

Papain-induced asthma: diagnosis by skin test, RAST and bronchial provocation test

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Summary

Seven out of eleven workers occupationally exposed to airborne papain developed immediate hypersensitive reactions, predominantly asthma and rhinitis. Skin tests and RAST with papain were positive in all symptomatic workers, but not in the four asymptomatic workers. Furthermore, out of forty non-exposed asthmatics, thirty-eight had negative RAST results and all had negative skin test results. Bronchial provocation tests with 0.15–0.5 mg papain performed in five patients with a positive case history showed in each case an immediate asthmatic reaction; in addition to that, one patient developed signs of a dual asthmatic reaction.

Our results suggest that airborne papain is a highly immunogenic agent in humans, which induces type I allergic reactions in a large percentage of the exposed subjects.

Introduction

Papain is a sulphhydryl protease, extracted from the latex of the fruit of *Carica papaya* (mol. wt. 23,000). Because of its broad substrate specificity it is widely used, e.g. in medicine as a digestive, or in the treatment of food (meat, beer), or in research/industrial biochemistry. Its use has become important to the structural analysis of immunoglobulins. As reported by Arnon & Shapira (1967), papain is highly immunogenic in rabbits. Until today only a few cases of allergic reactions in man have been reported (Osgood, 1945; Beecher, 1951; Milne & Brand, 1975; Marchioli *et al.*, 1977; Tarlo *et al.*, 1978).

Materials and Methods

Study population

We investigated eleven workers, who had been exposed to airborne papain for a period of 2 months to 10 years. Nine of them worked in a big industrial kitchen where

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a meat tenderizer containing papain was used, usually once or twice per week for a few hours at a time. The two others worked in different pharmaceutical plants, in which digestives containing papain were produced at irregular intervals.

The two purified papain preparations which were used in the industrial kitchen and in the pharmaceutical plants (M, Merck, Darmstadt; E, Extrakt Chemie, Stadthagen; both West Germany), as well as a further purified extract (S, Sigma Chemical Co., St Louis, U.S.A.) not used in the above-mentioned institutions were dissolved to get the following concentrations: 0.1, 1.0 and 10 mg/ml. Diluent was 0.9% saline with 0.4% phenol. Designed potencies as stated by the manufacturers: M=1200 Elg; E=60000 Elg; S=500Ulg.

Skin test

Skin tests were performed with a modified prick method: the allergens were introduced into the epidermis by the tip of a disposable needle (size 19), with a gentle lifting motion (Pepys *et al.*, 1973). We tested eighteen common inhalent and food allergens and the diluent, as well as histamine (1.0 mg/ml) and the papain solutions in increasing concentrations. Weal and flare reactions were measured after 20 min; the weal sizes of the allergen reactions were related to the histamine reactivity and scored from 0 to 4+ (Aas & Belin, 1972).

All three papain preparations gave comparable skin reactions. See Table 1 for the findings of the tests using papain M.

Radioallergosorbent test (RAST)

0.25, 0.5 and 1.9 mg of papain M were coupled to 500 mg cyanogen bromide-activated paper discs (Munktell 00) according to Wide's method (1969), as modified by Ceska & Lunkvist (1972). The same was done with papain E and S. Incubation time was 4 hr at room temperature. 50 μ l serum, diluted and undiluted, were added to a conjugated disc. After overnight incubation the IgE antibody activity was measured with a commercially available ^{125} I-labelled rabbit anti-human IgE (Pharmacia, Uppsala, Sweden). The radioactive count was taken with a gamma counter over a 2 min period. RAST results were scored from 0 to 4+ on the basis of the results obtained with a commercially available reference system, using serially diluted birch-positive serum and birch discs (Pharmacia, Uppsala, Sweden). All three papain preparations produced nearly identical reactions; see Table 1 for the results of the tests using papain M.

Reproducibility of bindings to the discs was $\pm 8\%$. No significant variation of RAST results was seen when the papain was site-blocked by phenylmethylsulphonyl fluoride or tosyl-L-lysine chloromethyl ketone as described by Whitaker & Perez-Villasenor (1968).

