

Radioallergosorbent test with conjugates specific for 'minor' haptenic determinants in the diagnosis of IgE-mediated penicillin allergy in man

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Summary

Penicillamine-polylysine, benzylpenicillanyl-human serum albumin and ampicillin-polymers were coupled to CNBr-activated paper discs and used in addition to penicilloyl G and penicilloyl V discs in RAST investigations. Sera from sixty patients with case histories of penicillin allergy and with positive or negative skin tests to different penicillin determinants and from seventeen subjects with atopic diseases caused by allergens other than penicillin were tested. The penicilloyl-(‘major’ determinant) specific RAST (Phadebas RAST® Penicilloyl G and V) was positive in twenty out of twenty-four patients with positive skin tests to penicilloyl-polylysine, in eight out of twelve patients with sole skin reactivity to ‘minor’ determinants, and in five out of twenty-four patients with negative skin tests to all antigens used. The penicillamine-specific RAST was the only positive *in vitro* test in four patients with negative skin tests (two) or sole positive skin reactivity to ‘minor’ determinants (two), whereas benzylpenicillanyl and ampicillin-polymer discs added no more information than the penicilloyl structures in all patients tested. Antibodies specific to ‘minor’ determinants apparently show strong *in vitro* cross-reactivity with the penicilloyl determinant. It is, therefore, concluded that ‘minor’ determinant specific conjugates are of marginal importance for *in vitro* diagnosis of penicillin-allergic patients.

Introduction

The usefulness of radioallergosorbent test (RAST) investigations with benzylpenicilloyl (BPO) and/or phenoxymethylpenicilloyl (PMPO) determinants in the detection of IgE-mediated penicillin allergy is well established (Wide & Juhlin, 1971; Juhlin *et al.*, 1977). Results achieved by this *in vitro* test showed good correlation with results of skin tests obtained with penicilloyl-polylysine, especially when *in vitro* tests were performed at the time of the acute reactions and the weeks following (Kraft *et al.*, 1977). However,

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previous work with skin tests and haemagglutination techniques has shown the existence of penicillin-reactive antibodies which are not related to penicilloyl which is the 'major' determinant involved in penicillin allergy (Parker *et al.*, 1962; Levine, 1965; de Weck & Blum, 1965). Therefore, the existence of other antigenic determinants has been suggested and are summarized under the generic name 'minor' determinants, (MD) (Levine & Price, 1964; Siegel & Levine, 1964; Thiel, Mitchel & Parker, 1964; Levine & Redmond, 1969). They are formed in only small quantities but may be important in allergic reactions to penicillin in some genetically determined patients (de Weck, 1978).

The aim of the present study was to evaluate conjugates with specificity for penicilloyl, penicillamine and other specificities to be used in RAST investigations. Results were compared with skin test patterns obtained with benzylpenicilloyl-polylysine (PPL) and/or 'minor' determinants. The importance of 'minor' determinants in radioallergosorbent test was investigated to provide an adequate *in vitro* diagnosis of IgE-mediated penicillin allergy.

Patients

Fifty-six patients with a history of symptoms suggesting penicillin hypersensitivity or with present adverse reactions in connection with penicillin treatment and four patients with a vague suspicion of penicillin allergy were included in the study. The observed clinical patterns were anaphylactic shock (four), urticaria and/or angioedema (thirty-three), polymorphic or maculopapulous exanthemas (18), and serum sickness-like reaction (1). Seventeen patients with proven allergic rhinitis or bronchial asthma and/or atopic dermatitis but without case histories of penicillin allergy were studied as a control group.

Materials and methods

Sera

Blood samples were drawn from each patient during the first 6 months after the onset of the clinical manifestation and/or at the time of skin testing. After allowing to clot for 2 hr at room temperature the serum was separated and stored at -25°C until the *in vitro* tests were performed.

A serum pool from blood samples of twenty-one healthy laboratory workers was prepared for control purposes.

