

Assessment of histamine release from basophils in whole blood by benzylpenicilloyl poly-L-lysine in penicillin-sensitized patients

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Histamine release from basophil granulocytes in whole blood by benzylpenicilloyl poly-L-lysine (PPL) was investigated in 7 patients with penicillin allergy. All patients presented with systemic immediate hypersensitivity reactions after i.v. administration of penicillin G. Total histamine (of 7 patients) ranged from 27.5 ng/ml to 62.1 ng/ml (mean 43.2 ng/ml). The spontaneous histamine release ranged from 0.15% to 5.1% (mean 1.8%) of the total content. Addition of PPL in various concentrations resulted in values between 0.8 and 9.6%. Although PPL is a reliable allergen for prick- and intradermal testing in the diagnosis of penicillin allergy – demonstrating a histamine liberation in the skin – the *in vitro* experiment using the same allergen showed no histamine release above 10%. Using a threshold of 5% out of 7 patients, 4 (57%) would show a positive histamine release. Therefore it might indicate that in penicillin allergy a threshold of 5% must be used. In addition, basophils in whole blood and skin mast cells may be activated differently.

Clinical aspects

After cell challenge with inhalant allergens in whole blood it is recommended that more than 10% of total histamine must be released to obtain a positive result. We suggest that in penicillin allergy a threshold of 5% should be recommended.

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Penicillins are the most frequently used antibiotics and the most frequent cause of drug allergy. The risk of a sensitization depends on the kind of application: oral therapy in 0.1%, iv or im application in 1% and topical use in 10% lead to sensitization (6).

Penicillin allergy manifests itself, for instance, in type I allergy, but types II, III and IV allergies are also described (2).

Although penicillin allergy occurs frequently, in spite of intensive research there are many points in question about the pathomechanisms of this drug allergy and about the sensitizing metabolites of penicillin and their binding to human plasma protein.

The diagnosis of penicillin allergy is based on clinical symptomatology, on an *in vitro* test (assessment of specific IgE-antibodies in serum) and on *in vivo* tests (skin tests and provocation tests) (7). All of them have disadvantages:

- The diagnosis by clinical symptomatology in outpatients is frequently based on shaky grounds.

- Specific IgE antibodies become reduced after a while, and false negative results may occur (8).
- The disadvantages of skin testing with benzylpenicilloyl-poly-L-lysine (PPL) and minor determinant mixture (MDM) must be seen in the amount of time required (titration) – unfavourable in children – as well as in risks of unwanted side-effects – hypersensitivity reactions – which could lead to serious situations, particularly in patients with a high degree of sensitization (4, 13). In addition, in skin tests false positive results may occur (13).
- Provocation testing should be done at an intensive care facility and is not practicable as a routine method.

Therefore we tried to establish an additional, inexpensive *in vitro* test that was easily managed and had a high degree of reproducibility – all these factors are important for a method which should be done in a routine laboratory.

In skin testing PPL and MDM cause an activation and degranulation of sensitized skin mast cells, resulting in a formation of a wheal and flare reaction. Basophils should be activated by the same mechanisms; thus we investigated the effect of PPL on histamine release from basophils in whole blood, which has not been described so far.

Material and methods

Seven patients (age 17–70 years), mean 43.14; $SD \pm 20.83$) whose hypersensitivity reactions against penicillin G had occurred within the last 2 months were investigated.

All patients (7/7) showed systemic immediate hypersensitivity reactions after i.v. injection of penicillin G. All of them reported pruritus; 6 of them presented with urticaria. Of these 6 patients one (1/6) reported shortness of breath, 2 (2/6) with additional swelling of the lips. One patient (1/7) presented with a rash and shortness of breath. None of the patients had taken drugs 2 months prior to the drawing of blood, where an influence of histamine release might be expected (steroids, theophyllin, calcium antagonists, etc.). Pregnant women were excluded as they show increased diamine oxidase (a histamine metabolizing enzyme) activities (9). Four patients (age 26–28 years; mean 26.75; $SD \pm 0.96$) who had previously taken penicillin G or V and shown no hypersensitivity reactions and demonstrated no specific IgE-antibodies against penicillins were used as controls.

