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Enzyme-potentiated desensitization in children with asthma and mite allergy: A double-blind study

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SUMMARY

The aim of this study was to evaluate the efficacy and safety of enzyme-potentiated desensitization (EPD) in children with asthma. Twenty asthmatic children (14 males and 6 females; median age: 8.5 years) were included in the study. They had positive skin tests to Dermatophagoides pteronyssinus *(Dpt), no history of other allergy and had suffered from asthma for at least two years. The children were examined before starting the trial, at the first EPD dose, after 8 weeks, at the second EPD dose and 3 months after the second EPD dose. Blood samples for PRIST and RAST were drawn before the first and at the second EPD dose, and at the last follow-up. Conjunctival provocation tests (CPT) and skin test endpoint determinations were performed with dilutions of a freeze-dried Dpt extract (10-100,000 SQ-U/ml) at the start of the trial and at the last follow-up. Parents kept a diary record of the days with asthma and daily drug usage. The children were randomized to receive either two intradermal placebo injections or the active material with an 8-week interval (November 1991 and January 1992). Ten children received EPD and 10 children placebo. The intradermal injection of EPD (0.05 ml) contained 0.01 ml of ß-glucuronidase (40 Fishman units) and 0.04 ml of a mixture of inhalant allergens (1 Noon unit). The placebo injection consisted of buffer solution only. The EPD-treated children had significantly fewer days with asthma (p = 0.00000). In addition, the EPD-treated children used significantly less medication for the management of asthma attacks (p = 0.00000). At the start of the trial, three out of 10 children in the EPD group and two out of 10 in the placebo group reacted only to the highest dose of allergen used in the CPT (100,000 SQ/ml) (NS). At the last follow-up, the threshold dose in the CPT was 100,000 SQ/ml or more in nine out of 10 children in the EPD group and in four out of 10 children of the placebo group (p = 0.0349). At the last follow-up, one child in the EPD group had a negative CPT with all doses tested. Global clinical evaluation by the investigators showed that eight out of 10 EPD-treated children improved, in comparison with three out of 10 children in the placebo group (p = 0.0349). Assessment by the parents was six out of 10 and four out of 10 improved, respectively (NS). Specific IgE to Dpt, total IgE and skin prick test endpoints before and after EPD showed no significant changes. One child in the placebo group experienced mild urticaria several hours following the second injection. No other local or systemic side effects were reported. The results of the present study provide further data on the effectiveness and safety of EPD in patients with asthma.*

Key words: Enzyme-potentiated desensitization - Immunotherapy - Asthma - Allergy

INTRODUCTION

A number of controlled studies have shown that specific immunotherapy (SIT) significantly reduces both the severity of symptoms and the use of concomitant medication in asthmatic children sensitive to grass pollen (1-5), house dust mite (6-8), cat and dog (9-11), and molds (12, 13). However, a limitation to the use of SIT is the risk of serious side effects. Severe side effects and even death have been reported in patients treated with SIT (14). In order to minimize the potential risks of fatal reactions, the Committee of Safety of Medicines in the United Kindgom (U.K.) recommends that SIT should be given only where full resuscitation apparatus is available and that the patient should wait two hours after each injection (15). In addition, a recent position paper by U.K. experts has practically restricted SIT to allergic rhinoconjunctivitis and *Hymenoptera*-allergic patients (16). These strict guidelines have almost stopped SIT in the U.K., thus depriving many patients of an effective therapy. Although guidelines of other societies recommend that patients should wait 30 min after each injection (17-19), in the last decade a remarkable reduction in the use of SIT has been reported in other European countries, mainly due to possible adverse reactions to such treatment. Several factors related to the patient, such as recent respiratory infections, bronchial hyperreactivity

and asthma, have been identified as significantly increasing the risk of severe reactions to SIT (14, 18, 19).

