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Occupational Asthma Caused by *Serratial* Peptidase and Lysozyme Chloride in a Pharmaceutical Industry

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There have been very few documented cases of occupational allergies caused by serratial lysozyme and peptidase. In this paper, we report a case of occupational asthma and rhinitis caused by *Serratial* peptidase and lysozyme chloride which are used as anti-inflammatory agents. The patient had strong positive responses to peptidase and lysozyme extracts on skin-prick tests. Bronchoprovocation tests showed a dual asthmatic response to peptidase but only an early asthmatic response to lysozyme. Serum specific IgE antibodies to peptidase and lysozyme were detected by enzyme-linked immunosorbent assay (ELISA). In order to further characterize the allergenic component of these extracts, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotting studies were also performed. Two IgE binding components (67, 10.9 kDa) were detected within the lysozyme extracts, and more than ten components ranging from 7.3 to 83.1 kDa found in peptidase extracts. The findings suggest that inhalation of peptidase and lysozyme can induce IgE-mediated bronchoconstriction in an exposed worker.

Key Words: Peptidase, Lysozyme, Occupational asthma, Pharmaceutical industry

INTRODUCTION

There have been a few reports of occupational asthma induced by biologic enzymes inhaled in the course of work in the pharmaceutical industry[1~7]. An IgE-mediated mechanism has been implicated in most cases[2~7]. To the best of our knowledge, occupational asthma due to peptidase and lysozyme in a pharmaceutical industry has not been reported. The peptidase (60 kDa) figuring in this study is a proteolytic enzyme derived from *Serratia spp.* and widely used as an anti-inflammatory agent, and lysozyme (lysozyme chloride, 14.5 kDa) is also used as anti-inflammatory agent for allergic patients. We report herein on a case of occupational rhinitis and asthma caused by peptidase and lysozyme powder in an worker handling these powders in a pharmaceutical industry, a diagnosis which was confirmed by bronchoprovocation tests.

Furthermore, serum specific IgE antibodies were detected and their IgE binding components were identified.

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SUBJECTS AND METHODS

Case summary

The patient was a 23- year-old female worker, who was admitted to the Allergy Clinic of Ajou University Hospital, Suwon, Korea. Three years earlier, she had been employed at a pharmaceutical company and her job responsibilities included handling drug powders to make tablets. She began to cough and experience shortness of breath 1 year before this study, and suffered from profuse rhinorrhoea and sneezing for the last 26 months. She complained that the above symptoms were aggravated after handling lysozyme and peptidase pow-

ders, however, improved when she was on vacation. On admission, physical examination showed clear breathing without wheezing.

A skin-prick test demonstrated negative responses to 80 common inhalant allergens (Bencard, Breford, U.K.). Immediate and late skin response was observed with peptidase and lysozyme extracts as shown in table 1. Total IgE level by PRIST was 877 IU / ml. The total eosinophil count was 1,365 /mm^3. Methacholine bronchial challenge test showed a positive result at 2.5 mg/ml. A chest X-ray revealed no abnormal findings.

Preparation of peptidase and lysozyme extracts

Peptidase and lysozyme powders were obtained from the patient's workplace. They were extracted with phosphate-buffered saline [(PBS, pH 7.5), 1: 1 w/v] at 4℃ for 1 h. The supernatants were used for the bronchoprovocation tests at a concentration of 1 : 100 (lysozyme) and 1 : 10 (peptidase) w/v. For the skin-prick test, the 1 : 5 w/v extracts were mixed with an equal amount of sterile glycerine.

Some of the supernatant was dialyzed (cut-off molecular weight was 6,000 Da) against 4*l* of distilled water at 4℃ for 48 h, and lyophilized at −70℃.

Bronchoprovocation test with peptidase and lysozyme

Airway responsiveness to methacholine was performed according to the method described previously[8]. Bronchoprovocation tests were performed according to standard procedure in occupational asthma studies[9]. Normal saline was inhaled for 2 min from a nebulizer 646 connected to a dosimeter (Devilbiss Co. USA). The subject was asked to breathe the aerosol for 2 min with tidal breathing. Antigen extracts were inhaled from 1:1000 w/v to 1:10 w/v. The forced expiratory volume in one second (FEV1) and peak expiratory flow rate (PEFR) were measured with a spirometer (Chest, Japan) before and 10 min after each inhalation. Then, the FEV1 and PEFR were measured every 10 min during the first hour, and pulmonary function tests were performed every hour for 7 h after the challenge.

