Abstracts & Posters

AAAAI/WAO 2018 Joint Congress
Orlando, FL, United States

ALK

CONTENTS

Dear AAAAI delegate

ALK welcomes you to the AAAAI/WAO 2018 Joint Congress in Orlando, FL.

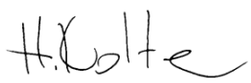
As the world leader in allergy immunotherapy (AIT), we are proud to present eight abstracts focusing on house dust mite allergy and the recently introduced SQ HDM SLIT-tablet with demonstrated efficacy in patients suffering from house dust mite induced allergic rhinitis.

The clinical performance of the SQ HDM SLIT-tablet is in focus for four of the abstracts. Improvement in quality of life and eye symptoms after treatment are subjects of discussion as well as the occurrence of adverse events, such as systemic allergic reactions, severe swellings and eosinophilic esophagitis.

Other abstracts focus on consistency in composition and potency of the SQ HDM SLIT-tablet and a comparative study on bioavailability of different products for sublingual house dust mite immunotherapy. Finally, one abstract looks at the prevalence of different house dust mite species across the United States.

ALK is committed to sustain, develop and disseminate allergy immunotherapy and anaphylaxis management worldwide.

Enjoy the congress and please join us at our stand in the exhibition area to learn more about our concepts and ongoing research activities at ALK.



Hendrik Nolte, MD, PhD
Senior Vice President
Research & Development

Occurrence of systemic allergic reactions and severe swellings with SQ HDM SLIT-tablet

Bernstein DI[1], Maloney J[2], Svanholm Fogh B[3], Nolte H[2], Epstein T[4]
[1]University of Cincinnati and Bernstein Clinical Research Center, OH, USA; [2]ALK, Bedminster, NJ, USA; [3]ALK, Hørsholm, Denmark; [4]Allergy Partners, Indiana, US & University of Cincinnati, OH, USA

Rationale

Systemic allergic reactions and severe swellings are potential risks during at-home treatment with sublingual allergy immunotherapy (SLIT). This abstract presents treatment-related systemic allergic reactions and severe swellings from 2 double-blind, randomized, placebo-controlled trials with the SQ house dust mite (HDM) SLIT-tablet (ALK, Denmark) conducted in North America (trial A, clinicaltrials.gov: NCT01700192) and Europe (trial B, EudraCT: 2011-002277-38).

Methods

Subjects (N=741 in trial A, N=318 in trial B) with moderate-to-severe HDM allergic rhinitis were treated with the SQ-HDM SLIT-tablet (dose of 12 SQ-HDM/day) for up to 1 year. Treatment-related adverse events are summarized.

Results

In trial A, there was 1 systemic allergic reaction (non-serious, assessed as moderate, occurring on day 1, treatment: epinephrine IM and desloratadine/pseudoephedrine) and 1 severe swelling (nasal edema, non-serious, day 66, treatment: antihistamine). In trial B, there were no systemic allergic reactions and 2 severe swellings (lip edema, non-serious, day 274, treatment: antihistamine; and edema mouth, non-serious, day 15, treatment: none).

Conclusions

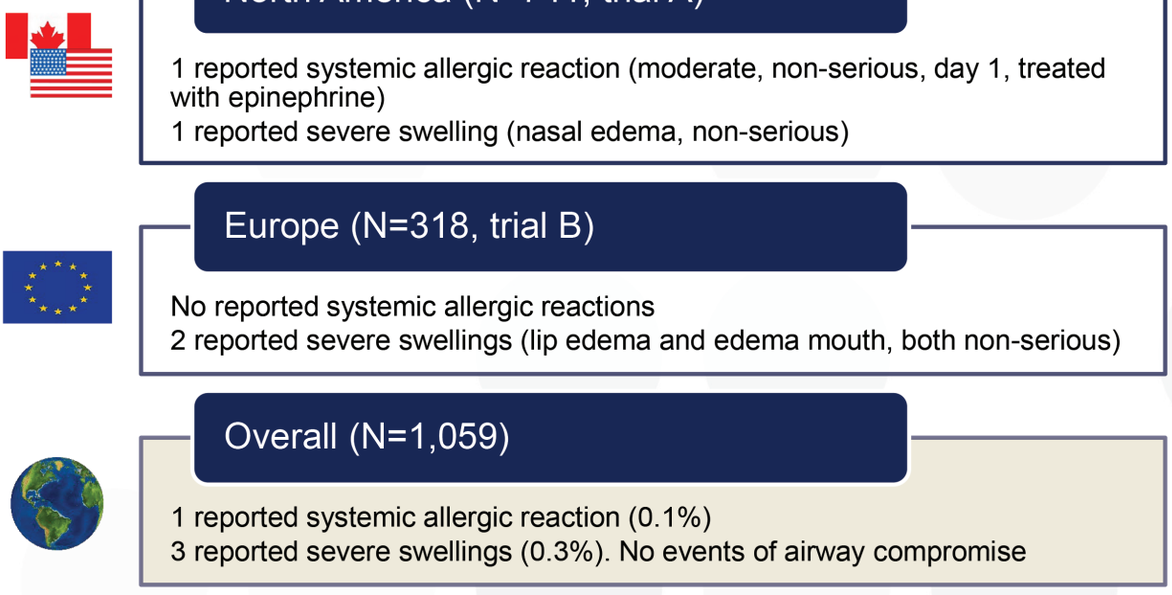
In the 2 phase III trials in North America and Europe, 1 non-serious, treatment-related systemic allergic reaction was reported. This event occurred on day 1 of treatment with the HDM SLIT-tablet within the recommended 30 minutes observation period at the clinic. There were 3 events of severe swelling, none of these resulted in airway compromise. All events were assessed as non-serious. Overall, this is a prevalence of 0.1% for systemic allergic reactions and 0.3% for severe swellings. Treatment with the SQ HDM SLIT-tablet appears to be safe for at-home administration.

Abstract Number: 598
Session Number: 3606
Session Title: Immunotherapy
Presentation Date: Sunday, March 4, 2018
Presentation Time: 2:00pm to 3:15pm

Occurrence of Systemic Allergic Reactions and Severe Swellings with SQ HDM SLIT-tablet

D I Bernstein[1], J Maloney[2], B Svanholm Fogh[3], H Nolte[2], T Epstein[4]

[1]University of Cincinnati and Bernstein Clinical Research Center, Ohio, US; [2]ALK, US; [3]ALK, Denmark; [4]Allergy Partners, Indiana, US & University of Cincinnati, Ohio, US



References: Nolte et al, JACI 2016; Demoly et al, JACI 2015

Treatment with SQ HDM SLIT-tablet improves Quality of Life in house dust mite induced allergic rhinitis

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Objective

To determine the improvement in QoL for people with HDM induced AR treated with SQ HDM SLIT-tablet assessed by the Rhinitis Quality of Life Questionnaire (RQLQ).

Methods

The RQLQ was included in 3 randomized, double-blind, placebo-controlled clinical trials (P001, P003 and MT-06) evaluating the efficacy and safety of SQ HDM SLIT-tablet in North American and European subjects with HDM induced AR, with or without allergic asthma. The RQLQ was assessed and recorded at multiple time points throughout the trials.

Results

QoL measured by the RQLQ consistently improved across all 3 trials. The groups receiving SQ HDM SLIT-tablet had larger increases in their QoL as compared to the placebo groups. The differences in improvement from baseline between the placebo and active groups were statistically significant in all 3 studies; P001: 0.28 (1.84 vs. 1.56), p<0.001, P003: 0.56 (2.47 vs. 1.91), p=0.010 and MT-06: 0.19 (1.58 vs. 1.38), p=0.031. Consistent results were also seen across the individual domains of RQLQ. In P001, the difference in improvement from baseline for all domains reached statistical significance for SQ HDM SLIT-tablet compared to placebo. This was also the case for all domains but "eye symptoms" in P003, whereas MT-06 showed statistically significant differences in 4 domains: "nasal symptoms", "non-nose/non-eye symptoms", "practical problems" and "sleep" domains.

Conclusions

The RQLQ results from 3 randomised, double-blind, placebo-controlled clinical trials show that the SQ HDM SLIT-tablet consistently improves the QoL of people suffering from HDM induced AR across slightly different populations and in different settings.

