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Occupational allergy to phytase: case series of eight production workers exposed to animal feed additives

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Summary

Background: In agricultural meat production, adding enzymes such as phytase to animal feed is widespread, but there is little awareness of the allergenic potential and health risks of these fungal enzymes.

Patients and Methods: We report on eight patients working in a plant producing phytase granulates. All patients complained about work-related rhinitis occurring within six months of the onset of exposure to phytase dust. Asthmatic symptoms and contact urticaria also occurred. To detect sensitizations to phytase, skin prick-, patch-, and basophil activation test were carried out with the factory product. Levels of IgE and IgG against phytase were also measured.

Results: There was a positive reaction to phytase with skin prick testing in seven of the eight patients. IgE specific to phytase was detectable in four of the eight patients, and IgG specific to phytase was detectable in six of the eight patients. The basophil activation test was positive in four out of seven patients tested, but the patch test was negative in all patients tested. Transfer to a different workplace with no exposure to phytase completely eliminated the symptoms.

Conclusions: Mold enzymes such as phytase are highly potent occupational allergens. Occupational safety measures must be strictly implemented in order to protect the health of workers.

Introduction

Enzymes such as phytase have been added to commercial animal feed since the beginning of the 1990s in order to enhance the nutritive value of plant material. In recent years the use of genetically engineered enzymes as biochemical catalysts has increased significantly in the food, detergent, textile, pharmaceutical and animal feed industries [1]. These enzymes are produced by genetically modified microorganisms (for example *Aspergillus*, *Trichoderma* species).

Phytase is a phosphatase and is used commercially to increase the bioavailability of phosphorus of plant origin in animal feeds. It catalyzes the hydrolysis of phytate to lower

order phosphate esters and inorganic phosphate. Unlike ruminant farm animals, pigs and poultry (monogastric animals) do not produce phytase and are therefore incapable of hydrolyzing sufficient amounts of phytate-bound phosphate from plant food. Supplementation of animal feed with fungal phytase leads to an increased release of phosphate, which makes it possible to reduce the amount of inorganic phosphate added to the feed and accelerate the weight gain of the animals. This also reduces excretion of fecal phosphorus and the phosphate load on the soil [2].

The allergenic potency of the industrial enzymes in the airborne dust aerosol and the associated health risk for workers in these industries has been known since the 1950s [3–5],

and for workers in the animal feed industry since early 2000 [6–10]. Despite knowledge of this high allergenic potency, severe allergic reactions continue to occur in workers who come into contact with these enzymes. We report on eight employees working in a factory in which phytase is microencapsulated. They were referred to our allergy unit because of the suspected allergic symptoms that they developed during and after work.

Material and Methods

Patients

Seven of the eight employees were exposed to phytase dust during the processing of phytase granules. One employee worked with phytase on sieve analyses as part of quality control. An extensive medical history was recorded, with a focus on the presence of respiratory and skin atopy (defined as previously known atopic dermatitis, rhinitis or asthma in the medical history), the type of symptoms, and the time exposed to phytase until the first occurrence of symptoms.

Skin prick tests

The phytase used in the factory was also available in liquid form. From this crude phytase, liquid dilution series were prepared with NaCl 0.9 % to a dilution of up to 1 : 100,000. Skin prick tests were performed as reported previously [7]. A positive (histamine) and negative (NaCl) control was used. A positive skin prick test was defined as a wheal with a mean transverse diameter of at least 3 mm and a positive histamine control [11, 12].

Patch tests

For patch tests, the phytase granulate was rubbed in petrolatum and serial dilutions were made to a dilution of 1 : 100 (pH 4.7). Patch tests were performed using the Finn Chambers technique [13]. The duration of occlusion on the upper back was 24 hours. The tests were read at 0.5, 24, 48 and 72 hours after the start of exposure.

Specific IgE and IgG determination

For the determination of IgE and IgG specific to phytase, extracts were prepared from the phytase granulate. They were subsequently biotinylated and coupled to streptavidin ImmunoCAPs at the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of Ruhr-University Bochum (IPA). Determination of IgE and IgG specific to phytase was carried out with the ImmunoCAP 250 system (Thermo Fisher Scientific, Phadia AB, Uppsala,

Sweden) as described by Sander and colleagues [14]. IgE values ≥ 0.35 kU/l were defined as positive, indicating sensitization. For the evaluation of the IgG concentrations, a limit value (maximum value of the control sera) was determined on the basis of six control subjects.