Circulating IgE antibodies

IgE antibodies to penicillin in the sera were assayed in duplicate by the radioallergosorbent test, RAST (Wide, Bennich & Johansson, 1967). Benzylpenicilloyl (BPO) and phenoxymethylpenicilloyl (PMPO)-human serum albumin (HSA)-coated paper discs (Phadebas RAST® Penicilloyl G and V, Pharmacia Diagnostics AB, Uppsala, Sweden) were used and tests performed with ^{125}I -antihuman IgE (D&2) according to the instructions given by the manufacturer. For experimental purposes penicillamine-polylysine, benzyl-penicillanyl-HSA, and polymerized ampicillin were coupled to CNBr-activated paper discs. The coupling procedures for the different 'minor' determinants were as follows:

Penicillamine-polylysine was kindly supplied by Dr C.H. Schneider and Professor

Dr A.L. de Weck and coupled to CNBr-activated paper discs as described by Ceska, Eriksson & Varga (1972).

The D-penicillamine-specific test antigen was prepared by binding D-penicillamine via thioether links to a partly succinylated poly-L-lysine (Schneider, Pfeuti & de Weck, 1973).

Sodium benzylpenicillin (penicillin G) as penicillanyl was coupled to HSA according to the carbodimid method (Sheehan & Hess, 1955; Khorana, 1955). After dialysis against 0.05 M NaHCO₃ and distilled water the complex was coupled to CNBr-activated paper discs according to the procedure for coupling of penicillamine-polylysine.

Ampicillin-polymers were kindly supplied by Astra Läkemedel, Södertälje, Sweden. The average residues per molecule were from two to ten and polymers were coupled directly to the paper discs according to the above mentioned procedure (Ceska *et al.*, 1972).

Calculation of results was done by expressing the mean count rate (a) for each individual test as per cent of the mean count rate of the total activity of ¹²⁵I-anti-IgE added, minus the value (b) obtained from HSA-discs with the same serum sample. A value higher than 0.8 was recorded as positive, between 0.6 and 0.8 as a borderline result, and lower than 0.6 as negative. For ampicillin-polymers, the value b was multiplied by 2.5 to compensate for high background levels.

Serum IgE levels

The determination of the total IgE concentration in the sera was made with Phadebas® IgE Test (Pharmacia Diagnostics AB, Uppsala, Sweden). The assays were performed and the results evaluated according to the instructions for use.

Skin tests

These tests were performed with different antigens at different concentrations on the inner side of the forearm as reported in detail elsewhere (Kraft & Wide, 1976). Benzylpenicilloyl-polylysine (PPL)*, sodium benzylpenicillin (Biochemie, Kundl, Austria), benzylpenicilloic acid* and ampicillin (Binotal®) were used for scratch and intradermal tests. The diameter of weal and flare reactions were measured after 20 min and the reaction considered positive when the diameter was greater than 5 mm. Controls with isotonic NaCl solution and with the histamine solution (10 mg/ml for scratch and 0.1 mg/ml for intradermal tests) were used.

Results

Skin tests

According to skin test results with PPL and 'minor' determinants, four groups of patients could be distinguished (Table 1): nineteen patients showed positive skin tests to both PPL as well as derivatives from benzylpenicillin and/or benzylpenicilloic acid; five patients reacted to PPL only; twelve patients showed sole skin reactivity to 'minor' determinants and twenty-four patients with strong case histories suggesting penicillin allergy were negative to all antigens used.

* The test substances were kindly supplied by Professor Dr A.L. de Weck, Bern and Hoffman - La Roche, Basel, Switzerland.

RAST investigations

RAST investigations led to different patterns of results. By means of the BPO/PMPO-specific RAST, thirty-three patients showed positive results whereas twenty-seven patients were negative. In the former group thirteen gave positive RAST with penicillamine-polylysine-coated discs (Table 1) whereas four patients showed positive results only when a penicillamine-specific RAST was performed.

When the benzylpenicillanyl-HSA conjugate was used in RAST, fifteen patients showed antibodies reacting with this conjugate but they also showed reactivity to BPO/PMPO-coated discs (Table 2). Patients with sole reactivity to benzylpenicillanyl-HSA were not observed.

With the addition of ampicillin-polymer discs, eighteen patients showed positive RAST values but also exhibited positive results with BPO/PMPO discs. Therefore patients with sole reactivity to ampicillin-polymer discs could not be detected (Table 3).

Correlation between skin test and RAST results

Twenty-four patients showed positive skin reactions to PPL and twenty of them had circulating IgE antibodies against penicilloyl conjugates. In four cases, positive skin tests to PPL were combined with negative RAST results (Table 1). In thirteen other cases RAST for BPO/PMPO was positive or gave borderline results despite negative skin tests with PPL. On the basis of penicilloyl-specificity, the overall correlation between skin tests and RAST results was 71.7 %.