Bronchial provocation test (BPT)

Airway resistance and thoracic gas volume were determined with a body plethysmograph (Jaeger, Würzburg, West Germany) according to the method of Ulmer & Reif (1965) and specific conductance (SG_{aw}) was calculated. Aerosols of diluent and test solutions were given with a nebulizer (model H/V, Heyer, Bad Ems, West Germany) in increasing concentrations and amounts at 10 min intervals.

Results

Clinical manifestation

Six workers reported nasal pruritis, rhinitis, non-productive cough, chest tightness and

Table 1. Papain-exposed symptomatic workers: IgE levels, duration of papain exposure, clinical manifestations and results of skin tests, RAST and bronchial provocation tests

Patient	Total IgE* (u/ml)	Papain exposure (years)	Symptoms induced by airborne papain†	Results of papain tests			Other allergens to which there was a positive case history, ¹ skin test, ² RAST ³ and/or BPT ⁴
				Prick test (0.1 mg/ml)	RAST (0.25 mg/500 mg)	BPT (mg papain necessary for positive results)	
L.B.	169	4.7	A	++++	++++	0.50	—
N.K.	127	4.7	A, M, C	++	+++	0.30	Spice ^{1,2}
S.H.	396	4.7	A, R	++++	++++	0.40	Flour, ^{2,3} milk, ^{2,3} grass pollen ^{2,3}
P.J.	168	4.7	R	+	+	n.d.	House dust mite ^{2,3}
M.R.	180	4.1	A, R	+++	++++	0.15	Flour ^{1,2,3,4}
G.A.‡	117	0.5	A, R	+++	++	n.d.	Flour, ^{1,2,3} house dust, ² grass pollen, ² house dust mite ²
F.M.	530	10.0	A, R	+++	+++	0.40	Penicillin V, ^{1,2,4} tree pollen, ^{2,3} bromelain ^{1,2,3,4,§}

n.d. = Not done.

* Determined with a Phadebas IgE kit (Pharmacia, Uppsala, Sweden). The normal range is 4 to 150 u/ml, mean = 25 u/ml.

† A = Asthma; R = rhinitis; C = conjunctivitis; M = migraine.

‡ Results for patient G.A. were obtained 5 years after papain exposure; all the other patients were exposed until point of investigation.

§ Bromelain allergy was confirmed by skin test, RAST and BPT in this patient, who has also been exposed to bromelain dust at her place of work; studies under way in our laboratory with RAST inhibition support the view that there is an immunological cross-reaction between bromelain and papain.

wheezing when exposed to airborne papain. In addition to that, one worker complained of conjunctivitis and migraine; a young female worker had rhinitis only. Dyspnea—appearing several hours after heavy papain exposure—was reported by one person (G.A.).

Skin test

Positive skin test reactions to a low concentration of papain (0.1 mg/ml) were obtained in all seven symptomatic workers, in most of them to a severe degree. Four asymptomatic co-workers and forty non-exposed asthmatics showed negative skin reactions to 1.0 mg/ml papain solution. In six of the symptomatic workers positive skin test reactions to other allergens were observed (Table 1).

RAST

The sera of six patients with a history of asthma, when exposed to airborne papain, produced strong positive RAST results. The serum of patient P.J., who complained

only of rhinitis, and that of two unexposed asthmatics with multiple allergies and high IgE levels gave weak positive RAST results. The sera of thirty-eight unexposed asthmatics showed negative results, even when paper discs coupled with larger amounts of papain (1.9 mg/500 mg) were used. The serial dilutions of the sera of symptomatic patients, when inserted into the RAST procedure, produced dose-response curves which were parallel among themselves as well as in relation to those of the reference serum (Fig. 1).

