

Skin Prick Test Reactivity to Supplemental Enzymes in Cystic Fibrosis and Pancreatic Insufficiency

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ABSTRACT

Background: Patients with cystic fibrosis and pancreatic insufficiency take supplemental pancreatic enzymes of porcine or plant origin on a daily basis.

Objective: The frequency of immediate skin prick hypersensitivity reactions to such enzymes and the correlations to gastrointestinal symptoms and nutritional status were assessed in a single center prospective descriptive study.

Methods: Skin prick tests using five different freshly prepared porcine pancreatic enzyme preparations, one enzyme preparation of fungal origin, porcine meat and cat epithelia were performed in patients with cystic fibrosis and pancreatic insufficiency and in healthy control subjects.

Results: Positive skin tests in the 48 patients with cystic fibrosis were no more frequent than in 21 control subjects. No correlations with the frequency of gastrointestinal symptoms, nutritional status and lung function were found.

Conclusion: Although immediate hypersensitivity reactions to pancreatic enzyme preparations in subjects with cystic fibrosis are not common, individuals with possible allergic reactions to supplemental enzymes should be identified by skin prick tests and in unclear cases by double-blind placebo-controlled provocation tests. *JPGN* 40:194–198, 2005. **Key Words:** Skin prick tests—pancreatic enzymes—*Aspergillus oryzae*—gastro-intestinal complaints. © 2005 Lippincott Williams & Wilkins

INTRODUCTION

Immediate hypersensitivity reactions to pancreatic extracts have been reported in subjects exposed to powdered products by inhalation. The exposures were mainly from occupational (1) or from everyday contact. In the latter cases the powdered enzymes were prepared by parents for children with cystic fibrosis (CF) and pancreatic insufficiency (2–6). This exposure resulted in sensitization and asthmatic symptoms in the caregivers but very rarely in the patients themselves. Of 36 children with cystic fibrosis and exposure to pancreatic extracts, there was only one with mild rhinorrhea (2). Another child reported in the literature to have intolerance to pancreatic enzymes was a 33-month-old girl who had consistent vomiting 1–2 hours after the administration of the enzymes. She was treated successfully by oral desensitization with increasing doses of Pancrease MT 16 (7). No skin tests were done in these patients.

Symptoms of hypersensitivity reactions to pancreatic enzymes, such as rhinitis, conjunctivitis, urticaria and angioedema or asthma, are rarely noted by patients with

cystic fibrosis. However, everyday clinical experience indicates that some patients attribute their gastrointestinal symptoms to an intolerance of the pancreatic enzyme preparations they take.

We hypothesized that subjects with cystic fibrosis would have a higher frequency of immediate skin hypersensitivity reactions to enzyme extracts than a comparison group not taking such preparations. The goal of this preliminary study was to assess for immediate skin hypersensitivity reactions and to correlate them to gastrointestinal symptoms reported by the patients and the patients' nutritional status.

MATERIALS AND METHODS

Subjects

The CF patients were recruited during regular outpatient visits over a 3-month period. Of 71 patients with pancreatic insufficiency asked to participate, 48 were interested in participating in the investigation. Twenty-nine of the CF patients were $\Delta F508$ homozygous, 12 were heterozygous with G542X, R553X and G551D on the other allele; in nine cases the other mutation was unknown. One subject had R347P and another N1303K on one allele whereas the other allele was unknown. In five patients the mutations were not known. All patients had two positive sweat tests and all subjects were in stable clinical condition. The mean age was 20 ± 10 (range, 4–43) years, 29 subjects were female and the average weight was 45 ± 16 (range, 18–78) kg. The weight for length was $94 \pm 13\%$ of

Received April 22, 2004; accepted September 29, 2004.

Supported by the cf-Selbsthilfe, Germany.

