Efficacy and safety of honeybee and wasp tyrosine-adsorbed venom immunotherapy

Maurizio Severino\textsuperscript{a}, Livio Simioni\textsuperscript{b}, Patrizia Bonadonna\textsuperscript{c}, Renato Cantone\textsuperscript{d}, Gabriele Cortellini\textsuperscript{e}, Stefano Crescioli\textsuperscript{f}, Anna D’Angelo\textsuperscript{d}, Luigi La Rosa\textsuperscript{d}, Donatella Macchia\textsuperscript{g}, Irene Martignago\textsuperscript{b}, Alessandro Massolo\textsuperscript{h,i}, Federico Reccardini\textsuperscript{k}, Diego Bagnasco\textsuperscript{l} and Giovanni Passalacqua\textsuperscript{l}\*.

ABSTRACT

Introduction: It is acknowledged that any claim of efficacy of allergen immunotherapy must be done for each specific product, and this remains true also for venom immunotherapy (VIT). Thus, we evaluated the efficacy and safety of a specific tyrosine-adsorbed VIT for \textit{vespula} spp. and honeybee in real-life.

Methods: Consecutive patients diagnosed with hymenoptera allergy, and receiving VIT for either \textit{vespula} or honeybee with a tyrosine-adsorbed preparation were observed to evaluate the grade of reaction (according to Muller) at the first field re-sting. A modified ultra-rush protocol was used.

Results: A total of 247 patients (73 female) were observed (102 honeybee, group H, 145 wasp, group V). Seventy-five patients in group H had a re-sting, and 74/75 had a lower grade reaction at re-sting as compared to the pre-VIT reaction. Considering systemic reactions, protection was achieved in 89% of patients. In group V 118 patients were re-stung, and 76/118 patients with previous grade III-IV reaction had no more systemic reaction under VIT. Overall, considering systemic reactions, protection was achieved in 92% of subjects. Of note, in both groups there was a clear inverse correlation between the severity of pre-VIT and during VIT reactions. The duration of VIT at the time of re-sting did not affect the efficacy. The safety was overall good, with 18% ad 15.4% local reactions in groups H and V, respectively.

Discussion: Modified extracts, including tyrosine-absorbed, have the aim of improving the safety of VIT still yet maintaining the efficacy. Field re-sting is the best way to assess the efficacy in real life. In this observational study we could confirm the protective efficacy of the tyrosine-adsorbed extract, with a good safety especially in the build-up using a modified-rush protocol.

Conclusion: The tyrosine-adsorbed VIT used herein is a viable and advantageous form of treatment for hymenoptera allergy.

Keywords: Hymenoptera venom allergy, Venom immunotherapy, Tyrosine adsorbed, Efficacy, Safety, Systemic reaction, Field re-sting

\*Anallergo S.p.A, Florence, Italy
\*Corresponding author. Allergy and Respiratory Diseases, IRCCS Policlinico San Martino, University of Genoa, L.go R.Benzi 10, 16133, Genoa, Italy. E-mail: passalacqua@unige.it

Full list of author information is available at the end of the article

http://doi.org/10.1016/j.waojou.2019.100086

Received 12 July 2019; Received in revised from 9 October 2019; Accepted 16 October 2019

1939-4551/© 2019 The Author(s). Published by Elsevier Inc. on behalf of World Allergy Organization. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
INTRODUCTION

Hymenoptera stings can provoke a wide range of reactions in patients with hymenoptera venom allergy (HVA),\(^1\, 2\), ranging from large local reactions to systemic anaphylaxis and death. In particular, in large local reactions the wheal-flare is larger than 10 cm and can persist for days, whereas systemic reactions (SRs) may be generalized urticaria (with/without angioedema), overt asthma, and hypotension. Patients with a previous large local reaction have a probability up to 25% of developing a SR at subsequent stings, whereas in those with a previous SR there is a risk of about 50% of having another reaction of the same or higher degree of severity.\(^1\, 3\, 4\)

Hymenoptera venom immunotherapy (VIT) is the only effective treatment able to prevent the onset (or to reduce the degree of severity) of SRs at re-sting in subjects with HVA, and shows a very favorable safety profile.\(^5\) VIT is effective in more than 95% of patients with wasp allergy and is only slightly inferior for honeybee.\(^6\) Venom extracts are commercialized either as aqueous or “retard” (adsorbed to alum hydroxide or other substances) preparations. The clinical efficacy is similar with the 2 kinds of extracts. The aqueous formulation allows a prompt availability of the allergen, but this may increase the number and severity of local reactions at the site of injection.

