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OCCUPATIONAL RESPIRATORY ALLERGY TO α -AMYLASE IN BAKERS

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Introduction

Occupational respiratory allergy among workers in the bakery industry and associated activities is quite prevalent and may affect up to 10-20% of those profes-

nals¹⁻³. Although no epidemiological data have been published, we suspect that baker's asthma is one of the most frequent occupational respiratory diseases in Spain. Most of the bakers with occupational asthma are sensitized to wheat flour and to storage mites; however, over the last few years we have seen a growing importance of some flour additives, particularly α -amylase, as a cause of rhinitis and asthma. Fungal α -amylase (1,4- α -D-glucanase-glucanohydrolase), usually derived from *Aspergillus oryzae*, is a 52-kD glycoprotein that catalyses the hydrolysis of internal α -(1,4)-glycosidic bonds to simpler polysaccharides. It is commonly and routinely added to wheat flour, in a mg-to-kg-flour proportion, for accelerating the panification process and improving the quality of the resulting bread.

The first cases of occupational asthma due to α -amylase allergy were reported by Flindt⁴ in workers of the pharmaceutical industry; he based his diagnosis on the anamnesis and on skin tests. Some years later, Wiessman and Baur⁵ demonstrated that pancreatic α -amylase was responsible for the respiratory allergy symptoms in a group of workers exposed to pancreatic extracts through an IgE-mediated immediate hypersensitivity mechanism. This finding was later confirmed by Aiken *et al.*⁶. The first description of allergy to enzymes in bakers with occupational asthma was published by Baur *et al.*⁷, who demonstrated a 34% prevalence of α -amylase sensitization through *in vitro* and *in vivo* tests among symptomatic workers. In Spain, the first case of occupational asthma due to α -amylase and demonstrated by means of skin tests, specific IgE and bronchial challenge

tests was reported in 1991 in a baker⁸. One year later, Losada *et al.*⁹ demonstrated α -amylase sensitization through skin tests and specific IgE in 26 among 83 (31%) workers exposed to α -amylase powder in a pharmaceutical manufacturing plant. The bronchial challenge tests with α -amylase were positive in 6 of the 14 cases tested, and only an immediate response pattern was observed. In that same year, another Spanish group¹⁰ elegantly demonstrated type I hypersensitivity to α -amylase and cellulase in five bakers through skin tests, histamine release tests and reverse enzyme-linked immunoassay (REIA) for specific IgE. The bronchial challenge tests were specific with α -amylase, cellulase or both, with immediate or dual asthmatic responses. The REIA inhibition study did not reveal cross-reactivity between these two enzymes, but there was cross-reactivity between α -amylase and *Aspergillus oryzae*.

We here report the results of a clinical study performed on seven bakers exposed to the dust of wheat flour and its additives, among them fungal α -amylase, who consulted because of symptoms of rhinitis and asthma related to their occupational activity.

Material and methods

Patients

In the period between November 1994 and June 1998 seven bakers were studied at our Allergy Service because of respiratory symptoms related to their work. Their ages ranged between 22 and 54 years (mean, 31.6

Table 1. Clinical and functional features of the seven patients

Patient	1	2	3	4	5	6	7
Age (years)	35	54	22	26	29	31	24
Sex	M	M	F	M	M	F	M
Occupation	Baker	Baker	Baker	Baker	Baker	Baker	Baker
Symptoms	R-A	R-A	R-A	R-A	R-A	R-A	R-A
Atopy	+	-	+	-	+	+	-
FEV ₁ (%)	53	55	116	100	104	84	101
BDT (%)	38	61	Neg.	12	Neg.	Neg.	Neg.
PC-20 metacolina (mg/ml)	NP	NP	0,23	NP	0,74	5	1,1

R-A = rhinitis and asthma; NP = not performed; BDT = bronchodilator test; PC-20 = PC-20 in the nonspecific methacholine test (mg/ml).

years); four of them were males and three females. The clinical features of the patients are presented in Table I. All of them evidenced increasingly severe episodes of cough, dyspnoea, chest tightness, wheezing, nasal blockage, watery rhinorrhoea and sneezing bouts, with a latency period ranging between several months and over 20 years. The occupational pattern was also typical in all cases, with symptomatic improvement up to being almost completely free of symptoms during holidays and work absence periods. In patients 1 and 2 the symptoms became chronic and severe, requiring emergency medical treatment on several occasions.

