

Preparation of Benzylpenicilloyl-Polylysine: A Preliminary Study

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ABSTRACT

The manufacturing of PrePen (benzylpenicilloyl-polylysine) was suspended from September 2000 through November 2001 because of Food and Drug Administration intervention. PrePen is penicilloyl bound to polylysine and is considered to be the major determinant of penicillin metabolism; it is used as a skin-testing reagent to detect immunoglobulin E antibodies in people with a history of penicillin allergy. Because of the uncertainty of whether PrePen would be made available in the future, we attempted to make benzylpenicilloyl-polylysine in our laboratory using a modification of a previously described method. Subsequently, we compared our preparation with PrePen by enzyme-linked immunosorbent assay and skin testing. Using a patient previously documented to have a positive reaction to PrePen by history and intradermal skin testing, a puncture test to PrePen as well as dilutions of benzylpenicilloyl-polylysine was negative. Positive intradermal tests were performed on PrePen and benzylpenicilloyl-polylysine at 5 mg/mL. The enzyme-linked immunosorbent assay was performed to compare the antigenicity of PrePen and benzylpenicilloyl-polylysine and revealed similar binding to both. Subsequently, cutaneous testing on 10 additional penicillin-allergic people was performed, with corroborative results. Our benzylpenicilloyl-polylysine production methodology could prove useful to those who do not have access to PrePen or another similar reagent. (Allergy and Asthma Proc 25:165–168, 2004)

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From September 2000 to November 2001, the manufacturer of PrePen (Hollister-Stier, Spokane, WA) was put on hold because of production irregularities alleged by the Food and Drug Administration. The majority of penicillin (95%) *in vivo* is metabolized into the major determinant (benzylpenicilloyl). The rest is metabolized into the minor determinants (benzylpenilloate and benzylpenicilloate) or remains unmetabolized. Approximately 10–20% of inpatients report an immunoglobulin E (IgE)–mediated allergic reaction to penicillin.^{1,2} *In vivo*, penicillin metabolites form hapten-protein complexes that are antigenic. PrePen (benzylpenicilloyl-polylysine), which is produced by Taylor Pharmaceuticals (Decatur, IL) and distributed by Hollister-Stier, is penicilloyl bound to polylysine that can be used for skin testing for IgE antibodies to the major determinant. Macy *et al.*, reported that testing with penicilloyl poly-L-lysine was able to identify 80% of penicillin-allergic patients, and omission of testing with this reagent would fail to identify 20% of patients.³ Thus, investigators have concluded that people with a negative skin test to the major determinant are at significantly decreased risk of an immediate IgE-mediated allergic reaction to penicillin as compared with those who are skin-test positive.⁴ Without the use of PrePen, it is difficult to risk stratify patients with a history of an allergic reaction to penicillin. This may lead to classifying many people as penicillin allergic, although they could tolerate penicillin. Consequently, these patients may receive alternative antibiotics that may be more toxic, expensive, or breed antibacterial resistance. Alternatively, people would be given penicillin without the benefit of skin testing, and this may increase the number of people who experience anaphylactic reactions. For this study, we attempted to make benzylpenicilloyl-polylysine, which can be used to skin test for IgE antibodies against the major determinant, and we compared our benzylpenicilloyl-polylysine to PrePen by skin testing and enzyme-linked immunosorbent assay (ELISA).

TABLE I

Results of Skin Tests with PrePen and the Benzylpenicilloyl-Polylysine Manufactured in Our Laboratory

	Prick (Wheal/ Erythema)	ID (Wheal/ Erythema)
PrePen 3 mg/mL	2/2 mm	11/21 mm
BPO-PL* 2.5 mg/mL	0/0	0/10 mm
BPO-PL 5 mg/mL	0/0	10/20 mm
BPO-PL 10 mg/mL	0/0	14/25 mm

* BPO-PL = benzylpenicilloyl-polylysine; ID = intradermal.

METHODS

Benzylpenicilloyl-polylysine was prepared as described by Parker *et al.*⁵⁻⁸ Some modifications were made to this method. First, 100 mg of poly-D-lysine with a molecular weight (MW) between 3000 and 4000 kDa (Sigma, St. Louis, MO) was dissolved in 0.4 mL of sterile water and neutralized to a pH of 7.0 with one drop of 5 N NaOH. Then, 1.2 g of clinical grade K⁺ penicillin

G (Sigma) was dissolved in 1 mL of 1 M carbonate buffer (pH, 10.4) and mixed with the poly-D-lysine solution. The pH was adjusted to 9.6 with sterile water, and the mixture was incubated at room temperature for 6 hours. An additional 600 mg of penicillin G was then added at 6 hours and again at 16 hours. The pH was adjusted to 11.0 with 5 N NaOH after each addition of penicillin G. The final solution was allowed to sit at room temperature for 6 hours. Then, the benzylpenicilloyl-polylysine solution was dialyzed using a SpectraPor #6 filter dialysis tubing (MW cutoff 1000) in 4 L of 0.001 M phosphate buffer at 4°C. The solution was dialyzed over 5 days with frequent changes of the 0.001 M phosphate buffer. The final dialysis was performed using 4 L of 0.15 M NaCl in 0.01 M phosphate buffer to bring the solution to a physiological pH of 7.4. Finally, the benzylpenicilloyl-polylysine solution was sterile filtered through a 0.2- μ m syringe filter into a sterile vial. The solution was dried in the hood to determine the dry weight (105.1 mg) and then reconstituted in 0.01 M phosphate-buffered saline (PBS) to a final concentration of 50 mg/mL. Benzylpenicilloyl-polylysine was stored at 4°C.

