

Humoral Immune Response to Some Benzylpenicillin Preparations

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In this study the humoral immune responses to long-term administration of benzylpenicillin preparations with and without adjuvants were compared. In 44.4% of the patients on long-term treatment with a benzylpenicillin preparation containing oil and alum monostearate, an induction of benzylpenicilloyl (BPO)-specific IgG, IgM and IgE was demonstrated during and after the course. Patients treated with a benzylpenicillin preparation containing no oil and alum monostearate showed only a very weak BPO-specific IgM and IgG response during the course. In patients in whom long-term treatment with a benzylpenicillin preparation without adjuvants was initiated by a benzylpenicillin preparation containing oil and alum monostearate, not only BPO-specific IgM and/or IgG but also IgE were demonstrated in 13.0%. The differences in immune response in the various long-term courses were significant ($P < 0.05$). The data suggest that the presence of such adjuvants as oil and alum monostearate has an influence on the synthesis of BPO-specific antibodies. However, it can not be excluded that the difference in immunogenicity had some unknown connection with the differences in penicillin/blood levels.

Key words: adjuvant effect; benzylpenicilloyl; drug hypersensitivity; IgE response; immunogenicity.

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CLINICAL ASPECTS

A comparison of the immune response to three different benzylpenicillin courses showed that in the long-term treatment of syphilis the favoured preparation was benzylpenicillin without adjuvants (oil and alum monostearate).

In a previous study (3) it was shown that long-term administration of benzylpenicillin (Almocillin®, Mycofarm, Delft) may induce a humoral immune response resulting in the production of benzylpenicilloyl (BPO)-specific antibodies (Abs): IgG, IgA, IgM and IgE. As Almocillin contains oil and alum monostearate it might be presumed that this humoral immune response is due to the presence of these adjuvants. In order to test this hypothesis we have compared the humoral immune response in patients before, during and after three different benzylpenicillin courses: one course consisted of benzylpenicillin in oil with alum monostearate (Almocillin), one of benzathin benzylpenicillin (Penidural®, Mycofarm, Delft), and

the last course was initiated with Almocillin followed by Penidural.

The investigations were performed using the ELISA and the passive hemagglutination test.

MATERIAL AND METHODS

Benzylpenicillin courses

A. Penidural course

This benzylpenicillin course consisted of injections with a solution of benzathin benzylpenicillin in *aqua dest.* After an i.m. injection of 2.4 mill. IU benzylpenicillin the concen-

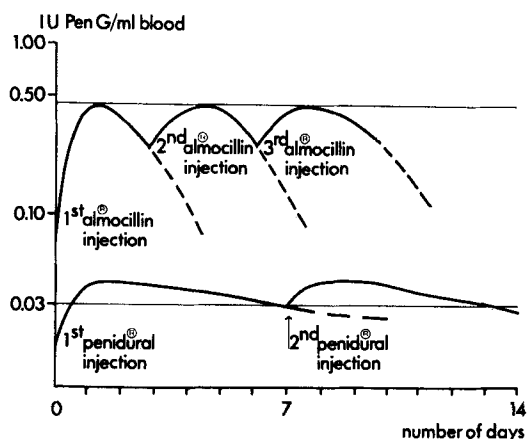


Fig. 1. Concentration of free benzylpenicillin (Pen G) in blood after an injection with Almocillin® (1 mill. IU benzylpenicillin) or with Penidural® (2.4 mill. IU benzylpenicillin). Y axis logarithmic division in 1-100.

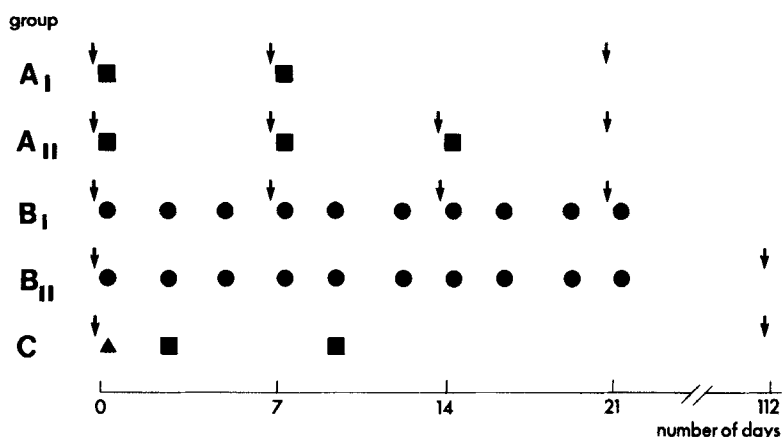


Fig. 2. Long-term treatment of syphilis with benzylpenicillin and serology.

Group A_I: Two injections of Penidural® (2.4 mill. IU benzylpenicillin).

Group A_{II}: Three injections of Penidural (2.4 mill. IU benzylpenicillin).