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predicted (range, 73–128%). Forced expiratory volume in 1 second was 62 ± 22 (range, 17–111); forced vital capacity was 72 ± 20 (range, 30–114); and total serum immunoglobulin (Ig)E was 666 ± 1461 (range, 0.2–7845) IU/mL.

All subjects were asked about chronic (>3 months duration) gastrointestinal complaints that were not explained by failure to take the correct dose of enzyme preparations or by distal intestinal obstruction syndrome. All subjects were asked if they attributed any gastrointestinal symptoms either chronic or immediate to the intake of enzyme supplements. Patients with previous abdominal surgery (i.e., meconium ileus) did not complain of any chronic gastrointestinal symptoms.

The control group included laboratory and clinical staff who volunteered for skin prick testing. Six of these 21 subjects had a personal history of allergy to pollen and house dust mite. The mean age was 38 ± 2 (range, 27–56) years, 18 subjects were female and the average weight was 63 ± 2 (range, 46–80) kg. The study protocol was approved by the institutional review board.

Skin Prick Tests

The presence of specific IgE antibodies to the various enzyme preparations, pork and cat epithelia (a common allergen), is indicated by immediate wheal and flare reaction in the skin at the injection site. Each of the following six enzyme preparations were tested at 1 mg/mL and at 0.1 mg/mL. Pankreon, Kreon and a pure pancreatin preparation were from Solvay Arzneimittel, Hannover, Germany; Panzytrat was from Abbot, Wiesbaden, Germany; Pangrol from Berlin-Chemie, Berlin, Germany; and Nortase was from Asche, Hamburg, Germany. The compositions including additives are given in Table 1. The appropriate solutions were freshly prepared at the indicated concentrations in 0.9% NaCl and sterilized by filtration through 0.4-μm filters. Various concentrations were tested beforehand in a subject known to be sensitized to porcine pancreatic enzyme preparations (Fig. 1). Skin prick test solutions of pork and cat epithelia were obtained from Smith-Kline-Beecham, Düsseldorf, Germany. A positive reaction, read 15 minutes after pricking of the antigen, was defined as a wheal at least 1 mm larger than the negative control. All tests were done on the forearm. Proximal and distal negative controls (0.9% NaCl, the solvent used for the dilution of the enzyme preparations) as well as positive controls (histamine, 0.1% and 1%) were applied in parallel. The subjects were advised to look for late-phase reactions.

Statistical analysis

Data are given as means ± standard deviation and range. Frequencies were compared by Fisher's exact test.

RESULTS

Consumption of pancreatic enzymes

All of the controls and five of the 49 patients with CF were taking no pancreatic enzyme preparations. Of these five CF patients not taking enzymes at the time of the study, one adult woman had stopped taking enzymes as a result of repetitive gastrointestinal complaints

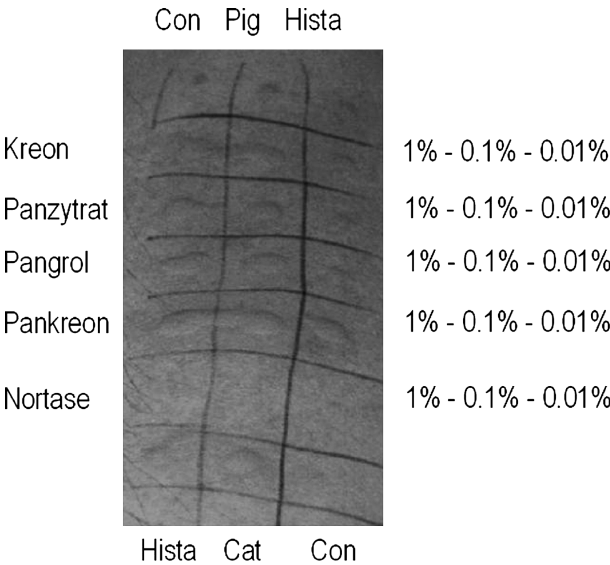


FIG. 1. Skin prick test with 3 concentrations of enzyme preparations, two negative control sites (con), two positive controls (Hista, Histamin 0.1%) and reactions to pig and cat. Note positive reactions to the pancreas derived enzymes, but not to Nortase, which is derived from *Aspergillus*. The test was reproduced with an identical result 5 months later. In this subject (Control 9) pure Pancreatin was not tested in the test shown, but in the one 5 months later, when it was positive for Pancreatin (not shown).