The benzylpenicilloyl-polylysine was diluted with 0.01 M PBS to the following concentrations for skin testing:

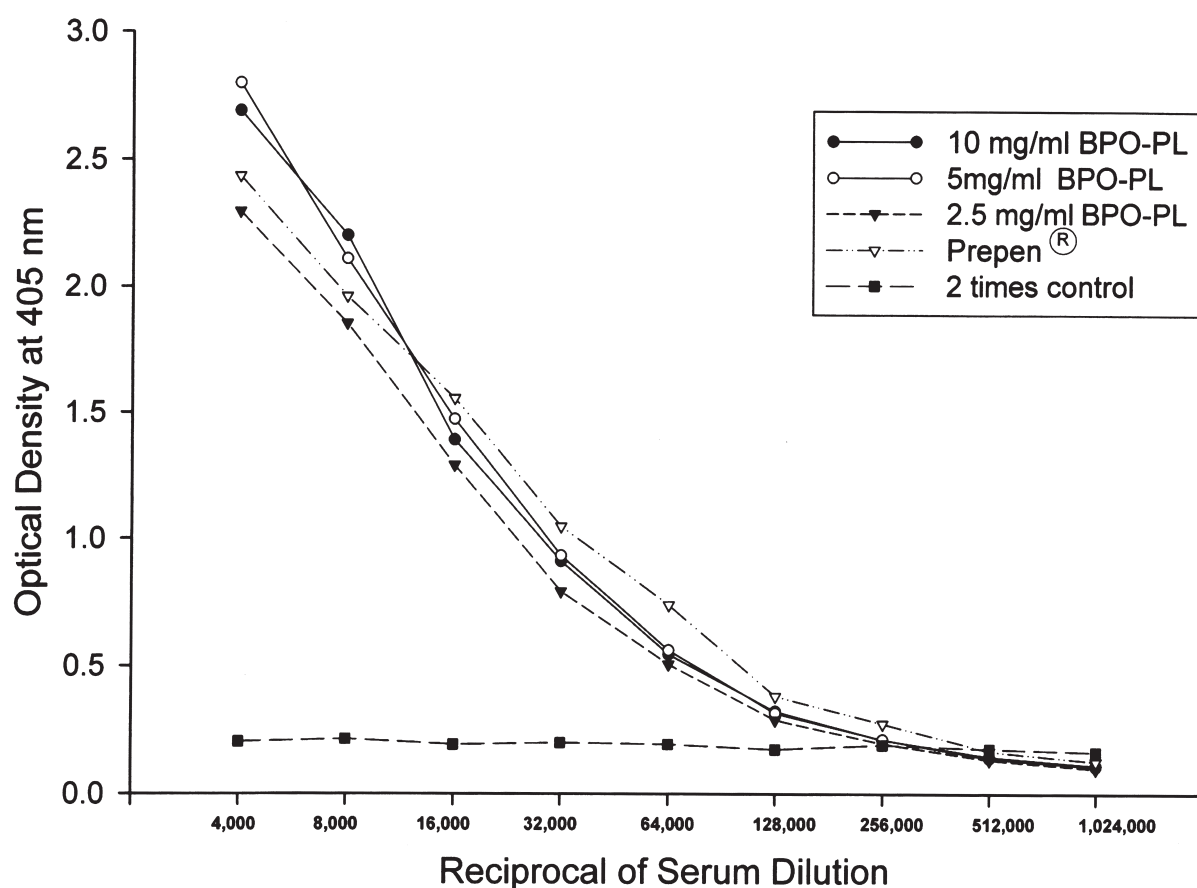


Figure 1. Results of an ELISA showing the comparison of the binding of serial twofold dilutions of polyclonal rabbit anti-penicillin antibody to PrePe and various concentrations of benzylpenicilloyl-polylysine (BPO-PL) made in our laboratory.

2.5, 5, and 10 mg/mL. Puncture and intradermal testing (with 0.01 mL of all dilutions) was preformed on a patient with a history of penicillin allergy and a documented positive reaction to PrePen by intradermal testing. Two subjects without a history of penicillin allergy were used as negative control patients. The study was approved by the Institutional Review Board and conducted according to the Declaration of Helsinki after informed consent was obtained.

An ELISA was performed using polyclonal rabbit anti-penicillin antibody (specific for the β -lactam ring) from Cortex Biochem, Inc. (San Leandro, CA). PrePen was compared *via* ELISA with the benzylpenicilloyl-polylysine solution as prepared previously. PrePen was diluted to 1:100 for use in the ELISA. A 1:100 dilution was made with each of the three concentrations (2.5, 5, and 10 mg/mL) of the benzylpenicilloyl-polylysine used for skin testing as well.

