

Short communication

Oilseed rape flour: another allergen causing occupational asthma among farmers

Background: Farmers are exposed to a wide variety of sensitizers. Since occupational asthma (OA) can lead to permanent disability, exposure discontinuation is the preferred treatment. When this is not possible, the identification of the causative allergen may allow an alternative therapy.

Methods: We present three farmers diagnosed with OA as a consequence of handling fodder. We carried out skin tests with common and occupational allergens and with oilseed rape (OSR) extract. Total and specific serum IgE levels were measured. The patients underwent the OSR-bronchial provocation test (OSR-BPT). The day before and 24 h after the OSR-BPT, the methacholine (M)-BPT and induced sputum were performed. Eosinophil percentages and ECP levels were measured in the sputum samples.

Results: OSR sensitization (skin tests and specific serum IgE) was detected in all the patients. The OSR-BPT elicited early responses in two subjects. Methacholine sensitivity, sputum eosinophils, and sputum ECP levels increased 24 h after the OSR-BPT in all the patients.

Conclusions: We have demonstrated that inhalation of OSR flour causes bronchoconstriction, induces an eosinophilic inflammatory bronchial response, and increases bronchial hyperresponsiveness in sensitized asthmatics. OSR flour contained in animal fodder should be considered another potential cause of OA among farmers.

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Over the past 12 years, the prevalence of asthma in both the general population (1) and in some groups of workers, such as farmers (2), has markedly increased. Longitudinal studies have shown that one-half of the occupational asthma (OA) patients removed from occupational exposure become asymptomatic in an average of 3.5 years (3). On the other hand, despite the various measures taken to decrease allergenic exposure (paper masks, change from daily to intermittent exposure or job reallocation) (4), most patients who stay at the workplace experience a worsening of their asthma symptoms and bronchial hyperresponsiveness (BHR) (3). Since OA can lead to permanent disability, its pathogenesis and the cessation of the causative exposure should always be stressed.

The diagnosis of OA is, firstly, based on the establishment of a cause-effect relationship between the airflow obstruction episodes and the occupational exposure, and, secondly, on the identification of the causative agent. Farmers are exposed to a wide spectrum of substances such as animal danders, fodder (cereals, legumes), and grain contaminants (molds, storage mites, insects, enzymes) that can be sensitizers. Removal of the patient from the workplace

is the preferred treatment, but sometimes for financial reasons this is not possible. Therefore, the identification of the causative allergen is crucial, since patients can in some instances avoid allergen exposure while continuing with their jobs.

Besides cereals, other compounds, such as oilseed rape (OSR) flour, are usually contained in animal fodder. In the present report, we present the cases of three farmers diagnosed with OA asthma who were sensitized to OSR flour.

Material and methods

Patients and study design

We present three nonsmoking farmers diagnosed with OA who had respiratory symptoms related to fodder manipulation. They were daily exposed to fodder, with the exception of patient 3, who only helped at the farm on vacations. OA diagnosis was made according to Bernstein (5). Atopy constitution was defined as positive skin test response to one or more of the most common airborne allergens in our environment. Patients underwent physical and immunologic evaluation, and skin tests with the batteries of common airborne, occupational allergens and the OSR extract were made. Total and specific serum IgE levels were measured.

Patients underwent the following protocol, carried out over 3

consecutive days: on day 1, the methacholine bronchial provocation test (M-BPT) and induced sputum were done. On day 2, the OSR-BPT was performed. On day 3, the M-BPT and induced sputum were done. Sputum induction was performed after the M-BPT. Only a short-acting β -agonist used "as needed" was allowed in the previous 2 months. No patient had suffered from lower or upper respiratory tract infections during the previous 2 months. All patients were informed and gave their consent before starting the study. Ten healthy control subjects (50% male, mean age: 28 years) were also skin tested with the OSR extract.