The potential dangers of SIT have increased interest in alternative types of immunotherapy that are considered safer, such as oral (20, 22), sublingual (23, 24), nasal (25-27) and enzyme-potentiated desensitization (EPD) (28-30). This last desensitizing method was first introduced into clinical practice in 1975 (28), on the basis of several studies performed in animals (31-33). More recently, a few controlled trials have been published (29, 30, 34, 35). These studies have shown that EPD significantly reduces the use of concomitant medication such as terfenadine (p <0.05) and beclomethasone nasal puffs (p <0.02) in adults with summer hay fever (29). In addition, the patients who received EPD significantly felt better (p <0.01) in comparison to the previous year when they received treatment with only antihistamines (29). More recently, a controlled trial on patients with hay fever showed that EPD significantly reduces rhinorrea (p <0.05), nasal obstruction (p <0.007), sneezing (p <0.01) and total nasal symptoms score (p <0.001), as well as the number of days with symptoms (p <0.001) (30).

The aim of this study was to evaluate the efficacy and safety of EPD in children with asthma and mite sensitization. The results of the present study provide further data on the effectiveness and safety of EPD in patients with asthma.

PATIENTS AND METHODS

Twenty asthmatic children (14 males and six females; median age: 6) were included in the study. They had positive skin tests to *Dermatophagoides pteronyssinus* (Dpt), no history of other allergy and had suffered from asthma for at least two years. Before entry into the trial, asthma was graded according to Aas (36). No child had previously received SIT. The study design is summarized in Table 1. The children were examined before starting the trial (baseline), at the time of the first EPD dose in November 1991, 8 weeks later at the second EPD dose and 3 months after the second EPD dose. Blood samples for PRIST and RAST analyses were drawn from each child before the first EPD dose, at the second EPD and at the last follow-up. The children were randomized either to receive placebo or the active material by intradermal injection: 10 children received EPD and 10 children placebo. Details of children in the active- and placebo-treated groups are shown in Table 2.

Table 1
Scheduling of the various tests in the study design.

	1991	1992	
	Nov.	Jan.	Apr.
EPD injection	+	+	
Clinical visit	+	+	+
Skin prick test (end point)	+		+
CPT	+		+
Total IgE	+	+	+
IgE to Dpt	+	+	+
Symptoms	→	→	→
Medication	→	→	→

CPT: conjunctival provocation test; Dpt: *Dermatophagoides pteronyssinus*.

Table 2
Clinical features of the 20 asthmatic children enrolled into the study.

	Num. of cases	
	Placebo	EPD
Males	8	6
Females	2	4
Median age in years (range)	6 (4-13)	6.5 (4-13)
Severity of asthma (grade):		
2	7	6
3	3	4
Positive prick test and RAST to Dpt	10	10

The material was provided by McEwen Laboratories (London). The trial code was held by the laboratory which provided treatment for each subject in numbered tubes. The sealed emergency copy of the code held by one of us (LB), remained unopened at the conclusion of the trial. The intradermal injection of EPD (0.05 ml) contained 0.01 ml of ß-glucuronidase (40 Fishman units), 1.3 cyclohexane diol (50 pg), protamine sulphate (50 ng), chondroitin sulphate (30 µg) and 0.04 ml of a mixture of inhalant allergens (1 Noon unit). The ß-glucuronidase was of molluscan origin (Seravac Ltd., Johannesburg), further purified by column chromatography. The inhalant allergens were as follows: *Grass, Parietaria officinalis, Olea europea, Artemisia, Birch*, Dpt, *Cladosporium herbarum, Aspergillus fumigatus, Alternaria alternata*, and cat and dog dander (Pharmacia, Uppsala). The placebo injections consisted of an equal dose of buffer solution. In order to avoid possible influences on the results, local reactions, elicited by injections, were recorded by one of us (M.A.M.) who was not involved in the outcome of the trial and were evaluated at the end of the study. The intradermal injections of EPD and placebo caused small areas of transient erythema, which were similar immediately after administration; 30 min later the local reactions were more intense in EPD-treated children than in those receiving placebo and four out of 10 EPD-treated children showed an induration which persisted for up to four hours. No child in the placebo group had such a reaction.