First, the ELISA plate was coated overnight with the diluted PrePen and the various dilutions of benzylpenicilloyl-polylysine. Then, this plate was incubated for 30 minutes with serial twofold dilutions of the anti-penicillin antibody to assess maximal binding capacity of PrePen and benzylpenicilloyl-polylysine. The amount of anti-penicillin antibody bound to the plate was detected by alkaline phosphatase-labeled goat anti-rabbit antibody at an optical density of 405 nm. We performed mass spectroscopy with a technique called matrix-assisted laser desorption/ionization, which has been useful in other protein analyses. However, analyses of spectra were not able to be performed technically.

RESULTS

Skin Testing

Puncture and intradermal testings on two control patients (with no previous history of penicillin allergy) were negative to the PrePen and all dilutions of benzylpenicilloyl-polylysine. Histamine was applied as a positive control. A positive test was defined as a wheal that was at least 3 mm greater than the negative control. Prick testing on the subject with a history of penicillin allergy was negative to PrePen as well as all dilutions of benzylpenicilloyl-polylysine. Next, intradermal testing was performed with 0.01 mL of PrePen and 0.01 mL of the various dilutions of the benzylpenicilloyl-polylysine. Intradermal testing to PrePen was positive and equivalent to our benzylpenicilloyl-polylysine at 5 mg/mL (Table I).

Ten additional patients known to be positive to PrePen from previous testing were retested. Two patients were prick-test positive for both PrePen and our reagent, and eight patients were intradermally positive using PrePen full strength and our reagent at 5 mg/mL.

TABLE II

Procedure for Producing Benzylpenicilloyl-Polylysine

1. 100 mg of poly-D-lysine 3000–4000 kDa (Sigma, St. Louis, MO), dissolved in 0.4 mL sterile water
2. A pH to 7.0, with 5 N NaOH
3. 1.2 g of clinical grade penicillin G (Sigma) is dissolved in 1 mL of 1 M carbonate buffer (pH 10.4)
4. Mix two solutions
5. Adjust pH to 9.6 with sterile water
6. Incubate at room temperature for 6 hours
7. At 6 hours and 16 hours, add 600 mg penicillin G
8. Adjust pH to 11.0 with 5 N NaOH after each addition of penicillin G
9. Incubate 6 more hours
10. Dialyze solution for 5 days using Spectra Por #6 filter dialysis tubing, MW cut off at 1000 and frequent changes of 4 L of 0.001 M phosphate buffer at 4°C
11. Dialyze solution with 4 L of 0.15 M NaCl in 0.01 M phosphate buffer to bring solution to a pH of 7.4
12. Filter solution through a 0.2 μ m syringe filter into a sterile vial
13. Lyophilize; weigh
14. Reconstitute in 0.01 M PBS to a final concentration of 50 mg/mL
15. Store at 4°C

ELISA Testing

The ELISA revealed similar anti-penicillin antibody binding between PrePen and our benzylpenicilloyl-polylysine at all concentrations (Fig. 1).

DISCUSSION

There are some structural differences between PrePen and the benzylpenicilloyl-polylysine made in our laboratory (Table II). The chemical structure of PrePen is composed of poly-L-lysine with >40 subunits; ~50–70% of the epsilon groups have been substituted with benzylpenicilloyl groups. Parker *et al.*, determined that there were 8–11 penicilloyl groups per molecule.⁵ From this study, we assumed that our benzylpenicilloyl-polylysine had ~50% binding, and the poly-D-lysine (MW, 3000–4000 kDa) chain had 23–30 subunits per chain. There are certain limitations for this data on benzylpenicilloyl-polylysine. Because the weight/volume determination was based on dry weight after lyophilization from a salt buffer, this may have resulted in an overestimation of the benzylpenicilloyl-polylysine concentration. In the future, studies to determine the exact concentration and amount of penicilloyl binding to lysine will be needed so this agent can be standardized before use on the general population. In addition, further studies need to be done to determine the reproducibility and stability (at different storage conditions) of this product.

From our preliminary data, we have shown that it is possible for benzylpenicilloyl-polylysine for skin testing to be prepared in research laboratories. The ELISA data shows similar binding curves between PrePen and benzylpenicilloyl-polylysine. A patient with a known positive skin test to PrePen was tested with benzylpenicilloyl-polylysine and had an equivalent positive response. Before benzylpenicilloyl-polylysine can be used routinely, we need better characterization of this structure as compared with PrePen.

The use of an independently synthesized agent in place of a Food and Drug Administration-licensed product raises some legal issues in the United States. Practitioners must comply with all or any federal, state, or local laws regarding use.

From our study, we have shown that benzylpenicilloyl-polylysine manufactured in our laboratory is capable of detecting IgE antibodies by skin testing penicillin-allergic individuals and demonstrating binding similar to PrePen on ELISA. Additional studies need to be performed in a larger patient population to validate and standardize this skin-testing reagent. If PrePen is not available in the

future, it should be possible for other laboratories to produce reagents for skin testing to the major determinant of penicillin.

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