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Jacobs Journal of Allergy and Immunology

Research Article

Vitamin D3 plus *Lactobacillus reuteri* DSM 17938 as Adjuvant for Allergen Immunotherapy: A Preliminary Experience

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Received: 11-08-2014

Accepted: 01-30-2015

Published: 02-06-2015

Abstract

Background

Allergen immunotherapy (AIT) restores T regulatory cells function and reduces Th2 polarization. Vitamin D3 (VitD3) and *Lactobacillus reuteri* DSM 17938 exert immune-modulatory activities, including T regulatory cells stimulation. Thus, their use as adjunctive therapy to AIT might be useful in improving AIT effectiveness.

Objective

This preliminary experience aimed at investigating whether a food supplement containing VitD3 plus *Lactobacillus reuteri* DSM 17938 could be effective in improving the effectiveness of a single pre-co-seasonal AIT course to Parietaria in patients with seasonal allergic rhinitis.

Methods

Globally, 30 subjects (15 males) with a mean age of 35.63 (± 11.12) years were evaluated. All patients were treated with a single pre-co-seasonal course of sublingual immunotherapy. The food supplement (VitD3 800 UI/die and *Lactobacillus reuteri* DSM 17938 20^8 CFU/die) was taken in 15 patients for 30 days since the AIT start. Patients' perception of symptom severity, medication use, and AIT efficacy were assessed by visual analogue scale, comparing the previous pollen season (2013) with the present one (assessed in July 2014).

Results

Patients treated with food supplement perceived less symptom severity (p=0.033) and medication use (p=0.047), and better AIT efficacy (p=0.01) than patients treated with AIT alone.

Conclusion

This pilot study suggests that VitD3 plus *Lactobacillus reuteri* DSM 17938 supplementation might improve the effectiveness of a single pre-co-seasonal SLIT course in reducing symptom severity and medication use in patients with seasonal allergic rhinitis.

Keywords: Vitamin D3; *Lactobacillus reuteri*; Seasonal Allergic Rhinitis; Allergen Immunotherapy

Introduction

Allergic patients have paradigmatically an allergen-specific functional defect of T regulatory cells (Tregs) and a Th2-polarized immune response [1]. This characteristic immunological pattern promotes IgE production and mucosal inflammation, which is closely dependent on the causal allergen exposure. Indeed, after inhaling sensitizing allergen, allergic subjects develop inflammation and present symptoms [1]. This axiom constitutes the rationale for the optimal treatment of allergic disorders: the avoidance of allergen exposure. Unfortunately, this choice is rarely feasible. Therefore, allergen immunotherapy (AIT) is at present the unique possible cure for allergy, as it may induce immunological and clinical tolerance toward the causal allergen. AIT induces longstanding immunological changes, such as switching the imbalanced immune response toward a physiological Th1 polarization and restoring allergen-specific Tregs function [1]. Currently, Tregs are considered the most relevant actors responsible for AIT clinical success. In this scenario, it was proposed that strategies to facilitate Tregs induction using adjuvants combined with AIT might be promising to improve its efficacy [2]. AIT adjuvants are mainly immune-modulatory molecules. Recently, some interest was spent for vitamin D3 (VitD3) as adjuvant for AIT. The rationale for using VitD3 is based both on its immune activity and the demonstration that low serum VitD3 levels correlate with development of allergy, asthma onset, and impaired lung function, mainly in children [3]. After uptake in the gut, vitamin D is hydroxylated in the liver and then in the kidney so it is transformed in 1,25-dihydroxyvitamin D3 or calcitriol, which is the metabolically active form of VitD3. VitD3 exerts its biological activities through binding to the VitD3 (VDR) receptor, a nuclear hormone receptor, which dimerizes with retinoic X receptor, and this dimer binds to VitD-responsive elements in the promoter region of VD3-responsive genes. VDR is widely express on the surface of dendritic cells (DC) as well as other immune system cells. The main effect on AIT is the induction of DC with tolerogenic properties, consequently enhanced production of IL-10 occurs [4]. These tolerogenic DCs are able to induce Tregs subpopulation expansion by an increased production of IL-10 able to mature T cells toward Treg lineage [5]. On the basis of this background, some animal studies explored the possible VitD3 adjuvant effect on AIT [6-8]. Thereafter, few human studies addressed this issue with conflicting outcomes [9-11]. On the other hand, there is also growing interest concerning the possible immune-modulatory effects exerted by probiotics on allergic disorders [12]. In this regard, a murine model was used to investigate whether probiotic may exert an adjuvant effect on AIT [13].