Group B_I and B_{II}: Ten injections of Almocillin® (1 mill. IU benzylpenicillin).

Group C: One injection of Almocillin® (0.5 mill. IU benzylpenicillin) followed by 2 injections of Penidural (2.4 mill. IU benzylpenicillin).

↓ = Serological investigation.

■ = One injection of 1 mill. IU benzylpenicillin (Almocillin).

▲ = One injection of 0.5 mill. IU benzylpenicillin (Almocillin).

● = One injection of 2.4 mill. IU benzylpenicillin (Penidural).

tration of benzylpenicillin is maintained just above 0.03 IU/ml blood for at least 1 week (4).

One weekly injection (2.4 mill. IU benzylpenicillin) keeps the minimal serum concentration just above 0.03 IU/ml blood (Fig. 1).

B. Almocillin course

This benzylpenicillin course consisted of injections with a suspension of procainbenzylpenicillin in oil with 2% alum monostearate. After an intramuscular injection of Almocil-

lin (1 mill. IU benzylpenicillin) the serum concentration of benzylpenicillin, 24 h after injection, is 0.5 IU/ml blood (4). Three to 5 days after the injection this concentration has decreased to 0.03 IU. To keep the concentration of benzylpenicillin above 0.03 IU/ml blood it is necessary to give a new injection after 2 to 3 days (Fig. 1).

C. *Combined course*

This course consisted of one i.m. injection Almocillin (0.5 mill. IU) followed by two i.m. injections of Penidural, 2.4 mill. IU benzylpenicillin each.

Patients and patient's sera

One hundred patients with syphilis were each treated with one of the three long-term benzylpenicillin courses. The patients were divided according to treatment course, differences in treatment schedules and times of serological investigation (Fig. 2).

A. *Patients treated with Penidural*

Eight patients (group A_I) received two i.m. injections of 2.4 mill. IU benzylpenicillin. Sera were obtained before, during and after the course (Fig. 2).

Eight patients (group A_{II}) received three i.m. injections of 2.4 mill. IU benzylpenicillin. Sera were investigated before, during (twice) and at the end of the course (Fig. 2).

B. *Patients treated with Almocillin*

Fifteen patients (group B_I) were given 10 injections of Almocillin (1 mill. IU benzylpenicillin each) over a period of 3 weeks. Sera were obtained before, during and at the end of the course (Fig. 2).

Forty-six patients (group B_{II}) received 10 injections of Almocillin (1 mill. IU benzylpenicillin each) over a period of 3 weeks. Sera were obtained before and 1–4 months after the course (Fig. 2).

C. *Patients treated with combined course*

Twenty-three patients (group C) received a combined course. Sera were investigated be-

fore and 1–4 months after the course (Fig. 2).

In vitro tests

I. *ELISA*

This test was done with a modification in the preparation of the conjugate, as described before by de Haan et al. (2). If a BPO-specific Ig class is not included in one of the tables, this particular Ig-class was not found.

II. *Passive hemagglutination test*

This test was performed according to Thiel et al. (7). IgM and IgG were identified by pre-incubation with beta-mercapto-ethanol (8).

RESULTS

The serological findings were as follows:

Patients treated with Penidural

No humoral immune response could be demonstrated in group A_I patients (total dose 4.8 IU benzylpenicillin) during or after the course with Penidural (Table 1, group A_I).

Two out of eight patients from group A_{II} (7.2 mill. IU Penidural) showed a weak humoral response (IgM and IgG respectively). This response had disappeared in the 3rd week after the start of the course.

Patients treated with Almocillin

As seen in Table 1, patients treated with Almocillin (group B_I) showed an immune response 3 weeks after the start of the course. It was remarkable that BPO-specific IgE was always found in combination with BPO-specific IgG and/or IgM. A sero-positive conversion was demonstrated in six out of 14 (35.7%) of the patients without BPO-specific Abs before the course.

Five out of 46 patients, with pre-existing Abs, treated with Almocillin (group B_{II}), showed BPO-specific IgE response.

This response was also found in four patients with a sero-positive conversion (Table 2). BPO-specific IgE was always found in combination

Table 1

Results of *in vitro* tests (ELISA and/or HA) before and after the start of a course

Group	No. of sera	Before course		
		IgM	IgE	IgM and/or IgG
A _I	7	—	—	—
	1	+	—	—
A _{II}	8	—	—	—
B _I	1*	+	—	↑
	3*	—	+	+
	2*	—	—	+
	9	—	—	—

↑ Increase of titre.

* Patients with an immune response.

Group A_I: patients on a Penidural® course (4.8 mill. IU benzylpenicillin i.m.),

group A_{II}: patients on a Penidural® course (7.2 mill. IU benzylpenicillin i.m.) and

group B_I: patients on a Almocillin® course (10 mill. IU benzylpenicillin i.m.).