Thirty-six patients gave positive skin tests to PPL and/or 'minor' determinants, but only twenty-eight of them showed positive BPO/PMPO-results in RAST (Table 1). Amongst the latter group eight out of twelve patients with sole skin reactivity to 'minor' determinants were positive to BPO/PMPO *in vitro*. Five patients with negative skin tests to all antigenic determinants used also gave positive results with the BPO/PMPO-specific RAST. The BPO/PMPO-specific RAST showed an overall correlation of 78.33 % with skin tests using PPL and 'minor' determinants.

Thirty out of thirty-six patients with positive skin tests to PPL and/or 'minor' determinants gave positive RAST results when BPO/PMPO- as well as penicillamine-specific conjugates were used (Table 1). The addition of the penicillamine discs increased the number of patients with positive RAST results from thirty-three to thirty-seven, but the overall correlation was again 78.33 % because of detection of specific IgE in two patients with negative skin tests to all antigens used.

Total serum IgE

Investigating the sixty sera from patients with suspected penicillin allergy for total IgE seventeen samples showed levels above 250 i.u./ml; fifteen out of them had significant concentrations of IgE antibodies to BPO/PMPO or penicillamine conjugates. On the other hand about 60 % (22/37) of the cases positive in RAST had IgE levels below 250 i.u./ml.

Control individuals

Among the seventeen atopsics used as controls, thirteen had no detectable IgE antibodies against the penicillin metabolites or ampicillin polymers tested in RAST. In the remaining four cases, the total serum IgE levels were elevated, ranging from 750 to 1250 i.u./ml (Table 4) and the HSA test background was higher than normal (between

Table 1. Results of skin tests and RAST with BPO/PMPO and penicillamine conjugates

Skin test reactivity	Number of patients	RAST			
		Positivity with:			Negativity
		BPO/PMPO only	BPO/PMPO and penicillamine	Penicillamine only	
PPL+ MD+	19	9	6	0	4
PPL+ MD-	5	4	1	0	0
PPL- MD+	12	4	4	2	2
PPL- MD-	24	3	2	2	17
Total	60	20	13	4	23

Table 2. Results of skin tests and RAST with BPO/PMPO and penicillanyl conjugates

Skin test reactivity	Number of patients	RAST			
		Positivity with:			Negativity
		BPO/PMPO only	BPO/PMPO and penicillanyl	Penicillanyl only	
PPL+ MD+	19	7	8	0	4
PPL+ MD-	5	3	2	0	0
PPL- MD+	12	5	3	0	4
PPL- MD-	24	3	2	0	19
Total	60	18	15	0	27

Table 3. Results of skin tests and RAST with BPO/PMPO-conjugates and ampicillin-polymers

Skin test reactivity	Number of patients	RAST			
		Positivity with:			Negativity
		BPO/PMPO only	BPO and ampicillin-polymers	Ampicillin-polymers only	
PPL+ MD+	19	4	11	0	4
PPL+ MD-	5	3	2	0	0
PPL- MD+	12	4	4	0	4
PPL- MD-	24	4	1	0	19
Total	60	15	18	0	27

Table 4. Controls. Total serum IgE levels and RAST results from seventeen patients with allergic rhinitis, asthma bronchiale, atopic dermatitis and urticaria

Total IgE (i.u./ml)	Number of controls	RAST positivity with:			
		BPO/PMPO and penicillamine	Penicillamine only	Penicillanyl only	Ampicillin-polymers only
20-124	5	0	0	0	0
125-749	5	0	0	0	0
750-1 250	7	3	1	0	0

0.8 and 3.76 % of total activity). These patients exhibited borderline (1) or positive (3) results with penicillamine discs and three of them also gave positive results in RAST with the BPO/PMPO-HSA conjugates.

The human serum pool showed a total IgE level of 110 i.u./ml and was negative in RAST with all conjugates used.

Discussion

Several methods are in use for the diagnosis of IgE-mediated penicillin allergy in man. Skin tests with different antigens and the penicilloyl-specific RAST are now accepted as the most reliable procedures (de Weck, 1979). Skin tests are usually performed with PPL, benzylpenicillin and benzylpenicilloic acid (de Weck & Jeunet, 1975). Despite useful results the procedures still have some disadvantages due to being time-consuming and they carry a definite risk of generalized reactions.