Skin prick test

The allergens tested were Dpt, *Alternaria alternata, Lolium, Olea europea* and *Parietaria officinalis* (SARM, Rome). Skin prick tests were performed on the volar

surface of the forearm. The reactions were read at 20 min. A negative control test was performed with glycerosaline solution and a positive one with histamine hydrochloride (1 mg/ml). The prick tests were considered positive when the wheal was at least 3 mm greater than the negative control.

Total IgE

Total serum IgE was determined by PRIST (Pharmacia Diagnostics AB, Sweden), and results were expressed in International Units per ml.

Specific IgE antibodies

Specific IgE antibodies for the above-reported allergens were assayed using Phadebas RAST (Pharmacia Diagnostics AB, Sweden). The results were expressed in arbitrary RAST units (PRU/ml) as recommended by the manufacturer.

Conjunctival provocation test (CPT)

The CPT was performed by a member of the team experienced in this technique (VR). The freeze-dried Dpt allergen extract (Pharmacia-ALK) was reconstituted with diluent (albumen HSA 0.3 mg/ml and phenol 0.5%) immediately before each testing session. Prior to testing, all medication which might influence the result was withdrawn. The conjunctivae were inspected for vascular congestion and itching, and if not present, CPT was begun by instillation of diluent into the lower fornix of one eye. If no response developed, one drop of the weakest dilution of Dpt extract was instilled. Provocation doses were increased in 10-fold steps from 10 SQ-U/ml to 100,000 SQ-U/ml, or until a positive response occurred. The CPT was considered positive if vascular congestion occurred in at least 50% of the conjunctival area 15-20 min after provocation. The controlateral eye was challenged by instillation of drops of diluent only.

Skin prick test endpoint

The skin prick test endpoint was determined with the extract used for the CPT. The same concentrations (10 to 100,000 SQ-U/ml) were tested. Histamine hydrochloride 1 mg/ml (positive control) and the diluent (negative control) were used. The endpoint was the lowest dose which gave a positive response. The skin tests were performed by the same investigator (V.R.) who had previously demonstrated reproducibility of skin tests within the recommended ± 20% of the mean.

Diary cards

The parents kept a record of the days with asthma and drug usage. The medications permitted for asthma attacks were as a first line: salbutamol nebulization (Broncovaleas) 20-100 μg/kg 4-8 times/day, theophylline tablets (Paidomal) 5 mg/kg 4 times/day; and as a second line: betamethasone tablets (Bentelan) 0.1 mg/kg. Preventive drugs such as sodium cromoglycate, nedocromil sodium and ketotifen were not permitted, nor were topical steroids or antihistamine drugs. All children received the same environmental measures for reducing the proliferation of house dust mites in the home.

The parents gave informed consent. The study was initiated and planned by one of us (L.B.) and supported by local funds.

Statistical analysis

Data were statistically evaluated using χ^2 and Fisher exact tests.

RESULTS

Analysis of the diary cards shows that the EPD-treated children had significantly fewer days with asthma in comparison with the children in the placebo group (p = 0.0000) (Fig. 1). In addition, the EPD-treated children used significantly less medication to manage asthma attacks (p = 0.0000) (Fig. 2).

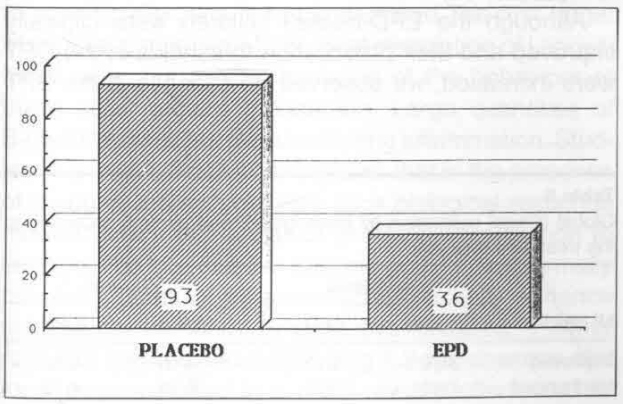

Fig. 1. Number of days with asthma in 20 children with mite allergy: EPD/placebo p = *0.00000.*

