

Cereal α -amylase inhibitors cause occupational sensitization in the wood industry

R. LÓPEZ-RICO, I. MONEO*, A. RICO, G. CURIEL*, R. SÁNCHEZ-MONGE† and G. SALCEDO†

Servicio de Alergia, Hospital Juan Canalejo, La Coruña, *Centro de Investigación Clínica, Instituto de Salud Carlos III, Madrid and †Unidad de Bioquímica, Departamento de Biotecnología, ETS Ingenieros Agrónomos, UPM, Madrid, Spain

Summary

Background Cereal flours are used in the wood industry to improve the quality of the glues necessary to produce veneer panels. However, up to now, no cases of sensitization to cereal flour in this kind of industry have been reported. Cereal α -amylase inhibitors have been previously described as important occupational allergens responsible for baker's asthma.

Objective To determine whether cereal allergens were responsible for occupational sensitization in three wood industry workers.

Methods The diagnosis was made by clinical questionnaire, physical examination, skin-prick tests to cereals, CAP and immunoblotting.

Results The three patients had positive skin prick tests and CAP to cereal flours. An IgE-immunoblotting revealed that only low molecular weight proteins (under 20 kDa) were detected by the three sera. These main IgE-binding proteins were members of the α -amylase inhibitor family which have been described as one of the group of main allergenic proteins in rye, barley and wheat. The three patients changed their workplace and remain asymptomatic in spite of the fact that they are still in contact with different woods and exposed to high concentrations of wood dust and other chemicals such as formaldehyde.

Conclusion Proteins from cereal flours are important occupational allergens in some wood industries.

Keywords: cereal, wood, α -amylase inhibitor, IgE, flour, glue

Clinical and Experimental Allergy, Vol. 28, pp. 1286–1291. Submitted 30 September 1997; revised 16 February 1998; accepted 23 March 1998.

Introduction

Occupational asthma is, at present, the most prevalent occupational respiratory disease [1]. Baker's asthma is one of the more frequent type of occupational asthma [2]. This affection is an IgE-mediated type I allergic reaction [3]. The allergens frequently responsible for this disease are flour proteins, additives (such as enzymes) and storage mites [4–9]. Occupational asthma produced by flour is well-known in professionals, such as bakers and millers, who work daily with these substances. There are less severe types of baker's allergies such as rhinitis, conjunctivitis and dermatitis. Epidemiological studies among bakery workers

show prevalences of asthma ranging from 5–7% to 15–30% [10–14].

Veneer panels are usually covered by a thin sheet of a high quality wood (pine, oak, teka, cedar, etc.) by means of an urea-formaldehyde resin that acts as a glue. The viscosity of this glue is increased by adding cereal flours to avoid the glue leaking through the pores of the wood sheet. This addition reduces the cost and improves the look of the final product. The objective of this study is to report three cases of occupational sensitization caused by flours (barley and rye) in workers from a wood factory that produces veneer panels.

Subjects and methods

The survey included three workers of a wood factory in the north of Spain.

Correspondence: Dr I. Moneo, Centro de Investigación Clínica, Sinesio Delgado 10, 28029 Madrid, Spain.

Patient 1—a 32-year-old atopic male, non-smoker with positive skin prick tests 4×4 mm for major mites (CAP class 3 to *Dermatophagoides pteronyssinus*). Two years after starting work in the factory he started to experience dyspnoea, rhinitis and ocular itching on work days, which worsened throughout the week. When on holiday and at weekends he was asymptomatic and required no medication in spite of his mite hypersensitivity.

Patient 2—a 41-year-old-atopic male, non-smoker, without any relevant health problems. He had been working in the factory for 4 years. While cleaning the machine that mixes flours and glue, he had an episode of dyspnoea, tachycardia, dizziness, palm itching, nausea and a fall of blood pressure. He needed adrenaline, corticosteroids, and salbutamol.

Patient 3—a 28-year-old non-atopic male, non-smoker, without previous health problems before starting work at the wood factory. After the first year in his work place he started suffering from rhinitis and ocular itching and some months later dyspnoea while working. He suffered rhinitis, dyspnoea and nausea after drinking beer; cutting some types of bread produced the same symptoms, although he had no problem after eating all types of bread.

