

Induction of benzylpenicilloyl specific antibodies, including IgE, by long-term administration of benzylpenicillin

P. DE HAAN* and G. L. KALSBECK†

**Department of Dermatology, Free University Hospital, Amsterdam, the Netherlands, and* †*Department of Dermatology, State University Hospital of Utrecht, the Netherlands*

(Received 18 June 1982; revision accepted for publication 31 August 1982)

Summary

Using the ELISA and passive haemagglutination test we investigated the humoral immune response induced by long-term administration of benzylpenicillin in patients. After the second week of the course in twenty-four out of sixty-one patients, synthesis of benzylpenicilloyl (BPO) specific IgG, IgA, IgM and/or IgE could be demonstrated. BPO-specific IgE was always found in combination with BPO-specific IgG and/or IgM. No difference in the humoral immune response was found between patients who developed clinical hypersensitivity reactions and those who did not.

In a follow-up study the disappearance of BPO-specific immunoglobulins of all classes was observed. Results of the epicutaneous tests showed that a long-term course of benzylpenicillin did not evoke delayed hypersensitivity. However intracutaneous tests with benzylpenicilloyl-polylysine or benzylpenicillin showed positive immediate type reactions. From the results it can be concluded that a BPO-specific IgE response is not necessarily associated with the development of a clinical hypersensitivity reaction.

Introduction

Data on the humoral and cellular immune response (IR) to penicillin are in most instances obtained only from patients showing clinical manifestations of penicillin hypersensitivity. However data on the IR induced by administration of benzylpenicillin in patients not showing any clinical sign of penicillin hypersensitivity are scarce. In most investigations antibodies were only determined in serum samples after completion of the course with benzylpenicillin. Levine *et al.* (1966) demonstrated by the passive haemagglutination test (HA) after the course of treatment, a humoral IR in all (114) patients with or without a hypersensitivity reaction. The intracutaneous test with benzylpenicilloyl-polylysine was positive in 100% of the patients with and in 8.7% of the patients without a hypersensitivity reaction.

Correspondence: Dr P. de Haan, Department of Dermatology B 347, Academic Hospital Free University, de Boelelaan 1117, 1007 MB Amsterdam, the Netherlands.

Using the HA, Schwartz & Vaughan (1963) demonstrated benzylpenicilloyl (BPO) specific antibodies in nine out of twenty-seven patients after completion of a long-term course with benzylpenicillin. The intracutaneous test with benzylpenicillin was positive in one out of the nine patients.

The occurrence of IgE and IgG after penicillin administration was demonstrated in six out of fifty-five patients by Adkinson (1977). This author also found BPO-specific IgG in twenty-seven out of the remaining forty-nine patients.

No data are available of the humoral IR before, during and after the benzylpenicillin course in patients with and without clinical hypersensitivity reactions. We therefore investigated the humoral IR before, during and after long-term administration of benzylpenicillin in patients who developed a hypersensitivity reaction and those who did not. Using the ELISA and the HA (de Haan, Boorsma & Kalsbeek, 1979) we could investigate the appearance and disappearance of BPO-specific Abs of all Ig classes.

Materials and methods

Patients

Sixty-eight patients treated for syphilis with a standardized long-term course with benzylpenicillin (procain-benzylpenicillin in oil with 2% alum monostearate, Almocillin®, Mycofarm, the Netherlands) were investigated. Sixty-one patients received 10 million IU benzylpenicillin—three times weekly 1 million IU intramuscular according to Idsoe, Gurthe and Willcox (1972) in 3 weeks. In the remaining seven patients the course was stopped after six or more injections because an allergic reaction occurred.

Sera

The sera of the patients could be divided into two main groups: sera of patients without a clinical hypersensitivity reaction (sixty-one); and sera of patients with a clinical hypersensitivity reaction (seven).

Sera of patients without signs of clinical hypersensitivity.

- Group I. Sera of forty-six patients were investigated with *in-vitro* tests before, and 1–4 months after completion of the course. Moreover thirty-four out of these forty-six sera were reinvestigated 2–7 months after the second blood sampling.
- Group II. Sera of fifteen patients were investigated before and during the course, as well as at the moment of the last injection. These sera were reinvestigated 1 month after completion of the course.

Sera of patients with a clinical hypersensitivity reaction.

