

Detection of penicillin allergy of the immediate type by radioimmunoassay of reagins (IgE) to penicilloyl conjugates

LEIF WIDE *Department of Clinical Chemistry, and*

LENNART JUHLIN *Department of Dermatology, University Hospital, Uppsala*

Summary

Reaginic antibodies (IgE) to penicilloyl were detected by an *in vitro* method, a radio-immunological technique (RAST), in the sera of nine patients out of eleven with a recent history of penicillin hypersensitivity of the immediate type. Provocation tests in two patients with negative RAST were negative as well as in twelve patients having had a possible penicillin hypersensitivity reaction 7 months to 14 years ago. Results from skin tests and RAST agreed. Skin tests and RAST reactions were negative for thirty-two patients with a history of penicillin reactions of the delayed type. The results of this study indicate that the RAST is a valuable alternative to the more dangerous intracutaneous test with penicilloyl-polylysine for detection of penicillin hypersensitivity of the immediate type.

Introduction

Allergic reactions to penicillin are a clinical problem (for references see Stewart & McGovern, 1970). In particular, anaphylactic shock is a serious complication which may have fatal consequences. Penicilloyl is regarded the principal allergenic determinant in the penicillin group (de Weck & Eisen, 1960; Levine & Ovary, 1961). Intracutaneous testing with penicilloyl-polylysine is used to detect allergy to penicillin. A serious drawback of this test is that it sometimes causes a violent reaction and may, therefore, be dangerous to perform (Resnik & Shelly, 1965; Heaton, Posey & Lentz, 1970).

Anaphylactic shock and acute urticaria are allergic reactions of the immediate type where reaginic antibodies belonging to immunoglobulin class E (IgE) are expected to be involved. The radioallergosorbent test (RAST) (Wide, Bennich & Johansson, 1967) is an *in vitro* method for the detection of such reaginic antibodies to particular allergens. The method is here applied for the detection of reagins in serum to penicilloyl and the results are compared with those of skin tests and provocation tests in patients suspected of being sensitive to penicillin.

Correspondence: Dr Leif Wide, Department of Clinical Chemistry, University Hospital, Uppsala, Sweden.

Patients

Fifty-four patients with histories of a possible penicillin allergy were studied. The patients were divided into groups according to their allergic reactions: *Group A-1*: Eleven patients with symptoms suggesting a reaction to penicillin of the 'immediate' type, i.e. anaphylactic shock or urticaria. They were investigated shortly after their drug reaction had subsided. *Group A-2*: Twelve patients with a similar history of drug reaction 7 months to 14 years ago. *Group B*: Thirty-one patients who had suffered from so-called late reactions such as morbilliform or scarlatiniform exanthemas and purpuric reactions. Almost all of them were investigated in close relation to their skin eruption.

Materials and methods

The following allergens were used: phenoximethyl-penicilloyl-polylysine (V-PPL, Kabi 1444), supplied by KABI, Stockholm, Sweden; penicilloyl conjugated to albumin (EBS 93/1A) supplied by ASTRA, Södertälje, Sweden; benzylpenicillin, supplied by ASTRA, Södertälje.

The *radioallergosorbent test* (RAST) was used to assay reaginic antibodies to penicilloyl in serum. The principle of RAST is the following: Reaginic antibodies of the IgE type in serum react with an allergen chemically coupled to an insoluble matrix and the allergen bound reagins are then detected by their ability to bind ^{125}I -labelled antibodies directed against IgE.

Penicilloyl in the form of V-PPL (110 units) or as albumin-penicilloyl complex (10 mg) was coupled to 100 mg of cyanogen-bromide-activated 'Sephadex' (1–10 μ , especially prepared by Pharmacia, Uppsala, Sweden). Alternatively microcrystalline cellulose (Merck, Darmstadt, West Germany) could be CNBr-activated and used as an insoluble matrix for allergens (Wide, 1969). The results were obtained in 36–48 hr and the capacity of the method was 700 tests per technician and week.

The RAST was performed as previously described (Wide *et al.*, 1967) with a few recent modifications (Stenius & Wide, 1969) including the use of 'sorbent units' (SU) to express the strength of the reaction. The inhibition tests were made by a preincubation for 20 hr before assay with RAST of 20 μl of serum from a penicillin-sensitive patient with 2 mg of various penicillin preparations dissolved in 0.75 M phosphate buffered saline, pH 7.4, to 10 mg/ml. The results of RAST were compared with those obtained when the preincubation was made with buffered saline solution.

