

Diagnostic validation of specific IgE antibody concentrations, skin prick testing, and challenge tests in chemical workers with symptoms of sensitivity to different anhydrides

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Background: The diagnostic possibilities of sensitization to various acid anhydrides are limited because of the lack of standardized allergens for the different test systems. This makes the diagnosis of IgE-mediated sensitization caused by occupational exposure difficult.

Methods: We prepared conjugates of human serum albumin with phthalic, maleic, trimellitic, and pyromellitic anhydrides to be used for IgE estimation by enzyme-allergosorbent test, skin prick tests, and nasal and bronchial challenge tests. Nine anhydride workers, who complained of various respiratory symptoms, were studied.

Results: Of the nine workers, four had immediate-type skin test responses to one or more conjugates. All four subjects had elevated IgE concentrations in addition to two other workers. Three of six nasal challenges and four of nine bronchial challenges resulted in positive responses. All but one of the positive nasal or bronchial test responses were associated with elevated IgE levels. The seven positive challenge test results included five positive skin test responses. On the other hand, in all but two of the subjects with negative challenge test results, no specific IgE could be detected. In these two subjects the negative results were associated with low levels of IgE, and in one, with the absence of asthma. None of the results of tests with unconjugated anhydrides were positive.

Conclusions: Anhydrides investigated in this study can induce IgE-mediated hypersensitivity, which can be diagnosed by using the respective human serum albumin in estimation of specific IgE and in skin, nasal, and bronchial challenge tests. Estimation of IgE was demonstrated to be more sensitive than skin prick testing. (*J ALLERGY CLIN IMMUNOL* 1995; 96:489-94.)

Key words: Acid anhydrides, IgE antibody, skin prick test, nasal challenge test, bronchial challenge test, occupational asthma

Anhydrides are frequently used for the production of various resins, varnishes, and adhesives. They are well known to cause rhinitis, bronchitis, asthma, conjunctivitis and, cutaneous hypersensitivity,¹⁻¹⁰ in addition to IgG-associated hemorrhagic alveolitis.¹¹ Various proportions of affected

Abbreviations used

EAST:	Enzyme-allergosorbent test
HSA:	Human serum albumin
MA:	Maleic anhydride
PA:	Phthalic anhydride
PMDA:	Pyromellitic dianhydride
TMA:	Trimellitic anhydride

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subjects show specific IgE antibodies to anhydride-human serum albumin (HSA) conjugates.

The aim of this study was to determine whether anhydride-HSA conjugates prepared in our laboratory, as well as aqueous solutions of these chemicals, are suitable for IgE antibody assays, skin

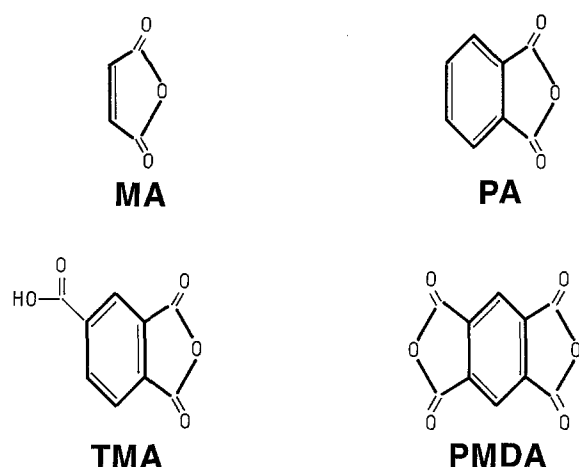


FIG. 1. Chemical structures of investigated anhydrides.

testing, and nasal or bronchial challenge tests and for correlating the results with clinical symptoms. This applies especially to pyromellitic dianhydride (PMDA), an anhydride that has been investigated in only one report.⁵ In this study evaluation of the clinical relevance of immunologic sensitization in exposed workers was of special interest to us.