If an Ig class is not presented in the table this class was not found.

Table 2

Results of *in vitro* tests (ELISA and/or HA) before and 1–4 months after an Almocillin® course (10 mill. IU benzylpenicillin) in 46 patients (group B_{II})

No. of sera	Before course		After course	
	IgE	IgM and/or IgG	IgE	IgM and/or IgG
1*	+	+	+	↑
1	+	+	—	↑
5*	—	+	+	+
4*	—	—	+	+
7*	—	—	—	+
3	—	+	—	—
2	—	+	—	↓
23	—	—	—	—

* Patient with an immune response.

↑ Increase of titre.

↓ Decrease of titre.

with BPO-specific IgM and/or IgG. In patients without Abs before the course a sero-positive conversion was seen in 11 out of 34 (32.2%).

Table 3

Results of *in vitro* tests (ELISA and/or HA) before and 1–4 months after a combined course of Almocillin® (0.5 mill. IU benzylpenicillin) Penidural® (4.8 mill. IU benzylpenicillin) in 23 patients (group C)

No. of sera	Before course		After course	
	IgE	IgM and/or IgG	IgE	IgM and/or IgG
1*	—	+	+	↑
1	+	+	—	+
1	+	+	+	+
2*	—	—	—	+
2	—	+	—	—
16	—	—	—	—

* Patients with an immune response.

↑ Increase of titre.

Patients treated with a combined course

In these patients (group C) a BPO-specific IgE response was found in one patient with pre-existing BPO-specific Abs (Table 3). A sero-positive conversion was seen in 11.1% of the patients without BPO-specific Abs before the course.

DISCUSSION

For practical reasons treatment of syphilis with benzylpenicillin is mostly done with long-term acting preparations such as benzylpenicillin in oil with alum monostearate or benzathin benzylpenicillin (4). Therefore we were only able to compare the humoral immune response to treatment with the three different benzylpenicillin courses.

In our patients a long-term course of Penidural (groups A_I and A_{II}) induced at the most a weak immune response of BPO-specific IgM or IgG in the 2nd week after the start of treatment. After the course the humoral immune response could no longer be demonstrated in these patients (Table 1, groups A_I and A_{II}). In contrast to these findings, patients who received first one injection of Almocillin (0.5 mill. IU benzylpenicillin) followed by two injections of Penidural (group C) showed a synthesis of BPO-specific Abs (Table 3). One injection of Almocil-

lin was able to induce a humoral response in 13.4% of the patients. Thus, as might be expected, long-term treatment with Almocillin (Table 2, group B_{II}) did induce a stronger immune response than that found after one infection of Almocillin followed by Penidural (Table 3).

Although high blood concentrations of benzylpenicillin after an injection of Almocillin enhance the coupling between benzylpenicillin and protein, this coupling occurs also at low concentration after an injection of Penidural. Therefore other factors seem to contribute to the immune response to Almocillin. Cirstea et al. (1) found that oil subcutaneously injected in rabbits at the same site as benzylpenicillin stimulates the penicilloylation of proteins. Thus the presence of oil in Almocillin might explain the immune response to this preparation.

In patients treated with a long-term course of Almocillin (groups B_I and B_{II}) an impressive BPO-specific IgE response was observed. This response occurred in patients both with and without pre-existing Abs (Table 1, group B_I and Table 2). Also in patients where the long-term course was initiated with one injection of Almocillin (group C) BPO-specific IgE Abs were found. In contrast to these findings no BPO-specific IgE Abs were demonstrated in patients treated with a long-term course of Penidural (Table 1, groups A_I and A_{II}). From this it can be concluded that treatment with Almocillin stimulates the synthesis of BPO-specific IgE Abs. This phenomenon can be explained by the presence of alum monostearate in Almocillin. Alum monostearate can be converted *in vivo* into alum hydroxide, which is known to have an adjuvant effect in the synthesis of specific IgE (5, 6, 9, 10).

Using the chi-square method, statistically significant differences in the post-treatment immune response were observable (Tables 1-3) between patients in groups A_I, A_{II} and group B_I well as between those in groups B_{II} and C.

Although not shown here 10 patients in group B_{II}, treated with Almocillin and demonstrating a specific IgE response, were tested intracutaneously with Cilligen® and benzylpenicillin (2). In eight out of these 10 patients

the test with Cilligen was positive, while in one of the remaining two patients the test with benzylpenicillin was positive. Thus these patients are in a state of hypersensitivity, and are therefore considered able to develop a clinical hypersensitivity reaction.

In summary, the results of this investigation demonstrated clearly that a long-term course of treatment with Almocillin is more immunogenic (induces the synthesis of BPO-specific IgM, IgG and IgE) than a long-term course with Penidural or a combined course with Penidural initiated by one injection of Almocillin.

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