Poster Number: 521
Session Number: 3209
Session Title: Rhinosinusitis: QOL and Newer Therapies
Poster Hall Location: Convention Center, South Concourse, Level 1, South Hall A2
Presentation Date: Sunday, March 4, 2018
Presentation Time: 9:45am-10:45am

Treatment with SQ HDM SLIT-Tablet Improves Quality of Life in House Dust Mite Induced Allergic Rhinitis

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Background

It is well established that symptoms of allergic rhinitis (AR) are associated with poor quality of life (QoL).[1] AR symptoms impact daily activities, incl. both sleep and work.[1,2] Failure to get a good night's sleep has profound impact on functioning during the day, thus adding to the burden of AR.[1] The SQ HDM SLIT-tablet has been shown to effectively reduce AR symptoms.[3,4,5] The aim of this analysis was to determine the improvements in QoL assessed by the Rhinitis Quality of Life Questionnaire (RQLQ) for people with house dust mite (HDM) induced AR treated with the SQ HDM SLIT-tablet.

Methods

The RQLQ was included in three randomized, double-blinded, placebo-controlled clinical trials (P001[3], P003[4] and MT-06[5]) evaluating the efficacy and safety of the SQ HDM SLIT-tablet in North American and European subjects with HDM induced AR, with or without allergic asthma. The RQLQ was assessed and recorded at multiple time points throughout the trials. Using the RQLQ values recorded during the efficacy evaluation period (P001: last 8 (of 52) weeks, P003: after 24 weeks, MT-06: last 8 (of up to 52) weeks) this analysis assesses the difference in RQLQ between the SQ HDM SLIT-tablet and placebo using an ANCOVA with treatment, asthma status (P001) and region as a fixed effect, baseline RQLQ scores as a covariate and adjusted for different error variation for each treatment group.

Results

The analysis showed that QoL measured by the RQLQ consistently improved across all three trials. The groups receiving the SQ HDM SLIT-tablet had larger increases in their QoL as compared to the placebo groups.

Table 1: RQLQ during efficacy evaluation period, P001

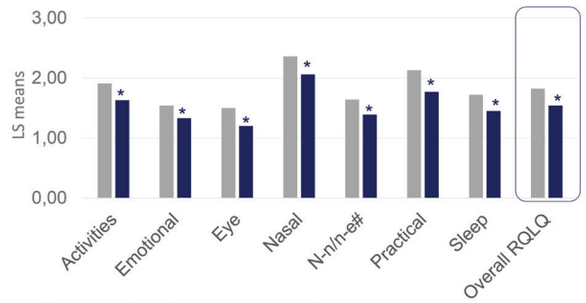


Table 2: RQLQ end of trial , P003

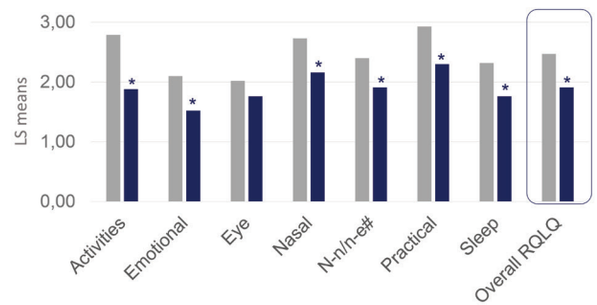
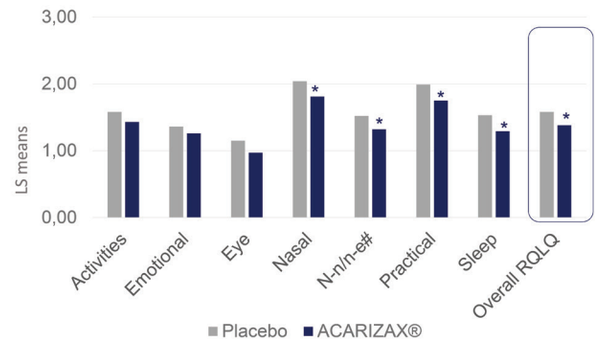


Table 3: RQLQ during efficacy evaluation period, MT-06



#non-nose/non-eyes *p<0.05

The RQLQ measured at end of trial showed a statistically significant difference between the placebo and active groups in all three studies; P001: 0.28 (1.84 vs. 1.56), p<0.001 (table 1), P003: 0.56 (2.47 vs. 1.91), p=0.010 (table 2) and MT-06: 0.19 (1.58 vs. 1.38), p=0.031 (table 3). Consistent results were also seen across the individual domains of RQLQ. In P001 the difference in all domains of RQLQ reached statistical significance for the SQ HDM SLIT-tablet compared to placebo. This was also the case for all domains but "eye symptoms" in P003, whereas MT-06 showed statistically significant differences in four domains: "nasal symptoms", "non-nose/non-eye symptoms", "practical problems" and "sleep" domains.

References

[1]Blaiss M, *Allergic rhinoconjunctivits: Burden of disease*, Allergy Asthma Proc 28:393-397, 2007

[2]Valovirta E et al, *The voice of the patients: allergic rhinitis is not a trivial disease* Current Opinion in Allergy and Clinical Immunology 2008, 8:1-9.

[3]Nolte H et al, *Efficacy of House Dust Mite SLIT-Tablet in North American Adolescents and Adults in a Randomized, Placebo-Controlled Trial* , J Allergy Clin Immunol *2016; 138:1631-1638.*

[4]Nolte H et al *Onset and dose-related efficacy of house dust mite sublingual immunotherapy tablets in an environmental exposure chamber*, J Allergy Clin Immunol 2015;135:1494-501).

[5]Demoly P et al *Effective treatment of house dust mite-induces allergic rhinitis with 2 doses of the SQ HDM SLIT-tablet: Results from a randomized, doubled-blind, placebo-controlled phase III trial* J Allergy Clin Immunol 2016;137:444-51.

Conclusion

The RQLQ results from three randomized, double-blinded, placebo-controlled clinical trials show that the SQ HDM SLIT-tablet consistently and significantly improves the QoL of people suffering from HDM induced AR across slightly different populations and in different settings.

High consistency in allergen composition of SQ house dust mite (HDM) tablet for sublingual immunotherapy (SLIT)

Henmar H[1], Mikles A[2], Grosch K[1], Larsen JN[1]
[1]ALK, Hørsholm, Denmark; [2]ALK, Bedminster, NJ, USA

Rationale

HDM immunotherapy products have traditionally been based on purified mite bodies or whole mite culture with little or no possibility for adjusting the allergen composition. For the SQ-HDM SLIT-tablet, high consistency was achieved in a fractionation process.

Methods

The 2 most important HDM species causing respiratory allergic disease, *Dermatophagoides farinae* and *pteronyssinus*, were grown separately under controlled conditions. After termination, the cultures were separated using an automated mechanical sieve. The fraction containing the smallest particles predominantly contained fecal particles rich in group 1 major allergen, whereas an intermediate fraction contained predominantly mite bodies rich in group 2 major allergen.

Fractions were mixed 1:1 based on µg major allergen forming one drug substance (DS) for each mite species. Quality control included total IgE binding capacity by Centaur assay, major allergen determination by radial immunodiffusion; protein and antigen profile by SDS-PAGE and crossed immunoelectrophoresis, respectively.

Results

Data were normalized relative to the mean, and the standard deviation of Der f 1 in DS was 11.7% (14.7% and 17.7% in source material, SM) and for Der p 2 12.3% (12.1% and 16.7% in SM), respectively. The corresponding figure for Der p 1 was 9.0% (12.7% and 16.8% in SM) and for Der p 2 9.9% (17.8% and 15.8% in SM).

The analyses of the DS showed that fractionation resulted in a consistent DS without compromising the complexity in the protein and antigen profiles.

Conclusions

Variation in allergen content was observed in the source material, but fractionation enabled a process resulting in a highly standardized composition of the SQ-HDM SLIT-tablet.

Poster Number: 868
Session Number: 4212
Session Title: Allergic Rhinitis: Diagnosis and Treatment
Poster Hall Location: Convention Center, South Concourse, Level 1, South Hall A2
Presentation Date: Monday, March 5, 2018
Presentation Time: 9:45am-10:45am

868 High consistency in allergen composition of SQ house dust mite (HDM) tablet for sublingual immunotherapy (SLIT)

Henmar H[1)], Mikles A[2)], Grosch K[1)], Larsen JN[1)]
ALK A/S, [1)]Hørsholm, Denmark and [2)]Post Falls, Idaho.

