A modified schedule of multiple aeroallergen ultra-rush immunotherapy in perennial allergic rhinitis: safety, efficacy, and T lymphocyte cell population studies

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ABSTRACT

Background: This study assessed whether a modified immunotherapy schedule for allergic rhinitis could be safe and efficient. Ultra-rush immunotherapy (URIT) rapidly desensitizes patients to aeroallergens.

Objective: We aimed to develop a modified URIT protocol in 3 days to achieve the target dose while observing whether it could improve this situation and decrease the time to achieve the maintenance dose.

Methods: The URIT was exercised in 21 patients with perennial allergic rhinitis. Premedinations were given to the patients 3 days prior to the immunotherapy and during the 3 days injections immunotherapy: prednisolone, ranitidine, and Airokast/montelukast. Finally, the T cell population frequencies of patients prior to and after immunotherapy, including T helper 1, T helper 2, cytotoxic T lymphocytes, and regulatory T cells, were studied using flow cytometry. During the URIT protocol, 21 patients received 291 injections.

Result: Six patients (28.6%) showed systemic reactions in our study. All systemic reactions occurred on the third day by the 1:1 dilution of the maintenance dose. These systemic reactions occurred in three patients after 13 injections, and the three remaining patients showed systemic reactions following the last injection. No systemic reaction was observed on the first and second day of the therapy, and the risk of systemic reaction with every injection was about 2%. Among the T cell populations, CD3+ and CD8+ cells decreased significantly.

Conclusion: The findings emphasized that URIT, alongside premedication with a high dose of antihistamine, helped to achieve the maintenance dose and control clinical manifestations. © 2024 Codon Publications. Published by Codon Publications.

KEYWORDS
allergens; allergic rhinitis; immunotherapy; hypersensitivity

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Introduction

Allergic rhinitis causes several disorders, such as sleep disorders, weakness, depression, reduced attention, and impaired performance at work and school. It results in the loss of work and study performance, significantly impacting the quality of life of people suffering from this disease. The global prevalence of allergic rhinitis approximately 10-15%, of which 17-28% is in European countries. Also, the prevalence of allergic rhinitis in Iranian children and adults is about 18% and 25%, respectively.

Allergic rhinitis can be a risk factor for exacerbating other diseases, such as asthma, sinusitis, and otitis media. It has also been found that between 15% and 38% of asthma patients have symptoms of allergic rhinitis. Moreover, expensive treatment of allergic rhinitis and its related disorders, such as asthma, sinusitis, and otitis media, are challenging issues for the healthcare system. Subcutaneous immunotherapy is a modern approach to allergic rhinitis. It has several benefits, including clinical decrease in disease period. However, routine subcutaneous immunotherapy protocols have several drawbacks that have led to the evaluation of different aspects of allergic rhinitis treatments to achieve the most apparent therapeutic results and safety.

Several subcutaneous immunotherapy protocols, including a conventional protocol with weekly increasing doses to achieve optimum level within few months, are approved as an appropriate route of subcutaneous immunotherapy. Cluster protocol usually within 8 weeks reaches to optimum dose. Rush immunotherapy protocol allows eight injections in one day, then increasing the dose in 8-11 weeks to achieve optimum dose. On the other hand, ultra-rush immunotherapy (URIT) is used to obtain an effective dose within a short duration, and is effective in in the case of insect venom (wasps and bees); however, no reference is determined to use it in aeroallergens immunotherapy. Compared to conventional immunotherapies, rush immunotherapy and URIT have several priorities, such as reducing the time to achieve optimum dose in less than 1 week and reducing the production of allergen-specific immunoglobulin G4 (IgG4) antibody. In addition, it is cost-effective because of reduced injection period.

However, in spite of the benefits of rush immunotherapies, one of the biggest concerns related to these procedures is the occurrence of adverse systemic reactions. To address this drawback, administering premeditations prior to and during rush and ultra-rush immunotherapies could be effective. Therefore, the present study aimed to assess the clinical effectiveness of the mentioned immunotherapy and its generated immune responses reactions. Related adverse systemic reactions were also evaluated and compared with conventional immunotherapy.

Methods and Materials

Patients

The present clinical trial study was conducted from May 2015 to September 2016, comprising 21 patients with allergic rhinitis with an age range of 15-55 years from the immunology and allergy ward of Mashhad University of Medical Sciences, Mashhad, Iran. The patients were enrolled in the study after obtaining their written consent. The inclusion criteria of participants included clinical manifestations of allergic rhinitis, in which the prick skin test was positive by the prevalent commercial extracts of the region’s aeroallergens (Greer Laboratories, Lenoir, NC). The exclusion criteria from the study were uncontrolled asthma with forced expiratory volume 1 (FEV1) below 70% of the predicted value, remarkable cardiovascular diseases, usage of beta-blocker medications, insulin-dependent diabetes, autoimmune diseases, any history of previous anaphylactic shock, and dissatisfaction with participation and noncooperation in the study.