Besides being exposed to wheat flour, all our patients also manipulated further foodstuffs used as flour additives, all of them containing α -amylase (*T-500*®, *Puratos*® and *MP-Vital Extra G*®). The causal relationship between the inhalation of powdery material derived from the flour and its additives and the immediate apparition of symptoms was evident. Few minutes after the exposure the patients developed acute symptoms of rhinoconjunctivitis, cough, dyspnoea and wheezing, which often worsened toward the end of the working day. Patients 2 and 4 had been previously studied at other Centres, with negative skin tests and specific IgE to storage mites and cereal flours. Despite the fact that an aetiological diagnosis of occupational respiratory allergy, none of our patients had quit their work; the reason given was an unstable labour situation. They were repeatedly counselled, as an alternative measure, to pay the utmost attention to hygienic measures and precautions at their workplaces, avoiding exposure whenever possible, installing efficient ventilation-extraction systems and giving permanent antiinflammatory and bronchodilator drug therapy.

Preparation of the antigenic extract

Pure α -amylase derived from *Aspergillus oryzae* was supplied by Merck-Igoda, S. A. (Barcelona, Spain) as a fine powder. The allergenic extract was prepared by dissolving 2 g α -amylase in 20 ml 0.9% isotonic saline at room temperature. After manual stirring for 60 minutes, the solution was filtered through filter paper and then dialysed against isotonic saline for 24 hours. Finally, it was filtered through a 0.22 μ m Millipore filter (Millipore Corp., Bedford, Mass., USA) for sterilisation. The final concentration of the solution was 10% (w/v); this was considered as the undiluted mother extract. Part of this extract was then glycerinated for skin tests, and

the remainder was diluted in isotonic saline for the bronchial challenge tests.

Skin tests

The skin tests, using the prick technique¹¹, were performed with the above extract. Histamine phosphate (10 mg/ml) and isotonic normal saline (0.9%) were used as positive and negative controls, respectively. The responses to the tests were read after 15 minutes, measuring the largest diameter of the elicited wheal. A reaction was considered to be positive when the wheal was equal to or greater than 3 mm in diameter and accompanied by erythema, in the presence of a negative prick test with the diluent. Ten non-exposed atopic subjects who served as controls were tested with the highest concentration of the α -amylase extract (10% w/v). Skin tests were also performed with a panel of common airborne and occupational allergens (Alergia e Inmunología Abelló, Madrid, Spain) which included pollens (grasses, olive tree and shrubs), mites (*Dermatophagoides* spp., *Lepidoglyphus*, *Tyrophagus*, *Acarus siro*, *Gohieria*, *Euroglyphus*, *Glycyphagus* and *Blomia*), cat and dog danders, moulds (*Alternaria*, *Cladosporium* and *Aspergillus fumigatus*) and cereal flours (wheat, barley, rye, oat and maize).

Specific IgE quantitation

The concentration of α -amylase-specific IgE antibodies was determined by the FEIA-CAP System (Pharmacia Diagnostics AB, Uppsala, Sweden) according to the manufacturer's instructions.

Bronchial challenge tests

The non-specific inhalational bronchial challenge tests with methacholine were performed using the technique described by Cockcroft *et al.*¹² with some modifications¹³. In order to demonstrate the presence of bronchial asthma through the routine bronchodynamic tests (bronchodilator or bronchoconstrictor), this test was only performed when the foregoing bronchodilator test had been negative. The aerosol particles were generated through continuous nebulisation using a Hudson model 1720 nebuliser with a 7.5 l/min activating airflow and a 0.276 ml/min output.