Rationale

HDM immunotherapy products have traditionally been based on purified mite bodies or whole mite culture with little or no possibility for adjusting the allergen composition.
For the SQ HDM SLIT-tablet high consistency was achieved in a fractionation process.

Methods

The two most important HDM species causing respiratory allergic disease, *Dermatophagoides farinae* (Der f) and *pteronyssinus* (Der p), are grown separately under controlled conditions on a proprietary medium, which not only provides nutrition, but a suitable matrix in which the mites can move around in and live. Mites are grown in stainless steel cylinders, capped at either end with a permeable filter to facilitate gas exchange. The cylinders are held in an environmentally controlled chamber (Fig 1). After termination the cultures are dried and separated using an automated mechanical sieve (Fig 2).
The purity and content of group 1 and 2 allergens in the source material (SM) fractions are measured. The fraction containing the smallest particles predominantly contained fecal particles rich in group 1 major allergen, whereas an intermediate fraction contained predominantly mite bodies rich in group 2 major allergen. Figure 3 shows the ratio of group 1 and 2. Fractions are then mixed based on µg major allergen forming one drug substance (DS) for each mite species (Fig 4). Quality control includes total IgE binding capacity by Centaur assay, major allergen determination by radial immune diffusion; protein and antigen profile by SDS-PAGE and crossed immune electrophoresis, respectively.

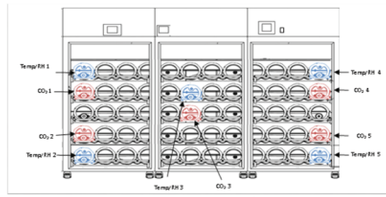


Figure 1 – Probed canister locations used to monitor T/RH inside the canisters. The chamber reacts to maintain desired T/RH setpoints inside the canisters

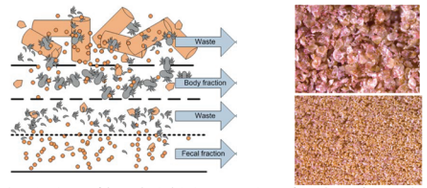


Figure 2 – Diagram of the mechanical sieving process. Picture of particle size of processed source materials, top picture containing primarily bodies particles and bottom containing primarily fecal particles

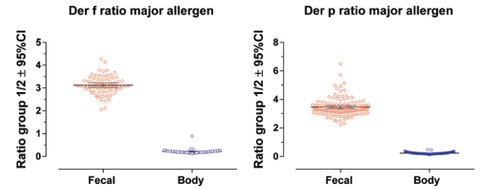


Figure 3 – Ratio of major allergen group 1 and 2, determined from measurement of the individual allergens in the fecal (red) and body (blue) fractions. Showing that the fecal fraction predominantly contain group 1 allergen and the body fraction group 2 allergen. Der f N=71, Der p N=120

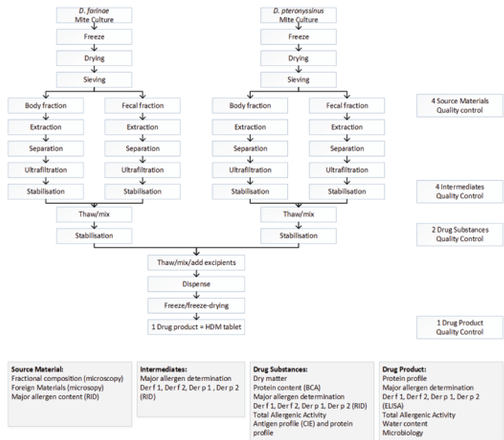


Figure 4 – Process diagram: production processes for the SQ-HDM SLIT-tablet. Quality controls are indicated below the diagram

Results

Data were normalised relative to the mean, and the standard deviation of Der f 1 in DS was 11.7% (14.7% and 17.7% in SM) and for Der f 2 12.3% (12.1% and 16.7% in SM), respectively. The corresponding figure for Der p 1 was 9.0% (12.7% and 16.8% in SM) and for Der p 2 9.9% (17.8% and 15.8% in SM) (Fig 5).
The analyses of the DS showed that fractionation resulted in a consistent DS without compromising the complexity in the protein and antigen profiles (Fig 6).

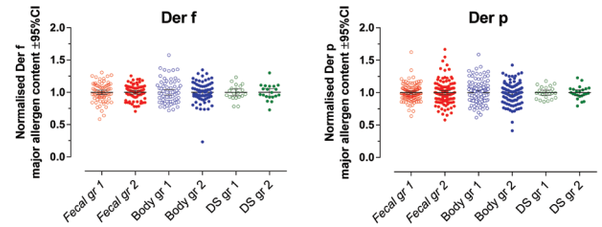


Figure 5 – Normalised Group 1 and 2 content in SM fractions, fecal (red), body (blue) and in DS (Green). N=71 for Der f SM and N=20 for Der f DS, N=119 for Der p SM and N=23 for Der p DS.

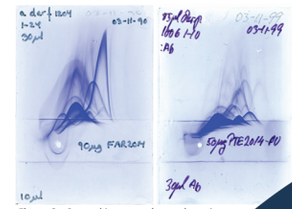


Figure 6 – Crossed immune electrophoresis (CIE) of Der f DS (left) and Der p DS (right)

Conclusion

Variation in allergen content was observed in the source material, but fractionation enabled a process resulting in a highly standardized composition of the SQ HDM SLIT-tablet.

Consistent composition and potency of freeze-dried tablets for sublingual immunotherapy

Henmar H, Sønderkær S, Nørby PN, Christensen LH, Larsen JN
ALK, Hørsholm, Denmark

Rationale

Consistency in composition and potency of allergen products is important for the clinical performance in everyday practice. Release kinetics of individual allergen components from source materials differ, and the production process must be optimised individually for every source material in order to secure consistent composition and potency of the final drug product.

Methods

Aqueous extraction of grass and ragweed pollens as well as house dust mite (HDM) particles was performed under chemical conditions resembling the conditions on the airway mucosa. Tablet disintegration experiments were performed in water. 69, 29 and 35 batches were analysed from grass, ragweed and HDM SLIT-tablets, respectively. Release kinetics were assessed by immunoelectrophoresis (IE), immunodiffusion, IgE binding, ELISA and mass spectrometry.

Results

Release kinetics from source material vary among allergen molecules and complete release can take up to 2 hours. Major allergen content released from tablets was normalised relative to the mean, and the standard deviation (SD) measured was 5.0% for Phl p 5, 6.6% for Amb a 1 and 9.8%, 6.7% and 11.4% for Der f 1, Der p 1 and Der 2, respectively. The SD of IgE-binding potency was 6.2%, 8.4% and 5.5% for grass, ragweed and HDM tablets, respectively. All relevant allergens were assessed semi-quantitatively by IE ensuring optimal complexity of the final products. Disintegration time for all 3 freeze-dried tablet formulations was within 2 sec.

Conclusions

The SLIT-tablets contain a consistent composition and potency, which is achieved in an optimized production process taking into account the variable kinetics of extration of individual allergen components.

Poster Number: 541
Session Number: 3212
Session Title: Allergic Rhinitis: Epidemiology, Diagnosis and Treatment
Poster Hall Location: Convention Center, South Concourse, Level 1, South Hall A2
Presentation Date: Sunday, March 4, 2018
Presentation Time: 9:45am-10:45am

541 Consistent composition and potency of freeze dried tablets for sublingual immunotherapy

Henmar H, Sønderkær S, Nørby P N, Christensen L H, Larsen J N.

ALK A/S, Hørsholm, Denmark

Rationale

Consistency in composition and potency of allergen products is important for the clinical performance in everyday practise. Release kinetics of individual allergen components from source materials differ, and the production process must be optimised individually for every source material in order to secure consistent composition and potency of the final drug product.