Study Design

This study was a prospective case series of a modified rush immunotherapy protocol to evaluate the efficacy, immunologic changes, and adverse events. All participants took the following premedication drugs started 3 days prior to the study and during 3 days of immunotherapy protocol: prednisolone 30 mg every 12 h, ranitidine 150 mg every 12 h, montelukast 10 mg once a day (OD), and telfast or fexofenadine 180 mg every 12 h. In order to assess the effects of premedication drugs or avoid using them by the patients, the histamine prick test was conducted by calculating the diameter of hives or swelling of the skin prior to the first injection of immunotherapy protocol. The demographic and clinical data and standard questionnaire of Sino-Nasal Outcome Test-22 (SNOT-22) and Mini Rhino Conjunctivitis Quality of Life Questionnaire (mini-RQLQ) of patients were recorded prior to the immunotherapy until 3 months after the intervention. The rush immunotherapy of patients based on was done using the following aeroallergens: (1) GS weed mix 1/20 w/v GP15AO3, (2) GS 7 grass mix 100.000 BAU/ML GTP27AO3, (3) GS 11 tree mix 1/20 w/v GPO714AO4, and (4) Salsola 1/20 w/v G59AO3.

Patients’ cardiopulmonary condition and local and systemic reactions were monitored during the injections until 1 h following the last injection. The systemic reactions were evaluated using the World Allergy Organization (WAO) guidelines. The patients were hospitalized up to 12 h following the last injection. Aerocast 10mg (Montelukast) and telfast 180 mg (Fexfenadine) were administered at least for 2 weeks, and prednisolone and ranitidine administrations were discontinued, following discharge from the hospital. Our protocol (Table 1) was modified based on the routine immunotherapy protocols. Immunotherapy was conducted by increasing the dose (adding 0.05 mg to the prevailing dose) of aeroallergens until attaining the optimum dose level. However, the rush immunotherapy was discontinued for patients with any sign of systemic reactions. The outpatient (after discharge) maintenance weekly therapy continued for 4 weeks and then every month.

Immunological assessment

To study immunological responses, 10 mL of ethylenediaminetetraacetic acid (EDTA) blood samples were collected...
from 15 randomly chosen patients. Intracellular and extracellular staining of peripheral blood mononuclear cells (PBMC) of the patient for flow cytometry analysis of the cytotoxic T lymphocyte (CTL \([CD3^+, \text{CD8}^+, \text{interferon gamma}^{+}, \text{IFN-}\gamma^{+}\]) , T helper 1 \((\text{Th1} \ [\text{CD3}^{+}, \text{CD4}^{+}, \text{IFN-}\gamma^{+}]\)) , T helper 2 \((\text{Th2} \ [\text{CD3}^{+}, \text{CD4}, \text{interleukin 4}^{+}, \text{IL-4}^{+}]\)) , and T regulatory cells \((\text{CD4}, \text{CD25}^{+}, \text{FoxP3}^{+}, \text{CD127}^{-})\) were performed by BD FACS Calibur flow cytometry using True-Nuclear™ Transcription Factor Buffer Set (BioLegend®, San Diego, CA, USA) and the following antibodies: anti-human CD8 FITC (BioLegend®), anti-human CD4 FITC (BioLegend®, USA), anti-human CD25 PerCP-cyanine 5.5 (BioLegend®), anti-human CD127 APC (BioLegend®), anti-human CD3 PerCP-Cyanine 5.5 (BioLegend®), anti-human IFN-\(\gamma\) PE (BioLegend®), and anti-human IL-4 PE PerCP-Cyanine 5.5 (BioLegend®).

### Statistical analysis

Descriptive statistics, paired T-test, one-way ANOVA, and post hoc tests were used with the SPSS software version 20. In addition, alternative nonparametric analyses of the above-mentioned statistical tests were considered. \(P < 0.05\) was considered as statistically significant.

### Results

#### Demographic data

In all, 21 patients, including 12 (57.1%) women and 9 (42.9%) men, with a mean age of 29.5 years, were included in the study. The clinical diagnosis of about 90% of the enrolled patients in the present study was allergic rhinitis (66%) and allergic rhinitis plus asthma (23.8%). According to the prick test results and clinical manifestations of patients, the most potential aeroallergens were selected, and the immunotherapy protocol was done on patients by the following aeroallergens: 19 (90.5%) by weed aeroallergen, 1 (4.5%) by grass aeroallergen, and 1 (4.5%) by tree aeroallergen. After 291 injections of aeroallergens, six patients showed manifestations of systemic reactions related to immunotherapy.