The specific bronchial challenge test (SBCT) was performed with the dilution of the α -amylase extract that had previously elicited positive skin tests. End-point titration skin tests were initially performed with sequential one-half dilutions of the undiluted mother

extract (1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640 and 1/1280 w/v, respectively) in order to establish the safe initial dose for the SBCT. Baseline lung function tests were performed prior to the inhalation of the antigen in order to establish the baseline FEV₁, FVC and PEFr values, followed by a control inhalational challenge test with normal saline. The patients then inhaled the aerosolised allergenic extract, increasing concentrations being administered in different days using the previously stated nebuliser and procedure; the inhalational challenge was performed with the patient breathing at his normal tidal volume, through a mouth-piece and with nasal occlusion, for 2 minutes. The FEV₁ and FVC were measured 5, 10, 15, 20, 30 and 60 minutes after the SBCT. A drop in the FEV₁ equal to or greater than 20% as compared to the baseline value was considered representative of a positive immediate asthmatic response. The patients were then instructed to monitor their PFR at hourly intervals for 24 hours (leaving out the sleep period) in order to detect an eventual late response, which was considered to be positive when the PEFr evidenced a $\geq 25\%$ drop as compared to the baseline value.

At the time of carrying out the SBCT's, all patients were under leave of absence from work, under good clinical and functional control, and without any medication that might have interfered in the test results. Four non-exposed atopic subjects with mild to moderate bronchial asthma submitted to SBCT with the maximum concentration of the α -amylase allergenic extract (10% w/v) as controls.

Results

Skin tests

All the bakers evidenced positive responses to the skin tests with α -amylase antigenic extract. Three of them also evidenced positive skin tests with wheat flour, and one with storage mites. Table II shows the results of the skin tests. The skin tests with the highest concentration of the α -amylase extract (10% w/v) were negative in the control subjects, thus ruling out a possible irritative mechanism induced by the allergenic extract.

Specific IgE quantitation

Specific IgE antibodies to fungal α -amylase were detected in all the patients' sera. The total and α -amylase-specific IgE concentrations are shown in Table III.

Bronchial challenge tests

The nonspecific methacholine bronchial challenge tests disclosed varying degrees of bronchial hyperreactivity in those patients in whom it was performed (0.23, 0.74, 5.00 and 1.10 mg/ml, respectively, in patients Nos. 3, 5, 6 and 7). As already stated, however, the aim of this test was to confirm the diagnosis of bronchial asthma in those patients in whom the lung function tests had been normal and the bronchodilator tests had been negative.

After the SBCT with α -amylase, five patients evidenced an isolated immediate asthmatic response and a further two a dual one. Figure 1 depicts one case of immediate response. The SBCT with the highest concentra-

Table II. Results of the prick tests with α -amylase (greatest diameter of the wheal, mm)

Patient	1	2	3	4	5	6	7
α -amilasa (1/10 w/v)	9	6	8	6	10	12	12

Table III. Total and α -amylase-specific IgE (kU/l)

Patient	1	2	3	4	5	6	7
Total IgE (IU/ml)	480	41	166	221	538	281	315
Specific IgE (α -amylase)	18,5	45,3	9,47	1,35	2,72	1,50	63,6

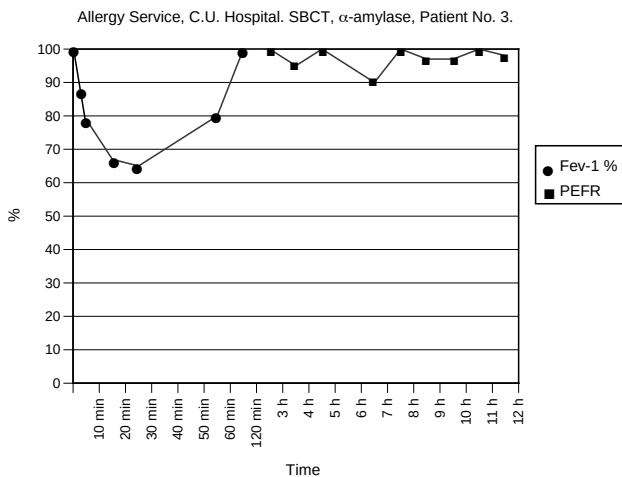


Fig. 1. Demonstration of an immediate asthmatic response after SBCT with fungal α -amylase at a 1/80 W/v concentration (in patient No. 3).

tion of the α -amylase extract (10% w/v) was negative in the control asthmatics, thus confirming the specificity of the test.

