

Allergic reactions, including asthma, to the pineapple protease bromelain following occupational exposure

X. BAUR and G. FRUHMANN

Pulmonary Section, Medical Clinic I, Klinikum Großhadern,
Ludwig-Maximilians-Universität, Munich, Germany

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Summary

A 58-year-old pharmaceutical worker regularly developed asthma and rhinitis when handling bromelain, a purified protease of pineapple (*Ananas comosus*), at her workplace, where she had been employed for about 10 years. RAST and prick test showed strong positive reactions to bromelain. Both inhalation test with 0.03 mg bromelain and peroral challenge by ingestion of 190 g pineapple resulted in asthmatic reactions; the latter challenge was accompanied by gastrointestinal symptoms. Five of six workers sensitized to papain, showed positive RAST and skin test results to bromelain, two of them also showed immediate asthmatic reactions after bronchial challenge with bromelain. Out of sixty asthmatics not exposed to airborne proteases but probably to these as constituents of foods, two had positive skin test results and eight had positive RAST results to bromelain; but in no case was there clear evidence for clinical sensitization.

The presented data prove conclusively that bromelain is capable of inducing IgE mediated respiratory and gastrointestinal allergic reactions. Furthermore, there is evidence for immunological cross-reaction between the two plant proteases bromelain and papain in human subjects.

Introduction

As previously published, we observed seven patients who developed respiratory hypersensitivity to airborne papain (Baur & Fruhmenn, 1979); in one of these patients, the case history supported the view that rhinitis and asthma were also induced by occupational exposure to airborne bromelain, which is a mixture of several closely related proteolytic enzymes derived from the stem and fruit of pineapple (Silverstein & Kezdy, 1975; Coorman *et al.*, 1976; Murachi, 1976).

To our knowledge only two cases of asthma caused by bromelain have been reported in the literature (Galleguillos & Rodriguez, 1978); in these cases, non-allergic

Correspondence: Dr X. Baur, Pulmonologische Abteilung, Klinikum Großhadern, D 8000 München 70, Germany.

effects cannot be completely ruled out, as the described skin and bronchial reactions might have been induced through the irritative character of the active enzyme.

The object of our study was to investigate the pathophysiological mechanism of the symptoms by different test methods including determination of specific antibodies.

Patients and methods

In addition to the one worker sensitized to bromelain and papain (F.M.) we investigated six other patients sensitized to papain as well as an asthmatic control group not exposed to airborne proteases but probably to bromelain and papain contained in different drugs and foods (e.g. in more than sixty patented medicines, in pineapple, pineapple juice, carica papaya fruit, and in mixed drinks like 'Coca Cola' made from extracts of the carica papaya fruit amongst other things).

Three commercially available bromelain preparations—one of which patient F.M. was exposed to at work—were used in this study: E, 3 FIP units* per mg, obtained from Extrakt-Chemie, Stadthagen, West Germany; M, 5 FIP units*/mg, purchased from Merck, Darmstadt, West Germany; R, 2,910 Rorer units/mg, obtained from Müller-Rorer, Bielefeld, West Germany. The latter was further purified by Sephadex G-75 gel filtration (kindly performed by Dr J. Seifert, Institute for Surgical Research, Department of Surgery, University of Munich).

Skin tests

Prick tests were performed as previously described (Baur & Fruhmann, 1979); besides eighteen common allergens, we tested the three bromelains in increasing concentrations up to 10 mg/ml.

Radioallergosorbent test (RAST)

The three bromelains were coupled to cyanogen bromide-activated paper discs using concentrations of 0.01 mg/ml. Further steps same as in the Phadebas RAST method (Pharmacia, Uppsala, Sweden). The results were expressed in Phadebas RAST units/ml serum (PRU/ml) using a reference system with a serially diluted birch positive serum and birch discs.

RAST values ≤ 0.35 PRU/ml are regarded as negative; values between 0.35 and 0.7 PRU/ml are regarded as doubtful.

In preliminary studies, it was shown that preincubation of the coupled discs with the protease inhibitors tosyl-L-phenylalanin or phenylmethylsulfonyl fluoride did not lead to a noticeable change in the RAST results.