The allergen consisted of PPL (Allergopharma Joachim Ganzer KG, Germany) in concentrations of 4×10^{-7} , 4×10^{-8} , 4×10^{-9} , 4×10^{-10} M (concentrations are with respect to the haptens). Histamine assessment was done by a radioimmunoassay (Immunotech Cat.1302, France) in a double-assay which has been shown to be reliable in previous studies (5, 11).

For histamine release after challenge with allergen, 50 μ l heparinized whole blood was diluted with 150 μ l histamine release buffer to which 100 μ l to allergen solution were added. The whole blood-allergen mixture was incubated at 37°C for 30 min. After centrifugation the supernatant was used for assessment. The procedure is described in detail elsewhere (11, 12). Histamine release was calculated by the formula of Lichtenstein (10). Activation of basophils can be assumed when histamine release is more than 10%.

Skin prick tests and intradermal tests were performed with PPL and MDM (Allergopharma Joachim Ganzer KG; Germany) only in patients with negative RAST results.

Specific IgE antibodies against penicillin G and V, as well as amoxycillin and ampicillin were measured

by radioallergosorbent tests (Phadebas RAST®, Pharmacia Diagnostics AB, Uppsala, Sweden). The results were expressed in Phadebas RAST classes 0, 1, 2, 3 and 4 according to a reference system established by running 4 reference sera (A, B, C and D) with reference discs during each assay in parallel to the unknown samples. Positive results were given for RAST-class 2 or higher.

Assessment of total serum IgE were done by IgE RIACT®, a solid phase immunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden).

Results

Of 7 patients, only 2 had slightly increased total serum IgE levels; the other patients (5/7) had normal values. Six patients (6/7) had positive RAST to penicillin G (RAST class 2–4; mean 2.8, $SD \pm 1.02$) and were thus not skin tested. Two of these 6 patients also had a positive RAST result to penicillin V, whereas only one patient (1/6) showed a positive result to ampicillin. One patient (1/7) with a negative RAST result to all penicillins showed a positive wheal and flare reaction in skin testing with PPL (Table 1).

The total histamine ranged from 27.5 to 62.1 ng/ml (mean 43.2 ng/ml; $SD \pm 14.1$). The spontaneous histamine release was 0.2–5.1% (mean 1.8%;

Table 1. Total and specific IgE antibodies in penicillin-sensitized patients

Pts	Total IgE (kU/l)	RAST-classes				Total histamine (ng/ml)	Spontaneous release (%)
		Pen G	Pen V	Amoxycillin	Ampicillin		
1	46	2.0	0	0	0	41.8	0.15
2	90	4.0	2.0	0	0	30.6	2.20
3	4	3.0	1.0	0	0	62.1	0.42
4	86	2.0	0	0	0	62.1	0.33
5*	43	1.0	1.0	1.0	0	42.6	0.30
6	153	2.0	1.0	1.0	0	35.4	4.30
7	185	4.0	3.0	0	2.0	27.5	5.10

* Patient with positive wheal reaction to PPL in intradermal testing but negative RAST result.

Table 2. Total histamine, spontaneous histamine release and allergen-induced histamine release expressed as mean $\pm$ SD

	Patients	Controls
Total histamine (ng/ml)	43.2 $\pm$ 14.1	49.0 $\pm$ 22.24
Spontaneous histamine release (% of total histamine)	1.8 $\pm$ 2.1	1.5 $\pm$ 1.83
Histamine release after challenge 4×10^{-7} M	3.3 $\pm$ 3.18	1.1 $\pm$ 0.81
with PPL in a 4×10^{-8} M concentration of	3.8 $\pm$ 3.39	1.4 $\pm$ 1.90
	3.4 $\pm$ 3.05	1.2 $\pm$ 0.59
	3.7 $\pm$ 2.61	1.6 $\pm$ 0.44

Normal values: total histamine: 20–200 ng/ml; spontaneous histamine release: <5% of total histamine; histamine release after challenge: <10% of total histamine.

SD $\pm$ 2.1) of the total histamine (Table 2). After challenge with PPL in various concentrations we obtained values between 0.8% and 9.6% (mean 3.6%; SD $\pm$ 2.9) (Fig. 1).

The controls (RAST class 0) showed total histamine ranging from 34.8 to 81.6 ng/ml (mean 49.0 ng/ml; SD $\pm$ 22.24), and a spontaneous release of 0.3–4.2% (mean 1.5%; SD $\pm$ 1.83) (Table 2). After challenge a histamine release between 0.2 and 4.2% (mean 1.2%; SD $\pm$ 0.99) was seen (Fig. 1).