To avoid this inconvenience, a tyrosine-absorbed extract has been introduced (Anallergo S.pA., Florence Italy). The advantage is, in fact, the improved local tolerability, still yet maintaining the clinical efficacy, that for the tyrosine-VIT (t-VIT) was demonstrated in vivo for Polistes dominula and Vespa crabro.\(^7\, 8\)

The aim of this study was to prospectively evaluate in real-life the efficacy and safety of t-VIT with honeybee and Vespula spp venoms in subjects receiving the treatment, according to guidelines, after a hymenoptera-induced SR.

METHODS

A total of 247 consecutive patients with SRs to Vespula spp (Group V) and to honeybee (Group H) were enrolled between January 1995 and January 2018 at various allergy units in Italy. VIT was prescribed according to current guidelines.\(^9\) The diagnosis involved, as recommended: skin prick test, intradermal test, and specific IgE assay (CAP system, Thermofisher Scientific, Upssala, Sweden) for honeybee, Vespula spp., Polistes dominula and Vespa crabro. SRs were graded according to Mueller’s classification.\(^10\)

VIT was given to all patients according to a modified rush protocol.\(^11\) An aqueous extract (100 mcg/mL) was used during the build-up phase (first

| Day | VIT concentration (mcg/mL) | mL injected | Dose in mcg |
|-----|---------------------------|-------------|-------------|
| 1   | 0,1                       | 0.1         | 0,01        |
|     | 1                         | 0.1         | 0,1         |
|     | 1                         | 1           | 1           |
|     | 10                        | 0.3         | 3           |
| 2   | 10                        | 0.5         | 5           |
|     | 100                       | 0.1         | 10          |
|     | 100                       | 0.2         | 20          |
| 3   | 100                       | 0.3         | 30          |
|     | 100                       | 0.35        | 35          |
|     | 100                       | 0.35        | 35          |
| 10  | 100                       | 1           | 100         |

Table 1. Build up phase of the modified-rush protocol
3 sessions), then a tyrosine absorbed extract was given (100 mcg/mL) in the maintenance period. The interval between injections administered on the same day was 30 minutes, and the patient stayed for 1 hour under observation after the last daily dose. The protocol is summarized in Table 1. The maintenance phase consisted of 1 injection per month during the first year, and thereafter every 6-8 weeks. The clinical efficacy was evaluated recording the severity of reaction(s) that occurred at field re-stings, and comparing them to the severity at baseline (before VIT prescription). Only those re-stings where the insect was clearly identified by the patient were considered. The first field-resting was considered, unless subsequent re-stings evidenced a higher grade of severity. The safety was evaluated at each injection, both in the build-up and maintenance periods.

Frequencies were tested by Pearson’s chi-square or binomial test, whereas means were compared by Student t test or ANOVA for paired or independent data, using the Tuckey’s correction for multiple comparisons. Moreover, the Sidak correction was applied, when appropriate. Statistical significance estimates were performed, for non-parametric tests, using the exact permutation \( p_{\text{Exact}} \) or the Monte Carlo method \( p_{\text{Monte-Carlo}} \). Analyses were made using IBM® SPSS® Statistics ver. 25 (IBM, Chicago, IL, USA).

The local Ethical Committees were simply notified about the data collection, and no specific approval was needed. All the still on VIT patients signed an informed consent for the sensible data protection and privacy. All the diagnostic and therapeutic procedures were part of the standard of care, with a commercialized product.

RESULTS

Group H involved 102 patients, 23 females and 79 males with a mean age of 49.1 ± 1.6 years. Males were more represented (Binomial test, \( p < 0.001 \)), with no difference in age between genders. Their baseline (before VIT) reactions had been grade I in 9.8% patients, grade II in 14.7% grade III in 31.3% and grade IV in 44.1% of patients. No significant difference according to grades of severity between men and women was detected (Mann Whitney test, \( U = 857.5, W = 4017.5, z = -0.436, p_{\text{Exact}}>0.050 \)), as well as no association was seen between age and severity of reactions (ANOVA, \( F_{3,98} = 1.354, p > 0.05 \)).