Methods

Aqueous extraction of grass and ragweed pollen as well as house dust mite (HDM) particles was performed under mild conditions resembling the conditions on the airway mucosa. Tablet disintegration experiments were performed in water. 69, 29 and 35 batches were analysed from grass, ragweed and HDM SLIT-tablets, respectively. Release kinetics were assessed by rocket immune electrophoresis (RIE), radial immune diffusion (RID), IgE binding (TACA), ELISA and mass spectrometry (MS).

Results

Release kinetics from source material vary among allergen molecules and complete release can take up to two hours. The allergens were identified by MS (grass fig 1 and 2, ragweed fig 3 and 4, house dust mite fecals fig 5 and 6 and house dust mite bodies Fig 7).

Major allergen content released from tablets was normalised relative to the mean and the standard deviation (SD) measured was 5.0% for Phl p 5, 6.6% for Amb a 1 and 9.8%, 6.7% and 11.4% for Der f 1, Der p 1 and Der 2, respectively (Fig 8).

The SD of IgE-binding potency was 6.2%, 8.4% and 5.5% for grass, ragweed and HDM tablets, respectively (Fig 8). All relevant allergens were assessed semi-quantitatively by immunoelectrophoresis ensuring optimal complexity of the final products. Disintegration time for all 3 freeze dried tablet formulations was within 2 sec.

A ragweed tablet was dissolved in water and the Amb a 1 content was measured over the first 20 minutes, showing instant release (Fig 9). Similar data are found for grass and mite (poster # 543)

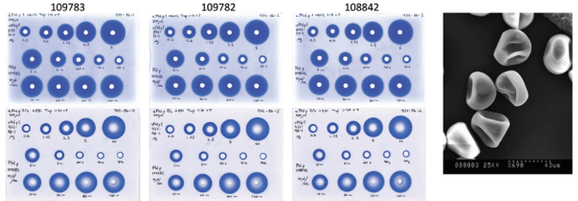


Figure 1 – RID showing the release of major allergen Phl p 1 (top) and Phl p 5 (bottom) from three different batches of Phl p pollen at different time points ranging from 20 seconds to 2 hours.

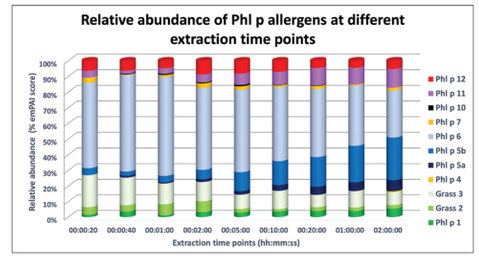


Figure 2 –Grass pollens (Phl p) were extracted for various time intervals between 20 seconds and 2 hours and the relative abundance of the grass allergens assed by LC-MS/MS.

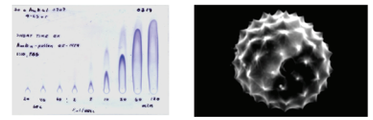


Figure 3 – RIE showing the release of major allergen Amb a 1 from ragweed (Amb a) pollen. From 20 seconds to 2 hours.

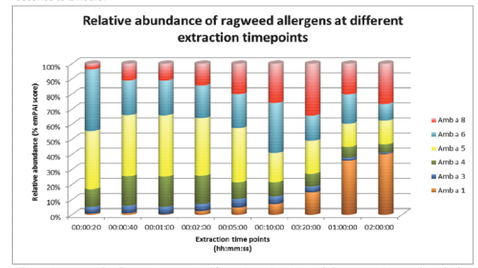


Figure 4 - Ragweed pollens were extracted for various time intervals between 20 seconds and 2 hours and the relative abundance of the ragweed allergens assed by LC-MS/MS.

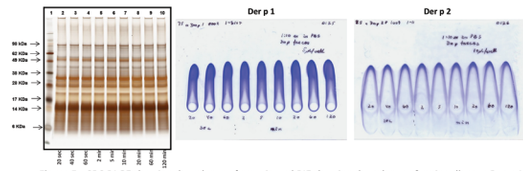


Figure 5 – SDS PAGE showing the release of protein and RIE showing the release of major allergen Der p 1 and Der p 2 from Der p fecals at different time points ranging from 20 seconds to 2 hours.

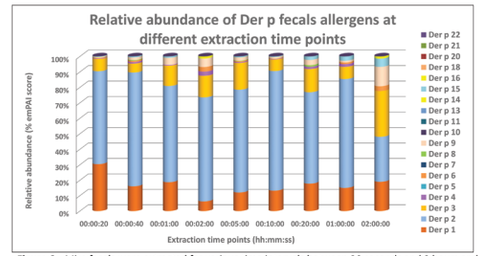


Figure 6 – Mite fecals were extracted for various time intervals between 20 seconds and 2 hours and the relative abundance of the mite allergens assed by LC-MS/MS.

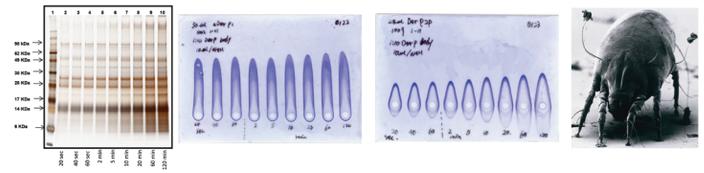


Figure 7 – SDS PAGE showing the release of protein and RIE showing the release of major allergen Der p 1 and Der p 2 from Der p bodies at different time points ranging from 20 seconds to 2 hours.

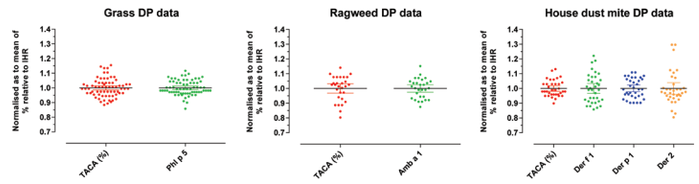


Figure 8 – Normalised Potency (TACA) and major allergen content (Phl p 5, Amb a 1, Der f1, Der p 1 and Der 2). Grass N=69 Ragweed N=29 and House dust mite N=35

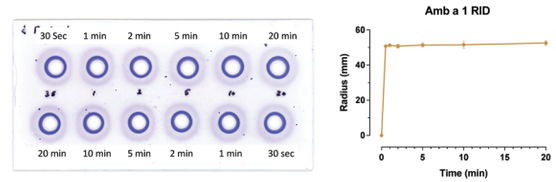


Figure 9 – RID showing the Amb a 1 content at different time points (30 seconds to 20 min) after a ragweed tablet was dissolved in water. Left raw data. Right the radius of the RID precipitates.

Conclusion

The SLIT-tablets contain a consistent composition and potency, which is achieved in an optimised production process taking into account the different kinetics of extraction of individual allergen molecules

The prevalence of eosinophilic esophagitis with use of SQ HDM SLIT-tablet

Maloney J[1], Sussman G[2], Svanholm Fogh B[3], Nolte H[1]
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Rationale

The SQ house dust mite (HDM) SLIT-tablet is approved in North America for treatment of HDM allergic rhinitis with/without conjunctivitis in adults (ODACTRA™, 12 SQ-HDM, ALK, Denmark).

Isolated cases of eosinophilic esophagitis (EoE) have been reported with sublingual allergy immunotherapy (SLIT), particularly in food-desensitisation trials; therefore EoE is monitored as a potential risk. We present cases of EoE reported with the HDM SLIT-tablet.

Methods

The clinical and post-marketing databases were searched for any reports of EoE.

Results

In the clinical program (4,175 subjects in active groups), 2 cases of EoE were reported (~48/100,000). 1 case was reported by a US, adolescent, male subject with a 3-year history of heartburn, dysphasia, and stomach discomfort. EoE (assessed as moderate) was diagnosed with biopsy 6 months after start of treatment with 12 SQ-HDM. Treatment included PPI and fluticasone. A French woman with medical history of gastrointestinal reflux-symptoms reported another case. This case was diagnosed with biopsy 3 months after start of treatment with 6 SQ-HDM and it was assessed as mild, with a possible causal relation to treatment. Treatment included PPI and antacid. Both subjects completed the 1 year of treatment without changes to investigational treatment. In worldwide post-marketing, 3 cases of EoE have been reported with exposure estimated to 20,102 treatment-years (~15/100,000 TY). 2 cases were reported as confirmed with biopsy.