Allergen extract

OSR raw material (Guissona Agropecuaria s.c.l., Barcelona, Spain) in powder form was extracted in 10% w/v of phosphate-buffered saline (PBS) (pH 7.3). After stirring for 30 min, solutions were passed through a filter paper and dialyzed in PBS (90 min at 4°C) with a Spectrapor dialysis membrane (molecular weight cutoff 10 kDa) (Spectrum Medical Industries, USA). After centrifugation at 3000 g for 15 min, supernatants were passed through a 0.22-mm Millipore filter (Millipore Group, Bedford, MA, USA) for sterilization. The protein amount of the OSR extract measured by the Lowry method (6) was 2.9 mg/ml. The extract was lyophilized and then dissolved so that the final protein content of the extract was 10 mg/ml.

Skin tests

Cutaneous tests were performed as described (7). We tested a battery of common airborne allergens (ALK-Abelló, Spain) including mites (*Dermatophagoides pteronyssinus*, *D. farinae*), pollens (*Lolium perenne*, *Cynodon dactylon*, *Plantago lanceolata*, *Chenopodium album*, and *Artemisia vulgaris*), and animal dander (cat and dog). We skin-tested the most common occupational allergens among grain workers, including storage mites (*Lepidoglyphus destructor* and *Tyrophagus putrescentiae* at 100 BU/ml [Abelló, Spain] and *Acarus siro* at 5000 E/ml [Aristegui, Spain]), flours (wheat, barley, rye, corn, soybean, and alfalfa) at 5% w/v (Abelló, Spain), molds (*Alternaria tenuis* at 100 BU/ml and *Aspergillus fumigatus*, and *Cladosporium herbarum* at 5% w/v [Abelló, Spain]), animal dander (cow, horse, and rabbit) at 5% w/v (Abelló, Spain), α -amylase at 1 mg/ml (Leti, Madrid, Spain), and the OSR extract at 10 mg/ml. Histamine phosphate at 10 mg/ml and PBS were used as positive and negative controls, respectively. Skin tests results were read at 15 min. Wheal diameters equal to or greater than 3 mm were considered positive in the absence of a response to PBS.

Bronchial provocation tests

Bronchial provocation tests were carried out as previously described (8) with methacholine (Provacholine, Roche Lab., Nutley, NJ, USA) and with the OSR extract. Dilutions of agonist or allergen were made in PBS and aerosolized through a De Vilbiss 646 nebulizer (De Vilbiss Co, Somerset, PA, USA) powered with an oxygen tank with a pressure of 20 psi, and output of 6 l/min. The same nebulizer was used in all the tests, and was calibrated before and after each inhalation. The patient was instructed to take slow vital capacity inhalations from a plastic mouthpiece attached to the nebulizer. The system was manually activated by the technician when the subject signaled readiness, and FEV₁ values were measured by spirometry. Tests were done after withholding the inhaled short-acting β -adrenergic agonist for 6 h. FEV₁ values higher than 70% predicted normal were required to start the tests. FEV₁ values at basal stage and 3 min after diluent (PBS) inhalation were measured, and a variability rate lower than 5% among them was required to start the tests.

Methacholine BPT

Methacholine was diluted in PBS at 25 mg/ml. The protocol consisted of the inhalation of one breath, followed by three courses of four breaths each of the 25 mg/ml methacholine solution. FEV₁ was measured 3 min after each inhalation. The test finished when a fall in FEV₁ values equal to or higher than 20% of the postdiluent value was achieved, or when the third series of four breaths of methacholine were inhaled. Results were expressed in terms of the cumulated methacholine units (one methacholine unit = one inhalation of the 1 mg/ml concentration) needed to decrease FEV₁ by 20% from the postdiluent values (M-PD₂₀).

Oilseed rape BPT (OSR-BPT)

Skin tests with twofold dilutions of the allergen extract were made to enable selection of a safe initial dose for the OSR-BPT (concentration of allergen that produces a 3-mm wheal). Peak expiratory flow rate (PEFR) was recorded at the baseline. Allergen extract was inhaled every 10 min with a twofold increasing allergen concentration at each step (FEV₁ was measured at 10 min after each inhalation), until the highest dose of the extract was attained or an early asthmatic reaction occurred. This was defined in terms of the provocation dose (given in mg/ml) needed to decrease FEV₁ by 20% of the postdiluent values (OSR-PD₂₀). When the last dose of allergen was administered, FEV₁ measurements were performed at 20, 30, 60, and 90 min. To record any late asthmatic reaction, PEFR measurements were made hourly until 10 h after the challenge. A late asthmatic reaction was considered as a fall of 25% or more from the basal value (9). To confirm the specificity of the PEFR falls obtained during the late response, patients were asked to record their PEFR values hourly following the baseline M-BPT.