Group V included 145 subjects, 50 females and 95 males, with a mean age of 57.7 ± 1.2 years, again with a prevalence of male subjects (Binomial test, \( z = -3.654, p < 0.001 \)). The grade of reaction before VIT was grade I in 7.5% patients, grade II in 15.1%, grade III in 22.7% and grade IV in 54.4% patients, again with no difference between men and women, and no correlation with age. The demographic and clinical data are summarized in Table 2.

Seventy-five patients in group H and 118 in group V had at least one re-sting during their VIT course. In the total of the recorded reactions, the stinging insect was clearly identified by patients

|                  | Group H N = 102 | Group V N = 145 |
|------------------|----------------|-----------------|
| M/F: N (%)       | 79/23 (77.4/22.6) | 95/50 (65.5/34.5) |
| Mean age (range) | 49 (16–78)       | 58 (22–85)       |
| Grade I          | 10 (9.8)         | 11 (7.6)         |
| Grade II         | 15 (14.7)        | 22 (15.2)        |
| Grade III        | 32 (31.3)        | 33 (22.7)        |
| Grade IV         | 45 (44.2)        | 79 (54.5)        |
| Field re-sting during VIT, N(%) | 75 (74) | 118 (81) |
| Mean duration of VIT, years (range) | 7(1–22) | 8 (1–23) |

Table 2. Demographic and clinical data
(sometimes also by an entomologist). In group H, in 74/75 re-stung patients, the reaction at re-sting was of a lower grade compared to the pre-VIT reaction (Wilcoxon paired test, $z = 7.547$, $p_{\text{Exact}} < 0.001$). Only 1 patient had no change in the severity of reaction (that remained of grade III). (Fig. 1a). There was a strong inverse correlation between the severity of reaction pre-VIT and that at re-sting ($r_s = -0.857$, $p < 0.001$). In other words effect was more apparent in those patients with the more severe reactions pre-VIT. Since only 8/75 patients had a systemic reaction at re-sting, the overall protection was 89%. In Group V 118/145 were field re-stung. Also in this case there was an overall significant decrease in the severity of reactions versus the pre-VIT situation: 76/118 patients with previous grade III or IV reaction had no more systemic reactions during VIT (Wilcoxon paired test, $z = -9.462$, $p_{\text{Exact}} < 0.001$). Only 3 patients were not protected, one maintaining a grade IV reaction, one reducing from IV to III, and one increasing from I to II. All the patients with pre-VIT grade III reactions had none or only local reactions during VIT (Fig. 1b). Globally, 9 patients still had SRs (6 grade I) during VIT, thus we can assume that t-VIT was protective in 92% of patients. Also for Vespula VIT, there was a clear inverse correlation between the severity of pre-VIT and during VIT reactions ($r_s = -0.609$, $p < 0.010$).

Concerning safety, the tolerability was overall satisfactory, considering that a modified rush protocol was applied. In group H local reactions accounted for 18% patients during the build-up and 3% during the maintenance phase (with overall 6 LLR, 10 grade 1 and grade 2). Females appeared more prone to experience adverse reactions during build-up, mainly with LLR and grade I reactions ($X^2_3 = 8.377$, $p_{\text{Exact}} = 0.044$), but this difference disappeared in the maintenance phase. In group V local reactions were noticed in 15.4% patients during build-up and in 2% patients during maintenance.

Of note, all those patients who experienced mild adverse reactions during the build-up phase could reach the 100 mcg maintenance dose. Also, there was no significant correlation between the duration of VIT and the re-sting reaction grade.