The IgE level of the sera was determined with a solid phase sandwich technique (Wide, 1971). Twenty μl of serum was mixed with 0.5 ml of a suspension of cellulose coupled anti-IgE-antibodies. IgE in the serum was bound to the solid phase antibodies during incubation for 24 hr. The solid phase was then washed and in the second step of the reaction ^{125}I -labelled anti-IgE-antibodies were added. These labelled antibodies reacted with the IgE bound to the antibodies on the solid matrix. The uptake of labelled antibody on the solid phase was directly correlated to the amount of IgE in the test solution. A serum sample with an activity of 40,000 ng of IgE per ml when tested in a competitive inhibition radioimmunoassay using purified myeloma IgE as a reference (Johansson, Bennich & Wide, 1968) was diluted in cord serum and used as a standard in the sandwich technique. The IgE level of this standard serum was set to 40,000 arbitrary units (arb.U) per ml. The IgE level of the cord serum was less than 0.5 arb.U/ml.

Skin tests were done by intracutaneous injection of 0.01–10 units of V-PPL in

0.1 ml saline solution. Most of the patients were also tested intracutaneously with 0.1–100 international units (I.U.) of benzylpenicillin. As a control 0.1 ml of saline solution was injected at the same time.

Provocation tests were done on group A patients who had a negative reaction to the skin tests. Benzylpenicillin in doses of 1000, 10,000 and 3,000,000 I.U. were successively injected subcutaneously.

Results

Group A-1

Positive skin tests with V-PPL and benzylpenicillin were obtained in nine of the eleven patients with symptoms of penicillin hypersensitivity of the immediate type and tested soon after the drug reaction had subsided (Table 1). Sera from these nine skin positive patients also gave a positive RAST reaction (Fig. 1). In one of these patients the intracutaneous test with 0.1 I.U. of benzylpenicillin caused an anaphylactic shock. Skin tests, RAST reactions and provocation tests were negative for the remaining two patients of this group.

Table 1. Patients with positive RAST and intracutaneous tests

Patient	Sex	Age	Type of penicillin causing reaction	Drug reaction		Minimum dose giving a positive reaction after i.c. injection (I.U.)	
				Type	Duration	V-PPL	Benzyl p-c
1	M	43	Phenoximethyl-pc	Urticaria	9 months	0.01	0.1
2	F	34	Benzyl-pc	Anaphylactic shock and urticaria	5 years	0.01	0.1*
3	F	24	Phenoximethyl-pc	Erythema multiforme and urticaria	7 days	0.01	0.1
4	F	44	Benzyl-pc	Urticaria	10 years	10	100
5	M	23	Benzyl-pc	Asthma and urticaria	1 day	0.01	10
6	F	36	Phenoximethyl-pc	Swelling of lips, throat and urticaria	2 days	0.01	100
7	M	33	Phenoximethyl-pc	Anaphylactic shock and urticaria	2 days	0.01	1
8	M	23	Phenoximethyl-pc	Urticaria	4 days	0.1	10
9	F	44	Phenoximethyl-pc	Asthma and urticaria	3 months	0.1	10

* This test caused an anaphylactic reaction.

Groups A-2 and B

All patients in these two groups showed negative RAST reactions and intradermal tests. Provocation tests on the twelve patients of group A-2 were also all negative.

Sera from patients with a positive RAST varied up to 100 times in 'reaginic antibody activity'. During the period of observation a decrease of this activity was observed in seven of the nine patients (Fig. 1). The skin test remained positive in all cases except in patient 4.

When V-PPL was compared to the albumin-penicilloyl conjugate as the coupled allergen in the RAST, stronger reactions were found with V-PPL. The relationship between the reagenic antibody activity of the different sera was similar when the two different allergosorbents were used.

The results from the assays of the serum IgE level of the fifty-four patients are shown in Fig. 2. The value of the first serum sample is plotted when several samples were obtained from the patient. The patients with positive skin and RAST reactions