Conclusions

The background prevalence of EOE is 57/100,000 persons in the US. Thus, the prevalence of EoE with SQ HDM SLIT-tablet does not support a potential risk related to treatment at this point in time.

Poster Number: 548
Session Number: 3212
Session Title: Allergic Rhinitis: Epidemiology, Diagnosis and Treatment
Poster Hall Location: Convention Center, South Concourse, Level 1, South Hall A2
Presentation Date: Sunday, March 4, 2018
Presentation Time: 9:45am-10:45am

The Prevalence of Eosinophilic Esophagitis with use of SQ HDM SLIT-tablet

J Maloney[1], G Sussman[2], B Svanholm Fogh[3], H Nolte[1]
[1]ALK, Bedminster, NJ, US; [2]Clinical Research, Inc., Toronto, Canada; [3]ALK, Hørsholm, Denmark

Rationale

The SQ house dust mite (HDM) SLIT-tablet is approved in North America for treatment of adults (18-65 years of age) with HDM allergic rhinitis with/without conjunctivitis (ODACTRA™, 12 SQ-HDM, ALK-Abelló, Denmark).

Isolated cases of eosinophilic esophagitis (EoE) have been reported with sublingual allergy immunotherapy (SLIT), particularly in food-desensitization trials; therefore EoE is monitored as a potential risk.

We present cases of EoE reported with the SQ HDM SLIT-tablet (N=4,175 in active groups).

Methods

The clinical and post-marketing databases were searched for any reports of EoE.

Results

In the SQ HDM SLIT-tablet clinical development program (4,175 subjects in active groups), 2 cases of EoE were reported (~48/100,000 subjects) (see **Table 1**).

No events of EoE was reported from the placebo groups (2,536 subjects).

In worldwide post-marketing exposure, 3 cases of EoE have been reported with exposure estimated to 20,102 treatment-years (~15/100,000 TY). Of these, 2 cases were reported as confirmed with biopsy.

The background prevalence of EoE is estimated to 57/100,000 persons in the US.

Thus, an association between treatment with SQ HDM SLIT-tablet and onset of EoE has not been established.

Table 1. Cases of EoE reported in active groups in SQ HDM SLIT-tablet clinical program (N=4,175)

Subject	History	Diagnosis	Treatment
13 year old boy	• Medical history of dysphagia, heartburn, and stomach discomfort prior to SQ HDM SLIT-tablet treatment • 16 pound weight gain while in the trial • On day 204 of treatment with SQ HDM SLIT-tablet, EoE was diagnosed • The subject completed the trial	• Endoscopy with biopsy 6 months into SQ HDM SLIT-tablet therapy revealed 10-20 eosinophils/hpf	• PPI • Fluticasone
34 year old woman	• Medical history of reflux symptoms prior to SQ HDM SLIT-tablet treatment • Treated with SQ HDM SLIT-tablet for 1 year • The subject completed the trial	• Endoscopy with biopsy 3 months into SQ HDM SLIT-tablet therapy; an eosinophil count/hpf was not reported • Pathology revealed thickened mucosal membranes, intracellular edema, and eosinophils	• PPI • Antacid

Eosinophilic esophagitis

The diagnosis of EoE is established on the basis of 3 criteria (Furuta and Katzka, 2015):

- Symptoms of esophageal dysfunction, such as dysphagia, food impaction, chest pain, heartburn, vomiting, abdominal pain, or failure to thrive
- Esophageal eosinophil count of ≥15 eosinophils/high power field (hpf)
- Exclusion of other causes

Reference
Furuta and Katzka. 2015. *N Engl J Med, 373*(17): 1640–1648.

Conclusion

The background prevalence of EoE is 57/100,000 persons in the US.
Thus, the prevalence of EoE with SQ HDM SLIT-tablet does not support a potential risk related to treatment at this point in time.

Efficacy of the SQ HDM SLIT-tablet on house dust mite induced allergic conjunctivitis

Nolte H[1], Nelson HS[2], Demoly P[3], Smith IM[4], Bernstein DI[5]
[1]ALK, Bedminster, NJ, USA; [2]National Jewish Health, Denver, CO, USA; [3]University Hospital of Montpellier, Paris, France; [4]ALK, Hørsholm, Denmark; [5]Bernstein Clinical Research Center and University of Cincinnati, OH, USA

Rationale

Co-existence of allergic conjunctivitis is well recognized in patients with allergic rhinitis although the co-reporting frequency may be as low as 40%. Under-recognition and treatment of house dust mite (HDM)-induced allergic conjunctivitis may be due to the under-appreciation of eye symptoms in patients with HDM-induced allergic rhinitis. We present clinical data from 2 North American trials with the SQ HDM sublingual immunotherapy (SLIT)-tablet evaluating treatment effect on ocular symptoms.

Methods

Trial 1 included 124 adult subjects with HDM-induced allergic rhinitis/rhinoconjunctivitis (AR/C) randomized to SQ HDM SLIT-tablet (6 or 12 SQ-HDM) or placebo for 24 weeks. Efficacy was assessed during HDM exposure in an environmental exposure chamber. Trial 2 included 1,482 adult/adolescent subjects with HDM-induced AR/C randomised to SQ HDM SLIT-tablet (12 SQ-HDM) or placebo for up to 12 months. A conjunctivitis symptom score (range 0-6) was constructed based on subjects' rating of 2 ocular symptoms (watery eyes and itchy eyes).

Results

In trial 1, 40% reported perennial allergic conjunctivitis. The average conjunctivitis symptom score during the HDM exposure session after 24 weeks of treatment showed a relative difference between both active treatment groups and placebo of -41% for 6 SQ-HDM and -68% for 12 SQ-HDM. In trial 2, 65% reported perennial allergic conjunctivitis. The average conjunctivitis daily symptom score obtained during the last 8 weeks of treatment showed a difference between treatment groups of -33.3% (95% CI, -47.1%, -18.5%).

Conclusions

The SQ HDM SLIT-tablet had a significant treatment effect on ocular symptoms in patients with HDM-induced AR/C.

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Session Number: 4212
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Poster Hall Location: Convention Center, South Concourse, Level 1, South Hall A2
Presentation Date: Monday, March 5, 2018
Presentation Time: 9:45am-10:45am

Efficacy of the SQ HDM SLIT-tablet on house dust mite induced allergic conjunctivitis

H Nolte[1], HS Nelson[2], P Demoly[3], IM Smith[4], DI Bernstein[5]

[1]ALK, Bedminster, NJ, USA; [2]National Jewish Health, Denver, CO, USA; [3]University Hospital of Montpellier & Paris, France; [4]ALK, Hørsholm, Denmark; [5]Bernstein Clinical Research Center and University of Cincinnati, OH, USA

Rationale

Co-existence of allergic conjunctivitis is well recognized in patients with allergic rhinitis although the co-reporting frequency may be as low as 40% (Williams et al. 2013). Underrecognition and undertreatment of house dust mite (HDM)-induced allergic conjunctivitis may be due to the underappreciation of eye symptoms in patients with HDM-induced allergic rhinitis.

Here, we present clinical data from 2 randomized controlled trials with the SQ HDM sublingual immunotherapy (SLIT)-tablet evaluating treatment effect on ocular symptoms.

Methods

Trial 1 (NCT01644617; Nolte et al. 2015)
- Randomized, double-blind, placebo-controlled, dose-finding phase II trial
- 124 adult subjects (18-65 years of age) with HDM-induced allergic rhinitis/rhinoconjunctivitis were randomized (1:1:1) to SQ HDM SLIT-tablet (6 or 12 SQ-HDM) or placebo for 24 weeks
- Efficacy was assessed during HDM exposure in an environmental exposure chamber

Trial 2 (NCT01700192; Nolte et al. 2016)
- Randomized, double-blind, placebo-controlled phase III trial
- 1,482 adult/adolescent subjects (12-65 years of age) with HDM-induced allergic rhinitis/rhinoconjunctivitis were randomized (1:1) to SQ HDM SLIT-tablet (12 SQ-HDM) or placebo for up to 12 months
- Efficacy was assessed during the last 8 weeks of treatment

In both trials, a conjunctivitis symptom score (range 0-6) was constructed based on subjects' rating of 2 ocular symptoms (watery eyes and itchy eyes).