ELISA for specific IgE and IgG4 antibodies to peptidase and lysozyme extracts

The presence of specific IgE and IgG4 antibodies to lysozyme and peptidase was determined by ELISA. Microtitre plates (Dynatech, USA) were first coated with 100 ul of peptidase (5 ug / well) and of lysozyme (12.5 ug / well) extracts, respectively, left at 4℃ overnight. Each well was washed 3 times with 0.05% Tween phosphate buffered saline (PBS-T), and the remaining binding sites were blocked by incubation with 250 ul of 10% new-born calf serum for 1 h at room temperature. The wells were then incubated for 2 h at room temperature with 100 ul of either the patient's serum (undiluted) or control sera from ten patients who showed negative skin-prick test responses to common inhaled allergens as well as to lysozyme and peptidase. After washing three times with PBS-T, 100 ul of 1 : 1000 v/v biotin-labeled goat anti-human IgE antibody (Sigma Co. USA) were added to the wells and incubated for 2 h at room temperature. The wells were then washed three times with PBS-T and incubated with 1 : 1000 v/v streptavidin-peroxidase (Sigma Co. USA) for 30 min before another washing step followed by incubation with 100 ul ABTS (2.2'-azinobis-3-ethyl-benzthiazoline sulfonic acid in a citrate phosphate buffer) for 10 min at room temperature. The reaction was stopped by the addition of 50 ul 2 mM sodium azide and the absorbance was read at 410 nm by an automated microplate reader. All assays were performed in triplicate.

SDS-PAGE and immunoblotting

Peptidase (10 mg / ml) and lysozyme (10 mg / ml) were each dissolved in a buffer (0.5 M tris-HCl, pH 6.8, 2.5 ml, glycerol 2.0 ml, 10% w/v SDS 4.0 ml, 0.1% bromophenol blue 0.5 ml and distilled water 1.0 ml) and boiled. Ten microlitres of standard marker (6-200 kDa) (Novex, San Diego, CA, USA) and antigen solutions were applied to a Novex precast tris-glycine gradient gel (4～20% acrylamide) for the separation of antigens. Electrophoresis was performed with a Novex Mini-cell for 90 min at 125 constant voltage. The gel was fixed and stained with Coommassie brilliant blue. For immunoblotting, the gel was soaked in a transfer buffer (12 mM tris, 96 mM glycine in 20% methanol) for 10 min. Electroblotting was carried out for 90 min at 200 mA using a Novex western transfer apparatus. The blotted nitrocellulose membrane was then treated with a 0.5% tris-bovine serum albumin solution for 1 h to block non-specific protein binding. The patient's serum and control sera were diluted to 1 : 2 v/v with a PBS-T buffer (tris 10 mM, NaCl 150 mM, 0.05% Tween-20).

The membrane was then incubated with the patient's and

control sera for 2 h at room temperature. It was then washed with 0.1% triton in TBS-T for 10 min, and followed by washes with 0.5% triton in TBS-T for 10 min, and 0.5% NaCl in TBS-T for an another 10 min. Biotin-conjugated anti-human IgE antibody (Vetor, USA) was diluted to 1 : 200 with PBS-T and used as a second antibody solution. The membrane was incubated with the second antibody for 1 h at room temperature and washed again as above. A substrate solution (NBT/BCIP kit, Sigma Co., USA) was applied to the membrane until positive bands appeared. After the reaction, the membrane was washed with distilled water and observed.

RESULTS

Bronchoprovocation test with peptidase and lysozyme

As shown in Fig. 1, when bronchial challenge test was performed with diluent, there was no significant changes of FEV1. A dual asthmatic reaction was noted after inhalation of 1 : 10 w/v peptidase extracts. Figure 2 shows the early asthmatic reaction to the bronchoprovocation test using 1 : 100 w/v lysozyme extracts.

