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The Effect of Local Nasal Immunotherapy in Allergic Rhinitis: Using Strips of the Allergen *Dermatophagoides pteronyssinus*

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ORIGINAL ARTICLE

The Effect of Local Nasal Immunotherapy in Allergic Rhinitis: Using Strips of the Allergen *Dermatophagoides pteronyssinus*

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Local nasal immunotherapy (LNIT) has been reported to be effective for mite-induced allergic rhinitis. However, its compliance is poor due to persistent nasal reactions and difficult application. The main objective of this study was to develop a *Dermatophagoides pteronyssinus* (Der p)-coated strip for self-application and to evaluate its clinical efficacy. Randomized, double-blind, placebo-controlled trials were carried out to evaluate the efficacy and compliance of LNIT. A total of 35 patients with allergic rhinitis from the allergy clinic were recruited to receive four months of LNIT. Clinical efficacy was evaluated by the symptoms of sneezing, rhinorrhea and nasal stuffiness. Local and systemic effects were evaluated by measuring histamine and eosinophil cationic protein (ECP) in nasal discharge and allergen-specific IgG1, IgG4 and IgE in sera. LNIT with Der p-coated strips could reduce nasal symptoms and modulate the serum levels of Der p-specific IgG1, IgG4 and IgE. The secretion of both histamine and ECP was reduced within four months after treatment. When the symptomatic and immunological changes were compared between active treatment and placebo groups, the improvements were greater in the active treatment groups for all three symptom scores. There were no severe adverse events except nasal response persisting within the first month in some cases. LNIT with a steady dose of Der p-coated strips can be self-administered at home without any systemic reactions. Both local and systemic immune responses were observed after LNIT. This therapeutic modality may serve as a good alternative treatment for allergic rhinitis.

Keywords allergen strip, allergic rhinitis, *Dermatophagoides pteronyssinus*, nasal immunotherapy, immunomodulation

INTRODUCTION

Allergic rhinitis is one of the most common allergic diseases with a prevalence rate of around 20% in the industrialized world. The disease comorbidity of sinusitis and asthma can cause significant lifestyle impact (1–4).

Allergic rhinitis is an inflammatory nasal disorder in which different cells participate. Mediators released from activated cells can account for symptom expression, such as histamine from mast cells, and eosinophil cationic protein (ECP) from eosinophils, and the allergen-specific immunoglobulin E (IgE) and IgG1 from B lymphocytes can account for hypersensitivity (5). House dust mites have been found to be the most important aeroallergens to trigger cell activation and more than 80 percent of allergic asthmatics are hypersensitive to the dust mite, *Dermatophagoides pteronyssinus* (Der p), in Taiwan (6).

Allergen-specific immunotherapy (IT) is effective in ameliorating the symptoms (7), and several immunological changes have been observed, including increase of allergen-specific IgG and reduction of allergen-specific IgE (8). Effector inflammatory cells in mucosal tissue are also reduced following IT (9). Although IT is a standardized procedure

that is administered subcutaneously, there may be complications such as unpredicted anaphylactic reaction (10–12). Non-injection routes, such as nasal and sublingual administration, have been investigated and have been shown to reduce symptoms and drug intake significantly (13, 14).

Investigation of the compliance with non-injection allergen IT has revealed that the most common causes for treatment withdrawal are the discomfort of repeated nasal reactions from local nasal IT (LNIT) and the large amount of allergen intake for sublingual IT (15). There are two forms of allergen preparation, dry powder and solution, for LNIT and both forms have to be sprayed into a nostril during vocalization. This technical limitation is another drawback of LNIT. Since LNIT with steady doses of Der p has been demonstrated to be clinically effective (16, 17), it is important to attempt to improve the application technique and relieve the discomfort of the repeated nasal reaction.

