath. Their durations of employment ranged from three to five years. None had eosinophilia.

Immunologic evaluation

The highest degree of radioallergosorbent test binding to himic anhydride-human serum albumin and other acid anhydride protein conjugates was found for worker 1 (table 1). Although a serologic evaluation of six other symptomatic workers was performed, serum samples from a large group of asymptomatic, similarly exposed individuals in the same plant could not be obtained. A borderline elevation of 6.1 % binding to himic anhydride-human serum albumin was also observed in worker 7. Interestingly, this worker also showed significant binding (2 SD > mean of the unexposed controls) of 4.3 and 4 %, respectively, to phthalic anhydride-human serum albumin and hexahydrophthalic anhydride-human serum albumin. Worker 5 exhibited no elevation in specific IgE to himic anhydride-human serum albumin but had significant binding to phthalic anhydride-human serum albumin (3.7 %) and hexahydrophthalic anhydride-human serum albumin (5.7 %). Thus significant radioallergosorbent binding to two or more phthalyl-human serum albumin discs was detected only in those workers who reported symptoms of wheezing. No significant radioallergosorbent elevations were demonstrated in workers 2, 3, 4, and 6, who had had only nasal symptoms.

Radioallergosorbent inhibition studies

The radioallergosorbent inhibition studies were performed with the use of the serum of worker 1, who showed the highest degree of radioallergosorbent binding to himic anhydride-human serum albumin, for an evaluation of the specificity and cross-reactivity between allergenic determinants of himic anhydride-human serum albumin and other phthalyl ligands (phthalic anhydride and hexahydrophthalic anhydride or their respective conjugates). Specific IgE binding to himic anhydride-human serum albumin in worker 1 was inhibited best by himic anhydride-human serum albumin and himic anhydride-lysine. The results of the complete radioallergosorbent inhibition studies of the himic anhydride-sensitive worker are listed in table 2. They are expressed as moles of phthalyl ligands contained in protein carriers required to achieve 50 % inhibition (I_{50}). Lysine and human serum albumin conjugates of hexahydrophthalic anhydride also inhibited specific IgE binding to himic anhydride-human serum albumin. However, phthalic anhydride and trimellitic anhydride reagents failed to show a similar inhibitory capacity. The data on the radioallergosorbent test inhibition from the same experiment is shown in figure 2. Homologous reagents of himic anhydride exhibited significant inhibition of binding to discs of himic anhydride-human serum albumin, but heterologous inhibiting reagents with the exception of hexahydrophthalic anhydride compounds had little inhibitory activity.

Table 2. Radioallergosorbent inhibition studies of himic anhydride-human serum albumin specific for immunoglobulin E binding by different conjugates of phthalyl human serum albumin and lysine expressed as the molar concentration required to achieve 50 % radioallergosorbent inhibition (I_{50}).

Inhibiting reagent	Molar concentration
Himic anhydride-human serum albumin	5×10^{-11}
Hexahydrophthalic anhydride-human serum albumin	1×10^{-9}
Phthalic anhydride	..[a]
Trimellitic anhydride	..
Himic anhydride-lysine	5×10^{-9}
Hexahydrophthalic anhydride-lysine	9×10^{-9}
Phthalic anhydride-lysine	..
Trimellitic anhydride-lysine	..

[a] I_{50} not obtained with maximum concentration of inhibiting reagents.

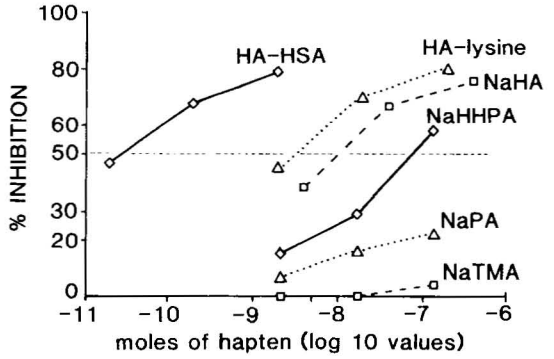

Figure 2. Radioallergosorbent inhibition studies of worker 1 (the index case). The inhibition of specific immunoglobulin E binding to himic anhydride-human serum albumin discs by homologous and inhibiting reagents. (HA-HSA = himic anhydride-human serum albumin, HA-lysine = himic anhydride-lysine, NaHA = sodium himic anhydride, NaHHPA = sodium hexahydrophthalic anhydride, NaPA = sodium phthalic anhydride, and NaTMA = sodium trimellitic anhydride)

Discussion

This is the first report of allergic sensitization to the compound himic anhydride. The chemical structure of himic anhydride is similar to that of other acid anhydrides (figure 1). Therefore, it is not surprising

that himic anhydride can induce IgE-mediated clinical sensitivity. Although the constraints of this study did not permit controlled bronchoprovocation testing, the combination of clinical history, temporal association with work, and positive in vitro immunologic tests supports a causal association.

Because of the possible cross-reactivity between allergenic determinants of structurally similar acid anhydride compounds, a direct radioallergosorbent test was performed with a panel of several phthalyl human serum albumin allergens. Radioallergosorbent binding to himic anhydride-human serum albumin was elevated in two workers with histories of occupational asthma. However, radioallergosorbent binding to hexahydrophthalic anhydride was elevated in all three of the workers (numbers 1, 5 and 7) who reported wheezing, and it appeared to discriminate them from individuals with rhinitis or no symptoms. Thus serum-specific IgE to hexahydrophthalic anhydride was more sensitive for detecting symptoms of occupational asthma associated with exposure to himic anhydride. This unexpected observation from workers with no known exposure to hexahydrophthalic anhydride could best be explained by a cross-reactivity of allergenic determinants formed by himic anhydride and hexahydrophthalic anhydride on protein carrier molecules. This phenomenon was clearly confirmed by radioallergosorbent inhibition studies in which hexahydrophthalic anhydride-human serum albumin and hexahydrophthalic anhydride-lysine inhibited himic anhydride-human serum albumin IgE binding in worker 1. Cross-reactivity between hexahydrophthalic and himic anhydride allergenic determinants was found. In contrast, significant cross-inhibition of himic anhydride-human serum albumin binding in worker 1 was not achieved by phthalic anhydride conjugates despite significant elevations in the direct radioallergosorbent binding to phthalic anhydride-human serum albumin. This explanation is also consistent with data obtained previously in an evaluation of workers sensitive to phthalic anhydride (1). In that study no evidence of cross-allergenicity was detected between phthalic anhydride-human serum albumin and other structurally similar acid anhydride compounds. The finding of serum-specific IgE responses to phthalic anhydride-human serum albumin in these workers could be related to possible concurrent exposure and sensitization to phthalic anhydride dust from an adjacent building or from clothes of other employees who worked in that building.

The precise prevalance of significant immunologic responses to himic anhydride in this plant could not be determined due to incomplete access to all of the exposed workers. However, it has been documented in previous studies of workers sensitized to a related acid anhydride compound, trimellitic anhydride, that the combined prevalence of IgE- and IgG-mediated sensitization is approximately 20 %. Because of significant cross-allergenicity between phthalyl compounds demonstrated in this and other studies, sensitization of a worker to himic anhydride could elicit significant allergic symptoms upon reexposure to a structurally related acid anhydride compound (1, 5).

Acknowledgments

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