**DISCUSSION**

VIT is overall effective in protecting at further stings those subjects with HVA, as confirmed by numerous trials where different extract preparations were employed. The occurrence of local (even systemic) reactions still represents one of the main concerns, especially with Honeybee-VIT. In this regard, the use of adsorbed (depot or retard) formulations overall showed a less

---

**Fig. 1** Number of patients with reactions by severity at re-sting (y axis) according to the reaction experienced before VIT (x axis). A: Honeybee Group; B: Vespula group
occurrence of reactions, when compared to aqueous extracts. Several attempts to improve the safety and tolerability of VIT were made, mainly using the adsorption approach. There are reports on the use of alum-adsorbed extracts and Rueff et al.\(^7\) showed that alum-adsorbed formulations of VIT achieved a reduction in the severity and frequency of reactions at the site of injection. Quercia et al.\(^8\) reported that alum-adsorbed VIT was better tolerated during a rush induction protocol, and this was confirmed by Alessandrini et al.\(^9\) with Vespula VIT absorbed with alum salt.

The tyrosine absorption of VIT was already described with various VIT protocols,\(^7\),\(^8\) showing that this formulation is effective, safe and modulable. The present study, conducted as observational, in patients with HVA and treated with a t-VIT, showed that this formulation is effective for both Vespula spp and honeybee allergy, with a protection rate overall around 90% of patients and with a slightly better efficacy and tolerability for Vespula venom. The strengthness of the study resides that no surrogate biomarker of efficacy was used, but the field re-sting with the culprit insect, and that the VIT course was prolonged enough, to assess the real-life effects. Moreover, as an additional observation, the extension to 3–4 months of the interval between maintenance doses allowed to maintain the protection, as already previously suggested.\(^10\) Similarly, no relationship between the duration of VIT and the time to the first re-sting was observed and the modified rush protocol was well tolerated.\(^21\)

Since there is currently the need for the demonstration of the efficacy of each single VIT product, to avoid an unjustified claim for a generic “class-effect”,\(^22\) we attempted to clinically confirm the clinical efficacy and safety of a specific t-VIT product, evaluating only very selected patients, and using the field re-sting assessment criteria.

**Abbreviations**

HVA: hymenoptera venom allergy; VIT: venom immunotherapy; t-VIT: tyrosine-adsorbed venom immunotherapy; SR: systemic reaction

**Disclosure of interest**

Dr. Maurizio Severino is consultant for Anallergo S.p.A., Florence, Italy. No conflict of interest to disclose for all the other authors.

**Ethics approval**

None required. All the described procedures were part of the standard of care in this field.

**Authorship ethics**

All the signing Author contributed to the clinical work, collection and analysis of the data and drafting the MS. MS, GP and AM designed the study. AM was the biostatistician.

**Consent for publication**

All Authors approved the final version and its submission.

**Declaration of competing interest**

None to declare for the present work.

**Acknowledgement**

The Authors wish to thank the Professional Nurse Mrs Sabrina Del Zenero for her valuable help in collecting the data.

**Author details**

\(^a\)Anallergo S.p.A, Florence, Italy. \(^b\)Allergy Unit, Medicine Department, Ospedale S.Maria del Prato, AULSS 1 Dolomiti Regione Veneto, Feltre, Italy. \(^c\)Allergy Dept, Integrated University-Hospital of Verona, Italy. \(^d\)Allergy Unit, ASL, Novara, Italy. \(^e\)Internal Medicine and Rheumatology Dept, Rimini Hospital, Rimini, Italy. \(^f\)Allergy Unit, Bassano del Grappa, Italy. \(^g\)Allergy and Clinical Immunology Unit, Ospedale s. Giovanni di Dio, Florence, Italy. \(^h\)Ethology Unit, Dept of Biology, University of Pisa, Italy. \(^i\)UMR CNRS 6249 Chrono-environnement, Université Bourgogne Franche-Comté, Besançon, France. \(^j\)Dept of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Canada. \(^k\)Azienda Sanitaria Universitaria Integrata di Udine SOC Pneumologia - Fisiopatologia Respiratoria, Udine, Italy. \(^\dagger\)Allergy and Respiratory Diesases, IRCCS Policlinico San Martino, University of Genoa, L.go R.Benzi 10, 16133, Genoa, Italy.

**REFERENCES**

1. Bonifazi F, Jutel M, Biló BM, Bimbaum J, Muller U. EAACI Interest Group on Insect Venom. Prevention and treatment of Hymenoptera venom allergy: guidelines for clinical practice. Allergy. 2005;60:1459-1470.

