EXPERIMENTAL IMMUNE-MODULATING THERAPY FOR AERO-ALLERGENS IN ALLERGIC CONJUNCTIVITIS TREATMENT.

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INTRODUCTION

Several controlled clinical trials have proven the effectiveness of specific immunotherapy (SIT) in the treatment of many allergic diseases [1,2,3].

However, clinical trials showing its efficacy in treating allergic diseases localized to the eye, such as vernal and atopic kerato-conjunctivitis, are not available.

Risks of side effects have led to some recommended limitations of SIT [4].

Enzyme Potentiated Desensitisation (EPD) is a proposed immunotherapic method for which irrelevant or no side effects have been claimed [5,6].

This method is based on the proved ability of the enzyme β-glucuronidase (β-G) to increase the hyposensitizing effect of small doses of mixed allergens (antigens) in the treatment of clinical (inhalants and chemical substances) allergy [7].

Being allergic and immune-mediated origin of paediatric allergic conjunctivitis proven [8] and following the confirmed hyposensitizing action of EPD, we assessed its efficacy and safety in the treatment of severe allergic conjunctivitis forms of young patients, when poorly controlled by conventional pharmacotherapy.

MATERIALS AND METHODS

Fifty three allergic patients (37 males and 16 females), aged between 7 and 20 (mean age 11.5 $\pm$ 3.2), affected by the major forms of allergic conjunctivitis (AC), not controlled by drugs or conventional SIT, were included in the study.

The selected patients came from an "observational period" of at least 2 years, during which they were treated with local treatment (Cyclosporine A, steroids and mast-cell stabilizers/antihistamines eye-drops) and with systemic therapy (antileukotrienes, if conjunctivitis is associated with asthma, antihistamines and short courses of oral steroids).

They had not received any immuno-therapy within the previous 12 months.

23 (43.4%) patients suffered from Vernal Kerato Conjunctivitis (VKC), 18 (34%) from seasonal Rhinitis and AC, 12 (22.6%) from Atopic Kerato-Conjunctivitis (AKC).

Ocular disease was diagnosed on the basis of IOIS allergic conjunctivitis classification [9] and of careful allergological history and ophthalmological examination (slit-lamp examination, Shirmer I tear test and Fluorescein staining, Rose Bengal, Lissamin Green colour tests and scraping test). From the ophthalmological point of view, at each examination we tested: dense mucus secretion, conjunctival hyperemia, papillary conjunctivitis, epithelial punctate keratitis and limbal Trantas nodules or dots.

Ocular examination was made by 2 different ophthalmologists at the same time.

Allergological routinary study (skin PRICK-test, blood cell count with total eosinophilia, blood PRIST and RAST for food and inhalant allergens) was scheduled in association with specific ocular tests, such as Conjunctival Allergen Challenge test (CAC), Eosinophil Cationic Protein (ECP), tryptase and specific IgE assay (RAST) in tears (for dust mites: Dermatophagoides Pteronissimus and Farinae, cat, cypress, olive tree, grass and pellitory pollens). Tear specific IgE assay was carried out at the same time in tears and in blood. The selected allergens tested were chosen from those that are the most common in our area (North-West of Italy).