After these events the patients' workplace was changed and at present they remain asymptomatic. The three patients have required medical attention on various occasions after entering the zone where the flours were used. The bronchoconstriction observed by the company physician has always disappeared shortly after use of salbutamol.

IgE determination and skin prick tests

Total IgE was measured with the CAP system (Pharmacia, Uppsala, Sweden). Specific IgE to formaldehyde, wheat, rye and barley was performed using the CAP method according to the manufacturer's instructions. Skin prick tests were carried out with routine inhalant allergens including house and storage mites, mixtures of pollens, moulds, cat and dog danders, as well as wheat, rye and barley flours (ALK-Abelló, Madrid, Spain) and 5% extracts in PBS of pine, oak and chestnut sawdust. Histamine was used as positive control and saline as negative control. Results were evaluated after 20 min and the diameter of the weals recorded.

Antigens

Barley, rye and wheat flours were purchased at a local food shop. Additionally, rye flour collected in the factory was also analysed. All the flours were extracted at a 5% w/v ratio in PBS shaking overnight at room temperature, centrifuged at 1500 g for 10 min and the supernatants stored at -20 °C until use.

Thirteen members of the α -amylase inhibitor family were purified from wheat, barley or rye flour following isolation procedures previously described [15–17]. Briefly, salt extracts enriched in inhibitors by $(\text{NH}_4)_2\text{SO}_4$ precipitation were separated by gel filtration and then the appropriate fractions subjected to high performance liquid chromatography. Purity of the isolated proteins was checked by SDS-PAGE and by two-dimensional electrophoresis. Protein concentration was quantified by the bicinchoninic acid assay [18]. All subfamilies described according to the amino acid sequence identity among the α -amylase inhibitors family [19] are represented in the 13 purified proteins tested in this study.

SDS-PAGE and immunoblotting

SDS-PAGE of rye, wheat and barley flour extracts, as well as purified proteins was performed in 16% acrylamide minigels as described [20]. Specific IgE detection by immunoblotting was performed using transfer of the separated proteins by a diffusion method and detection was carried out using alkaline-phosphatase labelled anti-IgE as in Moneo *et al.* [20]. Four unrelated sera were used as a negative control.

Immunoblotting inhibition

A crude rye extract was separated by SDS-PAGE and transferred to nitrocellulose as described above. Thereafter, the membrane was inserted in a MiniProtean II Multiscreen device (Bio-Rad, Richmond, CA, USA). Aliquots of the human serum (0.1 mL) were mixed with 0.025 mL of serial 1/5 dilutions of the allergens or 0.025 mL of PBS as a control together with 0.5 mL of diluent and dispensed in the different lanes of the device. These volumes corresponded to 40, 8 and 1.6 μg of crude rye extract or to 8, 1.6 and 0.32 μg of purified allergens. After overnight incubation, the membrane was taken out of the blotter, washed and revealed as described above.

Results

As shown by the clinical questionnaire, the three workers referred to respiratory and ocular symptoms clearly related with work and were asymptomatic during weekends and holidays. After changing their workplace they had no health problems, despite the fact that they work in contact with different woods. Table 1 shows the results of the skin prick tests, total IgE and specific IgE to cereal flours' measurements performed in the patients. With the exception of two low positive skin tests to mites, the three patients were negative to common allergens and wood extracts and clearly positive to the cereal flour extracts. Furthermore, specific

Table 1. Results of skin prick tests, total and specific IgE

Case	Skin prick test (mm)				CAP(kU/L)			
	Wheat	Rye	Barley	Others	Total IgE (kU/L)	Wheat	Rye	Barley
1	4×4	6×6	6×6	4×4#	111	2.5	16	6
2	6×6	10×10	6×6	—	236	10	21	9.7
3	6×6	8×8	6×6	4×4#	94	5.7	25	10

#*Dermatophagoides pteronyssinus*.

IgE to these cereals was confirmed by CAP, but results with formaldehyde were negative.