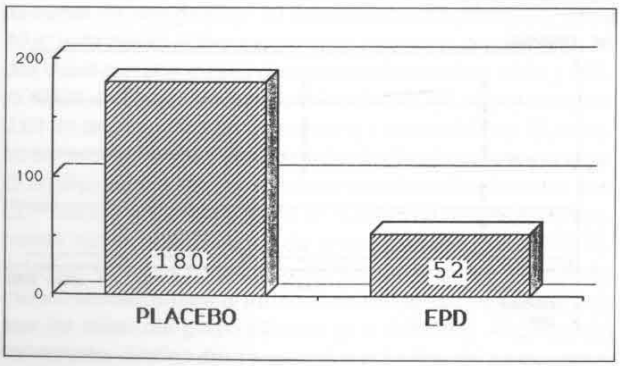

Fig. 2. Total days with drug consumption for asthma attacks: EPD/placebo p = *0.00000.*

Table 3
Conjunctival provocation test threshold dose according to the treatment regimen.

	Num. of cases			
	Placebo		EPD	
	Baseline	Last follow-up	Baseline	Last follow-up
Dpt SQ-U/ml				
10	0	0	0	0
100	1	1	0	0
1,000	0	0	0	0
10,000	7	5	7	1
100,0000	2	4	3	8
Negative with 100,000	0	0	0	1

Placebo baseline *vs.* EPD baseline: N.S.; placebo last follow-up *vs.* EPD last follow-up: $p = 0.0349$.

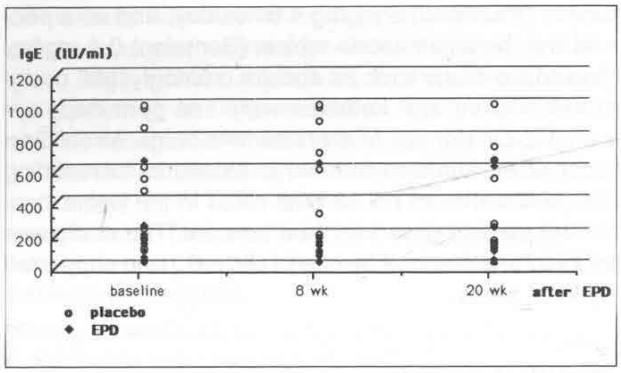

Fig. 3. Total serum IgE (IU/ml) in the children studied according to the treatment regimen.

The conjunctival provocation test threshold dose at the start of the trial was 100,000 SQ-U/ml in only three out of 10 children in the EPD-treated group and two out of 10 in the placebo group. The other children all reacted to lower doses. At the final follow-up session, the threshold dose was 100,000 SQ-U/ml or more for nine out of 10 of the actively treated children and four out of 10 in the placebo group ($p = 0.0349$) (Table 3). No significant differences were observed in total IgE (Fig. 3), specific IgE to Dpt (Fig. 4), or SPT endpoints before

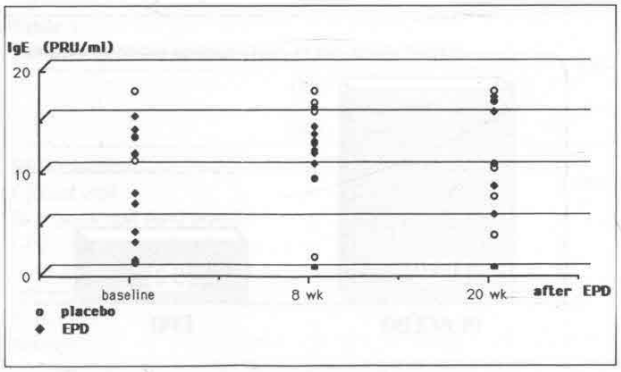

Fig. 4. IgE antibodies to Dermatophagoides pteronyssinus *(PRU/ml) in the children studied according to the treatment regimen.*

Table 4
Skin prick test endpoint according to the treatment regimen.

	Num. of cases			
	Placebo		EPD	
	Baseline	Last follow-up	Baseline	Last follow-up
Dpt SQ-U/ml				
10	0	0	0	0
100	0	0	0	0
1,000	0	0	0	0
10,000	7	8	6	6
100,0000	3	2	4	4
		(NS)		

and after EPD, or between actively treated and placebo groups (Table 4).