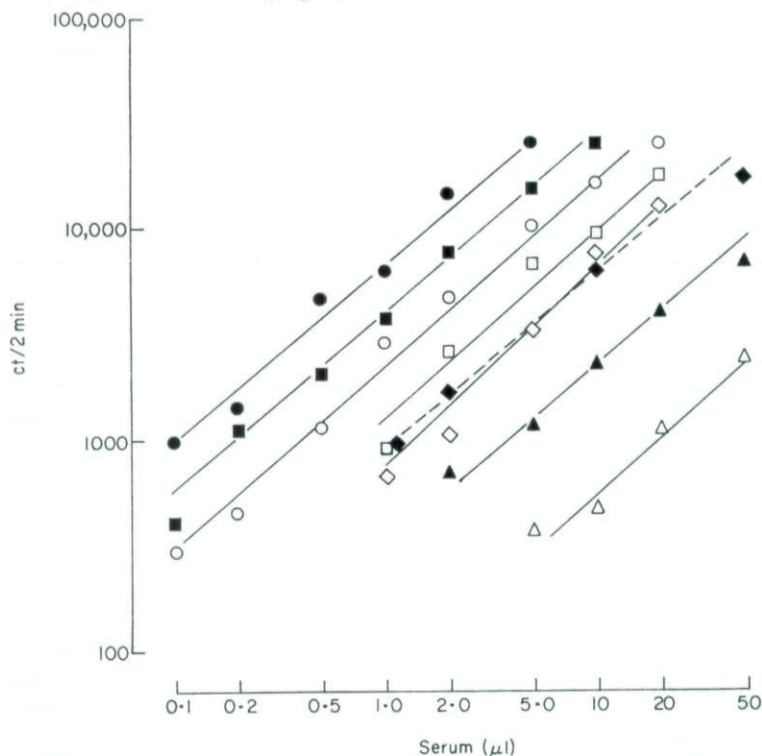


Fig. 1. Papain RAST: dose-response curves of seven positive sera. The results are expressed as radioactive counts over a 2 min period after subtraction of the mean count of five control sera (taken from unexposed asthmatics with light to moderate elevated total IgE). Discs coupled with 1.9 mg papain M per 500 mg were used for this study. Solid lines are sera of symptomatic workers; dashed line is the reference serum measured with reference discs (birch pollen). Patients: (●) S.H.; (■) M.R.; (○) L.B.; (□) N.K.; (◇) G.A.; (▲) F.E.; (△) P.J.; (◆) reference serum. All the correlation coefficients were +0.98 or greater.

Bronchial provocation test

Five patients underwent BPT. In all five cases, inhalation of diluent caused no significant variation of SG_{aw} , whereas inhalation of papain (0.15 to 0.5 mg) in a concentration of 0.1 and 1.0 mg/ml, respectively, resulted in a fall of SG_{aw} of at least 50% (Fig. 2). None of the patients complained of a late asthmatic reaction after papain inhalation; however, in one of the patients a significant fall of SG_{aw} in the fourth hour was observed, which was completely reversed by inhalation of salbutamol.

Follow-up study

Patient M.R. and patient G.A. changed their occupations because of their complaints