METHODS

Patients

Nine consecutively investigated chemical workers who came to our outpatient department for consultation took part in this study. All of them had been occupationally exposed to dust of different anhydrides in the production of these chemicals for at least 0.7 years (Fig. 1, Table I). A trained occupational physician was responsible for physical examinations and obtaining case histories. Only symptoms that appeared repeatedly in workplaces were considered. Clinical investigations were performed when the workers were free of symptoms and not receiving medication for at least 10 days. All subjects underwent skin prick tests and specific IgE estimation with PMDA-HSA, phthalic anhydride (PA)-HSA, maleic anhydride (MA)-HSA, and trimellitic anhydride (TMA)-HSA.

Materials

HSA, MA, and PA were obtained from Sigma (Munich, Germany). High purity grade anhydrides (TMA and PMDA) were purchased from Merck (Darmstadt, Germany). For skin test solutions, HSA suitable for human use (Blood Donor Service, Institut Hagen, Germany) was conjugated to the various anhydrides.

PMDA-HSA, PA-HSA, MA-HSA, and TMA-HSA were prepared by a method modified from that of Zeiss et al.⁹ and by a method previously described,¹¹ except that the calculated molar ratio of PMDA-HSA and TMA-HSA of 300:1 was used. This large molar ratio takes into account the numerous lysine residues in HSA, which are possible reaction sites for anhydrides. The

mixture was then ultrafiltered with an Amicon filtration unit with a molecular cutoff limit of 25 kd.

Paper disks (Schleicher and Schuell, Dassel, Germany) were activated with CNBr; and anhydride-HSA conjugates, as well as HSA alone, were immobilized on the paper disks in 0.1 mol/L sodium bicarbonate, pH 8.4, at a concentration of 1 mg of protein per milliliter. The disks were stored at 4°C until use. HSA was also coupled to paper disks at the same protein concentration to serve as a negative control.

To estimate anhydride-HSA-specific IgE antibody concentrations, we used the allergen-coated paper disks in a solid-phase assay. The enzyme-allergosorbent test (EAST) was then carried out with 50 µl of each serum sample by using the Phadezym RAST kit (Pharmacia, Freiburg, Germany) according to the recommendation of the manufacturer. Values above 0.35 kU/L and at least twice the HSA reference value (the latter always resulted in values <0.6 kU/L) were interpreted as positive. To determine the EAST specificity, inhibition tests were done with the anhydride-HSA conjugates. EAST inhibition was performed as mentioned previously, except that 10 µg (in 10 µl of phosphate-buffered saline) of the same allergen was mixed with the patient's sera before the assay and 10 µl of phosphate-buffered saline served as a negative control. An autoinhibition of greater than 95% was obtained for each of the four allergens.

For skin prick testing, pasteurized HSA for human use (Blood Donor Service, Institute) was conjugated to the various anhydrides as mentioned previously. The lyophilized conjugates were reconstituted in saline solution with 0.4% phenol at a concentration of 1 mg/ml.

Wheal-and-flare reactions equal to or less than one fourth of that obtained with 10 mg/ml of histamine were considered to be negative as recommended by Aas and Belin.¹²

Nasal challenge tests

Freshly made aqueous solutions of solvent (saline solution) and subsequently conjugates and free anhydrides (without phenol) were applied in the nostril, with higher airway flow during expiration, by using a commercially available Wagener's atomizer. We measured the airway flow by anterior active rhinomanometry at a fixed reference pressure of 150 Pa (Rhinotest MP 441; Allergopharma, Reinbek, Germany) at rest, 20 minutes after saline solution administration, and at 15 to 20, 45, 90, and 180 minutes after allergen challenge. The result was considered to be positive and the test was stopped if nasal airway flow in the challenged nostril dropped by more than 40% and clinical symptoms, such as rhinorrhea and sneezing (according to a score of ≥ 4),¹³ occurred.