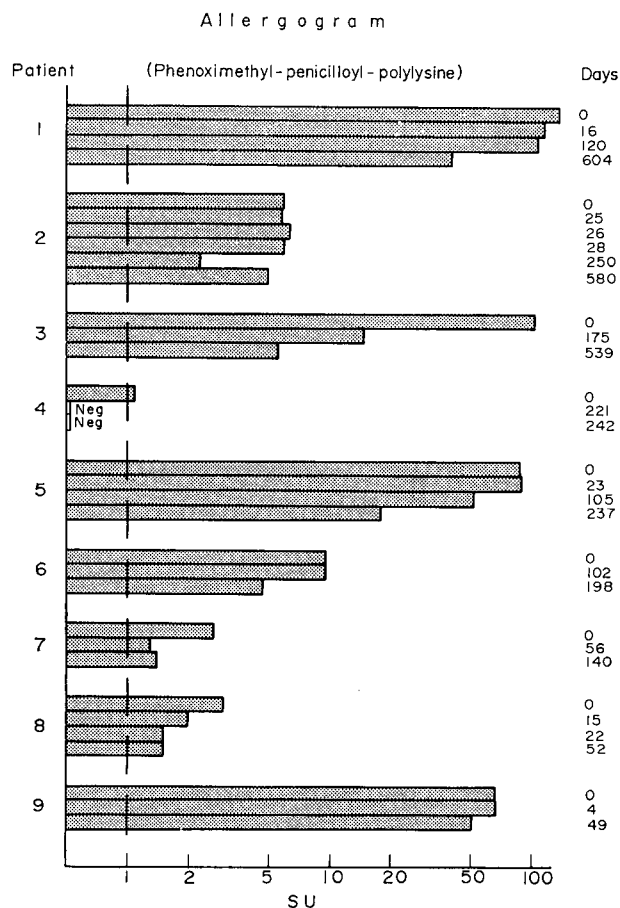


Fig. 1. Reagenic antibody (IgE) activity to penicilloyl as assayed by RAST in sera obtained at different time intervals after the first serum sample for each patient with a positive reaction for penicillin allergy. The results obtained are expressed in sorbent units (SU).

have a higher IgE level than those in group A with negative tests. The difference between the mean values of these two groups was highly significant ($P < 0.001$). The serum IgE level decreased during the period of observation for the nine positive patients and the mean value for the last day of observation of this group of patients was not significantly different ($P > 0.05$) from that of group A with a negative reaction.

There is one patient in group B who has a high IgE level (2800 arb.U/ml). This patient has atopic dermatitis caused by other allergens than penicillin.

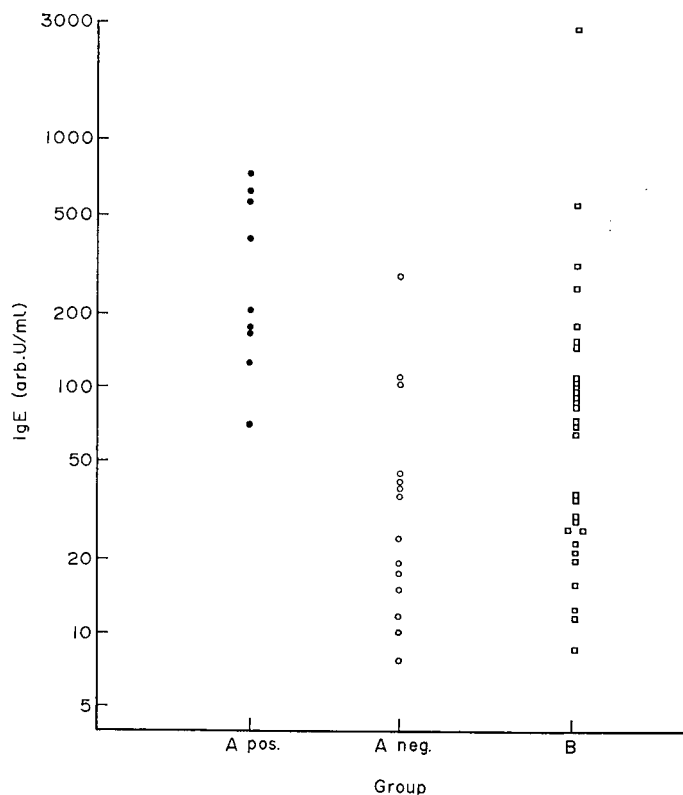


Fig. 2. Serum IgE levels for patients with a history of penicillin allergy of the immediate type with a positive and negative RAST reaction (group A) and of the delayed type of reaction (group B).