Inhalation tests

Airway resistance (R_{aw}) and thoracic gas volume (TGV) were determined with a body plethysmograph before and after inhalation of diluent (0.9% saline with 0.4% phenol) as well as at intervals of 10 to 60 min up to the fifth hour after inhalation of bromelain solutions, which were given in increasing concentrations with a nebulizer. A challenge test is regarded as positive if SG_{aw} after challenge is reduced by more than 50%, reaching values below 0.8 l/sec/kPa/l.

* Fédération Internationale Pharmaceutique, Gent, Belgium

Peroral challenge tests

The patients were given 190 to 250 g pineapple to eat. Measurements of R_{aw} and TGV were done before and at regular hourly intervals after ingestion.

Case report and immunological studies in a patient with hypersensitivity to bromelain

A 58-year-old female worker in a pharmaceutical plant had been handling bromelain and papain at irregular intervals for about 10 years. For some years she had noticed nasal secretion, sneezing, shortness of breath and wheezing when exposed to one of these substances. As these symptoms got worse, she consulted our pulmonary outpatient department. Physical examinations showed a hyper-resonant percussion note, faint breath sounds and some expiratory rhonchi.

The blood eosinophil count was normal. α_1 -Antitrypsin was 424 mg/100 ml (normal range 200–400); total IgE was above the laboratory normal range at 535 u/ml (normal 25–150). Lung function tests and a chest radiograph showed signs of emphysema. Hypersensitivity to papain was confirmed by skin test, RAST and inhalation test as previously described (Baur & Fruhmman, 1979).

Skin test and RAST showed positive immediate reactions to birch pollen, alder pollen and to all three bromelain preparations. The results with bromelain M are shown in Table 1.

In studies by Ouchterlony tests, diffusion of the patient's serum against the bromelain solutions did not cause precipitin lines.

The inhalation test with a very low amount of bromelain E (0.03 mg) resulted in an immediate asthmatic reaction; specific airway conductance (SG_{aw}) only gradually returned to initial values in the course of 5 hr (Fig. 1).

Peroral challenge by eating pineapple resulted, half an hour later, in abdominal distension and nausea. After 40 min, the patient complained of shortness of breath; on auscultation, expiratory rhonchi were pronounced. SG_{aw} was significantly reduced between the first and third hour (Fig. 2). The CO gas transfer, which was measured at hourly intervals after challenge, did not show a significant change. From the second to the fourth hour there was abdominal pain and diarrhoea. Blood pressure, pulse rate and temperature were stable all the time. The white blood cell count rose slightly from 6.4 to $8.4 \times 10^3/\text{mm}^3$ in the sixth hour.

In studies with RAST inhibition, freshly prepared pineapple juice was investigated; as shown in Fig. 3, there was a strong, dose-dependent inhibitory effect on the antibody reaction to bromelain on the discs, suggesting that pineapple contains large amounts of bromelain, which competes with the protease on the discs for the same IgE antibodies. The pineapple juice did not influence the antibody reactions on discs with papain, birch pollen and cat epithelium to a comparable degree.

Studies on immunological reactions to bromelain in patients sensitized to papain and in an asthmatic control group

Positive RAST results to bromelain (≥ 0.7 PRU/ml) were found in five of six workers with Type I hypersensitive reactions to papain (for details see: Baur & Fruhmman, 1979) as well as in eight of sixty asthmatics not exposed to airborne proteases (Table 1). It is of interest that all but three of these positive patients had IgE antibodies to grass pollen too.

Table 1. Results of RAST, skin test, inhalation test with bromelain and of peroral challenge test using pineapple in patients with or without previous exposure to airborne proteases.
All the patients were suffering from bronchial asthma except patient P.J. who had rhinitis only

Patients	Airborne proteases patients were exposed and clinically sensitized to	Tests with bromelain		Inhalation test		Peroral challenge by pineapple (190-250 g)	
		RAST (PRU/ml)	Skin test*	mg bromelain inhaled	Result (Reduction in SG_{aw})	Result (Reduction in SG_{aw})	Further symptoms
F.M.	Bromelain and Papain	76.7	++	0.03	positive	positive	Dyspnoe, nausea, distension, abdominal pain, diarrhoea
M.R.	Papain	25.5	+				
S.H.	Papain	6.6	++	0.50	positive	negative	0
L.B.	Papain	4.5	++	0.50	positive		
N.K.	Papain	2.4	(+)				
G.A.	Papain	1.5	+				
P.J.	Papain	0	0				
K.L.	—	12.1	+				
H.L.	—	25.0	++	0.50	negative	negative	0
R.C.	—	10.6	(+)				
U.K.	—	5.8	0	0.50	negative	negative	0
M.J.	—	4.8	0				
D.E.	—	2.5	0				
S.J.	—	2.1	0				
M.U.	—	1.6	0				
A.J.	—	0.5	0	0.50	negative		
Fifty-one further asthmatics	—	<0.7	0				