Results

At screening in trial 1, 50 of 124 subjects (40%) had a history of perennial allergic conjunctivitis (Fig 1A). At the screening HDM exposure session, 88 subjects (71%) reported ocular symptoms (Fig 1B). In trial 2, 956 of 1,482 subjects (65%) had a history of perennial allergic conjunctivitis at screening (Fig 1A).

After 24 weeks of daily treatment in trial 1, the average conjunctivitis symptom score obtained during the HDM exposure session showed a relative difference between both active treatment groups and placebo of -41% for 6 SQ-HDM and -68% for 12 SQ-HDM (Fig 2).

After up to 12 months of daily treatment in trial 2, the average conjunctivitis daily symptom score obtained during the last 8 weeks of treatment showed a relative difference between treatment groups of -33.3% (95% CI, -47.1%, -18.5%) (Fig 2).

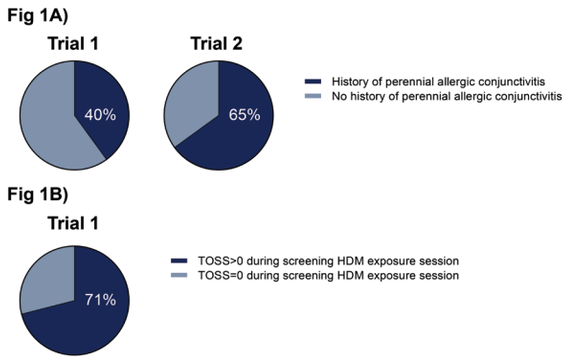

Figure 1. Proportion of HDM allergic subjects with ocular symptoms at screening. A) Proportions of subjects with a history of perennial allergic conjunctivitis at screening in trial 1 (50 of 124 subjects) and trial 2 (956 of 1,482 subjects). B) Proportion of randomized subjects reporting ocular symptoms (watery eyes or itchy eyes) during the screening HDM exposure session in trial 1 (88 of 124 subjects). TOSS, total ocular symptom score.

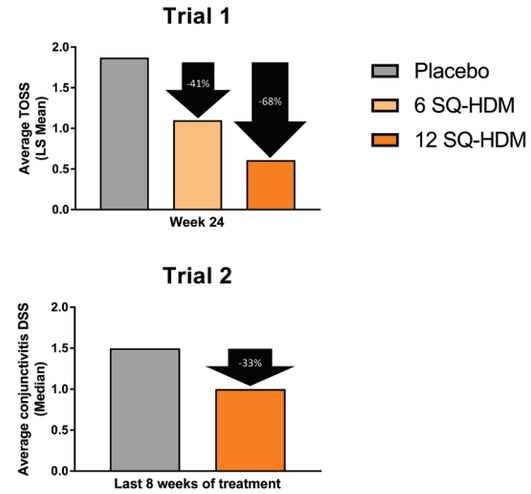

Figure 2. Average conjunctivitis symptom scores. Trial 1: Average total ocular symptom score (TOSS) during the HDM exposure session after 24 weeks of treatment. Trial 2: Average conjunctivitis daily symptom score (DSS) during the last 8 weeks of up to 12 months of treatment.

Conclusion

Ocular symptoms are common in patients with HDM-induced allergic rhinitis.

The SQ HDM SLIT-tablet had a significant treatment effect on ocular symptoms in patients with HDM-induced allergic rhinitis/rhinoconjunctivitis

References

Nolte et al. 2015. *Journal of Allergy and Clinical Immunology, 135*(6):1494-501.e6. doi: 10.1016/j.jaci.2014.12.1911.
Nolte et al. 2016. *Journal of Allergy and Clinical Immunology, 138*(6):1631-1638. doi: 10.1016/j.jaci.2016.06.044.
Williams et al. 2013. *The World Allergy Organization Journal, 6*(1), 4. http://doi.org/10.1186/1939-4551-6-4

Freeze-dried HDM SLIT-tablets provide maximal HDM allergen delivery and optimal potency

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Rationale

2 types of HDM SLIT-tablet formulations are currently available, a freeze-dried formulation and a compressed formulation. The impact of formulation on tablet potency and effectiveness of allergen delivery was assessed.

Methods

3 HDM SLIT-tablets, 2 freeze-dried (6 SQ-HDM and 12 SQ-HDM, respectively) and 1 compressed (300 IR), were dissolved in assay buffer under controlled and identical conditions, and the total potency of solubilized HDM allergens was measured at various time points from 0-5 minutes by inhibition ELISA, using a pool of HDM allergic patients' sera.

Results

The 2 freeze-dried HDM SLIT-tablets dissolved completely and reached full effective potency of solubilized HDM allergens within 15 seconds. The achieved potency was proportional to the relative nominal strengths (6 SQ-HDM and 12 SQ-HDM) of the freeze-dried tablets. In contrast, delivery of solubilized HDM allergens from the compressed tablet was slower and incomplete, even after 5 minutes in solution. In the time interval that corresponds to the recommended sublingual holding times, the release of HDM allergens was higher for both the 6 SQ-HDM and 12 SQ-HDM freeze-dried HDM SLIT-tablet compared to the compressed 300 IR tablet.

Conclusions

Only the freeze-dried HDM SLIT-tablets provided fast and complete allergen delivery, and achieved full potency of soluble HDM allergens within the recommended sublingual holding time (1 minute).

Poster Number: 543
Session Number: 3212
Session Title: Allergic Rhinitis: Epidemiology, Diagnosis and Treatment
Poster Hall Location: Convention Center, South Concourse, Level 1, South Hall A2
Presentation Date: Sunday, March 4, 2018
Presentation Time: 9:45am-10:45am

Freeze-dried HDM-SLIT tablets provide maximal HDM allergen delivery and optimal allergenic potency

K. Ohashi-Doi[1], W. Du[1], H. Kito[1], H. Nakazawa[1] and K. Lund[2]

[1]*Torii Pharmaceutical Co Ltd, Japan* [2]*ALK A/S, Denmark*

Background

Two types of HDM SLIT-tablet formulations are currently available, a fast dissolving freeze-dried formulation and a conventional compressed formulation. The importance of formulation for allergen delivery and effective tablet potency was examined.

Methods

Three strengths of the freeze-dried HDM SLIT-tablet (2 SQ-HDM, 6 SQ-HDM and 12 SQ-HDM) and two strengths of the compressed tablet (100IR, 300IR), were dissolved in assay buffer under controlled and identical conditions. Tablet disintegration times were measured according to the Japanese pharmacopoeia (Ph. JP.).

Allergen release kinetics and the achieved effective potency of the respective HDM SLIT-tablets were established by a dissolution test (Ph. JP.): Tablets were deposited in assay buffer and samples were collected at various time points. The allergenic potency of each sample was measured by inhibition ELISA, using a pool of HDM allergic patients' sera and in-house allergen standards.

Note: SLIT-tablet are traditionally labelled with company specific dose units using company-specific reagents and methods. The potencies of the freeze-dried and compressed tablets measured here can therefore not be compared on an absolute scale. The relative potencies reported here should be taken to indicate the relative reactivity of each dissolved tablet at specific time points with the use of the same pool of patient IgE and a common allergen standard.

Results

Tablet disintegration times

All strengths of the freeze-dried HDM SLIT-tablet (2 SQ-HDM, 6 SQ-HDM, 12 SQ-HDM) disintegrated immediately (≦ 1 second). Both strengths of the compressed tablet (100IR, 300IR) showed considerably longer disintegration times that increased with increasing HDM allergen content (Table 1).

	Sample	Unit	Time, s
Freeze-dried	Miticure (MTC)	2 SQ-HDM	1
		6 SQ-HDM	1
	Acarizax (ACZX)	12 SQ-HDM	1
Compressed	Actair (ACT)	100IR	27±1.5
		300IR	45±2.2
Values are presented as means ± SD.			