We have previously demonstrated that Der p-containing paper discs can be used as an effective tool for nasal provocation without causing lower airway spasms in Der p-allergic individuals (3). Despite the fact that many papers regarding LNIT have already published, there have still been no reports using Der p containing paper discs for LNIT. In this study, a Der p-coated strip was developed to be self-administered at home after the first application and instruction was provided. A randomized, double-blind, placebo-controlled trial was carried out to evaluate the efficacy and safety of LNIT using Der p-coated strips. The clinical efficacy was evaluated before and after LNIT by measuring symptom scores. Immunomodulation was evaluated by measuring the

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concentration of IgG1, IgG4 and IgE in the sera and histamine and ECP in the nasal discharge.

METHODS

Study Design

A double-blind, placebo-controlled trial was carried out in a group of patients with allergic rhinitis and all subjects were hypersensitive to Der p as determined by skin prick tests. All patients were recruited from the allergy clinic of Taichung Veterans General Hospital and were divided into two groups: one group received LNIT weekly for a total of four months with Der p-coated strips, and the other group received NS-coated strips for the same duration. Patients were assigned to receive LNIT with Der p-coated or placebo buffer saline (NS)-coated strips at a ratio of 2:1 according to a computer-generated number. Patients were to continue therapy with antihistamine throughout the whole course of IT. The study was started in January 2007 and lasted until December 2007. The study was conducted in compliance with the requirements of the Institutional Review Board of Taichung Veterans General Hospital, and written informed consent was obtained from each patient.

Inclusion and Exclusion Criteria

A total of 35 patients fulfilled the following inclusion criteria: aged 18 to 65 years, self-reported perennial rhinitis for the previous two years, and a history characterized by early morning and night symptoms after exposure to house dust. Exclusion criteria were: symptoms of bronchial asthma and seasonal rhinitis due to pollens, nasal polyposis or other nasal obstruction, systemic or local steroid treatment or specific immunotherapy during the previous three years, severe systemic diseases, e.g., diabetes mellitus, or hypertension and concomitant medication use that could affect allergen sensitivity during the trial.

Nasal Strip Application and Nasal Discharge Collection

The LNIT was performed in the allergy clinic the first time and then self-administered after instruction of usage. Standardized Der p extract and the dilution buffer saline (NS) with 50% glycerol were purchased from Allermid Laboratories, Inc. (San Diego, CA, USA). The sterile strips were prepared, coated with a steady dose of Der p (250AU/50ul), and sealed by aluminium-foil in a GLP grade laboratory by Dr. JL Sheu, Biomedical Engineering Research Lab., Industrial Technology Research Institute, Hsinchu, Taiwan. All strips were kept and stored in a refrigerator at 4°C before use. The Der p-coated strips were used for active treatment group and the strips coated with dilution buffer saline were used as control. The strip was applied to the nasal septum for 10 min and only one strip was used each time every week. Both used and unused strips were removed and dissolved with phosphate buffer saline for the Der p 2 measurements. The concentration of Der p 2 in the strip was measured using monoclonal antibody as previous described (18). The decreased Der p 2 was considered to be absorbed. The amount of Der p absorbed was extrapolated based on the concentration of Der p 2 measured. The unused Der p coated strip was used as a control. Approximately 86% of Der p was absorbed by the nasal mucosa. Nasal discharge was collected into a 4 ml

storage vial by blowing the nose at the beginning and at the end of the whole course of therapy. Samples were collected and stored at -80°C before analysis.

DETERMINATION OF HISTAMINE AND ECP IN NASAL SECRETIONS

Nasal discharge was dissolved in 0.1% dithiothreitol (DTT, Sigma Chemical Co., St. Louis, MO, USA) in phosphate buffer saline to break disulphide bonds in the mucus (19). After the treatment with DTT, the cell pellet and supernatant were separated by centrifuged at 500G for 10 min. Supernatants were collected for histamine and ECP measurement. For the histamine assay, secretions were reacted with O-phthalaldehyde (OPA, 5 mM) for 7 min. The reaction was stopped by adding H₂SO₄ (0.04M). Histamine in the supernatant was measured by a fluorescent spectrophotometer (F4500, Hitachi, Japan) (20). ECP in nasal secretions was measured by ELISA assay (21). The nasal secretions and 5% PEG6000 were mixed and coated on 96-well plates. For detection of ECP, we used EG2 to human eosinophils (Kabi Pharmacia Diagnostics, Uppsala, Sweden). Monoclonal anti-ECP antibodies diluted to 1:1000 and the secondary antibody, horseradish peroxidase conjugated rabbit anti-mouse Ig antibodies, diluted to 1:3000 were added separately in each assay. The bound enzyme substrate was detected using phosphate 3, 3', 5, 5'-tetramethylbenzidine (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD, USA). The reaction was stopped after 30 min and the optical density was measured at 450 nm in a multiscan spectrophotometer (Model A-5682; SLT Lab Instruments, Salzburg, Austria).