Bronchial challenge tests

Saline solution, followed by increasing concentrations of aqueous solutions of anhydrides or anhydride-HSA conjugates (both diluted in saline solution), were admin-

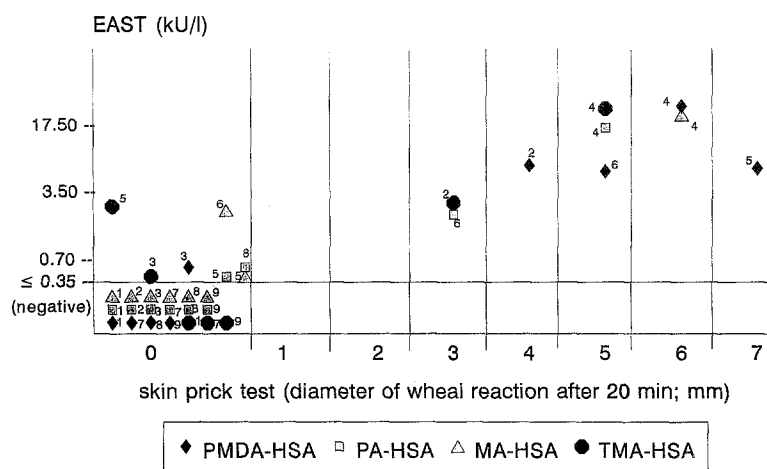


FIG. 2. Comparison between EAST and skin prick test results obtained with conjugates of HSA with four different anhydrides in nine workers. Numbers denote individual patients as in Tables I and II.

TABLE I. Data on workplace exposure and symptoms in the nine investigated anhydride workers

Patient No.	Exposure to anhydrides	Duration of exposure (yr)	Work-related symptoms	Duration of symptoms (yr)
1	PMDA, MA	0.7	C, P, D, R, K	0.4
2	PMDA, MA	0.7	C, D, R	0.5
3	PMDA, MA	0.7	C, P, D, R, K	0.6
4	PMDA, MA	0.7	C, P, D, R, K	0.2
5	PMDA, MA	0.7	C, R	0.3
6	MA, PMDA	16.0	D, R, K	14.0
7	MA, PMDA	0.7	C, R	0.3
8	PA, MA	2.0	D, R, K	1.0
9	PA	3.5	C, P, D, F	3.0

C, Cough; P, phlegm; D, dyspnea; R, rhinitis; K, conjunctivitis; F, fever.

istered by means of a nebulizer (Bronchoscreen; Jaeger, Wuerzburg, Germany) through a mouthpiece. Airway resistance was measured in a body plethysmograph (Masterlab, Jaeger) at rest and after inhalation of saline solution. Corresponding measurements were also carried out after anhydride or anhydride-HSA challenge and at intervals of 20 minutes within the first hour and thereafter every hour until the sixth hour after challenge. The results were regarded as positive and challenges were terminated when airway resistance increased by 100% or more, reaching values of at least 2 kPa · sec.

RESULTS

As shown in Table I, all but one of the nine anhydride workers complained of rhinitis and all but two complained of cough. Of the nine workers, six had both symptoms. Seven subjects reported

workplace-related dyspnea, four reported excess phlegm, five reported conjunctivitis, and one reported fever.

Correlation between skin prick test and IgE antibody estimation with anhydride-HSA conjugates

As shown in Fig. 2, seven workers had IgE antibodies to at least one HSA-conjugate, whereas the EAST results for two subjects were exclusively negative. Negative EAST results were always associated with a negative skin prick test response, and all positive skin prick test responses were associated with significant levels of IgE antibodies to the same conjugate. Seven positive EAST results, all but two in the low range, did not correlate with

TABLE II. Results of nasal and bronchial challenge tests with anhydride-HSA conjugates in correlation with IgE antibody concentrations and skin test results in nine workers