On the basis of this background, the aim of this study was to investigate whether a recently available food supplement containing the combination of VitD3 plus *Lactobacillus reuteri* DSM 17938 could exert adjunctive effect to pre-co-seasonal AIT course to Parietaria in improving symptoms and reducing medication use in patients with seasonal allergic rhinitis (SAR).

Materials and Methods

Sublingual immunotherapy (SLIT) is commercially available and is prescribed for indications that are recognized both nationally and internationally. Similarly, the food supplement containing VitD3 plus *Lactobacillus reuteri* DSM 17938 is regularly available. Therefore, both AIT and food supplement were prescribed on the basis of the common clinical practice. Inclusion criteria were: i) documented diagnosis of SAR, based on patient-reported symptoms and physical examination, ii) documented sensitization (such as positive skin prick test and/or presence of allergen-specific serum IgE) to Parietaria pollen, iii) clinically relevant allergic symptoms (such as really perceived and bothersome symptoms), iv) demonstration of a consistent relationship between inhalation of sensitizing allergen and occurrence of respiratory symptoms for defining the causal allergen (such as true allergy), and v) AIT prescription with Parietaria extract. Exclusion criteria were: i) suffering from other allergic diseases (i.e. atopic dermatitis, eczema), ii) clinically relevant anatomic impairment (such as septum deviation or nasal polyps), iii) acute or chronic disorders representing a contraindication to AIT (e.g. autoimmune disease, malignancy, etc), iv) poly-allergy (such as true allergy to more allergens) and v) non-allergic (vasomotor) rhinitis.

This was a retrospective clinical study conducted in patients followed in a private practice and with indication for Parietaria AIT. The study was conducted according to good clinical practice study guidelines. A written informed consent was obtained in all patients.

Globally, 30 subjects (15 males, [mean age: 35.63 ($\pm$ 11.12)] were visited and enrolled. SLIT (Staloral 300, Stallergenes Italia, Milan, Italy) was administered as a pre-co-seasonal course (starting in October-November and lasting 3 months) in all patients. The maintenance dose was of 2 pressures (corresponding to 105 mcg) administered 3 times a week on alternate days. The food supplement containing VitD3 plus *Lactobacillus reuteri* DSM 17938 (Reuterin D3, Noos S.r.l. Rome, Italy) was taken in 15 patients for 30 days as a single daily administration: 10 drops (VitD3 = 800 IU; *Lactobacillus reuteri* = 20^8 CFU) in the morning. This food supplement was taken during the first AIT month. All patients could not take other supplements or vitamin D anyway during the study period. The remaining 15 patients were considered as control group.

Patients' perception of symptom severity and medication use were assessed by visual analogue scale, comparing the previous pollen season (2013) with the present one (assessed in July 2014).

AIT efficacy was assessed by the patient's perception of improvement, using visual analogue scale (VAS) according to validated criteria [14]. The AIT effectiveness was evaluated, considering both clinical severity and drug use reduction [15]. VAS must assess a global evaluation including all symptoms (for eye: itching, tearing and redness; for nose: itching, sneezing, rhinorrhea and obstruction). Antihistamines and intranasal corticosteroids were prescribed on demand and the perception of their use was assessed by VAS. In this study, the VAS was a 10-cm horizontal line on which 0 implied no symptom or drug use, while 10 corresponded to very severe symptom or maximal drug use. With a movable marker, the patient could mark any point on the 10-cm segment which best described his/her perception. No interval marker was visible on the line. ANOVA followed by Sheffè post hoc test was used to compare VAS symptoms and VAS drugs before and after AIT in control and ancillary treatment groups. Unpaired t test was used to detect differences in: i) improment in VAS symptoms and in VAS medication use and ii) in VAS efficacy between the 2 groups i.e. control and ancillary treatment groups. Statistical analysis was performed with the GraphPad software package (GraphPad Prism Software Inc, San Diego, CA, USA). P values less than 0.05 have been considered as statistically significant.

Results

At baseline, the two sub-goups were homogeneous about age, gender, SAR duration, and severity of symptoms as well as medication use (Table 1). All allergic patients achieved a significant improvement after the pre-co-seasonal SLIT course.

Parameter	Treated Group	Control Group	p
Males	7	8	n.s.
Age	35.1 ± 2.2	36.1 ± 2.6	n.s.
VAS for symptoms	8.07 ± 1.03	8.0 ± 1.07	n.s.
VAS for medications	5.37 ± 1.3	5.27 ± 1.03	n.s.
SAR duration (years)	8.2 ± 3.5	8.4 ± 4.1	n.s.

Table 1. Demographic and clinical characteristics of patients at baseline (n.s. = non significant).