#### URIT affects clinical manifestations

The effects of URIT on clinical manifestations based on Mini-RQLQ and SNOT-22 questionnaires revealed that prior to and after the immunotherapy, the mean scores of SNOT-22 questionnaires were 45.2 ± 14.6 and 21.73 ± 18.67, respectively (\(P = 0.001\)). The mean scores of Mini-RQLQ questionnaire prior to and after the intervention were 35.66 ± 14.17 and 12.66 ± 10.03, respectively (\(P = 0.0001\)).

#### Role of URIT on immunological responses

Figures 1-3 present the flow cytometry analysis of patients pre- and post-URIT. URIT effects in case of 15 patients were evaluated on immunological responses by measuring...
As shown in Table 4, alterations in other cell groups were not statistically significant ($P > 0.05$).

**Discussion**

The present study was conducted to evaluate the effectiveness of a modified URIT and its generated immune responses and the related systemic adverse reactions,
Figure 3 Flow cytometry analysis of regulatory T lymphocyte cells. The PBMC of patients were collected along with the intracellular and extracellular markers of regulatory T lymphocyte cells (CD4+, CD25+, Foxp3+, and CD127-).

Table 3 Immunological response profiles prior to and after ultra-rush immunotherapy.

<table>
<thead>
<tr>
<th></th>
<th>Before immunotherapy (mean±SD)</th>
<th>After immunotherapy (mean±SD)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ MFI of CD3+ and CD4+-gated cells (Th1)</td>
<td>179.21±123.62</td>
<td>246.50±127.28</td>
<td>0.164</td>
</tr>
<tr>
<td>IFN-γ MFI of CD3+ and CD8+-gated cells (CTLs)</td>
<td>103.35±54.48</td>
<td>102.91±80.84</td>
<td>0.983</td>
</tr>
<tr>
<td>IL-4 MFI of CD3+ and CD4+-gated cells (Th2)</td>
<td>20.22±10.95</td>
<td>24.69±14.03</td>
<td>0.514</td>
</tr>
<tr>
<td>FoxP3 MFI of CD4+, CD25+, and CD127-gated cells (Th2)</td>
<td>18.52±20.00</td>
<td>23.34±9.67</td>
<td>0.519</td>
</tr>
</tbody>
</table>

*Statistical analysis was done by Independent T-test.

Table 4 Frequency of T lymphocyte subpopulations, pre- and post-ultra-rush immunotherapy data presented as mean percentage ± SD.

<table>
<thead>
<tr>
<th>Cell populations</th>
<th>Before immunotherapy</th>
<th>After immunotherapy</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+ and CD8+ cells</td>
<td>37.46±8.86</td>
<td>33.26±8.65</td>
<td>0.032</td>
</tr>
<tr>
<td>CD3+ and CD4+ cells</td>
<td>49.26±5.39</td>
<td>51.90±5.53</td>
<td>0.320</td>
</tr>
<tr>
<td>CD4+, CD25+, Foxp3+, and CD127 cells (Treg)</td>
<td>1.25±0.79</td>
<td>0.94±0.39</td>
<td>0.248</td>
</tr>
<tr>
<td>CD3+, CD4+, and IL-4+ cells (Th2)</td>
<td>1.54±0.49</td>
<td>2.06±0.69</td>
<td>0.059</td>
</tr>
<tr>
<td>CD3+ CD4+ IFN-γ+ cells (Th1)</td>
<td>11.40±4.41</td>
<td>11.56±5.38</td>
<td>0.923</td>
</tr>
<tr>
<td>CD3+, CD4+, and CD8+ cells (CTL)</td>
<td>23.19±7.27</td>
<td>24.01±6.97</td>
<td>0.607</td>
</tr>
</tbody>
</table>

*Statistical analysis was done by Independent T-test.
compared to the routine immunotherapy used for allergic rhinitis.\textsuperscript{18}

Our hypothesis was to reduce the time to attain maintenance dose to relieve symptoms sooner and convince the patients to continue with the therapy. Thus, a modified rush immunotherapy protocol was prepared to achieve a monthly maintenance dose. This protocol replaced the weekly build-up plan after the rush immunotherapy, and the patients attained the maintenance dose on the third day of the therapy. The purpose in this study was to assess the immunity level and immune responses of patients with allergic rhinitis after using new immunotherapy method, which to our knowledge was carried out for the first time in the clinic. Systemic reactions through this protocol were 28.6%, almost similar to the literature,\textsuperscript{18,19} which reported 20-33% systemic reactions. However, Cox et al. reported more systemic reactions in immunotherapy with inhaled allergens (in 27-100% of patients after rush immunotherapy and 0-79% of patients post cluster immunotherapy).\textsuperscript{20,21} In a rush immunotherapy study, systemic reactions were observed in 38% of patients.\textsuperscript{22} Bousquet et al. reported that 34.4% of dust mite-sensitive, allergic asthma patients demonstrated systemic reactions after rush immunotherapy.\textsuperscript{23} In all the above-mentioned studies, the rush immunotherapy protocol was achieved in 1 day (equivalent to the first day of our protocol), followed by a weekly build-up plan for attaining the maintenance dose.