Table 1 - Disintegration times for the freeze-dried (Acarizax, ACZX and Miticure, MTC) and compressed (Actair, ACT) tablets (data from Ohashi-Doi et al Int. Arch Allergy Immunology 2017;174:26-34)

Tablet dissolution and allergen release kinetics

At time=0 the HDM SLIT-tablets were deposited into assay buffer with continuous agitation. Samples were collected for ELISA at t=15, 30, 45, 60 and 90 seconds and 2, 3 and 5 minutes. The reactivity of dissolved allergen towards IgE in a common pool of HDM allergic patients' sera was measured at each time point with the use of a common HDM allergen extract standard. The results are shown as relative potencies with the reactivity of the freeze-dried Miticure (MTC, 6 SQ-HDM) = 100% (Figure 1).

Area Under the Curve (AUC) was calculated at different time points as a measure for the levels of the effective, cumulated allergenic potency over time (Figure 2, Table 2).

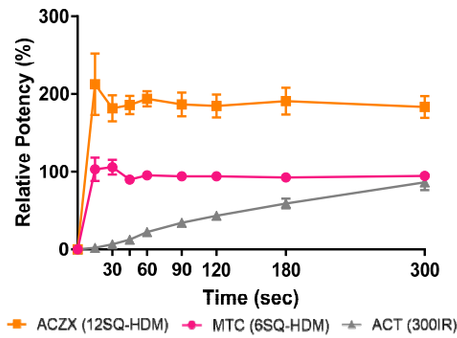


Figure 1 – HDM allergen release over time measured as allergenic potency, i.e. reactivity to a pool of HDM allergic patients' IgE using a common HDM allergen extract standard.

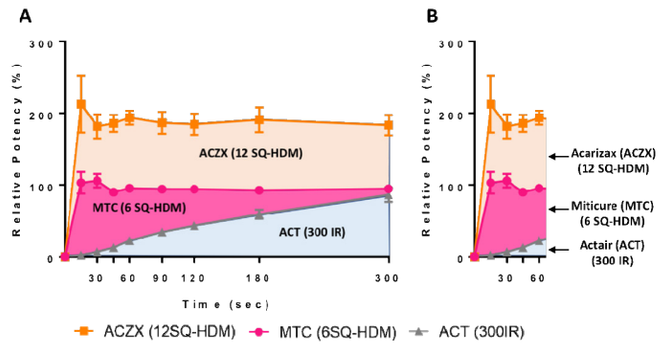


Figure 2 - Area Under the Curve (AUC) from t=0 to t=5 min (A) and from t=0 to t=1 min (B). The AUC is a measure for the effective allergenic potency obtained for each tablet at the indicated time points. One minute is the recommended sublingual holding time for the two freeze dried HDM SLIT-tablets Acarizax (ACZX, 12 SQ-HDM) and Miticure (MTC, 6 SQ-HDM).

Time (sec.)	Acarizax (ACZX) (12SQ-HDM)	Actair (ACT) (300IR)	*p*	Miticure (MTC) (6SQ-HDM)
15	2.06	0.02	.0054	1.00
30	1.94	0.04	.0013	1.00
45	1.92	0.06	.0003	1.00
60	1.95	0.09	.0001	1.00
90	1.97	0.17	<.0001	1.00
120	1.97	0.23	.0001	1.00
180	1.98	0.34	.0004	1.00
300	1.99	0.51	.0027	1.00
p values indicate the level of statistical significance between the absolute AUCs of ACT and MTC at the indicated time points. All ACZX and MTC AUCs were statistically significantly different at all time points (data not shown).				

Table 2 – Area Under the Curve (AUC) calculated for the freeze-dried Acarizax (ACZX, 12 SQ-HDM), Miticure (MTC, 6 SQ-HDM) and the compressed Actair (ACT, 300IR). MTC (6 SQ-HDM) = 100%

Discussion

The recommended sublingual holding time SLIT-tablet allergy immunotherapy (AIT) is traditionally from one to a few minutes (2). For effective sublingual administration of pharmaceuticals the sublingual holding time should be kept as short as possible to avoid reducing the effective potency of the drug by "saliva washout" (2). Consequently, the most effective formulation for SLIT-tablet is one that permits complete dissolution of the allergens contained in the tablet within the recommended sublingual holding time. Here we demonstrate that under identical and controlled conditions the release of soluble, IgE-reactive HDM allergens occurs much faster from the freeze-dried HDM SLIT-tablet formulation compared to the compressed tablet. In addition, the entire contents of the freeze-dried HDM SLIT tablets were solubilized after 15 seconds, regardless of strength, while only incomplete solubilization of IgE-reactive allergens had occurred from the compressed tablet, even after 5 minutes in solution.

(1) Moingeon et al Clin Dev Immunol 2012;2012:623474
(2) Kraan et al J. Control Release 2014; 190:580-592

Conclusion

Only the freeze-dried HDM SLIT-tablets provided fast and complete delivery of soluble allergen and achieved full effective potency within the recommended sublingual holding time

Detection of dust mite allergens in homes throughout the US

Plunkett G, Moore T
ALK, Bedminster, NJ, USA

Rationale

Dermatophagoides dust mites are known to vary across North America depending on location with factors like elevation and humidity influencing their presence. These studies have included microspopic identification of mites, DNA analysis and allergen protein measurements. In order to develop a current mapping of allergen protein prevalence, we have studied dust from homes in 16 regions around the US for major dust mite allergens. In order to explore variability within a region, multiple homes from the same region were studied.

Methods

Dust from vacuum cleaner bags from 24 homes was used to make 1:10w/v extracts using a 1% albumin, 0.9% saline phenol extraction fluid. The extracts were filtered using a 0.2 micron filter then analysed for species specific Der p/f 1 using direct binding monoclonal ELISAs (ALK, Madrid).

Results

19 of the homes had detectable mite allergens ranging from below 1 microgram/gram of dust to more than 20 micrograms/gram. 12 of these homes showed DF only, 1 in Southern California showed DP only, and 6 homes contained both mites. The DP containing homes were not confined to coastal areas. The homes in Spokane, Reno, Denver and Sonoma showed no mites. Out of 9 homes in Texas that contained mite allergens, 1 had no detectable dust mites.

Conclusions

This study confirmed widespread presence of dust mite allergens in homes across the US but are low in drier rocky mountain states. Many homes contain both species in New England and southern regions.

Poster Number: 400
Session Number: 3205
Session Title: Indoor Allergens
Poster Hall Location: Convention Center, South Concourse, Level 1, South Hall A2
Presentation Date: Sunday, March 4, 2018
Presentation Time: 9:45am-10:45am

Detection of house dust mite allergens in homes throughout the US

Tricia Sowers and Greg Plunkett, ALK, Inc., Round Rock, TX

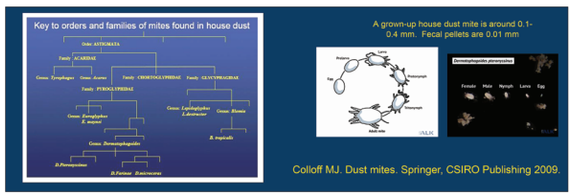
AAAAI 2018 Poster 400

Rationale

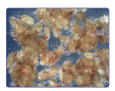
Dermatophagoides house dust mites (HDM) are known to vary across North America depending on location with factors like elevation and humidity influencing their presence.[1-8] These studies have included microscopic identification of mites, DNA analysis and allergen protein measurements. It is hypothesized that changes in our home environments could change the distribution and amounts of mites in our homes. The most recent report[6] which analyzed 17,000 homes is at least 10 years old and reported presence of mites in several US regions based on climate and elevation. In order to begin developing a current mapping of mite allergen protein prevalence, we have studied dust from homes in 16 regions around the US for major dust mite allergens. In order to explore variability within a region, multiple homes from the same region were studied.

Dust Mite Biology

HDM are part of the arthopods known as the class arachnids that include spiders, ticks, and scorpions. Dust mites share environments with many other mites such as food storage mites, but currently are considered to be the most important cause of allergic disease, hence are the only species available for immunotherapy in the US and Canada. Mites require certain amounts of humidity to survive as they absorb water through their bodies. Therefore they thrive in regions with high humidity. *D. farinae* (Df) can tolerate lower humidity therefore may be more prevalent than *D. pteronyssinus* (Dpt) in drier regions.