- Group III. The sera of seven patients with a clinical hypersensitivity reaction occurring after six or more injections were all investigated before the course (Table 1). Four sera were investigated at the time of the allergic reaction, and all sera 1–2 months after administration. Moreover from five out of the seven patients, sera for follow-up studies were obtained.

Skin tests

Patch tests were done in seven patients with, and in nine patients without, a hypersensitivity reaction and were performed as described by van Ketel (1975). The following penicillin preparations were used: benzylpenicillin (Mycofarm, the Nether-

Table 1. Results of the *in-vitro* test (ELISA and/or HA) before the course, during and 1-2 months after the hypersensitivity reaction in seven patients with a clinical hypersensitivity reaction (group III)

Type of hypersensitivity reaction	Number of injections received before manifestation of a hypersensitivity reaction	Before the course*		At the time of the hypersensitivity reaction				1-2 months after the hypersensitivity reaction			
		IgM	IgE	IgG	IgA	IgM	IgE	IgG	IgA	IgM	IgE
Arthus reaction	6	-	-	ND	ND	ND	ND	-	-	-	-
Arthus reaction	8	-	-	ND	ND	ND	ND	+	+	+°	+
Arthus reaction	8	+	+	ND	ND	ND	ND	-	-	↑	-
Arthus reaction	6	-	-	-	-	+	-	-	-	↑	-
Arthus reaction	6	-	-	-	-	-	-	+	+	+	+
Arthus reaction	6	-	-	-	-	-	-	+	-	+(HA)	-
Urticaria	9	-	-	+	-	+°	-	↑	-	↓	-

ND: Not done.

* No BPO-specific IgG or IgA could be demonstrated before the course in these seven patients. + = ELISA positive; +° = ELISA + HA positive; ↑ = increase of titre; +(HA) = HA positive; - = negative HA and ELISA; ↓ = decrease of titre.

lands); ampicillin (Beecham, England); amoxicillin (Beecham); pheneticillin (Beecham).

Intracutaneous tests were done if the patch tests were negative, with 0.1 ml of a benzylpenicillin solution (500 IU/ml) and with benzylpenicilloyl-polylysine (6×10^{-5} M Cilligen®, Sigma Chem. Co., Missouri, U.S.A.). Control tests were done with a physiological salt solution (Levine *et al.*, 1966).

ELISA

The ELISA was done with a slight modification in the HRP conjugate, as described previously (de Haan *et al.*, 1979). The sheep anti-rabbit IgG-HRP conjugate was prepared with periodate (Nakane & Kawaoi, 1974) in the modification of Wilson & Nakane (1978). Results with this conjugate are comparable with those obtained with the conjugate prepared with the two-step glutaraldehyde method (Boorsma & Streefkerk, 1979). The specificity of antibodies was proven by inhibition experiments with benzylpenicilloyl- ϵ -aminocaproate.

Moreover inhibition experiments were done with benzylpenicillin. The inhibition tests were performed as described previously (de Haan *et al.*, 1979).

Passive haemagglutination test

This test was done according to Thiel, Mitchel and Parker (1964). Discrimination between IgM and IgG was made by pre-incubation with β -mercapto-ethanol (Watson, 1964).

Results

In-vitro tests

Patients without clinical signs of a hypersensitivity reaction. Group I (forty-six patients): in nine patients, BPO-specific antibodies (IgM) were found at the beginning of the course, twice in combination with BPO-specific IgE. After the course a decrease

(four patients), as well as increase of the titre of BPO-specific IgM (five patients) was seen. BPO-specific IgE was found in six out of these nine sera after the course. Eleven patients showed a seropositive conversion. A BPO-specific IgE response in combination with BPO-specific IgM and/or IgG was observed in four out of these eleven sera. In three sera with BPO-specific IgM antibodies before the course, no BPO-specific antibody could be demonstrated after the treatment. In the remaining 23 sera no BPO-specific antibody was found.

Group II (fifteen patients): in general BPO-specific antibody, including IgE (three patients), could be demonstrated 3 weeks after the start of the course. Only in one patient a BPO-specific IR, also IgE was demonstrated 4 weeks after the course.