Discussion

Our results show that there is a good correlation between assessment of specific IgE antibodies and clinical symptomatology when determined within 2 months of the occurrence of hypersensitivity reactions. There is, however, no correlation between RAST results and total serum IgE.

According to previous studies, the abandonment of skin testing in RAST positive patients is justifiable (7, 8). Kraft et al. (8) showed that all patients with specific IgE antibodies to penicillins always showed positive skin test results. In addition, skin testing in patients with positive RAST results may lead to a higher incidence of adverse reactions.

None of the patients showed a positive histamine release (given as $\geq 10\%$ of total histamine). However, it could be demonstrated that the concentration of released histamine depends on individual concentrations of PPL in each patient or each control.

Although PPL is a reliable allergen for prick and intradermal testing in the *in vivo* diagnosis of penicillin allergy (13) where formation of a wheal and flare indicates liberation of mediators from skin mast cells, the same allergen was not able to induce a histamine release of $> 10\%$ from basophils in whole blood *in vitro*.

Therefore we tried to explain why skin mast cells could be activated by PPL while the same allergen

failed to induced histamine release of $> 10\%$ from leukocytes in whole blood.

1) Increase of histamine release by addition of deuterium oxide (D₂O) might be possible (1). As this is an unphysiological method, it was not used by us. In addition, it could be demonstrated that D₂O is not able to enhance histamine release from basophils in whole blood (14).

2) We did not attempt to prove the ability of our patients' basophils to release histamine *in vitro* (stimulation with anti-IgE antibodies) as this would be outside the scope of a routine laboratory and was not the aim of the study, i.e. establishment of an *in vitro* test easy to manage and not expensive. In addition, it seems statistically unlikely that all patients are non-responders.

3) We consider that 4 out of 7 patients (57%) might be able to demonstrate a positive histamine release if the threshold for positive results could be defined by 5% of total histamine (Fig. 1). As none of the controls released histamine above 4.2% of total histamine, it may be assumed that in penicillin allergy other thresholds for positive histamine release must be considered.

4) On the other hand, PPL could have lost its antigenic properties in whole blood by separation of the molecule, or the molecular structure of PPL might prevent bridging of cell-bound IgE molecules so that no basophil activation could occur.

5) Basophils and skin mast cells show different mechanisms on activation by osmotic stimuli (3). Hypothetically, different activation mechanisms of both cell types by PPL might be assumed. To confirm this, further investigations with isolated skin mast cells and isolated basophils are required.

In summary, PPL was not able to induce a positive histamine release from basophils in whole blood when the threshold was defined by 10%. However, with a threshold of 5%, a positive histamine release in 57% of our patients could be demonstrated.

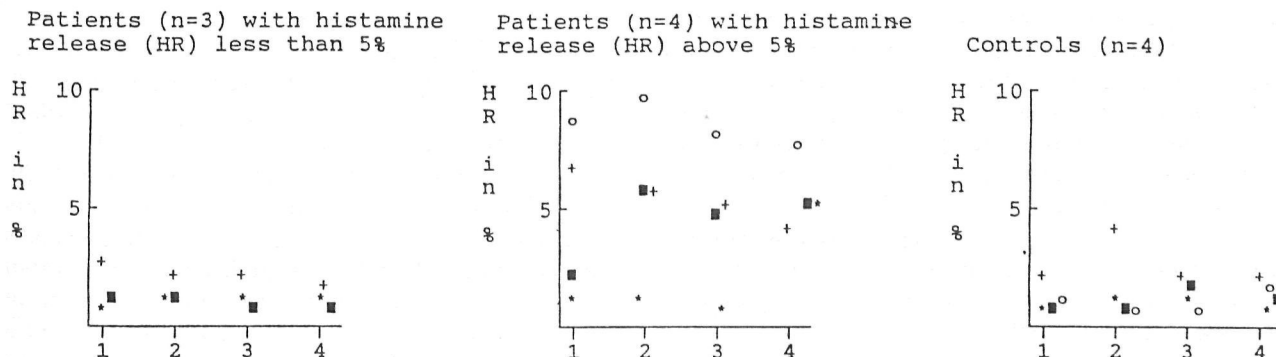


Fig. 1. Histamine release after challenge with PPL in various concentrations.

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