- **Two key species:**
  - ***Dermatophagoides pteronyssinus***
  - ***Dermatophagoides farinae***
- **Related with spiders and adult mites have 8 legs**
- **Found in most human habitats**
- **Mites can colonize a (brand) new home within a year**
- **Core source of indoor allergens**



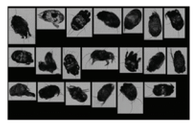


Figure 3: Laser Microscopy from ALK Source Material analysis of mite cultures used for allergenic extracts

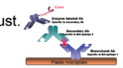
Figure 1: Taxonomic chart of various mites found in houses. Dust mite genetic relationships to other household mites such as storage mites are shown. There should be some cross reactivity to some of the proteins found in these various arachnids.

Figure 2: Life cycle of dust mites and size of mites in the environment. The mites predominate in the bedding and pillows of homes where higher humidity can sustain their life cycle in drier months.

Methods

How do we measure dust mite presence in houses?

Mite species can be detected in house dust microscopically, by specific PCR/DNA techniques and by immunoassays. Sampling bedding has been used in several studies as the mites can thrive in the mattresses and pillows due to the persistence of human body moisture. This study attempted to detect mite allergens from vacuum cleaner bags without specification for time or storage of the dust. We were testing if the allergens would survive sufficiently for detection in the monoclonal ELISA.



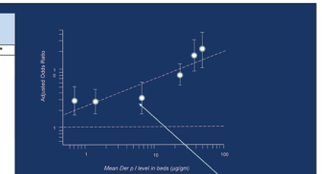
Fine dust, fibers and hair from vacuum cleaner bags from 24 homes were used to make 1:10w/v extracts using a 1% albumin, 0.9%saline phenol extraction fluid. The dust extracts were analyzed for species specific Der p/f 1 using direct binding monoclonal ELISAs from ALK, Madrid (see Table 2).

Why Measure Der 1

Dust mites feed on fibers, skin scales, fungi and other items, excreting waste as tiny pellets that populate their living spaces such as bedding and carpets. The tiny pellets are about 10 micron and can be airborne but easily settle in the house dust. These pellets contain many allergenic proteins. Mite bodies also contain unique allergens in addition to those in the feces pellets. A good extract for immunotherapy should contain both feces and body allergens. Table 1 below shows a list of known mite allergens. Studies have shown that Der 1 and Der 2 are very important allergens. Since our monoclonal antibodies are specific to each species, and studies with Der p 1 have identified important levels that can cause sensitization, Der 1 ELISAs were used for these studies.

House Dust Mites Allergens and Importance of Der 1

Allergen Name	Biochemical ID	MW kDa SDS-PAGE	IgE Prevalence(%)		
Dermatophagoides pteronyssinus (Dpt) and farinae (Df)			*Dpt**	*Df**	*Dpt-***
Der 1	cysteine protease	25	30-96	30-96	74
Der 2	NCP2 family	15	30-100	92-100	62
Der 3	trypsin	31	9-97	16	14
Der 4	amylase	60	25-75		44
Der 5		14	6-77		30
Der 6	chymotrypsin	25	41-65		
Der 7		26/30/31	15-60	50	31
Der 8	glutathione transferase	27	9-96		9.5
Der 9	serine protease	29	92		
Der 10	tropomyosin	36	5-55	25	8.6
Der 11	paramyosin	103	50-75	50-82	
Der 13	lipocalin (fatty acid binding)				
Der 14	apolipophorin	177	2-85	85	
Der 15	chitinase	98	70-88	47	
Der 16	gelsolin (actin regulation)	65			
Der 17	calcium binding protein			35	
Der 18	chitanase (dog allergy)	60		54	
Der 20	arginine kinase	40	15-44		15
Der 21		14	56		
Der 23	Peritrophin-like protein	14, 28 dimer	73		
Der 24	Ubiquinol-cytochrome c reductase binding protein	13			



- Studies suggest exposure that can be considered "safe" are levels less than 2 µg/g of HDM allergens for the primary prevention of sensitization in atopic children and young adults
- Greater than 10 µg/g are associated with current asthma
- Synergy of virus, pollution and house dust mites for acute asthma exacerbations

Table 1: List of known allergenic proteins from dust mites

The protein allergen content in dust mites was obtained from allergome.org and allergen.org. These websites also site references that describe the prevalence of IgE reactivity to the proteins. **Additional experiments from Australia show the prevalence of IgE to some of the allergens from patients that have been hospitalized due to asthmatic attacks from mites. The data show that Der 1 and 2 are the predominant allergen proteins by both prevalence and IgE quantity. **Hales *et al* JACI, 118, 361-367, 2008

Figure 4: Threshold levels of Der 1 in house dust

With the development of Der 1 immunoassays at the University of Virginia by Platts-Mills, Chapman, et. al., house dust was shown to contain Der 1 allergens. Several studies related the amount of Der 1 found in dust to atopic disease and asthma. Shown is a summary from studies in Germany and Australia that analyzed the major allergen in homes from atopic patients. These mite allergen ELISAs allowed determination of levels that caused sensitization for asthma.

References

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Results

Figure 5: Location of dust collection

Color of the star represents species detected in the house as shown in Table 2. The presence of *D. farinae* in drier climes is demonstrated by homes in Austin only contained *D. farinae*, but in a home closer to the gulf *D. pteronyssinus* was also detected. No mites were detected in several homes in Reno, NV and Spokane, WA. The color on the map shows various climate pollen zones.

Der p 1 and Der f 1 Content in Vacuum Cleaner Bag

Table 2a	Der p 1		Der f 1		
	+ or -	*Amount (ug/g dust)*	+ or -	*Amount (ug/g dust)*	Species
Dallas, TX	+/-		++	20.5	Df
Atlanta, GA	+/-		++	2	Df
Long Island, NY	+	<0.1	++	25	Df/Dpt
Charlotte, NC	+	<0.1	+	<0.1	Df/Dpt
Austin, TX	+/-		++	20.3	Df
Atlanta, GA	-		+	0.1	Df
Brooklyn, NY	-		++	11.8	Df
Cedar Park, TX	+/-		+	0.1	Df
Oxnard, CA	++	7.6	+	<0.1	Df/Dpt
Jacksonville, FL	+	<0.1	+	0.25	Df/Dpt
Houston, TX	+	<0.1	+	0.4	Df/Dpt
Washington DC	+/-		++	2.7	Df
Sonoma, CA	+/-		-		-
Georgetown,TX	+/-		++	27.8	Df
Spokane, WA	+/-		+	<0.1	-
Austin, TX	+/-		++	6.9	Df
Austin, TX	-		+/-		Df +/-
Austin, TX	+/-		+	0.15	Df
Philadelphia, PA	-		+	<0.1	Df
Denver, CO	-		-		-
Woodbine, NJ	+	<0.1	++	12.9	Df/Dpt+/-
Columbus, OH	+	<0.1	+	<0.1	Df/Dpt+/-
Austin, TX	-		-		-
Reno, NV	-		-		-

Table 2a & b: ELISA methods and results for Der p/f 1 in dust extracts

Samples of vacuum bags containing fine dust and fiber were extracted at 1:10w/v into extraction buffer containing 0.9% NaCl, 0.4% phenol and 1% bovine serum albumin. The extract was rotated overnight then filtered through 0.2 micron syringe filters.
Der p 1 and Der f 1 were measured by applying the extract neat and diluted 1:5w/v in 1% BSA, normal saline-phenol diluent into microplates absorbed with species specific Der 1 monoclonal antibodies. Following an hour incubation and wash, a secondary polyclonal rabbit antibody specific to Der 1 was applied for 1 hour. Following washing goat anti-rabbit antibody was used to detect bound Der 1 protein.
A +/- indication means that the absorbance in the ELISA was above the negative blank controls but below the 10 ng/mL standard (100 ng/g of dust).

The predominant species detected in this small study was *D. farinae* as summarized in the table below.

Table 2b	Df	Dpt	Both	No HDM
Total sites each species	18	7	6	5
Single species per site	12	1		

Conclusions

- This study confirmed widespread presence of dust mite allergen in homes across the US but is low or nonexistent in drier mountain areas.
- Many homes contain both species in New England and Southern regions. Some homes in areas with mites were negative, such as in the Austin area.
- Sampling of vacuum cleaner bags identified Der 1 allergen protein.
- Due to the limitations of small sample size and with changes in the home environments over the years, further studies are needed in other areas of North America such as Canada and where differences in local temperature and humidity exist.

NOTES

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