Patients with clinical signs of a hypersensitivity reaction. Group III (seven patients): before the course, only in one serum BPO-specific IgE in combination with IgM was found (Table 1). In the remaining six sera 1–2 months after the course, BPO-specific Abs could be demonstrated. In two of these six patients BPO-specific IgE was found, here also in combination with other Ig classes.

Serological follow-up study

A serological follow-up study was done in thirty-four patients without, and five patients with, a clinical hypersensitivity reaction. In twenty-one out of these thirty-nine sera BPO-specific antibody was found after the course: in thirteen sera IgG, in two sera IgA, in fourteen sera IgM and in ten sera IgE (Table 2, group a and b). In eighteen out of these twenty-one sera a decrease in the titre of the BPO-specific Ig class was found (Table 2, group a). In the remaining three sera the Ig patterns showed a shift (Table 2, group b).

Inhibition experiments in the ELISA

The specificity of BPO-specific Abs in the ELISA was proved by inhibition experiments with penicilloyl- ϵ -aminocaproate. Inhibition of colour development (100%) was found at concentrations at 10^{-5} M penicilloyl- ϵ -aminocaproate, the same concentrations as previously described (de Haan *et al.*, 1979).

Table 2. Results of the follow-up study (ELISA and/or HA). Group a: patients with a decrease in the titre. Group b: patients with a seroconversion

Group	First serological investigation after the course*	Second serological investigation after the course†
a	11 sera with IgG 2 sera with IgA 13 sera with IgM 9 sera with IgE	7 sera with IgG(↓) 0 sera with IgA 6 sera with IgM(↓) 3 sera with IgE(↓)
b	1 serum with IgM 1 serum with IgG and IgE 1 serum with IgG	1 serum with IgM(↓) and IgE 1 serum with IgM 1 serum with IgM

* 1–4 months after the course.

† 2–7 months after the serological investigation.

In case of inhibition with benzylpenicillin only high concentrations of benzylpenicillin (10^{-3} – 10^{-2} M) inhibited colour development in the ELISA.

In-vivo tests

Patch tests. In all sixteen patients investigated (nine without, and seven with, clinical manifestations of a hypersensitivity reaction) patch tests with the various penicillins were negative.

Intracutaneous tests. In four out of seven patients with a clinical hypersensitivity reaction positive intracutaneous tests with Cilligen® were found (Table 3, group 1). In two of these four patients the intracutaneous test with benzylpenicillin was also positive.

In seven out of the nine patients who did not show a clinical reaction, the intracutaneous test with Cilligen® was positive (Table 3, group 2). Moreover in four out of these seven patients the intracutaneous test with benzylpenicillin was also positive. In one of the remaining two patients only the intracutaneous test with benzylpenicillin was positive.

Correlation between the results of the in-vivo tests and the ELISA-IgE

In two out of the four patients with a clinical reaction and a positive intracutaneous test with Cilligen®, circulating BPO-specific IgE was found with the ELISA (Table 3, group 1).

In the three patients with a negative intracutaneous test no BPO-specific IgE was demonstrated. In the group of nine patients without a clinical reaction but with positive serological ELISA-IgE finding, the intracutaneous test with Cilligen® was positive in seven patients (Table 3, group 2).

Discussion

In contrast to the results of Levine *et al.* (1966), who found after the course of penicillin treatment in all his patients, agglutinating BPO-specific antibodies, we found agglutinating BPO-specific antibodies in only five out of sixty-eight patients. Probably

Table 3. Comparison of the results of the intracutaneous tests with Cilligen® and benzylpenicillin with those of the ELISA-IgE in patients with (group 1) and without (group 2) a clinical hypersensitivity reaction

Group	No. of patients	ELISA-IgE	Cilligen®	Benzylpenicillin
1	1	+	+	+
	1	+	+	ND*
	1	–	+	+
	1	–	+	ND*
	3	–	–	–
2	4	+	+	+
	3	+	+	–
	1	+	–	+
	1	+	–	–

* ND: not done.

less purified penicillin preparations used in the past were more likely to induce agglutinating BPO-specific antibodies. Our results showed that the ELISA detects antibody better than the HA. Therefore routine agglutination tests are unlikely to detect the immune response in all patients in whom it is induced.