* Skin tests were performed using concentrations of 1 mg/ml bromelain M (patients F.M., M.R. and S.H.) and 10 mg/ml bromelain M (all the other patients). Paper discs coupled with bromelain M were used for RAST assay.

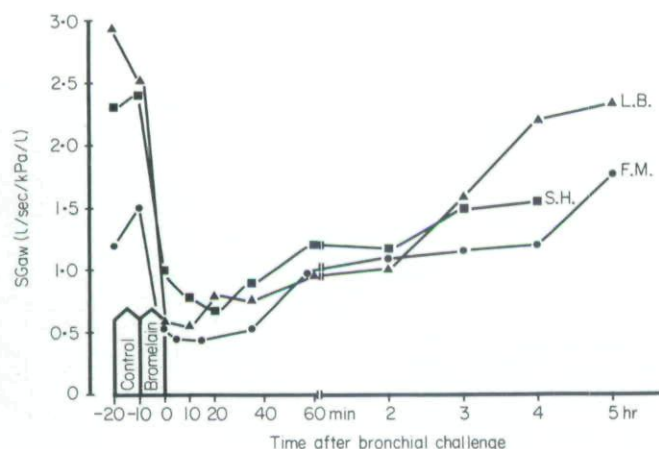


Fig. 1. Inhalation tests with bromelain in patients occupationally exposed to papain (L.B. and S.H.) and to both papain and bromelain (F.M.) Patient F.M. inhaled 0.03 mg bromelain E (concentration 0.1 mg/ml); the other two patients inhaled 0.5 mg bromelain M (concentration 0.5 mg/ml). 1 ml diluent (0.9% saline with 0.4% phenol) was used as control.

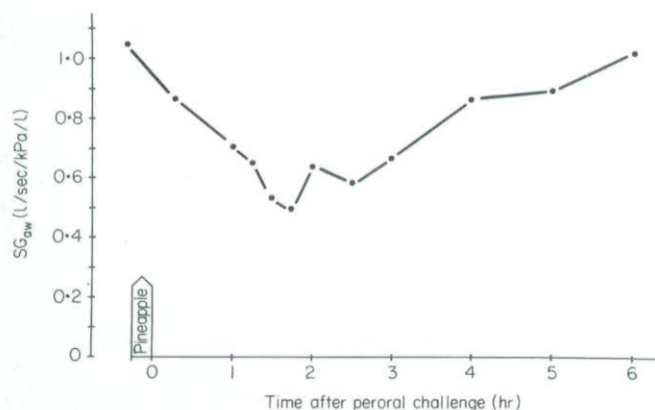


Fig. 2. Peroral challenge by ingestion of pineapple (190 g) in patient F.M. The bronchial reaction was accompanied by distension, nausea, abdominal pain and diarrhoea.

The skin test with bromelain seemed to be more specific: only two of the non-exposed patients but four of the papain group showed a positive result.

Inhalation tests with bromelain performed in two patients with hypersensitivity to papain resulted in an immediate asthmatic reaction (Fig. 1); the amounts of bromelain necessary for positive results were similar to that of papain, which was investigated in previous studies.

