

Clinical symptoms and results of skin test, RAST and bronchial provocation test in thirty-three papain workers: Evidence for strong immunogenic potency and clinically relevant 'proteolytic effects of airborne papain'

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Summary

Seventeen out of thirty-three workers who have been exposed to airborne papain at their place of work regularly developed asthmatic symptoms such as shortness of breath, wheezing, coughing, sneezing, rhinorrhea and conjunctival irritation upon contact with this proteolytic enzyme. Investigations by RAST, skin test and bronchial provocation test proved IgE-mediated hypersensitivity to papain in fourteen symptomatic workers. Ten of these were in a screening investigation involving twenty-nine of the thirty-three workers; i.e. the incidence of IgE-mediated sensitization was 34.5% of this group. Bronchial provocation of as little as 0.001–0.5 mg of papain was shown to elicit immediate or dual asthmatic reactions in all eight tested workers with RAST values > 3 u/ml. On the other hand, inhalation of 0.5 mg of papain did not cause any remarkable change in non-exposed asthmatics. Occupation-related blood-stained nasal secretions and/or cutaneous flare reactions in all four heavily-exposed papain workers, of whom three had negative skin test and RAST results, suggest a direct effect of the proteolytically active enzyme on human tissue.

There was a significant elevation ($P < 0.001$) of serum trypsin inhibitory capacity in papain workers which seems to depend on the degree of exposure. No significant differences between symptomatic and asymptomatic workers in α_1 -antitrypsin (Pi) phenotype subtypes were found.

Introduction

In a previous paper (Bauer & Fruhmann, 1979), we reported on IgE-mediated hypersensitivity to papain in workers handling this proteolytic enzyme. Now we have performed a screening investigation of twenty-two additional subjects (making up a total of thirty-three) who have been exposed at differing time intervals to varying amounts of papain.

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The aim of this study was to determine if there exist predisposing factors, especially pre-existing atopic diseases or deficiency of serum trypsin inhibitory capacity, which may favour the development of respiratory symptoms in this group. The role of α_1 -anti-trypsin is of special interest, as Martorana & Share (1976) Martorana, Share & Richards (1977) reported that this proteinase inhibitor prevents pulmonary emphysematous lesions in papain-exposed hamsters. Furthermore, incidence of clinically relevant sensitization to papain should be evaluated and related to the degree of exposure.

Materials and methods

Patients

Thirty-three papain workers, aged 24 to 58 years (mean 38), were investigated. Twenty-nine workers took part in a screening investigation comprising the whole staff at three work places. Four individuals from other firms consulted us as a result of respiratory difficulties (patients Nos. 9, 15, 20, 25) and can be considered as selected subjects. The thirty-three papain workers were divided into groups: (a) heavy exposure—four cases employed in a spice mill, packing large amounts of papain; (b) moderate exposure—twenty-two cases who used a papain containing meat tenderizer in two industrial kitchens or were engaged in the production of digestives with papain; and (c) low exposure—seven cases who had indirect contact with papain in working near the individuals of group (b).

Only one out of the thirty-three workers had been suffering from atopic diseases before he came in contact with papain (patient No. 23 had suffered from allergic rhinitis).

Radioallergosorbent test (RAST)

Crude papain (Sigma Chemical Co., St Louis, lot 45C-0297) and in some experiments also twice crystallized papain (Sigma Chemical Co., St Louis, lot 38C-8145) were coupled to cyanogen bromide-activated paper discs (Munktell 00); concentrations were 0.1 mg/ml in 0.1 M NaHCO₃. Each disc was then incubated with 50 μ l serum for 3 hr, washed three times with 0.9 saline-0.1% Tween 20, then incubated over night with 50 μ l of a D₆₂ specific ¹²⁵I-labelled rabbit anti-human IgE from Phadebas RAST (Pharmacia Diagnostics AB, Uppsala). After washing again, radioactivity was measured with a gamma counter over a 2-min period. The results were expressed in RAST u/ml by comparing the counts with those of Phadebas RAST references (Pharmacia Diagnostics AB, Uppsala) which were run in parallel.

The control mean value ± 2 s.d. of sixty asthmatics, never having been exposed to airborne papain, was 0.15 $\pm$ 0.39 u/ml; RAST values > 0.54 u/ml are regarded as positive.