In the specific IgE blot the sera from the three patients only recognized low molecular weight proteins (under 20 kDa) when the crude flour extracts of wheat, barley and rye were analysed (Fig. 1). In contrast, these crude extracts showed a high number of protein bands ranging from 10 to 150 kDa when stained with Coomassie Blue (Fig. 1). The same results were obtained with the rye used in the factory.

Table 2. Purified allergens of the cereal α -amylase inhibitor family tested in this study and their reactivities with the sera from the three patients in specific IgE immunodetection. Allergens are grouped in subfamilies according to their level of amino acid sequence identity [16,17,19]

Allergen ¹	G ²	Sera ³		
		Case 1	Case 2	Case 3
WMAI-1(syn 0.28)	No	+	+	—
BMAI-1	Yes	+	+	—
RAI-1	No	+	+	—
RAI-2	No	+	+	—
WDAI-1(syn 0.53)	No	—	—	—
BDAI-1	No	+	—	—
Sec c 1	No	—	+	—
WTAI-CM2	No	—	—	—
RAI-3	No	—	—	+
WTAI-CM3	No	—	—	—
WTAI-CM16	No	—	—	+
BTAI-CMb	No	—	—	+
BTAI-CMb*	Yes	—	—	+

¹ Abbreviations used to designate the allergens are as in Fig. 1.² G, glycosylation; ³+, positive and —, negative reaction.

Several members of the α -amylase inhibitor family with molecular weights 12–16 kDa have been identified as prominent allergens associated with baker's asthma in the three cereal species [15,21,22]. Therefore, representative members of all the subfamilies included in this protein group were purified and tested for their IgE-binding properties (Fig. 1 and Table 2). The three individual sera recognized four to five of the 13 purified proteins assayed, but each serum had its own detection pattern. Independently of their different cereal species origin, all the members of the same subfamily (i.e. WTAI-CM16/BTAI-CMb/BTAI-CMb*) were either detected as a group or not detected. The only exception was the case of WTAI-CM2/RAI-3 when probed with serum 3. Interestingly, glycosylated and non-glycosylated forms of the same (BTAI-CMb* vs BTAI-CMb) or of homologous allergens (BMAI-1 vs RAI-1 and -2) showed similar responses (Table 2). Figure 2 shows in a representative case how a purified allergen was able to inhibit the immunoblot performed with a crude rye extract.

Discussion

The three reported cases were considered as occupational sensitizations because in all of them the symptoms developed at the workplace. They were asymptomatic when out of the work environment, as for instance on weekends and holidays. The sensitization periods ranged from 2 to 5 years. Our first suspicion was that the patients had an occupational sensitization due to formaldehyde, which is one of the components of the glues [23], or was caused by some of the different woods [24] used in this factory. We therefore checked the different chemicals and woods used by the patients in the old and new jobs and found that the only difference was that they were previously in contact with flours. It is noteworthy that the patients are now exposed to formaldehyde as well as high concentrations of ambient sawdust.

The study of the three cases by skin prick tests and measurement of specific IgE antibodies showed a sensitization to cereal