Patient No.	Anhydride-HSA conjugate	Concentration (mg/ml)	Results of challenge tests		IgE antibodies (kU/L)	SPT diameter of wheal (mm)
			Nasal Δ NAF; %	Bronchial Δ sRaw; %		
1	PMDA	0.1	+29	—	<0.35	0
2	PMDA	1	-41*	—	3.52	4
3	PMDA	1	—	+14	0.48	0
4	PMDA	1	—	+187*	25.48	6
5	PMDA	0.1	-74*	0	5.61	7
6	PMDA	0.01	-95*	—	5.20	5
6	PMDA	1	—	+114*	5.20	5
7	PMDA	1	-38	—	<0.35	0
7	PMDA	0.1	—	-24	<0.35	0
8	PA	0.1	—	+203*†	1.10	0
8	PMDA	0.1	-18	+210*	<0.35	0
9	MA	2	—	-18	<0.35	0
9	PA	0.1	—	+6	<0.35	0

Δ indicates percent change compared with pretest value.

NAF, Nasal airway flow; sRaw, specific airway resistance; SPT, skin prick test.

*Significant change in NAF or sRaw (see Methods).

†Dual reactions at 45 to 75 minutes and 210 minutes.

responses to the skin prick test. Three of them occurred in response to the anhydride to which the workers were exposed and four in response to other anhydrides, indicating cross-reactions. This especially applies to TMA-HSA.

Results of nasal challenge tests with anhydride-HSA conjugates

Because data on challenge concentration were not available, we began at 0.001 mg/ml and gradually increased up to 0.1 mg/ml in the first part of the study, raising it to 1 mg/ml later on. In three of six nasal challenge tests performed with PMDA-HSA, a significant decrease in nasal airway flow occurred in association with clinical symptoms of rhinitis (Table II). All of the three subjects had positive skin test responses and specific IgE antibodies. Results of nasal challenges with PMDA-HSA were negative in the three subjects with negative skin test and EAST results (nos. 1, 7, and 8).

Results of bronchial challenge tests with anhydride-HSA conjugates

When doses similar to those in the nasal tests were used, three of six bronchial challenges with PMDA-HSA (nos. 4, 6, and 8) and one of two with PA-HSA (no. 8) resulted in asthmatic reactions. Two PMDA-HSA responders (nos. 4 and 6) and

one PMDA-HSA nonresponder (no. 5 who had conjunctivitis and rhinitis only) had positive skin test and EAST results (Table II). It is interesting to note that subject no. 8 did not demonstrate IgE antibodies or a skin test reaction to PMDA-HSA, which caused significant bronchial obstruction in the challenge test, but demonstrated a reaction to PA-HSA in EAST. One subject with negative results (no. 3) and one subject with positive results (no. 8) in the bronchial challenge test with PMDA had low IgE levels and no skin test response to PMDA-HSA and PA-HSA, respectively. The bronchial challenge test with PA-HSA (0.1 mg/ml) induced a dual bronchial obstruction with maxima at 45 to 75 minutes and at 210 minutes in the latter subject (no. 8). All other reactions were of the immediate type only (i.e., occurring within 60 minutes). Subject 9, who had negative EAST and skin test results, did not respond to either the MA-HSA or the PA-HSA challenge (Table II).

On the whole, results of five bronchial challenge tests were negative (three with PMDA-HSA, one with MA-HSA, and one with PA-HSA). These outcomes were in all but one case (a nonasthmatic subject, no. 5) associated with negative skin test results and three times with a negative EAST result, whereas one EAST result was weakly positive (subject 3) and one was moderately positive (subject 5, nonasthmatic).

Results obtained with unconjugated anhydrides in saline solution

Skin prick testing was performed in subjects 1 to 5 with all four anhydrides in aqueous solution and with PA in subject 8 (concentration, 1 mg/ml). Two subjects had borderline skin test responses, corresponding to one fourth of the histamine wheal (subject 3, MA; subject 8, PA), and all other test results were negative.