Sputum induction

Sputum samples were obtained as described (10). Before sputum induction, patients inhaled short-acting β agonists, and were asked to clean their mouth and nose. An ultrasonic nebulizer model Fisonneb was used to administer saline at 5% for three periods of 10 min. After each period, the patient was asked to cough and expectorate into a sterile container, while saliva was, when possible, collected in a different receptacle. The test finished when a macroscopically adequate sputum sample was obtained or when the three periods of inhalation were completed.

The volume of the entire sputum sample was measured, and 3 ml of the sample was mixed with an equal volume of dithiotreitol (DTT) (Sputasol, Unipath Ltd, Basingstoke, UK) at 1/100, and rocked at room temperature for 15 min. The mixture was then filtered through one 0.42- μ m Millipore filter (Millipore, Bedford, MA, USA) and centrifuged at 1500 g for 10 min. The supernatant was then aliquoted and frozen at -70°C until further analysis. The pellet was suspended in saline serum at 0.9%, cytocentrifuged, and stained with either Papanicolaou or Giemsa for differential cell counts. The sample was considered adequate for analysis when macrophages could be visualized and squamous cell contamination was lower than 20% (11). Percentual counts of macrophages, eosinophils, neutrophils, mast cells, and lymphocytes were made over a total count of 400 cells. A single person, blind to the clinical conditions of the patients, performed the evaluation.

IgE and ECP measurement

The concentrations of ECP in the sputum supernatant and of serum total and specific IgE were measured by a fluoroenzyme immuno-sorbent assay (UniCAP, Pharmacia Diagnostics, Uppsala, Sweden). The limit of detection for the fluid phase assays was 0.35 kU/l for

specific IgE, and 2.0 kU/l for total IgE and ECP. Results for sputum chemicals were referred to the sputum and DTT mix volume. All assays were performed blind to the clinical details.

Results

Demographic characteristics, the period between the onset of exposure and development of asthmatic symptoms (latency period), and the duration of OA are listed in Table 1. Baseline lung-function indices, skin tests, and serum IgE levels are shown in Table 2. Skin tests with OSR were positive in all the patients and negative in the 10 controls. Only patient 1 was monosensitized to OSR.

The OSR-BPT was well tolerated in all the cases. Early asthmatic responses were recorded in subjects 1 and 3 (Table 3). No late asthmatic responses were obtained (PEFR falls were 11%, 9%, and 18%, respectively). PEFR variability after the M-BPT was always lower than 10%; moreover, PEFR values were higher in the evening than in the morning. Methacholine sensitivity (M-PD₂₀ values), as well as eosinophil percentages and ECP levels in sputum, increased 24 h after the OSR-BPT in the three patients (Table 3).

Discussion

Cereal workers are exposed to a wide variety of substances that, by eliciting an IgE-mediated response, can act as occupational allergens (2, 12, 13). The different allergens manipulated, the type of exposure, and the environmental factors (such as humidity, temperature, and allergen load) can determine sensitization to one or more allergens.

OSR is a partially wind-pollinated crop which has been extensively cultivated in some regions of Europe. The allergenic role of OSR pollen is controversial since monosensitization has been described only in a small percentage of the subjects suffering from respiratory symptoms (14). Such symptoms, in some instances associated with BHR increase, have been attributed to both sensitization to other pollens and inhalation of irritant volatile substances released by the plant during the flowering season (15). However, recent data suggest that OSR pollen is a moderate but certain cause of allergy that can sensitize despite low exposure (16).