In preliminary experiments by RAST inhibition there was strong evidence for specificity of the RAST results. The addition of 1 μ g of fluid phase papain to papain discs incubated with positive sera of three clinically sensitized workers resulted in a loss of IgE binding of 85, 74 and 72% respectively; addition of 5 μ g of fluid phase papain produced inhibition rates of 94, 93, and 91% respectively. On the other hand, extracts of *Dermatophagoides pteronyssinus* and animal dander had no significant influence; inhibition rates ranged from -5 to $+6\%$ ($n=6$).

Skin test

Papain solutions (0.1 mg/ml), sixteen common allergens, histamine (1.0 mg/ml), and diluent were tested by a modified prick method (Baur & Fruhmenn, 1979).

Bronchial provocation test

Intrathoracic gas volume (IGV) and airway resistance (R_{aw}) expressed as its reciprocal specific airways conductance (SG_{aw}) were measured by a constant-volume variable-pressure plethysmograph (Jäger, Würzburg, West Germany) before challenge, immediately after inhalation of diluent and twice during, as well as immediately, and 10 min after inhalation of 0.001 mg/ml of papain solution. If inhalation of 1 ml of the atomized papain solution through a mouth piece (nebulizer: Heyer, model AV/3H) produced no significant reduction in SG_{aw} ($> 30\%$ from the baseline measurement reaching values below $0.08 \text{ l cm H}_2\text{O}^{-1} \text{ s}^{-1} \text{ l}^{-1}$), the papain concentration was elevated progressively to tenfold while measurements of SG_{aw} were repeated as mentioned above. If a significant fall of SG_{aw} occurred, inhalation was halted and measurement conducted at 20, 35, 60 min and thereafter hourly until at least the fifth hr after inhalation challenge.

Serum trypsin inhibitory capacity (STIC). STIC was measured functionally according to the method of Fritz, Trautschold and Werle (1970); 1 inhibitory unit (IU) is defined as the reduction of the trypsin-catalysed hydrolysis of the substrate N α -benzoyl-L-arginine-p-nitroanilide by $1 \mu\text{mol/min}$.

α_1 -antitrypsin (Pi) phenotypes. These, including the PiMM subtypes, were determined as described elsewhere (Bencze & Sabatke, 1980).

Results

Clinical symptoms (Table 1)

Occupational exposure to dusts of papain provoked clinical symptoms in all four patients of group (a), eleven of group (b), and two of group (c). The symptoms included itching of the nose, sneezing, and rhinorrhea (fifteen individuals); conjunctival irritation (five individuals); coughing, shortness of breath, and wheezing (fifteen individuals). In addition to these, all of the heavily-exposed subjects regularly developed a blood-stained nasal secretion lasting up to 24 hr after papain exposure. Three of this group also exhibited itching and flare reactions on uncovered skin areas. Duration of exposure to papain before symptoms were noted ranged from several days to eight years.

Test results

Skin test and RAST gave positive results in thirteen symptomatic subjects with low to moderate exposure, in one out of four with heavy exposure, and were negative in fifteen healthy workers (Table 1 and Fig. 1). Two subjects (Nos. 11 and 12), whose bronchitic and rhinitic symptoms could not conclusively be related to papain exposure, had a positive skin test, one of them also having a positive RAST result.

Bronchial provocation tests showed strong bronchial hyper-reactivity to papain in eight of nine symptomatic subjects with positive skin tests and RAST: five had immediate, and three dual or prolonged, asthmatic reactions (Table 2 and examples in Fig. 2).

One patient (No. 15) with weak positive RAST and skin test results exhibited a

Table 1. Clinical data and test results of thirty-three workers occupationally exposed to airborne papain