We performed five nasal and one bronchial challenge tests with aqueous solutions of unconjugated anhydrides (Table III). In none of the subjects did rhinomanometry or body plethysmography reveal any significant change in functional parameters, and significant clinical symptoms did not appear.

It is interesting to note that one sensitized subject (no. 6), who had a negative response to nasal challenge with aqueous unconjugated PMDA, had demonstrated a severe response to a low concentration of the corresponding anhydride-HSA conjugate (Tables II and III).

DISCUSSION

Our results demonstrate that unconjugated free anhydrides are not suitable for diagnostic testing. This is probably due to rapid hydrolysis of these chemicals in aqueous solutions reacting to the corresponding acids. The pathologic mechanisms clinically relevant in subjects with negative IgE results and symptoms of sensitization have not been clarified. It can be assumed, however, that anhydride dust and, to a minor degree, carboxylic acids formed on hydrolysis behave as irritants in the respiratory tract. On the other hand, there is convincing evidence that inhaled anhydride dust reacts with human proteins, resulting in the formation of partially cross-reactive new antigenic determinants. In a proportion of exposed workers corresponding IgE antibodies develop. As shown in these studies, skin prick test responses corresponded well with EAST results in highly sensitized subjects and in workers without detectable IgE antibodies. However, low or moderate IgE antibody concentrations were frequently associated with negative skin prick test results. It is also possible that immunologic cross-reactivity between the different HSA conjugates is not equally detectable in EAST or by skin testing.

Nasal and bronchial responses showed a good correlation with skin test results and EAST values. Of the seven positive nasal or bronchial challenge test results, six were associated with specific IgE antibodies and five with positive skin

TABLE III. Negative nasal and bronchial challenge tests with unconjugated anhydrides in aqueous solutions

Patient No.	Anhydride	Results of challenge tests	
		(Δ NAF; %)	(Δ sRaw; %)
1	PMDA (0.1 mg/ml)	-27	
2	MA (1.0 mg/ml)	-25	
3	PMDA (1.0 mg/ml)	-3	
4	PA (0.1 mg/ml)		0
6	PA (0.1 mg/ml)	-22	
6	PMDA (1.0 mg/ml)	-21	

Δ indicates percent change compared with pretest value.
NAF, Nasal airway flow; sRaw, specific airway resistance.

test responses to anhydride-HSA conjugates. In the exceptional IgE-negative case, IgE antibodies to other conjugates were present. The eight negative challenge test results were associated with the absence of IgE antibodies and skin test responses six times. There were also two negative bronchial challenge test responses, one in a subject with low IgE antibodies and one in a nonasthmatic subject. All positive challenge reactions were of immediate onset. One subject had a dual asthmatic response to PA-HSA in addition to an immediate asthmatic reaction to PMDA-HSA, to which he was not exposed in his workplace. He had significant levels of specific IgE antibodies to PA-HSA but no skin test response.

On the whole, EAST and the skin prick test exhibit an acceptable specificity with regard to clinical diagnoses, but the sensitivity of the EAST was superior to that of prick tests. Recent results of a study by Nielsen et al.,⁶ demonstrating significant reactions in nasal challenges with hexahydrophthalic anhydride-HSA in a similar group of workers with positive IgE and skin test results, support the interpretation of IgE-mediated allergic responses.

In conclusion, anhydride-HSA conjugates are useful for routine skin prick tests, IgE antibody estimation, and nasal or bronchial challenges, which are performed to evaluate immediate-type allergy. Formation of additional antigenic structures probably occurs on inhalation of anhydride dust by their reaction with proteins other than HSA. Further studies are necessary to determine whether such additional compounds will increase the sensitivity of diagnostic tests in affected anhydride workers. Our recent results indicate that these new antigenic determinants are predominantly of continuous (linear) nature in the case of

PMDA reaction with HSA; the haptenic involvement of the benzol ring must also be considered (Chen, Baur, and Czuppon. Personal communications).¹⁴

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