Intragroup analysis

Comparing 2013 to 2014 pollen season, the control group had a decrease in symptom severity perception from 8.00 (± 1.07) to 3.53 (±1.13) ($p<0.001$), and in perception of medication use from 5.27 (± 1.03) to 1.67 (± 0.90) ($p<0.001$).

Similar results were obtained in the patients who took the food supplement: comparing 2013 to 2014 pollen season, symptom severity perception decreased from 8.07 (± 1.03) to 2.47 (± 1.06), and in perception of medication use from 5.37 (± 1.3) to 0.87 (±0.92) ($p<0.001$ for both).

Intergroup analysis

The improvement in symptom severity and medication use after AIT was significantly higher for patients treated with adjuvant therapy compared to control group (p=0.033 and p=0.047 respectively), as reported in Figure 1A and 1B.

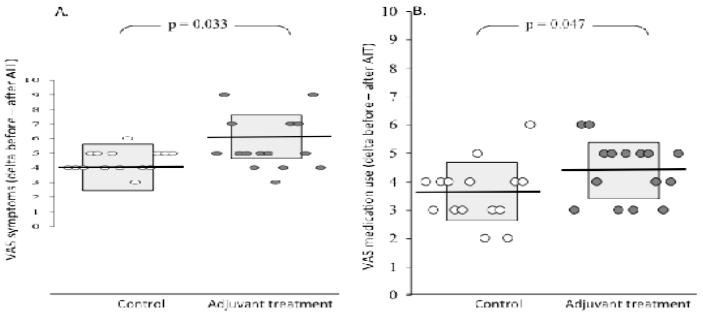

Figure 1. Improvement in symptom severity (panel A) or in medication use (panel B) assessed by VAS after allergen immunotherapy (AIT) in controls and in adjuvant treatment group.

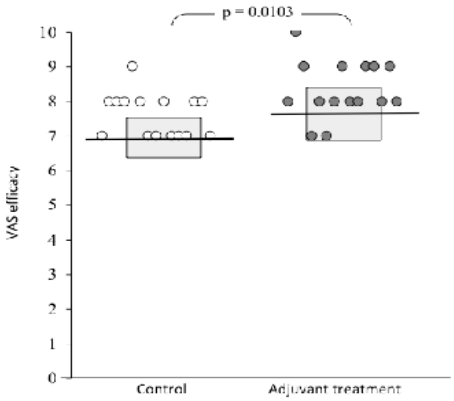

Figure 2. Perception of allergen immunotherapy (AIT) efficacy assessed by VAS in controls (white circle) and in group treated with adjuvant (grey circle).

The assessment of AIT efficacy showed that all patients perceived AIT as effective treatment, but patients treated with food supplement perceived a better effectiveness than control group (p=0.01), as reported in Figure 2.

Safety: all patients completed the treatment and there were no relevant side effects.

Discussion

AIT is at present the unique cure for allergic disorders as it may exert a longstanding modification of the natural history of allergic reaction. AIT is able to restore a physiological immune tolerance towards the causal allergen. AIT effectiveness is fast

insomuch as patients achieve clinical improvement just after the first course. The clinical outcome is closely associated with immunological changes, including increased IFN- γ production [16], reduced Th2-dependent cytokines release [17], and restored Tregs function [18]. On the other hand, there are some patients who do not respond to AIT or slowly respond. Therefore, the use of adjuvants in combination with AIT could be a suitable strategy to improve the AIT efficacy.