Patient No.	Papain exposure		Test results with papain		Sensitization to common allergens
	(yr)	Clinical symptoms induced by papain	Skin test	RAST (u/ml)	
(a) Patients with heavy exposure					
1	8.0	A, R, S	0	<0.54	+ (pollen)
2	8.0	R, C	0	<0.54	+ (pollen, flour)
3	4.0	A, R, C, S	1	0.85	
4	3.0	A, R, S	0	<0.54	
(b) Patients with moderate exposure					
5	20.0		0	<0.54	
6	20.0		0	<0.54	
7	15.0	A, R	2	3.00	++ (flour)
8	13.0	A, R	3	988.00	
9	10.0	A, R	3	4.80	++ (bromelain, penicillin)
10	10.0		0	<0.54	
11	8.0		2	0.60	
12	8.0		2	<0.54	
13	8.0	A, R, C	4	60.15	+ (pollen, house dust)
14	6.0	A, R	3	19.50	
15	5.5	A, R, C	2	0.69	++ (pollen, flour)
16	4.7	A, R	4	222.50	+ (pollen, flour)
17	4.7	A	4	60.00	
18	4.7	A, C	2	18.10	++ (spice)
19	4.5		0	<0.54	
20	4.1	A, R	3	183.00	++ (flour)
21	3.0		0	<0.54	
22	1.5		0	<0.54	
23	1.0		0	<0.54	++ (dander, house d. mite)
24	0.7		0	<0.54	
25	0.5	A, R	3	7.60	++ (flour)
26	0.2		0	<0.54	
(c) Patients with low exposure					
27	22.0		0	<0.54	
28	21.0		0	<0.54	
29	10.5		0	<0.54	
30	10.0		0	<0.54	
31	8.0	A, R	2	0.73	
32	4.7	R	1	0.80	+ (house d. mite)
33	4.0		0	<0.54	

A=asthma; R=rhinitis; C=conjunctivitis; S=irritation of the skin. RAST measurements were performed with crude papain; the control mean value + 2 s.d. of sixty non-exposed asthmatics was 0.15+0.39 RAST u/ml, RAST values ≤ 0.54 are regarded as negative. RAST values with twice crystallized papain were slightly higher: patient No. 8: 1090 u/ml, Patient No. 9: 4.9 u/ml, and patient No. 14: 21.0 u/ml. + Not clinically relevant; ++ Clinically relevant sensitization.

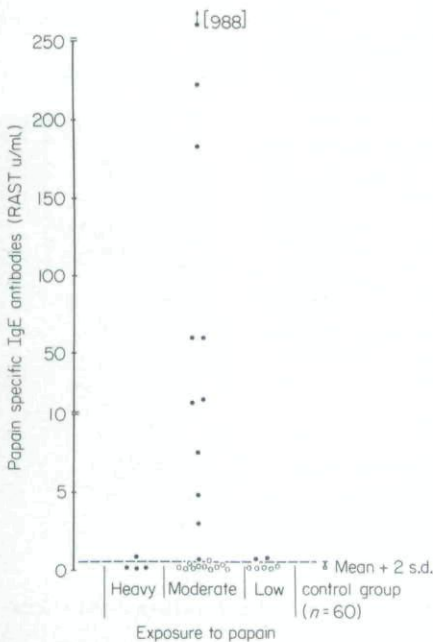


Fig. 1. Levels of specific IgE antibodies against papain in the sera of thirty-three workers occupationally exposed to airborne papain. ●, Symptomatic workers; ○, asymptomatic workers. Sixty asthmatics not exposed to airborne papain served as controls (geometric mean + 2 s.d. 0.15 + 0.39 RAST u/ml).

negative provocation test with 0.1 mg of papain. Bronchial challenges by 0.5 mg of papain were also negative in three non-exposed asthmatics (ΔSG_{aw} : -5, -4 and 8% respectively) who had shown bronchial hyper-reactivity in inhalation challenges by common allergens and/or 1% solution of acetylcholine.

The duration of papain exposure at the place of work was not correlated with the frequency of sensitization as evaluated by skin test, RAST, and case history.

Investigations by double immunodiffusion (Ouchterlony test), which were con-

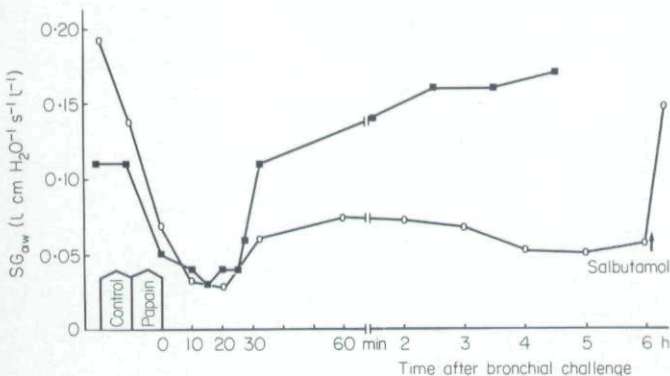


Fig. 2. Immediate and prolonged asthmatic reactions following inhalation challenges by 0.4 mg of papain, concentration 1 mg/ml (patient No. 9) and by 0.006 mg of papain, concentration 0.01 mg/ml (patient No. 14) respectively. ○, Patient No. 14; ●, patient No. 9.