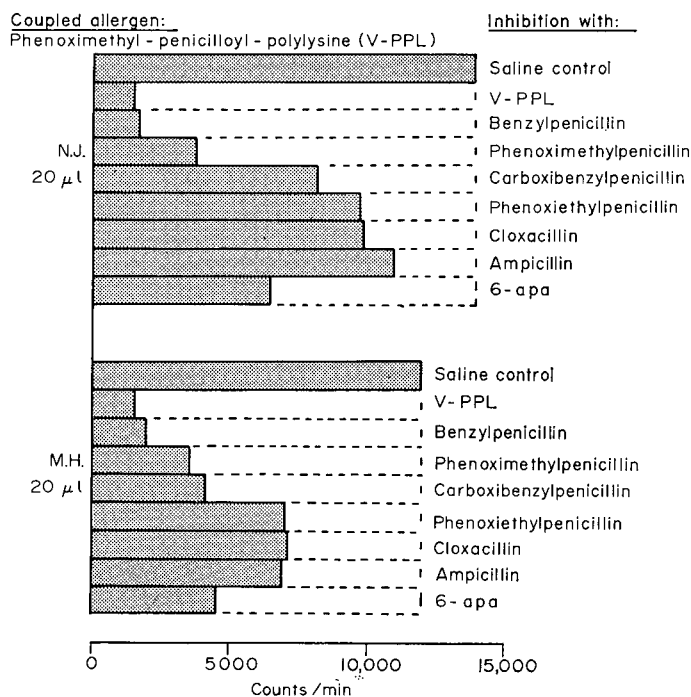


Fig. 3. Results of studies for inhibition of RAST with six different penicillin preparations, V-PPL and 6-apa on the reaction between V-PPL and reagins in serum from two patients sensitive to penicillin.

The reaction between reaginic antibodies and V-PPL was inhibited by preincubation of the serum with various penicillin preparations. The results of the inhibition studies with six different penicillin preparations and V-PPL and 6-aminopenicillanic acid (6-apa) on the reaction between V-PPL and reagins in the serum from two patients sensitive to penicillin are shown in Fig. 3. In both cases benzylpenicillin and phenoximethylpenicillin had the strongest capacity to inhibit the reaction, and the weakest capacity was found with phenoxiethylpenicillin, cloxacillin and ampicillin.

Discussion

The usefulness of skin testing with penicilloyl-polylysine in the diagnosis of penicillin hypersensitivity, first introduced by Parker *et al.* (1962), is now well established. However, the frequency of positive test reactions among patients with history of penicillin allergy varies from 17% to 99% in different investigations (Idsøe *et al.*, 1968). One reason for this variation may be the different criteria used for the diagnosis of penicillin hypersensitivity. In the present investigation the patients were divided into two groups: those with an 'immediate' type of reaction and those with a 'delayed' type. Only patients with the immediate type of reaction gave positive skin tests with V-PPL or benzylpenicillin.

A decreased rate of positive PPL tests has been reported when several years have elapsed since the penicillin reaction (Finke *et al.*, 1965). This is a likely explanation to most of the negative tests in this investigation on the patients who had symptoms of immediate type of penicillin hypersensitivity 7 months to 14 years ago. In some cases an intravenous penicillin injection or other drugs might have been responsible for the reaction.

The results of RAST agreed completely with those of the skin and provocation tests. The quantitative tests of RAST showed a decrease for seven of the nine subjects during the time of observation. It is of interest to note that the decrease in specific IgE antibodies against penicilloyl was reflected in a decrease of the 'total' IgE level in serum in five of the subjects. None of the patients with positive penicillin tests had any previous history of atopy or gave a positive RAST against various pollen, animal dandruff, moulds or house dust.

There was no obvious correlation between severity of the drug reaction and the strength of the RAST reaction with the use of V-PPL. The chemical basis for penicillin allergy is very complex with penicilloyl as a major allergen and with several minor determinant allergens (Siegel & Levine, 1964; Voss, Redmond & Levine, 1966). Therefore, it seems important to include also some of these minor determinants in the test battery. In the present investigation it is not likely that tests with such minor determinants would have given more positive reactions as all provocation tests with up to 3 millions I.U. of benzylpenicillin were negative on patients with symptoms suggesting an immediate type of reaction to penicillin and negative tests with penicilloyl-polylysine. However, reagins to allergenic determinants from penicillin other than penicilloyl may be present in some sera from the patients with positive reactions.

The inhibition experiments showed that all the penicillin preparations tested were capable of binding reagins against penicilloyl. This binding capacity varied between the different preparations and was highest for benzylpenicillin. The possibility of a correlation between this binding capacity of reagins against penicilloyl for a penicillin preparation and the potency to elicit an allergic reaction in sensitized patients has to be further studied.

The RAST has previously been successfully used for the detection of reagents against a great number of different allergens (Wide *et al.*, 1967; Stenius & Wide, 1969; Holford-Strevens *et al.*, 1970; Fagerberg & Wide, 1970). The results of this study indicate that RAST is a valuable alternative to the more dangerous intracutaneous tests with penicilloyl for detection of penicillin hypersensitivity of the 'immediate' type.

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