(abdominal pain, distention, loose and bulky stools). She had a positive immediate skin test reaction to Pangrol and to cat.

Among the 44 CF patients who were regularly taking enzymes, one patient was on Pankreatin and all the others were taking Kreon. In addition to Kreon, three patients took 30–60% of their total enzymes as Nortase, one patient 50% as Panzytrat, two patients 50% as Pankreon and one of the patients used Kreon, Nortase and Pankreon. It is not standard practice in our CF center to prescribe several enzyme preparations in one patient, but some patients thought the different enzymes improved their digestion and their stools. The composition of the different brands are listed in Table 1.

Skin Test Reactions

Nine of 21 control subjects showed 14 total positive reactions to the skin prick test and seventeen of the 49 CF patients had a total of 24 positive reactions. There was no difference in frequency between the two groups (Table 2).

Within the group of the CF patients, all cutaneous reactions to the pancreatic enzymes occurred in patients who were taking Kreon only or who were currently not taking any enzymes (one patient on Pangrol, as above). Half of all sensitizations to enzyme preparations were attributable to Nortase, which was not reactive in the control group. Of interest was that all four patients reactive to Nortase had elevated total IgE levels (5790, 641, 7845

TABLE 1. Contents of the enzyme preparations tested

Compound	Enzymes	Additives
Pankreatin	Pure triacylglycerollipase, amylase, proteases	None
Kreon 25,000	Pankreatin from pig (Triacylglycerollipase 25,000 Ph.Eur.-E, amylase 18,000 Ph.Eur.-E, proteases 1,000 Ph.Eur.-E)	Dibutylphthalat, dimeticon 1000, gelatine, hypromellosephthalat, macrogol 4000, paraffine, E 171, E 172, sodiumdodecylsulfate
Panzytrat 25,000	Pankreatin from pig (Triacylglycerollipase 25,000 Ph.Eur.-E, amylase 12,000 Ph.Eur.-E, proteases 800 Ph.Eur.-E)	E 171, E 172, crospovidon, gelatine, siliciumdioxide, methacrylacid-ethylacryl-copolymer-(1:1), magnesium stearate, cellulose, montanglycolwachs, talcum, triethylcitrate, ethoxyethanol, sodium-dodecylsulfat, simeticon-emulsion, soy-lecithin.
Pangrol 25,000	Pankreatin from pig (Triacylglycerollipase 25,000 Ph.Eur.-E, amylase 12,000 Ph.Eur.-E, proteases 1,250 Ph.Eur.-E)	Rizinus-oil, siliciumdioxide, magnesium stearate, croscarmellose-sodium, simeticon-emulsion, methacrylacid-copolymer type C, talcum, triethyl-citrate, cellulose, gelatine, E 104, E 132, E 171, E 172
Pankreon 10,000	Pankreatin from pig (Triacylglycerollipase 10,000 Ph.Eur.-E, amylase 8,000 Ph.Eur.-E, proteases 550 Ph.Eur.-E)	Macrogol 4000, eudragit, macrogol 6000, talcum, hypromellose, propylenglycole, titandioxide, Calciumcarbonate, Saccharin-sodium, povidon, E 171.
Nortase	Rizoli 7,000 F.I.P.-E, proteases 10,000 E, amylase 700 F.I.P.-E from <i>Aspergillus oryzae</i>	Gelatine, magnesium-stearate, E 171, E 172

and 3269 U/mL) and had had bronchopulmonary aspergillosis more than 1 year prior to the study. All these subjects were sensitized to *Aspergillus fumigatus*. Nortase is an enzyme preparation derived from *Aspergillus oryzae*. As none of these sensitized subjects had ever

taken Nortase, the positive skin test reactivity was likely to be a result of cross-reactivity or to similar epitopes in *Aspergillus fumigatus* and *Aspergillus oryzae*. Proteins of the latter are very likely to be contaminants of Nortase.