In the past studies were performed which suggest that RAST could work as an alternative or at least a complementary method to the skin test (Wide & Juhlin, 1971; Juhlin & Wide, 1972; Adkinson, 1975; Kraft & Wide, 1976; Kraft *et al.*, 1977; Juhlin *et al.*, 1977; Basomba *et al.*, 1979; Spath *et al.*, 1979) and also the ELISA-technique (de Haan, Boorsma & Kalsbeek, 1979). Results of RAST and skin tests showed an overall correlation between 80 and 100%. The applicability of RAST is limited to the extent that in most penicillin allergic patients specific IgE shows a tendency to fall below detectable limits during the months and years after disappearance of clinical symptoms (Kraft *et al.*, 1977).

Another limitation of RAST in the diagnosis of IgE-mediated penicillin allergy may be that conjugates with specificity for 'minor' determinants have not yet been included, although some authors found that RAST using penicilloyl determinants gave the same information as skin tests performed with several penicillin derivatives (Juhlin *et al.*, 1977). However, according to results of skin tests and of the red cell-linked antigen-antiglobulin reaction, it was suggested that a more complete diagnosis would have included both 'major' and 'minor' determinants in all test systems used (Levine & Redmond, 1969; Assem & Vickers, 1975).

To investigate the importance of 'minor' determinants (MD) for *in vitro* assays, sera from patients with sole skin test positivity to MD were tested. The sera had been carefully collected over 4 years and, therefore, these patients seem to be somewhat over represented in the present study in relation to the other patients. This fact may also explain the relatively low agreement (71.7 %) between skin test and RAST results.

Eight out of twelve patients with sole skin test positivity to MD were positive in a penicilloyl-specific RAST. These results indicate that the majority of patients with skin tests positive only to MD will react in a BPO/PMPO-specific RAST, although few penicillin-allergic patients were left who showed negative results. There is apparently *in vitro* a large cross-reactivity between MD-specific antibodies and the 'major' determinant-specific RAST conjugates. These results are in good agreement with results reported by Juhlin *et al.*, (1977).

Some work has been carried out suggesting that penicillamine can act as an antigenic and allergenic determinant (for review, see Dewdney, 1977). In only a few cases in our study, the penicillamine discs gave additional information to the penicilloyl structures. The four patients with a positive penicillamine-specific RAST response showed IgE levels between 90 and 550 i.u./ml in contrast to controls with high levels of IgE. Therefore they seem to be true positive cases (Table 4). The data presented indicate that in very rare instances penicillin allergy may be due to penicillamine-specific IgE which cannot be detected by a penicilloyl-specific RAST. However, the difficulties in preparing succinylated penicillamine-polylysine prevent the use of RAST with conjugates specific for the penicillamine determinant at acceptable costs in the near future.

The carbodiimide method used to get penicillanyl-HSA conjugates can also lead to penicilloyl structures. However, only fifteen out of thirty-three patients with a positive BPO/PMPO-specific RAST gave a positive penicillanyl response in RAST. This indicates some binding by the carboxyl group to human serum albumin. The fact that no additional information was obtained by the use of these discs limits their applicability in clinical routine work.

It was found that immunization of rabbits with ampicillin-polymers coupled to proteins led to antibodies partly specific to polymer structures (Shaltiel, Mizrahi & Sela, 1971). However, others found that the specificity of PCA reactions induced by ampicillin-polymers in various species (Munro *et al.*, 1976) and also the specificity of haemolytic plaque assays (Ahlstedt *et al.*, 1977) primarily are penicilloyl. In the present study, no evidence for the existence of ampicillin-polymer-specific antibodies was discovered. The ampicillin-polymer-coated discs contributed no more information than the penicilloyl structures did. Thus it seems to be unnecessary to include these haptenic determinants in the whole arsenal of RAST conjugates.

In conclusion, the data obtained from the use of discs with other antigenic determinants suggest that in only few penicillin-allergic patients a more detailed and more comprehensive diagnosis can be made by a penicillamine-specific RAST. According to skin tests results, RAST conjugates specific for 'minor' determinants seem to have limited importance for the *in vitro* diagnosis of IgE-mediated penicillin allergy in man.

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