Table 2. Results of bronchial provocation tests by papain

Patient No.	Amounts of papain inhaled ⁺⁺ (mg)	Immediate asthmatic reaction (% change of SG _{aw})	Late asthmatic reaction (% change of SG _{aw})
7	0.100	-55*	-46
8	0.001	-78*	-74*
9	0.400	-68*	+28
14	0.006	-88*	-85*
15	0.100**	-23	-10
16	0.400	-75*	-58*
17	0.500	-64*	
18	0.300	-63*	-18
20	0.150	-74*	

⁺⁺ Maximal doses given by a nebulizer via a mouth piece; * Regarded as positive result; ** This person did not accept to be exposed to higher doses.

ducted with the sera of twenty-eight patients, did not show precipitating antibodies against papain.

Total IgE was normal in all the asymptomatic individuals but was elevated in eleven out of the seventeen symptomatic subjects, predominantly in those with moderate exposure (Fig. 3). There was no direct correlation between total IgE levels and papain specific IgE values as determined by RAST.

STIC measured in twenty-two workers showed a significant elevation ($P < 0.001$) which seems to depend on the degree of papain exposure, although the differences between each exposed group were not statistically significant (Table 3). The mean STIC values of symptomatic and asymptomatic workers as well as of RAST positive and RAST negative workers were nearly identical.

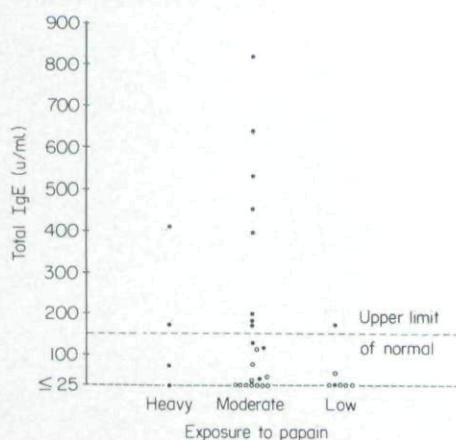


Fig. 3. Total IgE serum levels of thirty-three papain workers, measured by the PRIST assay (Pharmacia, Uppsala). Upper limit of normal: 150 u/ml (geometric mean + s.d.). ●, Symptomatic workers; ○, asymptomatic workers.

Table 3. Serum trypsin inhibitory capacity (STIC)* of twenty-two papain workers; arithmetic mean $\pm$ 1 s.e. mean: 1347 ± 51 ; *P* (healthy controls) < 0.001

	Occupational exposure to papain			Symptomatic workers	Asymptomatic workers	RAST positive workers	RAST negative workers	Healthy controls, not exposed to airborne papain (PiMM)
	Heavy	Moderate	Low					
Patient Nos.	4	12	6	10	12	8	14	257
Mean (arithmetic)	1465	1347	1268	1366	1332	1376	1331	1061
s.e. mean	63	62	135	67	77	88	64	13
<i>P</i> (healthy controls)	< 0.001	< 0.001	n.s.	< 0.001	< 0.001	< 0.001	< 0.001	

* STIC values in IU; Statistical analyses based on Student's *t*-test for the logarithmized STIC values exhibited no significant variations among the different groups of papain workers; nevertheless, there is a trend towards increased STIC values with higher exposure.

Determination of the Pi phenotype subtypes revealed a prevalence of the M_1M_3 subtype and a diminution of the M_1M_1 subtype in our study group: the distribution in the group was 39 and 24% respectively; 24 and 52% respectively, in healthy controls; 21 and 26% respectively, in patients with chronic obstructive pulmonary disease (Bencze, Sabatke & Fruhmann, 1980). There were no significant differences of Pi subtypes between symptomatic and asymptomatic, RAST positive and RAST negative workers, except that MS phenotypes were not present in the symptomatic or the RAST positive group.