Immunotherapy procedures reduce the activity of mast cells and basophils, such as cytokine secretion, mast cell priming by antigen-specific IgE antibodies, and degranulation of the granules containing allergy-induced components. This selective suppression is affected by alterations in different immune system parameters, including reducing allergen-specific IgE levels and specific Treg cells frequency. Suppression of high-affinity IgE receptor, FcεRI, activates basophils with selective suppression of H2 receptor-mediated histamine, and release of sulfide-leukotrienes (LTs) could be associated with fast induction of allergen tolerance and desensitization effect, particularly in venom immunotherapy.\textsuperscript{24}

Histamines are a low molecular weight monoamine components that bind to four different G-protein receptors with different effects on immune responses. Released histamine induces peripheral tolerance by several mechanisms. Stimulation of H1 receptors enhances Th1 responses,\textsuperscript{25} because CD4+. Th1 cells express H1 receptors in themselves.\textsuperscript{26} Because of different effects of histamines on immune responses via H1 and H2 receptors, injecting a high dose of allergens in URIT could release high amounts of histamines. However, the low frequency of systemic effects in our study could be due to the high dose of administered antihistamine, compared to the results of previous studies, in which suppression of H1 receptors stopped the production of histamines by basophils and mast cells.\textsuperscript{27}

The systemic reactions that occurred during premedication could be due to some unknown mechanisms, hence precautions must be taken. Our study's 3-month clinical results were similar to conventional subcutaneous immunotherapy, which was done by Dolz et al.\textsuperscript{28} In order to evaluate immune responses after rush immunotherapy, a study was conducted by Lack et al. involving 10 children with asthma, allergic to dust mites.\textsuperscript{29} It showed that after 2-4 weeks of rush immunotherapy, 1-2 days after attaining the maintenance dose, the allergen-specific IgE and IgG4 were reduced and T helper cells proliferation was suppressed, but the frequency of CD8+ cytotoxic T cells was increased.\textsuperscript{29} In the present study, induction of CTL frequency was observed, although not statistically significant (P > 0.05). Specific IgE and IgG4 were not evaluated in our study. Since the level of specific IgE and IgG4 can be induced by immunotherapy, evaluating specific IgE and IgG4 is strongly recommended for future studies.

In our study, the evaluation of immune responses and changes in clinical manifestation were monitored up to 3 months after the maintenance dose, which may not be a convenient time to observe changes in immune responses. We suggest studying various time points post-immunotherapy. The limitation of our study was the lack of control groups for conventional and rush immunotherapy in order to compare results. Therefore, amelioration in patients was compared with their first-day conditions prior to URIT.

Different unknown mechanisms may be involved in the induction of tolerance by the rapid protocols of immunotherapy, such as rush immunotherapy and URIT, which are different from conventional immunotherapy. It is obvious that with a bigger sample size, normalization of patients and standardized assays of immunological responses could obtain the exact effect of URIT.

Conclusion

The present study showed that URIT, combined with premeditations and continuing taking an antihistamine - Leukotriene inhibitor daily for 2 weeks after the rash phase could help lessen clinical manifestations. In our study, the timing and number of allergens injected to induce systemic reaction were not similar to previous studies. The reactions in our study were at much higher doses than in previous studies. Therefore, URIT with premeditations could be a candidate for optimal immunotherapy to treat allergic rhinitis, particularly for patients to whom advanced health facilities are unavailable.

Ethics approval and consent to participate

The study was approved by the Ethical Committee of Mashhad University of Medical Sciences (IR.MUMS. fm.REC.1394.362) and trial registration: Iranian Registry of Clinical Trials, IRTC2017010123235N8; Registered: 25 June 2017; retrospectively registered at: https://www.irtc.ir/trial/19853.

Availability of data and materials

The datasets generated and analyzed during the current study are not available publicly because of patients' data and ethical issues, but are available from the corresponding author on reasonable request.
Competing interests

The authors declared that they had no competing interests.

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Author contributions

Ali Fouladvand, Reza Farid Hosseini, Maryam Khoshkhui, and Farahzad Jabbari carried out treatments, selected patients, and followed. Nazila Ariaee, Mojgan Mohammadi, Amin Reza Nikpoor, and Jalil Tavakkol-Afshari performed immunological examinations. Nazila Ariaee analyzed data and designed the project. All authors read critically and approved the final manuscript.

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