The finding of BPO-specific IgM, together with BPO-specific IgE and/or IgG in 13 out of 61 sera of patients without clinical manifestations of a hypersensitivity reaction before the course with penicillin, indicates that these patients have had previous exposure to penicillin by treatment or in food (Boonk, 1981), resulting in a synthesis of specific antibody. Similar findings were reported by Wheeler (1971) using the red-cell-linked antigen-antiglobulin reaction. In the thirteen sera we examined, the titre of BPO-specific antibody decreased after penicillin administration ceased. No differences were found in the immune response between patients with and those without BPO-specific antibodies before the course.

In general no humoral immune response could be demonstrated during the second week of the course of treatment. This could not be attributed to inhibition by benzylpenicillin, as the maximum serum concentration during the course (0.5 IU ml^{-1} blood; $0.9 \times 10^{-7} \text{ M}$, Idsoe *et al.*, 1972) did not inhibit antibody of positive sera. However after the third week of the course an immune response was demonstrated in seven patients without a clinical hypersensitivity reaction. Serological findings in a follow-up study demonstrated in general that BPO-specific antibody declines in titre or disappears from the circulation. In this respect, no difference was found between patients with and those without signs of a hypersensitivity reaction. Disappearance of BPO-specific IgE after treatment with penicillin was also found by Kraft *et al.* (1977). Therefore negative serologic findings at the start of a penicillin course do not exclude the possibility that the patient has been immunized in the past.

The intracutaneous test was done primarily in patients showing clinical signs of a hypersensitivity reaction, and in patients without a hypersensitivity reaction the intracutaneous tests were done only if there was a positive ELISA-IgE finding.

In the literature mostly 'overall' correlations between the results of the intracutaneous test with Cilligen® and those of the *in-vitro* IgE tests are presented (Kraft & Wide, 1976; Kraft *et al.*, 1977; de Haan *et al.*, 1979). In our group of patients with a hypersensitivity reaction the 'overall' correlation was 71.4% which compares with the variations previously reported (Kraft & Wide, 1976, 95.1%; Kraft *et al.*, 1977, 91.1%; Basomba *et al.*, 1979, 62.5%). In our patients not showing clinical signs of hypersensitivity reaction we found an 'overall' correlation of 89.7%. Adkinson (1977) found a positive intracutaneous test with Cilligen® in all his patients without a hypersensitivity reaction, but with a positive BPO-specific RAST-IgE.

In patients with and without a hypersensitivity reaction no differences in synthesis of BPO-specific Ig classes was found. A clinical hypersensitivity reaction is therefore not strictly related to the presence of BPO-specific antibodies. It is possible that two phenomena may have a more or less independent role in the pathogenesis of a clinical hypersensitivity reaction: an immunization phase and an elicitation phase (Spengler & de Weck, 1972).

We conclude that our findings demonstrate clearly that a BPO-specific IgE response is not necessarily correlated with the development of a clinical hypersensitivity reaction. However in these patients there is a state of humoral hypersensitivity as can be shown the positive intracutaneous tests with Cilligen® and/or benzylpenicillin.

Acknowledgments

We are most grateful to Miss Janny van Nimwegen for typing the manuscript.

This study was supported by 'Het Praeventiefonds', the Hague, the Netherlands.