Detection of specific IgE antibodies to peptidase and lysozyme extracts

Fig. 3 shows the specific IgE binding to peptidase (Fig. 3a) and lysozyme (Fig. 3b) extracts in the patient's serum and control sera. In comparison to the control highly specific IgE binding to peptidase and lysozyme extracts was noted in the patient's serum. Fig. 4 demonstrated the inhibition of peptidase and lysozyme ELISA results with addition of these two extracts (Fig. 4a: peptidase ELISA inhibition test, Fig; 4b: lysozyme ELISA inhibition test). Significant inhibition in peptidase ELISA inhibition tests was noted with serial addition of peptidase extracts, but not lysozyme extracts. Similarly, significant inhibition in lysozyme ELISA inhibition tests was noted with serial addition of lysozyme extracts, but not with peptidase extracts.

Gel electrophoresis

In order to determine the protein components of peptidase and lysozyme extracts, the extracts were analyzed by a 4~20% gradient SDS-PAGE. Fig. 5 shows that the peptidase extracts resolved into nine bands ranging from 6 to 64.1 kDa and the lysozyme extracts into two bands (10.9, 15.1 kDa).

Immunoblot analysis

Fig. 5 shows the binding of specific IgE in patient's and control sera on a blotted nitrocellulose membrane. With peptidase extracts, there were more than ten bands bound to IgE antibody with molecular weights ranging from 7.3 to 83.1 kDa. With lysozyme extracts, two IgE binding components with molecular weight of 10.9 and 67.0 kDa, respectively, were noted.

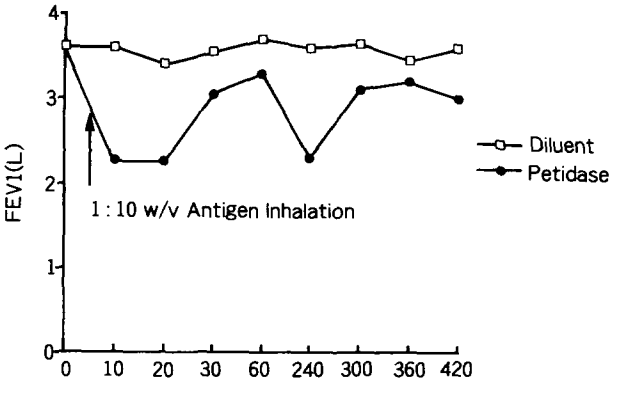

Fig. 1. The result of bronchoprovocation test with *Serratial* peptidase extracts.

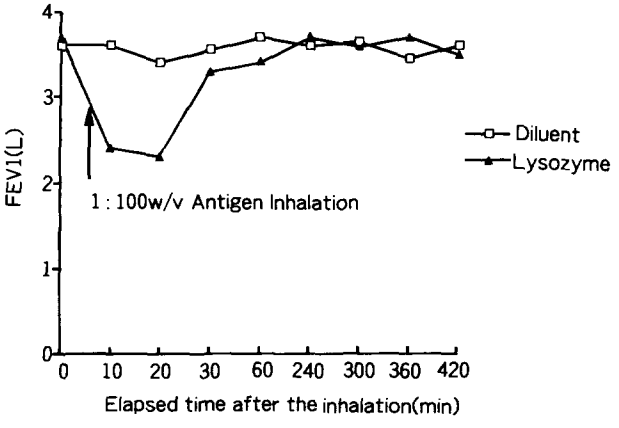

Fig. 2. The result of bronchoprovocation test with lysozyme chloride extracts.