Global clinical evaluation by the investigators showed that eight out of 10 EPD-treated children improved, in comparison with three out of 10 in the placebo group ($p = 0.0349$). Parental assessment showed that improvement occurred in six out of 10 in the actively treated group and in four out of 10 of the controls (NS) (Table 5).

One child in the placebo group experienced mild urticaria several hours following the second injection. No other local or systemic side effects were reported.

DISCUSSION

The work presented here is the first double-blind placebo-controlled study in asthmatic children of the efficacy of EPD.

The number of days with asthma and the need for medication were both significantly reduced in the EPD-treated children compared with the placebo group ($p \leq 0.01$). In addition, the threshold dose of Dpt antigen in the CPT was significantly increased in the actively treated subjects ($p \leq 0.01$).

These data are in agreement with the results of four double-blind placebo-controlled clinical trials in adults which have shown that EPD is effective and safe as a one-shot prophylactic immunotherapy for seasonal pollenosis (29, 30, 34, 35).

Although the EPD-treated children were clinically improved and their provocation thresholds in the CPT were increased, we observed no changes in the SPT

Table 5
Global clinical evaluation by investigators and parents according to the treatment regimen.

	Investigators		Parents	
Nº children	Placebo	EPD	Placebo	EPD
Improved	3/10	8/10	4/10	6/10
Unchanged	7/10	2/10	6/10	4/10
	($p = 0.0349$)		(NS)	

endpoints, nor in total or specific IgE of active- and placebo-treated groups. This might be expected since SIT also produces clinical improvement and changes in provocation thresholds, independently of cutaneous reactivity and specific IgE antibodies (37-39).

During the past ten years it has become generally recognized that the chief pathology of asthma is bronchial inflammation, leading to a secondary hyperresponsiveness to pharmacological transmitters which provoke bronchoconstriction. Aeroallergens derived from Dpt are powerful inducers of allergic responses in children (40). It has been proposed that the mean particle size of the mite's fecal allergen makes it particularly likely to impact in the larger bronchioles, setting up small foci of inflammation which take time to heal. These collectively contribute to bronchial hyperreactivity, although the effect of an individual focus would be imperceptible (41, 42). We suggest that following EPD, the reduction of reactivity to specific allergen (Dpt) reflected by the increased threshold dose in the CPT might contribute to the control of allergen-induced bronchial inflammation and lead to the clinical improvement of asthma observed by us. In this respect our data suggests that EPD and SIT may have similar effects. Successful SIT for ragweed pollenosis increases the threshold dose of pollen allergen in intranasal provocation testing and reduces the release of mediators at each stage of the challenge (39, 43). It has been suggested that the decreased sensitivity of target cells following successful SIT may be due to cytokines, and particularly to a reduction of the cytokine histamine releasing factor (38). The same final pathway may apply to the action of EPD. Unlike conventional SIT that elicits blocking antibody titers (44), EPD with inhalant allergens does not induce blocking antibodies. However, the correlation between clinical outcome and blocking antibody titers is said to be poor in patients treated with SIT. Actually no immunological modification induced by SIT parallels the clinical improvement.

Although SIT has been employed for the treatment of respiratory allergic diseases for almost a century, its mechanism of actions has not yet been elucidated. Several mechanisms have been proposed, but none is completely accepted and many are still unclear. Accordingly, the mechanism by which EPD produces tolerance to specific allergens is not understood The first target of the injection is likely to be local stimulation of Langerhans' cells, but *in vitro* modeling of the behaviour of these cells remains inadequate. Large quantities of ß-glucuronidase are released during inflammation. Studies in animals (31, 32) have shown that in the presence of a sugar, the enzyme acts as a biological response modifier which can enhance or inhibit sensitization to antigen. The quantities of enzyme and sugars normally present at sites of inflammation will usually enhance subsequent immunity to causative antigens. Further research showed that substituting 1,3-cyclohexane-diol for a sugar resulted in a more reliable effect, and to produce a hyposensitizing formulation, an exceedingly small but precise dose of the diol is required (28, 33). Antigen dose also controls the outcome, and again, a very small dose favors hyposensitization, suggesting that the final result may resemble the low-dose tolerance described by Mitchison (45).