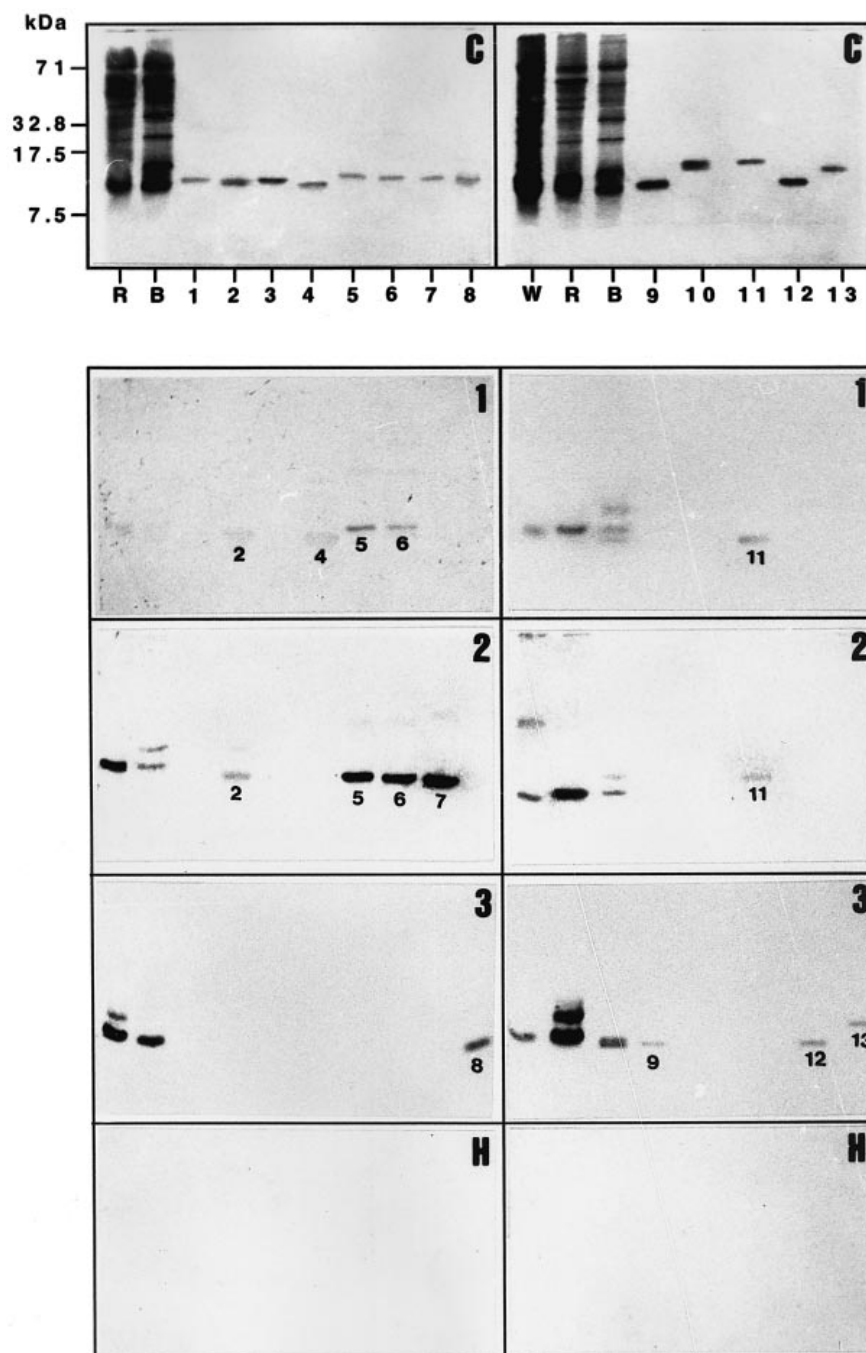


Fig. 1. SDS-PAGE and IgE immunoblotting of crude extracts from rye (R), barley (B) and wheat (W) flour and of purified allergens WDAI-1(1), WMAI-1(2), WTAI-CM2(3), BDAI-1(4), RAI-1(5), RAI-2(6), Sec c1(7), RAI-3(8), WTAI-CM16(9), WTAI-CM3(10), BMAI-1(11), BTAI-CMb(12) and BTAI-CMb*(13). Gels were stained with Coomassie Blue (C) or transferred to nitrocellulose membranes and treated with the appropriate serum (1–3) or a control serum (H) and IgE immunodetected. Abbreviations used to designate the allergens are as follows: wheat (W), barley (B), rye (R); monomeric (M), dimeric (D), tetrameric (T); α -amylase inhibitor (AI).