Basophil activation test

With the BÜHLMANN FlowCAST® assay, peripheral blood samples from the workers were collected in EDTA and a normal reference blood sample was incubated in serial dilutions with crude extracts of the phytase granules from the factory of the workers. Starting with an initial concentration of 1 mg/ml, the crude extract was diluted in factors of 10 from 1 : 10 to the 3rd to 1 : 10 to the 6th to 1 : 10. The degree of activation of the basophilic granulocytes was estimated based on the expression of CD63. In addition, a blank sample without allergen stimulation and positive controls was determined by stimulation with anti-FcεRI and N-formyl-methionyl-leucyl-phenylalanine (fMLP). The results were expressed as a percentage of the CD63-positive basophilic granulocytes compared to all basophilic granulocytes. A result of ≥ 5 % CD63-positive basophilic granulocytes in the corresponding dilution was considered positive.

Pulmonary function diagnostics and radiological diagnosis of the lung

All patients were offered a pulmonary function test and radiologic lung diagnostics. The pulmonary function test was performed with the Medical Device Masterscreen BODY/DIFFUSION SN 693963. The values of the European Community for Coal and Steel were used as reference values [15]. Vital capacity, total lung capacity and diffusing capacity of the lungs were determined. A value was defined as pathological if it was outside the reference range of the values listed by the European Community for Coal and Steel [15].

The radiological pulmonary diagnosis was based on X-ray or computed tomography examination.

Results

Patients' characteristics

The patients (6 men and 2 women) were between 27 and 49 years old (median age 34.1 ± 8.4 years) (Table 1). Three patients had a history of atopic dermatitis and two patients had a history of non-occupational respiratory allergies. Work-related allergic symptoms started two weeks to six months (mean: 3.6 months) after the onset of phytase dust exposure. One patient (#8) reported symptoms on the first day of work. All patients complained about work-related

Table 1 Patient characteristics.

Patient #	1	2	3	4	5	6	7	8
Age	43	30	49	31	30	25	38	27
Gender	Female	Male	Female	Male	Male	Male	Male	Male
Non-occupational allergies before starting work	None	Grass pollen allergy	None	None	N.d.	House dust mite allergy, grass pollen allergy, animal hair allergy	N.d.	N.d.
Respiratory allergic symptoms	None	Rhinoconjunctivitis, bronchial asthma	None	None	N.d.	Bronchial asthma	N.d.	N.d.
Atopic dermatitis before starting work	Yes	Yes	No	No	N.d.	Yes	N.d.	N.d.
Task	<i>Sieve analysis</i>	<i>Feed plant: encapsulation of phytase and addition to animal feed</i>						
Duration of allergen exposure until onset of symptoms	6 months	6 months	1 month	1–2 months	6 months	3 months	2 weeks	1 day
Work-related allergic symptoms	Rhinitis, asthma	Rhinitis, asthma, urticaria	Rhinitis	Rhinitis	Rhinitis, asthma, urticaria	Rhinitis, asthma, urticaria	Rhinitis, asthma	Rhinitis, asthma, urticaria

Abbr.: asthma, asthmatic complaints; N.d., no data.

rhinitis, six regarding asthmatic symptoms with severe respiratory distress during and after work and three regarding contact urticaria. The symptoms only occurred with exposure to phytase dusts and were reproducible at levels of occupational exposure. They did not appear on non-working days such as weekends or holidays.

Diagnostics

Seven of the eight patients had a positive reaction to phytase with the skin prick test (Table 2). Phytase-specific IgE (≥ 0.35 kU/l) was detected in four of the eight patients, and elevated phytase-specific IgG concentrations were found in six of the eight patients. In four of the seven workers tested, the basophil activation test was positive. The patch test was negative in all patients tested. Chest X-rays showed normal results in four tested patients. The results of basic pulmonary function testing were normal in three patients, but one patient's lungs had a reduced diffusion capacity as measured by a low transfer coefficient for carbon monoxide (TLCO) at

rest. In summary, type I sensitization to phytase was detected by at least one test in seven of the eight patients. Patient #8 already complained of work-related rhinitis symptoms on the first working day. No sensitization to phytase was detected and irritative rhinitis was diagnosed.

Course of disease

The workers' protective equipment consisted of protection masks (FFP3 protection mask with protection of at least 99 % from particles up to a size of $0.6 \mu\text{m}$; maximum total leak 5 %), 3M protective suits and nitrile gloves. However, this did not reduce symptoms. After providing the employees with 3M Jupiter® Blower Units with suitable particulate filters or transfer of the employees to a different workplace with no exposure to phytase, the symptoms disappeared completely. However, when working at workplaces with minimal exposure to phytase, for example working at the gate or at the phytase packaging station, the symptoms returned.