DETERMINATION OF ANTIGEN-SPECIFIC IMMUNOGLOBULINS IN SERA

The allergen specific Igs in the sera were determined by ELISA. The sera were diluted five times before use. One hundred microliters of the sample solution were added to ELISA plates (Nunc Lab, IL, USA) coated with Der p (30 µg/ml) and incubated overnight at 4°C. The plates were washed three times with PBS. For detection of antibodies, alkaline phosphatase-conjugated mouse anti-human IgE antibodies diluted 1:2000 (Southern Biotech Assoc., Inc., Birmingham, AL, USA) were added separately to each assay, and the bound enzyme substrate was detected using *p*-nitrophenyl phosphate (Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD, USA). The reaction was stopped after 30 min and the optical density was measured at 405 nm in a multiscan spectrophotometer (Model A-5682; SLT Lab Instruments, Salzburg, Austria).

Results were expressed as ELISA units. The Der p-specific IgG1 and IgG4 in the sera were measured using an ELISA method, as described above. Plates were coated with 100 µl of Der p at a concentration of 30 µg/ml and stored in a refrigerator at 4°C. Detection of antibodies was carried out by using alkaline phosphatase-conjugated goat anti-mouse IgG1 or IgG4 antibodies diluted to 1:4,000 (Southern Biotech Assoc.), which was added separately for each assay, and the bound enzyme substrate was detected using *p*-nitrophenyl phosphate (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD, USA).

CLINICAL EVALUATION BY SYMPTOM SCORES

All patients were enrolled in the study after a one month of observation period. The main clinical endpoints were evaluated during a window period of five months. Efficacy criteria were: patients' diary cards on which symptoms were recorded during the study.

The monthly symptom scores (nasal stuffiness, sneezing, rhinorrhea) for each patient were obtained from symptom severity recorded daily on the patients' diary cards. The patients were asked to describe the severity of nasal symptoms using visual analog scales on a 10 cm scale with marked intervals by indicating the most severe symptoms on the scale at 10 cm and the absence of symptoms on the scale at 0 cm. Three VASs were listed on the questionnaire about the severity of symptoms of nasal stuffiness, sneezing and runny nose. The recording method was explained to the study participants by a study clinician, but the VASs were self-recorded by patients at home. Symptom scores were evaluated before and after LNIT.

Any reaction and the concomitant presence of other events were recorded on the monthly diary card. Only those patients who had three nasal symptom scores before and after LNIT were included for analysis.

STATISTICAL ANALYSIS

Symptom scores were statistically analyzed by nonparametric tests. The mean values of symptom scores in the 1st and 4th month were analyzed with the Wilcoxon test for intragroup analysis. The differences between the 1st and 4th month (the value of the 4th month subtracted from the value of the 1st month) were analyzed with the Mann-Whitney U-test for intergroup analysis. SPSS Sample Power 2.0 was used for power calculation analysis.

RESULTS

Study Population

A total of 35 patients were recruited in the study, including 24 patients (13 male and 11 female with a mean age of 30.8 ± 2.1) who underwent active treatment with Der p strips, and 11 patients (6 male and 5 female with a mean age of 28.6 ± 1.8) who underwent placebo treatment with NS strips. The most common associated symptoms were sneezing after the application of nasal strips. All the cases of dropout were within the first month of therapy. Nineteen out of 24 cases in the Der p treatment group and 9 out of 11 cases in the NS treatment group completed four months of therapy. The main reason for discontinuing treatment was due to poor response to the therapy in the first month. There were no severe adverse events or systemic reactions during the period of LNIT.