Enzyme-potentiated desensitization has been criticized because it is claimed that there is no scientific basis for treatment involving the administration of allergen extracts in doses equivalent to conventional prick tests, but EPD is based on the action of a biological response modifier which dictates the necessary doses of allergens. In addition, it is generally agreed that the use of mixtures of allergen extracts for SIT increases the risk of anaphylaxis and reduces the efficacy of the treatment. However, EPD has been in a continuous process of development for more than 20 years, and long-term follow-up of a large number of patients has shown that the development of sensitivities to new allergens does not occur with this treatment (Dr. McEwen, personal communication).

In conclusion, although the mechanism of action of EPD is not understood, the favorable results of this study provide further evidence for the efficacy and safety of this treatment.

RESUMEN

El objeto de este estudio fue evaluar la eficacia y seguridad del EPD en el tratamiento del asma en niños con alergia a Dermatophagoides pteronyssinus. *Se estudiaron 20 niños (14 chicos y 6 chicas), con una edad media de 8,5 años, con asma recurrente y sensibilización exclusiva a* D. pteronyssinus *(Tabla 1). Los niños se examinaron al comienzo del estudio, con la administración de la primera dosis de EPD, con la de la segunda dosis (después de 8 semanas a partir de la primera dosis) y tres meses después de la segunda dosis. El PRIST y el RAST se efectuaron antes de la administración de las dos dosis de EDP y en la última revisión. El test de provocación conjuntival (TPC) y la titulación a punto final cutánea se efectuaron al comienzo del estudio y en la última revisión, utilizando un extracto liofilizado de* D. pteronyssinus *en diferentes concentraciones (10-100.000 SQ-U/ml). Durante el estudio los padres llevaron un diario en el que anotaban los síntomas asmáticos y el consumo de fármacos (estimulantes $ß_2$, teofilinas, corticosteroides). Los niños se dividieron aleatoriamente en dos grupos: 10 niños recibieron placebo y otros 10 el tratamiento activo mediante la inyección intradérmica en dos dosis con dos meses de intervalo (noviembre 1991 y enero 1992). La inyección intradérmica de EPD (0,05 ml) contenía 0,01 ml de alergenos por inhalación (1 unidad Noon). El placebo contenía una dosis igual de solución fisiológica tamponada. El análisis de los diarios demostró que los niños tratados con EDP habían presentado asma un número de días significativamente menor que los niños del grupo placebo (p = 0.00000). Además, los niños tratados con EPD usaron significativamente menos fármacos para el tratamiento de los ataques asmáticos que los niños del grupo placebo (p = 0.00000). Al comienzo del estudio, 3 niños del grupo activo y 2 niños del grupo placebo toleraban la dosis umbral más alta al TPC (100.000 SQ/ml)*

(NS). En la última revisión la dosis umbral al TPC era de 100.000 SQ/ml o más en 9 niños del grupo EPD y en 4 del grupo placebo (p = 0.0349). En la última revisión un niño del grupo EPD tuvo TPC negativo en todas las concentraciones de alergeno testadas. La evaluación clínica global efectuada al final del estudio demostró que 8 niños tratados con EPD habían mejorado, en comparación con 3 niños del grupo placebo (p = 0.0349), mientras que la efectuada por los padres demostró que los niños que habían mejorado eran 6 y 4, respectivamente (NS). No se observaron diferencias significativas en cuanto a IgE total, IgE específica frente a D. pteronyssinus *y la titulación a punto final cutánea antes y después del EPD y entre el grupo de EPD y el grupo placebo. Un niño del grupo placebo presentó urticaria leve algunas horas después de la inyección de la segunda dosis. No se refirieron otros efectos colaterales locales o sistémicos.*

Palabras clave: Desensibilización potenciada por enzimas - Inmunoterapia - Asma - Alergia

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