References

- ADKINSON, N.F. JR (1977) Quantitative studies of the IgE and IgG immune response to penicillin administration in man. *Annals of Allergy*, **39**, 73.
- BASOMBA, A., VILLAMANZO, I.G., CAMPOS, A., PELAEZ, A. & BERGLUND, A. (1979) IgE antibodies against penicillin as determined by Phadebas RAST. *Clinical Allergy*, **9**, 515.
- BOONK, W.J. (1981) Dermatologic hazards from hidden contacts with penicillin. *Dermatosen in Beruf und Umwelt*, **29**, 131.
- BOORSMA, D.M. & STREEFKERK, J.G. (1976) Peroxidase-conjugate chromatography. Isolation of conjugates prepared with glutaraldehyde or periodate using polyacrylamide-agarose gel. *Journal of Histochemistry and Cytochemistry*, **24**, 481.
- BOORSMA, D.M. & STREEFKERK, J.G. (1979) Periodate or glutaraldehyde for preparing peroxidase conjugates. *Journal of Immunological Methods*, **30**, 245.
- CIRSTEA, M., SUHACIA, G.H. & BOROS, I. (1975) Experimental studies on drug sensitization. I. Penicillin sensitization as an experimental model for drug allergy. *Revue Roumaine de Physique*, **12**(3), 243.
- DE HAAN, P., BOORSMA, D.M. & KALSBECK, G.L. (1979) Penicillin hypersensitivity: Determination and classification of anti-penicillin antibodies by the enzyme-linked immunosorbent assay. *Allergy*, **34**, 111.
- DE WECK, A.L. (1981) Regulation of IgE response by anti-idiotypes and adjuvants. In: *New Trends in Allergy* (ed. I. Ring & G. Burg), p. 288. Springer Verlag, Berlin, Heidelberg, New York.
- IDSOE, O., GURTUE, T. & WILLCOX, R.R. (1972) *Penicillin in the Treatment of Syphilis. The Experience of Three Decades*. World Health Organization, Geneva.
- KRAFT, D., ROTH, A., MISCHER, P., PICHLER, H. & EBNER, H. (1977) Specific and total serum IgE measurements in the diagnosis of penicillin allergy: A long term follow-up study. *Clinical Allergy*, **7**, 21.
- KRAFT, D. & WIDE, L. (1976) Clinical patterns and results of radio allergosorbent test (RAST) and skin tests in penicillin allergy. *British Journal of Dermatology*, **94**, 593.
- LEVINE, B.B., REDMOND, A.P., FELLNER, M.J., VOSS, H.E. & LEVYTSKA, V. (1966) Penicillin allergy and the heterogeneous immune responses of man to penicillin. *Journal of Clinical Investigations*, **45**, 1895.
- LEVINE, B.B. & VAZ, N.M. (1970) Effect of combinations of inbred strain and antigen dose on immune responsiveness and reagin production in the mouse. A potential mouse model for immune aspects of human atopic allergy. *International Archives of Allergy*, **39**, 156.
- NAGEL, J., SVEC, D., WATERS, T. & FIREMAN, P. (1977) IgE synthesis in man. I. Development of specific IgE antibodies after immunization with tetanus-diphtheria (Td) toxoids. *Journal of Immunology*, **118**, 334.
- NAKANE, P.K. & KAWAOI, A. (1974) Peroxidase-labeled antibody. A new method of conjugation. *Journal of Histochemistry and Cytochemistry*, **22**, 1084.
- SAURAT, J.H., PONVERT, C., BURTON, C., SOUBRANE, C., LEBEL, B., BEUCHER, L. & PAUPE, J. (1976) Lymphocyte transformation, leucocyte migration, specific IgE, IgG and IgM before, during and after penicillin treatment without adverse reaction: A follow-up study. *Acta Allergologica*, **31**, 1.
- SCHWARTZ, R.H. & VAUGHAN, J.H.V. (1963) Immunological responsiveness of man to penicillin. *Journal of the American Medical Association*, **186**, 1151.
- SPRENGLER, H. & DE WECK, A.L. (1973) Current concepts in penicillin allergy. *Folia Allergologica*, **20**, 360.
- THIEL, J.A., MITCHEL, S.R. & PARKER, C.W. (1964) The specificity of hemagglutination reactions in human and experimental penicillin hypersensitivity. *Journal of Allergy*, **35**, 399.
- VAZ, E.M., VAZ, N.M. & LEVINE, B.B. (1971) Persistent formation of reagins in mice injected with low dose of ovalbumin. *Immunology*, **21**, 11.
- VIJAY, H.M., LAVERGE, G., HUANG, H. & BERNSTEIN, T.L. (1979) Preferential synthesis of IgE reaginic antibodies in rats immunized with alum-adsorbed antigens. *International Archives of Allergy and Applied Immunology*, **59**, 227.
- WATSON, K.C. (1964) The nature of antiglobulin reactivity of antibody to penicillin. *Immunology*, **7**, 97.
- WHEELER, A.W. (1971) A method for measuring different classes of human immunoglobulins specific for the penicilloyl group. *Immunology*, **21**, 547.
- WILSON, B.M. & NAKANE, P.K. (1978) Recent developments in the periodate method of conjugation horseradish peroxidase (HRPO) to antibodies. In: *Immunofluorescence and Related Staining Techniques*, (eds W. Knapp, K. Holubar and G. Wick), p. 215. Elsevier, North Holland, Biomedical Press, Amsterdam.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.