Symptom Score Analysis

The three typical symptoms of allergic rhinitis, nasal stuffiness, sneezing and runny rhinorrhea were analyzed. Patients reported these symptom scores based on the degrees of severity. There were three subjects in the active treatment group and two subjects in the placebo control group who were discarded due to with oral prednisolone for acute exacerbation of nasal symptoms. There were 23 subjects included in the symptom score analysis. The mean scores were compared

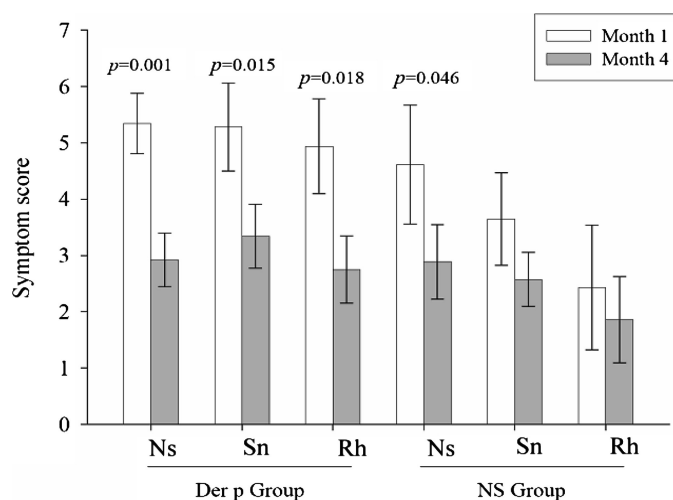


FIGURE 1.—Effects of LNIT on the symptom scores of nasal stuffiness (Ns), sneezing (Sn) and rhinorrhea (Rh). Data represented as mean $\pm$ SEM. *p*-value were determined by Wilcoxon signed-rank test.

between one month and four months of treatment. The improvements of symptoms were compared between placebo and Der p treatment groups. The results showed that all three symptoms scores were significantly improved after four months of LNIT in the Der p treatment group. However, there was no significant improvement in the NS treatment group except in nasal stuffiness (Fig. 1). When the changes of symptom scores were compared between the two treatment groups, the results showed that the improvement was greater in the Der p treatment group than in the placebo group. However, the differences were not statistically significant (Table 1).

Effect of LNIT on Der p-specific Immunoglobulins in the Sera

Der p-specific IgG1, IgG4 and IgE were measured before and after LNIT. The results showed that sera-specific IgE and IgG1 significantly decreased and IgG4 increased after LNIT in the Der p treatment group, while all three specific IgG in the sera remained similar in the NS group (Fig. 2). When the decreased amounts of IgG were compared between the two groups, the reduction of IgE and IgG1 and the increase of IgG4 were significantly higher in the Der p treatment group than in the NS treatment group with $p < 0.001$ for IgE, $p = 0.001$ for IgG1, and $p < 0.001$ for IgG4 (Table 2). Thus, LNIT with Der p can modulate Der p-specific immunoglobulin levels in the sera within four months.

TABLE 1.—The comparison of two groups on the changes of symptom scores before and after LNIT.

	Group	No. of cases	Month 4 – Month 1	<i>p</i> -value
Nasal stuffiness	Der p	16	-2.38 ± 0.66^a	0.769 ^m
	NS	7	-1.93 ± 0.85	
Sneezing	Der p	16	-1.94 ± 0.74	0.769 ^m
	NS	7	-1.07 ± 0.54	
Rhinorrhea	Der p	16	-2.19 ± 0.76	0.198 ^m
	NS	7	-0.57 ± 0.95	

^m Mann-Whitney U-test.

^a Changes of month 1 to 4. Data represented as mean $\pm$ SEM.