Table 2 Diagnostic results.

Patient #	1	2	3	4	5	6	7	8
Phytase skin prick test (threshold dilution of positive skin prick test; hives/erythema in mm)	1 : 10,000 4/10	1 : 1000 4/12	1 : 1000 3/5	1 : 2 4/40	1 : 100 8/25	1 : 1000 6/25	Undiluted 5/7	Neg.
Phytase patch test	Neg.	Neg.	Neg.	N.d.	N.d.	Neg.	N.d.	Neg.
Total IgE (kU/L)	655	975	7	24	136	344	16	10
Spec. IgE phytase [kU/L] (CAP-Class)	16.4 (3)	27.3 (4)	0.10 (o)	0.04 (o)	15.5 (3)	15.9 (3)	0.05 (o)	0.00 (o)
Basophil activation (Ref. <5)	N.d.	1.2	2.0	5.1	7.4	15.0	1.0	0.8
Stimulation with anti-FcεRI; % CD63 ⁺ (Ref. > 10)	N.d.	84.5	90.0	82.4	61.8	80.0	49.0	89.8
Stimulation fMLP; % CD63 ⁺ (Ref. > 10)	N.d.	59.0	75.4	68.5	49.3	62.8	71.6	87.1
Phytase stimulation in dilution series 1 : 1,000 up to 1 : 100,000,000; % CD63 ⁺	N.d.	80,1–14,5	89–29	< 5	70–50	75–57	< 5	< 5
Index stimulation with/ without phytase (cut-off: 2)*	N.d.	67–12	44–14	< 2	9–7	5–4	< 2	< 2
Phytase stimulation with blood from non-exposed control subject; % CD63 ⁺	N.d.	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Interpretation	N.d.	Sensitization to phytase	Sensitization to phytase	No sensitization to phytase	Sensitization to phytase	Sensitization to phytase	No sensitization to phytase	No sensitization to phytase
Phytase spec. IgG [mgA/L] (Cut-off: 2.75 mgA/L)	32.90	135	71.9	38.5	18.1	16.1	< 2	2.70
Pulmonary function tests	Wpf	Wpf	Wpf	TLCO 79 %	N.d.	N.d.	N.d.	N.d.
Radiologic diagnosis of lung	Wpf (CT-/chest X-ray)	Wpf (chest X-ray)	Wpf (chest X-ray)	Wpf (chest X-ray)	N.d.	N.d.	N.d.	N.d.

*An index value ≥ 2 indicates sensitization to phytase.

Abbr.: N.d., no data; Wpf, without pathological findings; TLCO, transfer coefficient for carbon monoxide.

Discussion

Fungal phytase is a highly potent occupational allergen. We investigated seven cases with occupation-related type I sensitization to phytase, leading to allergic rhinitis in seven patients, to bronchial asthma in five patients and contact urticaria in three patients. Table 3 summarizes the current literature on occupational allergy to phytase, and shows that up to 72 % of exposed workers reported occupational allergic symptoms. The prevalence of sensitization to phytase is similar to that of bakers against alpha-amylase, with up to 36 % of those exposed [16]. Sensitization occurs not only in highly exposed production workers, but also in office workers in enzyme processing factories with low exposures [1, 6, 9, 10].

As in the case of the patients presented here, symptoms were predominantly type I allergic reactions. However, one case of IgG-mediated hypersensitivity pneumonitis has also been reported after exposure to phytase [17].

Occupational safety measures must aim to avoid inhalation and skin exposure. Inhalation of allergens can be prevented effectively by wearing blower units with suitable filter systems. Care must be taken to ensure strict compliance with health and safety regulations. In order to avoid fine dust formation, microencapsulated enzymes should be used. It has been shown that this improves safety at work significantly [4, 18]. For workers involved in this process of microencapsulation, occupational safety measures are particularly important, as demonstrated by our patients.

Table 3 Literature overview: occupational allergies to phytase and other enzymes produced in molds.