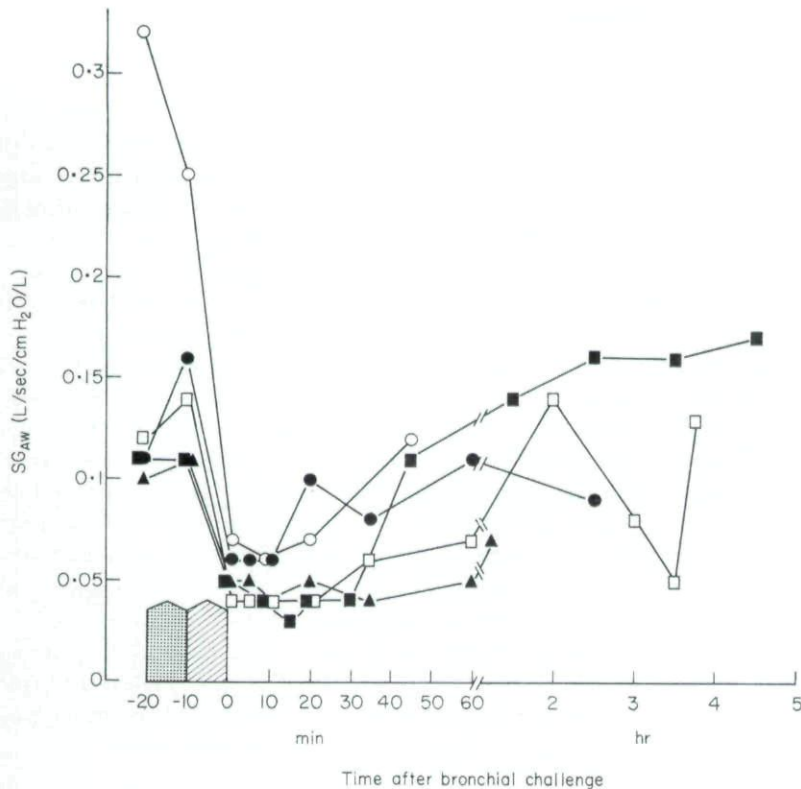


Fig. 2. Asthmatic reactions in five symptomatic workers to inhalation of papain (1.0 mg/ml). 1.0 ml of diluent (0.9% saline with 0.4% phenol) was used as control. Arrows show application of sulbutamol (0.2 mg). (□) Control; (▨) papain. Patients: (●) N.K.; (■) F.M.; (○) M.R.; (□) S.H.; (▲) L.B.

1 and 5 years ago respectively. Since then symptoms have not reappeared in patient M.R., whereas patient G.A. developed rhinitis when exposed to flour dust during her work as a housewife. Patient F.M. interrupted her work for 10 weeks and symptoms decreased remarkably. She showed only moderate symptoms after starting work again, provided she regularly inhaled cromolyn sodium and bronchodilators. The symptomatic workers in the industrial kitchen have complained only of milder symptoms after the introduction of a more highly granulated meat tenderizer producing less airborne papain.

Discussion

There are many reports and immunological studies on human allergies to *Bacillus subtilis* enzymes and to pancreatic extracts (Belin & Norman, 1977; Pepys *et al.*, 1973; Twarog, Weinstein & Colten, 1977; Zetterström, 1977). But to our knowledge only seven cases of papain-induced asthma are described in the literature. The diagnoses of most of them are based predominantly on case history and skin tests. Dolovich *et al.* (1977) were able to prove IgG and IgE antibodies to papain in two asthmatic workers. Also Marchioli *et al.* (1977) found IgG antibodies to papain but not those of the IgE type in the serum of an asthmatic patient working in the production of meat tenderizer. Furthermore, this was the only subject in which the causative role of papain was

confirmed by bronchial provocation test; the man developed an asthmatic reaction after inhalation of 1.0 mg papain. This is consistent with our findings obtained with the inhalation of 0.15 to 0.5 mg papain by five symptomatic workers. Furthermore, we were able to confirm positive skin tests and RAST results in all subjects with asthma and/or rhinitis when exposed to airborne papain. Only two out of forty sera of non-exposed asthmatics gave positive RAST results; this may be due to cross-reactions with other allergens. The latter problem is under study. We do not believe in non-specific reactions, for several sera with very high IgE levels gave negative results.

The high rate of sensitization among the exposed workers suggests that papain is highly immunogenic in man. Furthermore, many positive skin and RAST reactions to common allergens suggest that it may act as a trigger for further allergic reactions.

In rats, hamsters and guinea-pigs acute emphysema-like lesions following exposure to papain were reported by Gross *et al.* (1964, 1965), Palecek, Palecekova & Aviado (1967), Ackerman, Corkey & Perkins (1977) and Goldring, Greendburg & Ratner (1968). Also, in highly exposed human beings, 'loss of elastic recoil', that means signs of emphysema, have been described (Gandevia & Mitchell, 1971). In contrast to that, only two of our patients showed signs of emphysema, which in any case were no more extensive than in comparable non-exposed asthmatics. This is consistent with the findings of Milne & Brand (1975), who observed only minimal abnormality of ventilation distribution in two papain-exposed subjects.

These results suggest that long-term exposure to papain need not lead to destruction of the human lung if specific antibodies are present. But nevertheless airborne papain has to be regarded as a dangerous substance, both because of its proteolytic activity and because of the possibility of inducing hypersensitive reactions.

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