DISCUSSION

The use of allergen SIT, considering the reported risks (adverse reactions) associated with it and the need for prolonged treatment, has considerably diminished in the last ten years all over Europe [11].
IT with EPD vaccine is a relatively new method for treating allergic diseases; it was first developed in the late 60's on the basis of Popper's and McEwen's clinical observations [12,13].
Many experimental trials during the last twenty years proved that the enzyme β-G is able to enhance the degree of hypo-sensitization induced when small quantities of specific antigen are used in the treatment of allergies. [14,15] This peculiar mechanism of action may be explained by tolerance induction when small antigen doses are administered.
Tolerance induction may be induced by a wide range of antigens, but above a certain threshold this effect is merely masked by the immune response. [16]
The ability of β-G to prevent the response to a dose of antigen, which is high enough to provoke antibody production, indicates that at lower dose levels of antigen the induction of tolerance may be enhanced indirectly by suppression of simultaneous weak immunity and not by a direct effect [17].
Many long-term follow-ups and double-blind masked studies [18,19,20] have established the safety of this new method of hypo-sensitization and also its efficacy in reducing symptoms produced by a wide variety of clinical allergic conditions that could not be treated in the past years.
Recently hypo-sensitization has proved useful also in oto-laryngic allergy [21].
Therefore, our study confirms the efficacy and the very low level of side-effects of this treatment.
We want to stress that the group of patients studied was already the one not responsive to conventional therapies.
The effectiveness and safety of EPD in our cases illustrate how the method will extend the therapeutic capability of the allergologist but also of the ophthalmologist.
The mechanism by which desensitization has been produced in these patients is still uncertain, but whatever the immunological mechanism involved maybe, EPD has already proved to be an effective therapy.
Its immediate safety is greater than that of conventional hyposensitizing therapies tried in our patients in the years before EPD treatment and there have been no unwanted long-term effects.
On the basis of our observations EPD must not be compared to SIT which is specific for only one allergen.
This is a preliminary report of a new method for treating ocular allergy with the EPD.
In conclusion, EPD is clinically effective in the treatment of AC.
Some immunological modifications observed in our patients, like in Astarita's study [22], suggest an EPD-induced enhancement of tolerogenic mechanisms like "immune deviation".
From recent studies EPD is the only preventive symptomatic immunotherapy available and its efficacy in perennial and seasonal rhinitis has been demonstrated [23].
The results of Cantani's study [24] provide further data on the effectiveness and safety of EPD in patients with asthma. In this series, the shortest total period of treatment was 24 months, the longest 8 years.
Oral antihistamines reduction observed during the seasonal period and the long-term benefit after the discontinuation of treatment support the efficacy of this therapy.
More recent studies have shown an increase of IL-10 and IL-6 after treatment with EPD. This proves the direct action of EPD on the immune response, suggesting antigen-specific and non-specific mechanisms that produce clinical improvement and reducing inflammatory reactions related to allergic diseases, sometimes related to the worsening of symptoms [25].
Long-term action demonstrates that EPD is a preventive immunotherapy employing β-G as a biological response modifier.
But until now, like other kinds of immune-system acting drugs, its mechanism of action still remains unknown.

EPD administration was based on McEwen method: in the first year 3 subsequent (approx every 4 months) intra-dermal injections, in the second and third year 2 injections (every 6 months).
The injection site was the flexor surface of the forearm. After the injection the patients were observed in hospital for 2 hours.
The intra-dermal injection of EPD (0.05 ml) contained 0.01 ml of β-G (40 Fishman units) and 0.04 ml of a mixture of inhalant allergens (1 Noon unit) injected by means of an insulin syringe with integral needle.
All patients, the day before treatment (at least 24 hours before) had to discontinue local and systemic therapy and the day after the injection patients had to follow, for 15 days, a support therapy based on Zinc sulphate (25 mg/day), Folic acid (6 mg/day), Vitamin D (calcitryol: 0.25 μg/day). Children over 12 years had to intake a double dosage of these drugs.
Patients were examined at the same time by allergologist and ophthalmologist, before starting the trial, at each EPD dose and 6 and 12 months after the last EPD administration (four years follow-up). Mean follow-up was 48 ± 2 months.
Blood samples for PRIST and RAST, CAC tests and skin PRICK test were performed at the beginning of the trial and at the last follow-up.
Parents kept a diary record of the days with ocular and general characteristics of symptoms and daily drug dosage [10].
Long-term reduction or interruption of local and/or systemic therapy and persistent improvement of ophthalmological signs and symptoms were considered as success for treatment.
We developed a scoring system from 0 to 4 depending on the need of our patients for local or systemic therapies for more than 50% of follow-up: only artificial tears (0), systemic therapy with antihistamines, sometimes supported by anti-leukotrienes (montelukast) and antihistaminic eye drops (1), steroid eye drops (2), systemic steroids slowly tapered (3), and Cyclosporine A 1% manufactured solution in artificial tears (4).