flours. The immunoblotting demonstrated that all the patients were sensitized to low molecular weight proteins. The use of purified cereal proteins allowed us to show that α -amylase inhibitors were the main IgE-binding proteins, as described in the classical baker's asthma [15,21,22]. Allergic diseases are very common in the bakery industry. A large variety of allergens, including some additives and flour pests, have been described as inducers of occupational asthma, but the

most relevant are the salt-soluble proteins of flours [25]. The major fraction of these salt-soluble proteins is represented by a single protein family of 12–16 kDa polypeptides, which show inhibitory activity towards insect and mammalian α -amylases. Several members of this inhibitor family are the best-characterized allergens from cereal flours [15,21,22]. In our case the three sera showed positive reactions with four to five members of the α -amylase inhibitor family. Positive results

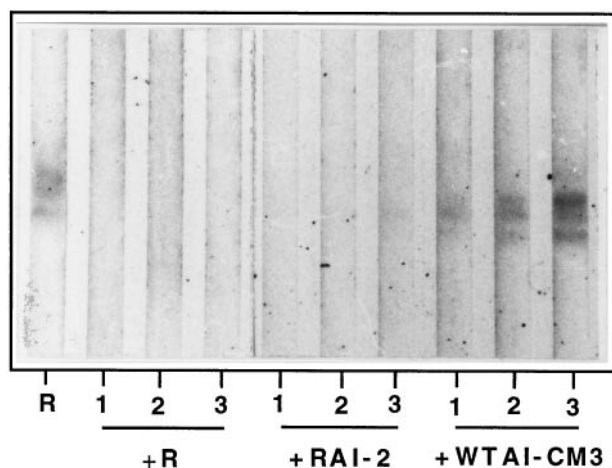


Fig. 2. Immunoblot inhibition assays using serum from patient 2. Crude rye extract separated by SDS-PAGE and transferred to nitrocellulose was immunodetected with the serum incubated with: buffer (R), with 40 (1), 8 (2), or 1.6 (3) μ g of the same extract (+ R) or with 8 (1), 1.6 (2) or 0.32 (3) μ g of purified RAI-2 (+ RAI-2) or WTAI-CM. 3(+ WTAI-CM3).

were related with the degree of primary structure identity, except in the pair of proteins WTAI-CM2/RAI-3. In this case, previous results indicate a significantly higher reactivity of the rye component (RAI-3) with respect to its wheat homologue (WTAI-CM2) in baker's asthma patients, both by *in vivo* and *in vitro* assays [22]. The covalent attach of asparagine-linked glycans has been reported to enhance the allergenicity of cereal α -amylase inhibitors [15,26]. The data presented in Table 2 strongly suggest that this is not the case with the patients included in this study, a result that can be of interest regarding potential cross-reactions with other glycosylated plant allergens [26]. The relevant role of several allergens which belong to the inhibitor family in the sensitization events reported here was fully confirmed by the immunoblot inhibition assays shown in Fig. 2. Moreover, the complete inhibition induced by RAI-2 indicated that no allergens other than the inhibitor family with molecular weights around 13–16 kDa were present in cereal flours.

Joint consideration of our results indicate that the cereal α -amylase inhibitor family is not only implicated in baker's asthma and in allergic diseases provoked by ingestion of cereal foods [27], but also in apparently unrelated activities such as in some wood industries. Preliminary evidence along this line has been obtained independently in another group of carpenters and sawmill workers showing occupational asthma and rhinoconjunctivitis [28].

Although 24 individuals were exposed to cereals in the factory, only the three affected workers were referred to us. Our study was a preliminary approach, but we estimate that the prevalence could be similar to the 5–30% of the exposed workers described for baker's asthma [10–14].

The use of cereal flours for improving the quality of the urea-formaldehyde resin in the veneer panel industry is, as far as we know, widely used in Europe and probably around the world. This means that a large number of workers in the wood industry are exposed to cereals and could therefore have an occupational asthma due to flours. It is possible that some of them are at present misdiagnosed and this fact is specially important because factories can adapt the industrial processes in order to avoid the exposure of the workers to these potent occupational allergens [29,30].

Acknowledgements

We thank Mary Harper for her assistance in improving the English usage and J. García-Guijarro for skilful technical assistance. This work was supported by Dirección General de Enseñanza Superior, MEC (grant PB95–0035) and by a grant of the Fundación de la Sociedad Española de Alergología e Inmunología Clínica.

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