Discussion

Over many years, bakers' asthma was considered to be a form of allergic asthma mediated by IgE antibodies directed specifically against cereal flour allergens¹⁴. However, the roster of causative allergens has grown with the passage of time, and at the present time up to seven different groups of occupational allergens have been described¹⁵ which are able to induce occupational respiratory allergy in bakers and in associated occupations. Besides the already-mentioned cereal flours, soybean flour¹⁶, buckwheat flour¹⁷, storage mites¹⁸, grain weevils¹⁹, moulds of the genera *Alternaria* and *Aspergillus*¹⁵ and enzymes derived from *Aspergillus* spp.^{10,20} have also been identified as causative antigens in IgE-mediated occupational respiratory allergy. Over the last 20 years, the manufacturing processes for bread and similar products have evolved considerably. This is to a great extent due to the general use of improving additives added to wheat flour in order to accelerate bread production and to improve the quality of the bread. Among these additives, α -amylase from *Aspergillus oryzae* is the most important one²¹. The exposure is usually occupational, through the inhalation of the powdered enzyme, in workers of

the pharmaceutical industry⁹, in laboratory workers⁶ and in bakers²³.

We have presented the results of a study of seven bakers with symptoms of bronchial asthma and rhinitis in relation to their occupational activity, all of them exposed to α -amylase. Four of these workers were also sensitized to other common and/or occupational airborne allergens (flours and storage mites), and they also evidenced high total serum IgE concentrations. Atopy thus appears to play an important role in the development of IgE-mediated α -amylase sensitization, a common observation with high-molecular weight occupational allergens²². However, three of our patients evidenced monosensitization to α -amylase. Although we have not measured the environmental exposure levels in the work environments of our patients, studies have been recently published demonstrating a strong positive correlation between the exposure levels (ng α -amylase/m³) and the probability of sensitization and later development of occupational asthma²³⁻²⁵. The positive skin tests with α -amylase in our seven patients, together with the positive specific bronchial challenge tests and specific IgE quantitations demonstrate that an immunologic IgE-mediated immediate hypersensitivity mechanism was responsible for the respiratory symptoms. The negativity of the skin tests and of the specific bronchial challenge tests in the non-exposed atopic controls demonstrates the specificity of these findings. Immunologic studies with sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and later immunoblotting using a pool of sera from allergic patients have identified a 52-kDa glycoprotein band with specific IgE-binding capacity^{7,10}. This important occupational airborne allergen has recently been isolated and identified as α -amylase. In accordance with the nomenclature of the International Union of Immunology Societies (IUIS), it has been suggested that this antigen be named "*Aspergillus oryzae* α -amylase", or Asp o 2²⁶.

In conclusion, it is clear that, besides cereals and other specific occupational airborne allergens, the fungal enzyme α -amylase (Asp o 2) is a common cause of occupational respiratory allergy among bakers and confectioners. On the other hand, the positivity of the skin prick tests and of the specific IgE suggest the sensitization of the patient and the degree of sensitization, but only the specific bronchial challenge test with α -amylase can provide the definitive diagnosis.

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IMMUNOCHEMICAL CHARACTERISTICS OF α -AMYLASE SENSITIZATION

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Amylases were the first enzymes to be added to bread dough, and they are still today the object of considerable interest in the research of enzymes used in bakery¹. Three sources of amylases have been used up to now: malt flour (the amylase from malt flour was historically the first one to be used), fungi (*Aspergillus oryzae*), and bacteria (*Bacillus subtilis*).

These enzymes were initially used for the production of fermentable sugars. Starch, the main component of cereal flours, contains 75% amylopectin and 25% amylose. α -amylase acts on these constituents by randomly breaking down the 1,4-glycosidic bonds (with the exception of the terminal ones), producing maltose and most importantly (and mainly) dextrins. β -amylase acts on the latter by sequentially hydrolysing the terminal 1,4-glycosidic bonds, thus releasing maltose. This is the fermented by yeast producing gas release (CO₂,