Ref.	Enzyme exposure	Collective	Symptoms	Diagnostics (positively tested persons/tested persons)
[10]	Phytase	11 production workers, 11 office-/ laboratory workers of the same factory (internal control) and 19 laboratory animal care staff (external control)	Asthmatic symptoms 6/11 (55 %) production workers; 2 of the non-exposed group	EIA: 4/11 (36 %) production workers, 1/11 internal control, 1/19 external control
[7]	Glucanase, amylase, xylanase, phytase	86 employees in animal feed production plants	All workers with proven sensitization (9 %): asthmatic symptoms, rhinoconjunctivitis	8/86 (9 %) sensitization to at least one enzyme (SPT, BAT, BLT)
[6]	Phytase	53 laboratory workers (23 highly exposed and 30 low exposed)	38/53 (72 %): asthmatic symptoms, rhinitis, conjunctivitis, dermatitis	EAST: patient with symptoms 14/38 (37 %), without symptoms 1/15
[9]	Phytase; glucanase	1 office worker of an animal feed production plant	Asthmatic symptoms	SPT, RAST and bronchial provocation test positive for phytase and β -glucanase
[14]	Phytase	1 employee of an animal feed production plant	Cough, dyspnea, fever, fatigue	IgG-mediated hypersensitivity pneumonitis: chest X-ray, HRCT, PFT, BAL; spec. IgG to phytase, no spec. IgE
[8]	Cellulase, glucanase, xylanase, Amylase; phytase	Exposed production workers (140); non-exposed office workers (78)	Work-related rhinitis: production workers (16 %) vs. office workers (8 %)	Sensitization to at least one enzyme (SPT, RAST): 10/140 (7 %) vs. 0/78 (0 %) of office workers
[1]	Phytase, xylanase, cellulase, and/or α -amylase	813 employees: examination of sensitization to exposed, genetically modified enzymes	Representative group of 134 workers: 64 % asymptomatic, 19 % rhinitis-/conjunctivitis and 17 % asthmatic symptoms	187/813 (23 %) detection of specific IgE against exposed enzymes

Abbr.: BAT, basophil activation test; BLT, immunoblot; EAST, enzyme allergosorbent test; EIA, enzyme immunoassay; RAST, radioallergosorbent test; SPT, skin prick test; PFT, pulmonary function test; HRCT, high-resolution computed tomography.

However, microencapsulation alone does not provide reliable protection against sensitization to enzymes, as Liss and colleagues have shown [19]. For example, at a company that used the serine protease subtilisin from the *Bacillus* genus in microencapsulated form to produce detergents, allergic symptoms were reported in 6 of 15 (40 %) workers. In three out of 15 (20 %), specific IgE was detected and in five out of 15 workers specific IgG (33 %) was found. As an added safety measure, Liss and colleagues recommend routine performance of a specific allergology work-up in workers who come into repeated contact with enzymes.

To improve occupational safety, it is necessary to control and minimize workplace exposure. This requires the development of standardized sensitivity test systems to determine the concentration of enzymes at the workplace of exposed persons. Optimal care of workers requires interdisciplinary cooperation with physicians and laboratory specialists from various disciplines [20]. Other protective measures include regular occupational medical examinations, regular instruction of the workers and training in the correct use of protective clothing.

A limitation of our study is that commercial secrecy restricted our access to further data, such as the number of exposed employees. We could only examine symptomatic workers sent to us from the factory. For this reason, the study lacks an exposed asymptomatic control group. However, unlike the other workers in our study, patient #8 reported symptoms on the first day of work. He was previously diagnosed with cancer and was very concerned about his health. We assume that he was aware of the occupational symptoms of his colleagues, and was striving for transfer to another department. Apart from a previously acquired non occupational sensitization, no sensitization to phytase may have been present on this first working day of the patient. Accordingly, the tests on this patient were all negative. This patient can therefore be seen as a negative control.

There was clear sensitization to phytase in the skin prick test in patients #1, #2, #5 and #6. This result correlates very well with the detection of IgE antibodies specific to phytase and a positive basophil activation test in these patients. Although only low levels of specific IgE antibodies against phytase were found in patient #3, there was a good correlation between the positive skin prick test and the positive basophil activation test, so that we also assume a clear sensitization against phytase in patient #3. Patients #4 and #7 only showed a positive skin prick test. In these patients, occupational symptoms occurred after a few weeks of exposure to phytase dusts. For this reason, we assume beginning sensitization.

Conclusion

It is well known that enzyme dusts have a strong sensitizing effect as published by the European Commission [21].

Despite knowledge of the allergenic potency of these fungal enzymes, cases of immediate-type allergies after occupational exposure continue to occur. Given the significant increase in the use of enzymes in various industries, the corresponding requirements must be implemented and occupational safety measures must be increased to avoid inhalation and skin exposure. Another problem is that no validated test methods exist to determine and predict sensitization via inhalation. It seems that the allergenic potency of phytase and other enzymes produced in molds continues to be underestimated by those responsible for the health of workers.

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