RESULTS

The EPD- treated patients had significantly fewer days with general symptoms ($p< 0.005$) and used significantly less medication for the management of ocular symptoms ($p<0.001$) compared with the previous two years with conventional treatment.
42 (79%) of the examined patients improved general and ocular symptoms and signs; 25 (47.1%) patients had complete remission of all of them and discontinued every treatment and 17 (32%) had partial but substantial improvement and needed only occasional local support with antihistamines and/or steroid eye-drops.
At the last follow-up some of the patients previously positive (15 out of 26, i.e. 28.3%), had a negative CAC with all doses tested.
Specific IgE, total IgE, and skin Prick tests before and after EPD showed no significant changes.
One patient experienced mild urticaria several hours after the second injection, 6 patients had a local asymptomatic localized oedema in the site of the injection lasting about 2 hours (solved by applying ice).
Two patients experienced a delayed mild headache lasting about 2 days.
Among the remaining 11 (20.7%) patients, 8 patients (15.1%) didn't show any clinical improvement related to EPD therapy and needed an addition of oral antihistamines, montelukast, and sometimes oral steroids but showed mild improvement of ocular symptoms (itching, tearing and photofobia) and signs (lids oedema, conjunctival hyperemia, punctate keratitis), sometimes with the appearance of Trantas' nodules.
Three patients (5.6%) had significant recurrences of ocular disease during the follow-up period, requiring Cyclosporine A (3-4 times a day) and steroid (dexamethasone 0.2%) eye-drops treatment combined with general antihistamines.
The life quality score recorded by parents improved in 47 patients (88.7%).

Leonardi [26] showed that VKC and major forms of SAC belong to a generalized disorder of the immune system, because many more allergens than those tested are involved in young patients' sensitization.
We know that children's immune system has developed partially. Consequently we observe quite often generalized allergic symptoms and signs, even in patients who are negative to classical allergological tests [27].
In our experience, EPD-based treatment of AC patients, who were under-treated by conventional therapies and who had severe ocular complications, has provided encouraging results with reduction of general and local symptoms and signs and sometimes with persistent remission.
About 2/3 of patients reduced the need for medications for the management of asthma.
Shrader [28] assumed that EPD desensitization lasts much more than any other immune therapy, since it is cell-mediated, acting on T-cells immune-suppressors as compared to the conventional IT that requires a monthly administration since it is antibody-mediated and to the "neutralization" therapy using the low-dose tolerance. These two last therapies require a long period of administration because of their short-acting duration and cannot be easily discontinued without a partial or complete symptoms' relapse.
These previous studies provide an explanation to only three/four administrations the first year and two administrations per year the subsequent years, instead of monthly.
However, our work is a preliminary report study since it will be completed in 2010 with all data of the long-term follow-up at least 5-6 years after therapy discontinuation.
In our study, those patients who were not responsive to EPD are probably affected by a different immune-system disease of not well defined origin or were not compliant with the EPD protocol or had Candida Albicans or Helicobacter Pilorii in their stomach (these micro-organisms partially or fully inactivate the product efficacy). [29]
Radcliffe's study [30].....
Based on our experience (we included in our study only patients who were not responsive to conventional therapies), the positive aspect of EPD-based desensitization therapy in AC patients consists in the reduction of systemic treatments and local steroid or Cyclosporin A-based therapy and consequently allows to avoid potentially blinding complications of the major forms of AC.
In addition, it is a not expensive and safe therapy with no side-effects compared to conventional SIT and permits a good quality of life because signs and symptoms are improved also because young patients are not submitted to dietary or environmental restrictions.

CONCLUSIONS

Our four years EPD experience showed good results from both the subjective point of view (patient's compliance and agreement) and the conventional drugs saving, despite the high difficulty in finding the EPD-vaccine and the look with suspicion of many allergologists for a new product on the action mechanism of which we have few data.

Key-words: Enzyme Potentiated Derivative, Specific Immuno Therapy, allergic conjunctivitis.

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