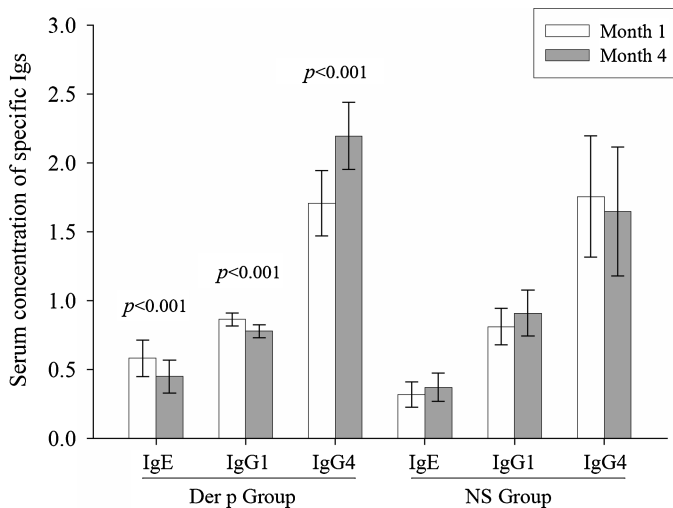


FIGURE 2.—Effects of LNIT on the serum concentration of Der p-specific IgE, IgG1 and IgG4. Data represented as mean ± SEM. *p*-value were determined by Wilcoxon signed-rank test.

Effect of LNIT on Histamine and ECP Secretion in Nasal Discharge

The inflammatory mediators in the nasal discharge before and after LNIT were measured. There were 11 samples in the Der p treatment groups and 3 samples in the NS group. In the Der p treatment group, both nasal histamine and ECP were significantly decreased after LNIT with $p = 0.003$ and $p = 0.003$, respectively, while in the NS group both nasal histamine and ECP remained similar (Fig. 3). When the decreased amounts of histamine and ECP were compared between the two groups, the reduction of histamine and ECP were significantly higher in the Der p treatment group than in the NS treatment group with $p = 0.011$ and $p = 0.038$, respectively (Table 3). LNIT with Der p can, therefore, reduce the secretion of histamine and ECP within four months; however, this was not detected in the NS treatment group.

DISCUSSION

Immunotherapy requires commitment from the patient, not only regarding safety but also regarding compliance that might be relevant to successful treatment. In this study, we developed a feasible modality for LNIT using Der p-coated strips and are the first to describe this in the English literature.

TABLE 2.—The comparison of two groups on the changes of serum concentration of specific Igs before and after LNIT.

	Group	No. of cases	Month 4 – Month 1	Power	<i>p</i> -value
Serum IgE	Der p	19	-0.13 ± 0.03^a	76%	<0.001 ^{bs}
	NS	9	0.05 ± 0.05		
Serum IgG1	Der p	19	-0.09 ± 0.02	72%	0.001 ^{bs}
	NS	9	0.10 ± 0.08		
Serum IgG4	Der p	19	0.49 ± 0.09	92%	<0.001 ^{bs}
	NS	9	-0.11 ± 0.07		

^aChanges of Igs in the sera. Data represented as mean ± SEM.

^bMann-Whitney U-test. * $p < 0.01$.

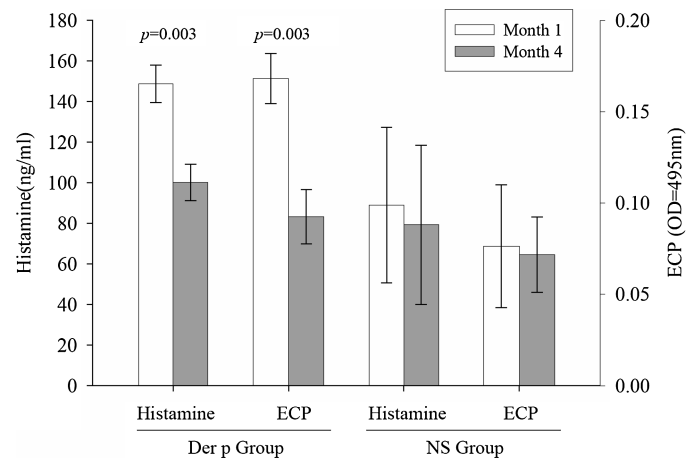


FIGURE 3.—Effects of LNIT on the histamine and ECP secretion in the nasal discharge. Data represented as mean ± SEM. *p*-value were determined by Wilcoxon signed-rank test.