Nine of the RAST positive and three of the RAST negative individuals had positive skin test and/or RAST reactions to common allergens. According to case history and bronchial provocation test these sensitizations were clinically relevant in seven cases and in six of these appropriate clinical symptoms became evident throughout the duration of papain exposure.

Discussion

Occupational exposure to airborne papain elicited respiratory, conjunctival, and/or cutaneous symptoms in more than half of the investigated workers. The proportion of symptomatic subjects was positively correlated with the degree of papain exposure: two of the seven workers with low exposure, eleven of the twenty-two with moderate exposure and all four with heavy exposure, were affected.

Significantly positive skin test and RAST results in all symptomatic subjects of groups (b) and (c) and in one subject of group (a), as well as asthmatic reactions following bronchial challenges by low amounts of papain in eight out of nine investigated workers with positive skin test and RAST results, but not in non-exposed asthmatics, are strong evidence for IgE mediated hypersensitivity. In the screening study, comprising twenty-nine individuals, the incidence of sensitization to papain was 34.5%. Only one of these patients had a pre-existing atopic history. So it is not likely that an atopic diathesis is a prerequisite for papain induced allergic reaction.

On the other hand, blood-stained nasal secretions, itching and flare reactions appearing on uncovered skin areas in heavily exposed subjects, of whom three had negative, and one weak positive, skin test and RAST results, suggest a direct irritative

effect and damage of human tissue by high concentrations of the active proteinase papain. This hypothesis is supported by the discovery of extensive pulmonary haemorrhage in animals after a single exposure to aerosolized papain (Martorana *et al.*, 1977) and by discovery of hemoptysis in one papain worker reported by Novey *et al.* (1980).

The low prevalence of type I allergic reactions in group (a) may be due to the selection of these patients since two subjects at this place of work had previously changed their jobs because of respiratory complaints.

Our results reveal that airborne papain has to be considered as a health hazard for workers both because of strong immunogenic potency and proteolytic activity.

The literature contains several reports on respiratory affections in workers exposed to papain which are suggestive of a type I allergic reaction (Beecher, 1951; Flindt, 1978; Keenan *et al.*, 1979; Milne & Brand (1975); Novey *et al.*, 1980; Osgood, 1945; Tarlo *et al.*, 1978). However, until now the existence of papain-specific IgE antibodies was documented in some cases only (Novey *et al.*, 1980; Tarlo *et al.*, 1978; Baur & Fruhmann, 1979). In contrast with the results of other investigators (Novey *et al.*, 1980; Tarlo *et al.*, 1978), we did not find precipitating antibodies to papain. Clinically and according to provocation tests there was no evidence of a type III hypersensitivity reaction in our study group. It is worth noting that active proteinases have been reported to produce false positive results in precipitin tests (Wicher & Dolovich, 1972).

As six subjects in our patient group developed clinically relevant hypersensitivity to common allergens during the time of papain exposure, it is thought that airborne papain may constitute a triggering effect to further sensitization. This is interesting as there is evidence for partial immunological cross-reactivity between papain and antigens of pollen and flour (Baur, 1979). In addition, it is noticeable that 33% of the papain workers had elevated total-IgE levels.

No heterozygous or homozygous individuals with respect to the α_1 -antitrypsin allele Z were observed in our study group. In none of the cases could a decrease of STIC values be detected. So, deficiency in trypsin inhibitory capacity does not seem to play a role in the pathogenesis of diseases induced by exposure to the proteinase papain. Significant elevation of STIC, as seen in our study group, is most probably a result of inflammatory reactions caused by the proteolytic activity of papain. As reported by several investigators, α_1 -antitrypsin is an acute phase reactant and rises in inflammatory conditions (Kueppers, 1968; Laurell & Jeppsson, 1975).

In vitro experiments showed that human and animal sera were able to inhibit the proteolytic activity of papain (Fisher *et al.*, 1976; Järvinen, 1976). According to the reports of Sasaki *et al.* (1977) and Ryley (1979) the antipapain activity of human serum should mainly be due to the presence of a recently discovered thiol proteinase inhibitor, and not to α_1 -antitrypsin or α_2 -macroglobulin, as it was previously thought (Barrett & Starkey, 1973; Martorana & Share, 1976; Martorana, 1977; Starkey & Barrett, 1977).

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