On the other hand, inhalation tests with bromelain were negative in three patients not exposed to purified proteases but having in two cases positive RAST results (≥ 0.7 PRU/ml), in one case also positive skin test reactions to bromelain (Fig. 4). Peroral challenges by pineapple performed in two patients of the asthmatic control group and

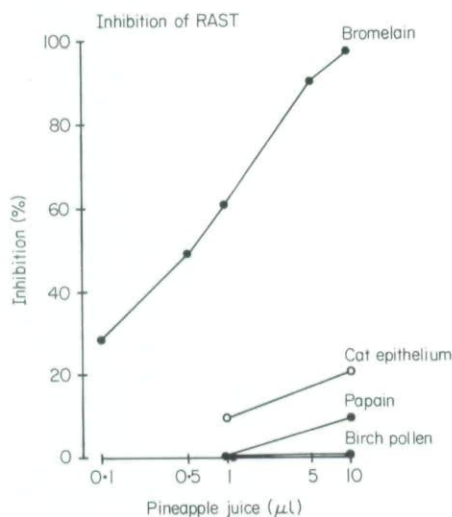


Fig. 3. Studies on cross-reactivity of pineapple juice with various allergens by RAST inhibition. The results are expressed as percentage inhibition of IgE binding on discs which were coupled with bromelain, papain or extracts of birch pollen or of cat epithelium. Patients: (●) F.M.; (○) P.K.

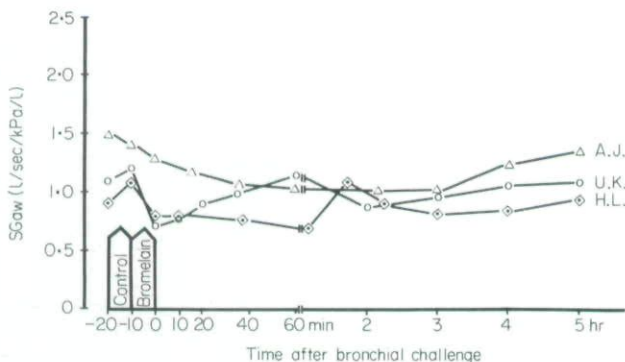


Fig. 4. Inhalation tests with bromelain in asthmatics not exposed to airborne proteases. Amounts of 0.5 mg bromelain M (concentration 0.5 mg/ml) were inhaled in each case. 1 ml diluent was used as control.

in one patient of the papain group showed negative results (Table 1). None of the subjects sensitized to papain and none of the control group had previously noticed abdominal or respiratory symptoms which could be definitely related to foods and drugs containing bromelain.

Discussion

Up to now the chemical structures and properties of most of the substances which are responsible for the antigenic character of common allergens have not been identified. To our knowledge, this is the first report of a defined protease, a constituent of everyday foodstuffs, which is capable of provoking hypersensitive reactions both by ingestion and by inhalation. According to the present clinical data and to further

studies with RAST and RAST inhibition (Baur, 1979), there is no doubt that the described respiratory and gastrointestinal symptoms in this patient are caused by specific IgE antibodies. Primary sensitization had obviously taken place via the respiratory tract. The bronchoconstriction following peroral challenge seems to be due to absorption either of the whole molecule bromelain or of immunologically active fragments of it. It is suggested that these substances may be transported to the bronchial system via the blood stream. There they may induce degranulation of the passively sensitized mast cells. As reported by Miller & Ophers (1964), Smyth, Brennan & Martin (1964), Uscavage, Gilman & Martin (1966), Izaka *et al.* (1972) and Seifert *et al.* (1979) there is evidence that in man and animal parts of perorally administered bromelain are absorbed without being digested.

The other way bronchoconstriction after peroral challenge might occur is an antigen-antibody reaction on the gastrointestinal mast cells or on the circulating white cells; the then released mediators might reach the lung and there induce bronchial constriction. But according to this mechanism signs of a general anaphylactic reaction would be expected. The absence of such symptoms speaks against the latter theory.

As shown by RAST, skin test and in two cases also by inhalation test, exposure to purified papain, a protease with structural homology to bromelain (Drenth *et al.*, 1971; Sasaki, Kato & Iida, 1973; Goto, Murachi & Takahashi, 1976), predisposes to hypersensitive reactions to bromelain too. This may be due to similar or identical antigenic determinants.

In the course of a screening investigation of asthmatics not exposed to airborne proteases, we could find no evidence for a clinically relevant sensitization to bromelain, despite the relatively wide consumption of foods, drinks and drugs containing this enzyme. Therefore it is suggested that weak to moderate positive RAST results to bromelain as seen in some of our patients may be due to a latent sensitization or due to immunological cross-reactions with common allergens. Further studies to examine this problem are proposed elsewhere (Baur, 1979).

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