OSR proteins are extensively used in animal feed formulas. The oil is extracted by crushing or by

Table 1. Demographic characteristics of patients

Patient no.	Age (years)	Sex	Atopy	Latency period (years)	Duration of AO symptoms (years)
1	45	M	No	15	2
2	25	M	No	15	1
3	51	F	No	20	2

Table 2. Baseline lung function and sensitization to OSR and other occupational allergens

Patient no.	FEV ₁ (%)	tlgE (kU/l)	OSR-SPT (mm)	OSR-IgE (kU/l)	Oal-SPT (mm)
1	96	32.8	8	4.55	-
2	96	387	4	0.95	WF:10
3	95	601	9	4.99	Leg: 3 WF: 4

tlgE: total serum IgE; OSR: oilseed rape; SPT: skin prick test; Oal: other allergens; WF: wheat flour; leg.: legumes.

chemical methods. The protein residue is then dried and used to increase the protein value of animal fodder. Recently, Suh et al. have described a grain industry worker suffering from OA who was sensitized to OSR flour (17). In this study we present three farmers who suffered from respiratory (upper and lower) symptoms related to handling fodder, and who were sensitized to OSR flour. In keeping with reports on OSR-pollen-sensitive subjects (14), OSR flour monosensitization was uncommon among our patients. In our area, farms are usually small operations in which all the family members occasionally work. Thus, intermittent allergen exposure begins at an early stage of life, a fact which might explain the long latency periods detected in our study.

To study the role played by sensitivity to OSR flour in our patients, we subjected them to allergen bronchial challenge. Induced sputum is a valid method to explore bronchial inflammation (11), and provides additional information to the bronchial challenges made with occupational allergens (18, 19). We investigated whether OSR inhalation elicited changes in the sputum eosinophilic inflammation. After patient 1 learned of his OSR sensitivity, he had used an OSR-free fodder formula, and his respiratory symptoms had progressively improved, so that at the time of the allergen challenge (6 months later), he was asymptomatic and BHR was not detectable. Unfortunately, BHR had not been recorded at the moment of the initial diagnosis. The lack of BHR at the baseline in subject 2 is attributable to the fact that he studied in the city and was exposed to fodder only during vacations. Allergen bronchial challenge elicited early responses in patients 1 and 3, and, as has been

Table 3. OSR-BPT results. Methacholine sensitivity and eosinophilic inflammation in sputum at baseline and 24 h after OSR-BPT

Patient no.	OSR-PD ₂₀ (mg/ml)	M ₁ -PD ₂₀ (mcu)	M ₂ -PD ₂₀ (mcu)	Sp ₁ Eosinop (%)	Sp ₂ eosinop (%)	Sp ₁ ECP (µg/ml)	Sp ₂ ECP (µg/ml)
1	0.36	103.5	34.7	2	8	34.5	93.9
2	Neg.	Neg.	36.3	2	10	18.3	57.0
3	0.54	45.28	14.15	8	15	45	> 200

OSR: oilseed rape; M₁-PD₂₀: baseline methacholine PD₂₀ values; mcu: methacholine cumulated units; M₂-PD₂₀: methacholine PD₂₀ values 24 h after OSR bronchial challenge; Sp₁: baseline sputum; Sp₂: sputum obtained 24 h after OSR bronchial challenge; eosinop.: eosinophils.

widely demonstrated, caused increases in BHR (10, 19, 20). The eosinophil counts and the levels of ECP in sputum samples also increased 24 h after the OSR-BPT (10, 19). Patient 2 showed a definite increase in sputum eosinophils and an increase in BHR to methacholine. This indicates that OSR inhalation induces BHR and eosinophilic bronchitis. Further bronchial challenge testing with OSR might have induced a positive bronchial response and confirmed OA to this allergen (21).

In this report we have demonstrated that inhalation of OSR flour increases bronchial eosinophilic inflammation and enhances BHR. Thus, OSR flour can be considered another cause of OA among farmers. In

some instances, as in the case of patient 1, it can be easily avoided, allowing the patient to continue with his job safely. Therefore, we conclude that the identification of the agent causing OA should always be stressed and that allergy to OSR flour should be considered in the investigation of OA among farmers.

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