VitD3 and probiotics are interesting candidates in this issue. VitD3 supplementation has been evaluated in some experimental and clinical studies, but with conflicting results [6-11]. Taher and colleagues showed in a murine asthma model that the co-administration of $1,25(OH)_2D_3$ potentiates the efficacy of ovalbumin immunotherapy as significantly inhibited airway hyperresponsiveness and potentiated the reduction of serum specific IgE, airway eosinophilia, and Th2-related cytokines concomitant with increase of IL-10 in lung tissue as well as of serum TGF-β and specific IgA [6]. Van Overtvelt and colleagues used a combination of VitD3 plus dexamethasone (Dex) as SLIT adjuvants in asthmatic mouse [7]. VitD3/Dex induced IL-10 production by DC. In addition, following stimulation with VitD3/Dex-pretreated DC, CD4+ naïve T cells exhibited a Treg profile. Therefore, these authors concluded that as VitD3/Dex induce a Tregs polarization, they may be adjuvants for enhancing SLIT efficacy in the murine asthma model. Grundstrom and coworkers used a mouse model of cat allergy and tested whether VitD3 could improve AIT effects [8]. Treatment with Fel d 1 immunotherapy plus VitD3 decreased bronchial hyperreactivity, cellular infiltrate, and Th2 cytokines in bronchoalveolar lavage fluids in comparison to untreated mice. Thus, these authors concluded that VitD3 is a novel immunomodulatory candidate which may improve efficacy and safety of AIT. Of course, the limitation of these studies is that they were conducted in animal models. Majak and colleagues performed a clinical study in asthmatic children treated with AIT [9]. The children received a single dose of oral steroid (20 mg of prednisone) with or without VitD3 (25 mcg of cholecalciferol) or placebo only on the day of the build-up phase of AIT. Children treated with the adjuvant reported less clinical improvement than the control group. However, VitD3 seemed to neutralize the negative effects provided by steroid. This study had some limitations, including the lack of dose-finding assessment, the single administration, and the timing and the route of administration of both medications that may be critical. The same group correlated the serum VitD3 levels with Foxp3 induction and steroid-sparing effect of immunotherapy in children with asthma [10]. This retrospective study demonstrated that children with higher serum VitD3 levels experienced more significant reduction in asthma symptoms and steroid use and had higher TGF-β and higher Foxp3 induction during AIT. These authors concluded that serum VitD3 levels > 30 ng/mL seem facilitate the optimal AIT effect. Therefore, this study indirectly underline the role of VitD3 in AIT effectiveness. Very recently, a Turkish study evaluated the adjuvant effect of VitD3 (650 IU/day) in children treated with subcutaneous AIT for house dust mites [11]. The findings showed that VitD3-supplemented AIT induced less asthma symptoms and higher proportion of Foxp3+ cells than AIT alone. The authors concluded that the favourable VitD3 outcomes warrant further investigations.

About probiotics, there is only a study performed using the murine model [13]. This study evidenced that *Bifidobacterium adolescentis, B. bifidum, and B. longum* induced DC maturation and polarized naïve T cells toward Th1 and Tregs phenotype. *B. bifidum* provided the most interesting activity. Thus, the authors concluded that *B. bifidum*, due to its capacity to reorient established Th2 responses toward Th1/regulatory T cell profiles, may be a valid candidate adjuvant for AIT.

Therefore, the present study aimed at verifying whether a food supplement containing VitD3 plus *L. reuteri* DSM 17938 could improve the efficacy of one pre-co-seasonal SLIT course on symptom severity and medication use in a group of patients with pollen-induced allergic rhinitis.

The present preliminary experience suggests that food supplementation with VitD3 plus *L. reuteri* DSM 17938 might significantly improve the patient's perception of AIT effectiveness. In fact, patients taking VitD3 plus probiotic reported a significantly more remarkable benefit both concerning symptom severity and medication use and perceived better AIT efficacy than patients treated with AIT alone.

The possible explanation of this effect may depend on the immune-modulatory activity exerted by both compounds. VitD3 may induce generation of adaptive Tregs [19]. In fact, the complex VitD3/VDR generate antigen-specific tolerance promoting maturation of DC toward a phenotype characterized by stimulation of Tregs [20,21]. This effect may improve the mechanism of action of AIT on the allergen-specific immune response. *Lactobacillus reuteri* DSM 17938 may exert some immunological activities, including increase of IL-10 levels and reduction of IL-2 levels in the exhaled breath condensate of asthmatic children [22]. Therefore, *Lactobacillus reuteri* DSM 17938 supplementation could exert an adjuvant benefit to AIT treatment, acting as a synergistic factor so improving the AIT effectiveness. This issue might suggest a practical implication about AIT management: patients starting immunotherapy course could adjunct VitD3 and *Lactobacillus reuteri* DSM 17938 to boost AIT.

However, this study has some limitations: i) it was conducted as retrospective study, ii) the number of patients was restrict-

ed, iii) the vitamin D status was not assessed, and iv) mechanism (such as Treg-Foxp3, cytokines, dendritic cells, etc) were not evaluated . For these reasons, further conclusions cannot be done as this report is a preliminary observation. In this regard, a further randomized, double blind, placebo-controlled, and multicenter study is mandatory for confirming the clinical value of this preliminary experience, for providing evidence to justify the use of the adjuvant to immunotherapy, and for adressing the considered limitations.

Conclusion

This pilot study seems to suggest that VitD3 plus *Lactobacillus reuteri* DSM 17938 supplementation might improve the effectiveness of a single pre-co-seasonal SLIT course to reduce symptom severity and medication use in patients with seasonal allergic rhinitis.

Disclosure

The authors state that there is no conflict of interest about this manuscript, but the publication fee was provided by an unrestricted fund by Noos, Rome, Italy.

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