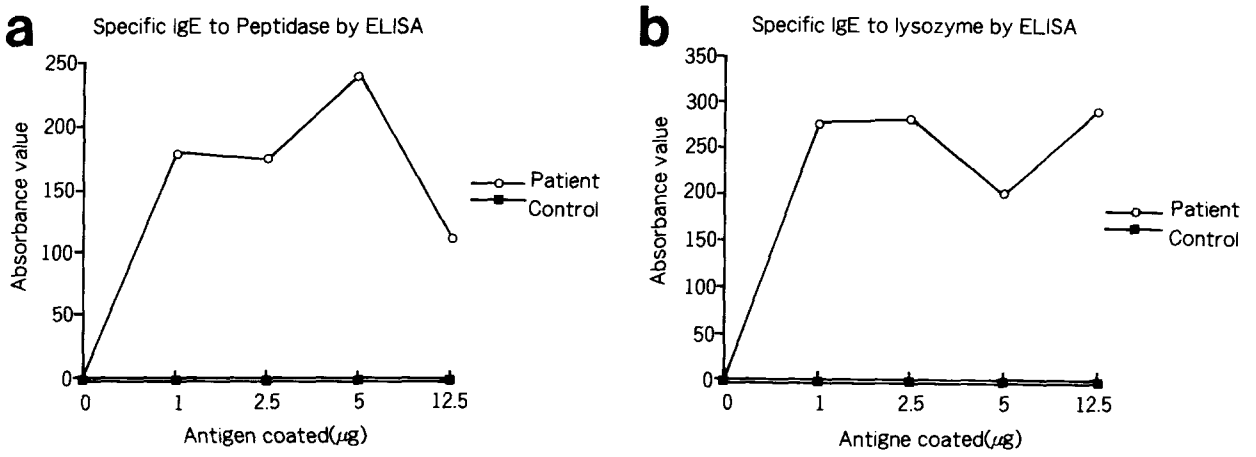

Fig. 3. Specific IgE binding to peptidase (a) and lysozyme (b) extracts in sera of the patient and control according to antigen concentrations.

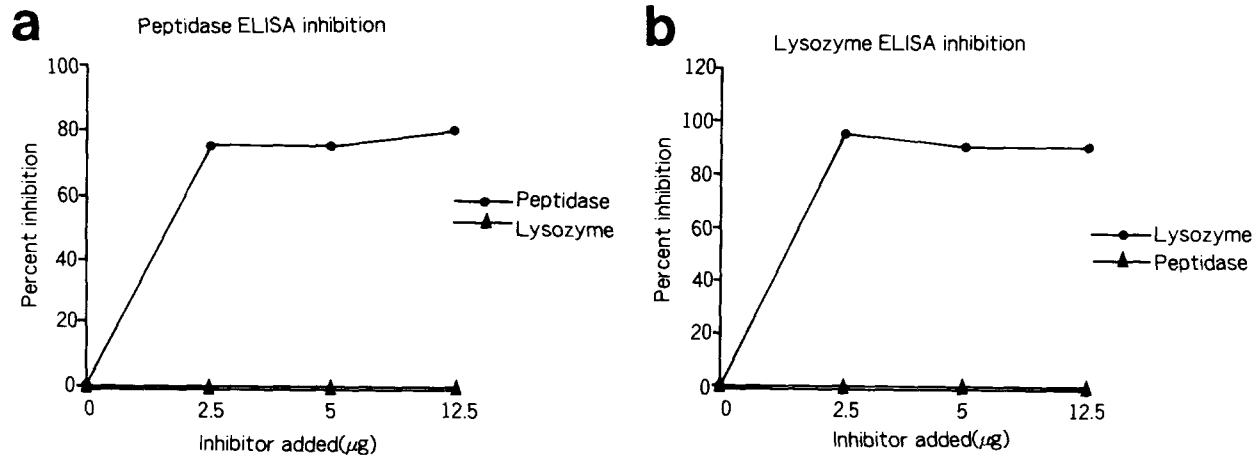

Fig. 4. ELISA inhibition test with addition of peptidase and lysozyme extracts: (a) peptidase ELISA inhibition test; (b) lysozyme ELISA inhibition test

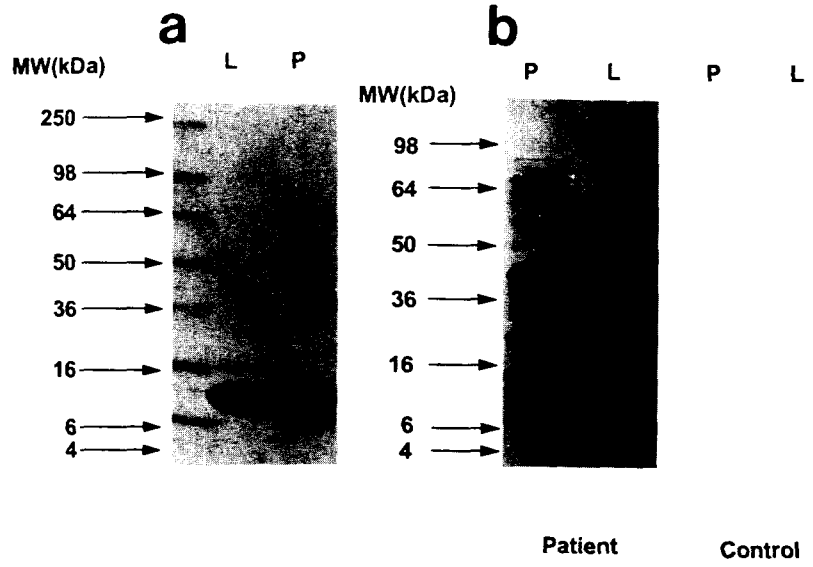

Fig. 5. Gradient SDS-PAGE (4-20%) and protein staining of lysozyme (L) and peptidase (P) extracts (a). Immunoblotting of lysozyme (L) peptidase (P) extracts using patient and control sera (b).