TABLE 2. Results of skin prick tests in CF patients and in healthy adult volunteers

	Pankreatin	Kreon	Panzytrat	Pangrol	Pankreon	Nortase	All enzymes	Cat epithelia	Pig meat	All allergens
CF 1						2 mm/n.r.				
CF 2						4 mm/2 mm			1 mm	
CF 3								1 mm		
CF 4				3 mm/n.r.					1 mm	
CF 5								7 mm	1 mm	
CF 6				n.r./1 mm				3 mm		
CF 7		1 mm/n.r.						1 mm		
CF 8								1 mm		
CF 9								3 mm		
CF 10						3 mm/n.r.		2 mm		
CF 11					2 mm/n.r.			2 mm		
CF 12								3 mm		
CF 13								4 mm		
CF 14								1 mm		
CF 15									2 mm	
CF 16						1 mm/n.r.				
CF 17								2 mm		
Con 1				2 mm/n.r.						
Con 2								5 mm		
Con 3				3 mm/1 mm						
Con 4								2 mm		
Con 5				2 mm/n.r.						
Con 6								1 mm		
Con 7									2 mm	
Con 8		3 mm/n.r.						5 mm		
Con 9	4 mm/2 mm	3 mm/3 mm	3 mm/2 mm	2 mm	3 mm/2 mm			3 mm		
All CF	0	1	0	2	1	4	8	12	4	24
All Con	1	2	1	4	1	0	8	5	1	14
P-value*	1	0.268	1	0.073	1	0.30	0.387	1	1	0.309

CF = cystic fibrosis.
Only patients with positive reactions are listed. The results are given in mm diameter of the wheels for 0.1% / 0.01% of each enzyme preparation.
n.r. – no reaction. The average positive histamine control was 2.9 ± 1.7 mm in diameter and the control was 0 ± 0 mm in diameter.
* Results of Fisher’s exact test for the comparison of the frequencies in the two populations.

The eight CF patients with positive reactions to pancreatic enzymes did not differ from those without skin prick reactions, with respect to amount of enzymes/kg body weight/day, weight for height, body mass index or lung function (forced expiratory volume in 1 second and forced vital capacity). Total IgE, however, was marginally increased in this subgroup of CF patients ($P = 0.08$, Wilcoxon's test). Most importantly, the frequency of gastrointestinal complaints in general and those attributed to enzymes by patients did not correlate with the results of the prick test for the pancreatic enzymes. Similarly, the frequency of a history of atopy or a lower body mass index did not differ with a positive enzyme prick test (Table 3).

Four, 8, 12 and 24 hours after the skin prick test all subjects were observed for late phase reactions. No such reactions were noted in any of the subjects tested.

DISCUSSION

In this study of patients with cystic fibrosis taking enzyme preparations for exocrine pancreatic insufficiency, we assessed the immediate cutaneous prick test reactions to various preparations and found no differences compared to a healthy control group. Within the group of patients with cystic fibrosis no correlations between prick test reaction, enzyme usage, gastrointestinal symptoms or nutritional status were found. The data suggest that there is no general link between positive enzyme prick tests and allergic reactions leading to gastrointestinal symptoms. Although our results presume that chronic exposure of patients to the enzyme preparations by the oral route does not appear to increase the risk for skin test sensitization, we need to emphasize the fact that although prospective, this study is a single center descriptive study and therefore the results are only preliminary. To determine the risk for hypersensitivity reactions against enzyme preparations by regular oral application, a prospective double-blind placebo-controlled study with a multicenter participation is needed.