Our study demonstrated the efficacy and safety of LNIT with Der p-coated strips for the treatment of allergic rhinitis without any technical limitation, and the results showed that not only nasal symptoms, but also immunological parameters could be modulated by LNIT. In comparison with placebo control groups, the improvement of nasal symptoms and changes of immunological parameters were greater after four-months of active treatment. Although there were no statistically significant differences of the subjective measured parameters of symptom scores between the two groups, the objective measured parameters of immunological changes were significantly differences after LNIT. This discrepancy might be due to the concurrent treatment with antihistamine. LNIT with Der p-coated strips can down-regulate Der p-specific IgE and IgG1 and up-regulate Der p-specific IgG4 in the sera of Der p allergic patients. The concentration of histamine and ECP in the nasal discharge was also significantly reduced. These results indicated that LNIT might have modulatory effects on both systemic immune system and local nasal airways. These results were compatible with previous reports that LNIT is capable of modulating the immunological response (22, 23). The production of local salivary IgA and systemic serum IgG2a was upregulated after LNIT in a mouse asthma model (24, 25).

The current medications for allergic rhinitis mainly act on the symptoms, including antihistamines, local steroids, cromolyn sodium, anticholinergic agents and so forth. However, therapy for individual allergic rhinitis patients requires treatment directed against the causes of allergy. Allergen-specific

TABLE 3.—The comparison of two groups on the changes of histamine and ECP secretion before and after LNIT.

	Group	No. of cases	Month 4 – Month 1	<i>p</i> -value
Histamine (ng/ml)	Der p	11	-48.63 ± 7.47^a	0.011 ^{ms}
	NS	3	-9.67 ± 2.20	
ECP	Der p	11	-0.08 ± 0.01	0.038 ^{ms}
	NS	3	-0.005 ± 0.01	

^mMann-Whitney U-test. * $p < 0.01$

^aChanges of month 1 to 4. Data represented as mean ± SEM.

IT is the only treatment recognized to be effective. It has been shown to prevent new sensitization, and the clinical efficacy of various specific nasal ITs has been documented either using aqueous extract (22, 23) or extract in powder form (26, 27). However, persistent local reaction and technical difficulty limit its application.

In this study, we demonstrated that the self-administered allergen-coated strip could improve treatment technique and adherence. Throughout the whole study period, there were no systemic adverse events. Although, there were five subjects (3 cases in active treatment group and 2 cases in control group) who had transient nasal symptoms while receiving LNIT, the symptoms could be controlled with oral prednisolone. It has been reported that LNIT frequently causes local adverse reaction (15) and the percentage of unpleasant symptoms was 56.6% and the withdrawal rate was 43.9% in children (28). However, only 21.8% of patients withdrew from this study. The discrepancy might be either due to the concurrent antihistamine treatment or due to the fact that a steady dose of allergen was used throughout the whole course of therapy. Adult patients might also be more tolerant to nasal strip therapy than children. In our study, clinical improvement was found after LNIT with a steady dose of allergen for four months. Similar clinical observations are reported in some double-blind placebo-controlled studies of LNIT within 4 months (29). The clinical efficacy and tolerability of the steady dosage schedule of allergen application has been demonstrated (28) and the effective maintenance dose at 240AU taken weekly has been reported (16). Although the statistic analysis and sample power was significant, the sample size was small. Therapy with Der p-coated strips for more patients and long duration should be further studied. Based on the issues of efficacy and safety, this study can prove the concept that the nasal mucosa is a suitable target organ for LNIT with Der p-coated strips.

In conclusion, the LNIT with a steady dose of Der p-coated strip can be self-administered at home without any severe local or systemic adverse reactions. Both local and systemic immune responses were observed after LNIT. This therapeutic modality may serve as a good alternative treatment for allergic rhinitis.

ABBREVIATIONS

LNIT	= Local nasal immunotherapy
Der p	= <i>Dermatophagoides pteronyssinus</i>
ECP	= Eosinophil cationic protein
Der p 2	= <i>Dermatophagoides pteronyssinus</i> group 2 allergen
NS	= Dilution normal saline

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