2. Golden D, Demain J, Freeman T, et al. Stinging insect hypersensitivity: a practice parameter update 2016. Ann Allergy Asthma Immunol. 2017;118:28-54.

3. Biló MB, Martini M, Pravettoni V, et al. Large local reactions to Hymenoptera stings: outcome of re-stings in real-life. Allergy. 2019 May 10. https://doi.org/10.1111/all.13863 (Epub ahead of print).
4. Reisman RE. Natural history of insect sting allergy: relationship of severity of symptoms of initial sting anaphylaxis to re-sting reactions. *J Allergy Clin Immunol*. 1992;90:335-339.

5. Boyle RJ, Eremeli M, Hockenhull J, et al. Venom immunotherapy for preventing allergic reactions to insect stings. *Cochrane Database Syst Rev*. 2012;10. CD008838.

6. Bilò MB, Antonicelli L, Bonifazi F. Purified vs. nonpurified venom immunotherapy. *Curr Opin Allergy Clin Immunol*. 2010;10:330-336.

7. Severino M, Bonadonna P, Bilò MB, et al. Safety and efficacy of immunotherapy with Polistes dominulus venom: results from a large Italian database. *Allergy*. 2009;64:1229-1230.

8. Macchia D, Cortellini G, Mauro M, et al. Vespa crabro immunotherapy versus Vespula-venom immunotherapy in Vespa crabro allergy: a comparison study in field re-stings. *World Allergy Organ J*. 2018;11:3.

9. Sturm GJ, Varga EM, Roberts G, et al. EAACI guidelines on allergen immunotherapy: hymenoptera venom allergy. *Allergy*. 2018;73:744-764.

10. Mueller HL. Diagnosis and treatment of insect sensitivity. *J Asthma Res*. 1966;3:331-333.

11. Díez Gómez ML, Quirce Gancedo S, Juliá de Páramo B. Venom immunotherapy: tolerance to a 3-day protocol of rush-immunotherapy. *Allergol Immunopathol*. 1995;23:277-284.

12. Sokal RR, Rohlf FJ. *Biometry. The Principles and Practice of Statistics in Biological Research*. 3 edition. New York: W.H. Freeman & C.; 1995. USA.

13. Siegel S, Castellan NJJ. *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill; 1988. USA.

14. Mehta CR, Patel NR. *IBM® SPSS® Exact Tests 20 for Windows®*. Chicago, USA: IBM; 2011.

15. Kosnik M, Korosec P. Venom immunotherapy: clinical efficacy, safety and contraindications. *Expert Rev Clin Immunol*. 2015;11:877-884.

16. Bilò MB, Pravettoni V, Bignardi D, et al. Hymenoptera Venom Allergy: management of children and adults in clinical practice. *J Investig Allergol Clin Immunol*. 2019;29:180-205.

17. Ruöff F, Wolf H, Schnitker J, Ring J, Przybilla B. Specific immunotherapy in honeybee venom allergy: a comparative study using aqueous and aluminium hydroxide adsorbed preparations. *Allergy*. 2004;59:589-595.

18. Quercia O, Emiliani F, Pecora S, Burastero SE, Stefanini GF. Efficacy, safety, and modulation of immunologic markers by immunotherapy with honeybee venom: comparison of standardized quality depot versus aqueous extract. *Allergy Asthma Proc*. 2006;27:151-158.

19. Alessandrini AE, Berra D, Rizzini FL, et al. Flexible approaches in the design of subcutaneous immunotherapy protocols for Hymenoptera venom allergy. *Ann Allergy Asthma Immunol*. 2006;97:92-97.

20. Simioni L, Vianello A, Bonadonna P, et al. Efficacy of venom immunotherapy given every 3 or 4 months: a prospective comparison with the conventional regimen. *Ann Allergy Asthma Immunol*. 2013;110:51-54.

21. Brown SGA, Wiese MD, van Eeden P, et al. Ultrarush versus semirush initiation of insect venom immunotherapy: a randomized controlled trial. *J Allergy Clin Immunol*. 2012;130:162-168.

22. Bachert C, Larché M, Bonini S, et al. Allergen immunotherapy on the way to product-based evaluation-a WAO statement *World Allergy. Organ J*. 2015;8:29.