These results do not preclude that in individual cases the ingestion of an enzyme preparation may provoke allergic gastrointestinal symptoms. Such a causal rela-

tionship can only be proven in carefully blinded and placebo-controlled tests. To our knowledge this has not yet been shown. Among other reasons, it can be speculated that a chronic ingestion of the preparations may induce tolerance (7). It is also of interest that none of our CF patients attributed symptoms other than gastrointestinal complaints, e.g. runny nose, itchy eyes, cough or bronchoconstriction, to their pancreatic extracts. The gastrointestinal complaints had a chronic characteristic and no one reported any symptoms suggesting gastrointestinal anaphylaxis (8). Among many other factors, the inefficacy of the pancreatic enzyme supplements at acid pH or a reduced pool of bile acids may have contributed to chronic gastrointestinal complaints of the patients. Cross-reactive proteins between cat epithelia and pork meat may lead to food allergy in patients with a pork-cat syndrome (9,10). Such a mechanism does not appear to be likely in the group of CF patients investigated, as there were no correlations between sensitization to cat epithelia and pork or the pork-derived enzyme preparations.

In the control group there was a relatively high number of positive skin prick tests to Pangrol 25,000. It is possible but unlikely that the positive reactions are a result of irritants in the preparation, as in the CF patients the number of reactions was low.

In this manuscript, we used a wheal size of more than 1 mm larger than the negative control as a positive skin prick test to be very sensitive. However, the guidelines of the European Academy of Allergology and Clinical Immunology define a positive reaction as a wheal size of 3 mm larger than the control (11). Based on this 3 mm wheal size limit, the number of positive skin prick test reactions to pancreatic enzymes would occur in only nine subjects compared with 17 subjects in our analysis. The fact that immediate skin prick test reactions occur less often when using the wheal size limit proposed by the European Academy of Allergology and Clinical Immunology strengthens our hypothesis that immediate hypersensitivity reactions to pancreatic enzymes in CF patients do not play a major role.

The skin test reactions to Nortase were very likely not related to the intake of this preparation, as all subjects with positive skin prick tests had not previously used

TABLE 3. Frequency of patients with cystic fibrosis in dependency of the results of the enzyme prick test

	Subjects with a positive enzyme prick test	Subjects with a negative enzyme prick test	P value
Positive history of atopy	3	9	NS
No history of atopy	5	31	
Positive history of gastrointestinal symptoms	2	9	NS
No history of gastrointestinal symptoms	6	31	
Body mass index ≤ 5 . percentile for age	5	18	NS
Body mass index > 5 . percentile for age	3	22	

Values are numbers of subjects in each category. Differences between categories were calculated by Fisher's exact test. NS = not significant

Nortase. All of the reacting subjects had an allergic sensitization to *Aspergillus fumigatus* (positive skin prick tests, specific IgE) and also had had an episode of an acute bronchopulmonary aspergillosis, giving an explanation for the observed skin test reactivity. It is of some theoretical concern that the use of Nortase in CF patients may result in the sensitization (or with long term usage desensitization) to *Aspergillus* species and as such may be one of the as yet unknown factors that may trigger the evolution of an allergic bronchopulmonary aspergillosis. Similarly, the application of Nortase to patients with an acute episode of an allergic aspergillosis might interfere with the clinical condition. We must stress that such a link is purely speculative and needs to be investigated further.

In summary, immediate skin test reactions to enzyme preparations do not seem to be more frequent among CF patients who use these preparations regularly and do not seem to be linked tightly to gastrointestinal complaints in this group of patients. Double-blind, placebo-controlled tests in individual patients may prove allergies that are clinically relevant, although no such associations have yet been demonstrated unequivocally.

Acknowledgment: The authors thank Valeria Maryuschenko for help with the data entering and Regine Göhlert and Maria Schlöder for performing the skin prick tests.

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