DISCUSSION

There have been a few reports that biologic enzymes can cause occupational asthma in exposed workers working in a pharmaceutical industry[1~7]. In this study, the patient had clinical presentation of occupational asthma and rhinitis due to high-molecular-weight agents, specifically lysozyme and peptidase, as confirmed by brochoprovocation tests.

The pathogenic mechanism of occupational asthma caused by peptidase and lysozyme may be an IgE-mediated reaction, since skin-prick tests with these two extracts showed an immediate and late positive response. Furthermore, IgE antibodies specific to each substance were detected by ELISA; the result of ELISA inhibition suggested specificity of IgE binding to these two antigens. No cross-reactivity between two antigens was suggested. The immunoblotting study also showed more than 10 IgE binding components within peptidase, and two in lysozyme. When the bronchial challenge tests with these two extracts were performed in two house dust mite-sensitive asthmatic patients, they showed no response, indicating that the brochoconstrictive response in this study's patient was a specific one. These results suggested that peptidase and lysozyme can induce IgE-mediated bronchoconstriction in an exposed patient.

Several reports that argue against important role of atopy in the development of IgE sensitization to occupational allergens with high molecular weight[10~12]. This lysozyme and peptidase antigen must be of macromolecular size, because compounds with a molecular weight of less than 6000 were excluded by the dialysis membrane which we used. The patient was non-atopic, showing all negative responses to common inhalant allergens, and was a non-smoker. In conclusion, this study demonstrates that two commonly used drug powders, lysozyme and peptidase, can induce occupational asthma in an exposed employee working in the pharmaceutical industry, and evidence is presented to indicate that an IgE mediated immunological mechanism may be involved.

REFERENCES

1. Colten HR, Polakoff PL, Weinstein SF and Strieder DJ. Immediate hypersensitivity to hog trypsin resulting from industrial exposure. N Engl J Med 292: 1050-3, 1975
2. Baur X, Konig G, Bencze K and Fruhmann G. Clinical symptoms and results of skin test in thirty-three papain workers: evidence of strong immunogenic potency and clinically relevant "proteolytic effects of airborne papain". Clin Allergy 12: 9-17, 1982
3. Carier A, Malo J-L, Pineau L and Dolovich J. Occupational asthma due to pepsin. J Allergy Clin Immunol 73: 574-7, 1984
4. Wiessmann KJ and Baur X: Occupational lung disease following long-term inhalation of pancreatic extracts. Eur J Respir Dis 66: 13-20, 1986
5. Pauwels R, Devos M, Callens L and Van der Straeten M: Respiratory hazards from proteolytic enzymes. Lancet i: 669, 1978
6. Galleguillos F and Rodriguez JC: Asthma caused by bromelin inhalation. Clin Allergy 8: 21-4, 1978
7. Bernstein JA, Bernstein DI, Warrington R, Bolin T and Warren CPW: Occupational asthma induced by inhaled egg lysozyme. Chest 103: 532-5, 1993
8. Chai H, Farr RS, Froelich LA, Mathison DA, Rosenthal RR, Shelter AL, Spector SL and Townley RLT: Standardization of bronchial challenge procedure. J Allergy Clin Immunol 56: 323-7, 1975
9. Park HS, Kim MJ and Moon HB: Occupational asthma caused by two herb materials, Dioscorea batatas and Pinellia ternata. Clin Exp Allergy 24: 575-81, 1994
10. Newhouse ML, Tagg B, Popck SL and McEwan AC: An epidemiologic study of workers producing enzyme washing powders. Lancet i: 689-93, 1970
11. Davies GE and McArdle LA: Allergy to laboratory animals: a survey by questionnaire. Int Arch Allergy Appl Immunol 64: 302-7, 1981
12. Block G, Tse KS, Kijek K, Chan H and Chan-Yeung M: Baker's asthma: clinical and immunological studies. Clin Allergy 13: 359-70, 1983