Vespa velutina nigrithorax venom allergy: inhibition studies approach for the choice of specific immunotherapy

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Summary

Vespa velutina nigrithorax (VVN), commonly known as Asian wasp because endemic in Asia, represents an alien species in Europe. VVN can induce allergic reactions similar to those caused by other *Hymenoptera* and deaths after VVN stings, presumably due to fatal allergic reactions, were reported. In the treatment of *Hymenoptera* venom hypersensitivity, specific immunotherapy (VIT) is highly effective, and the vaccine allergen-specificity plays a crucial role. Currently, there is no specific available VIT for VVN, so VVN stung patients with severe systemic reactions are treated with *Vespula spp* (Vspp) venom. It is also relevant to assess if patients stung by VVN and showing allergic reactions could be treated with the *Hymenoptera* commercially available extracts *Vespa crabro* (VC) and Vspp, or if they need the specific VIT with VVN venom extract. Our results suggested that both Vspp and VC venoms were able to inhibit the specific IgE for VVN, although the VC venom, compared to the venom of Vspp showed a higher inhibition.

Key words: *Vespa velutina nigrithorax;* immunotherapy; *Hymenoptera* venoms

Introduction

Vespa velutina nigrithorax (VVN), commonly known as Asian wasp because naturally present in Asia, is an alien species in Europe (1). VVN was introduced in France, probably in 2004 (2) and since then has spread into Spain (3), Portugal (4), Great Britain (5, 6), Belgium (7), Germany (8). The first well identified case of VVN in Italy was reported in 2012 (9) with different characteristics in terms of land cover and density. In particular, nests were found in Liguria and Piedmont and a monitoring system was established in 2007 in these regions close to the French border for detecting the first animal introduction in the country (10). VVN represents one of the most aggressive *Hymenoptera* species in China, where it is known as killer wasp because most affected people die after multiple stings due to organ dysfunction induced by toxins in the venom (11,12). Moreover, VVN can provoke allergic reactions similar to those caused by other *Hymenoptera* and deaths after VVN stings, presumably due to fatal allergic reactions were reported (13). In *Hymenoptera* venom hypersensitivity, specific immunotherapy (VIT) is highly effective, and the allergen-specificity of the vaccine plays a crucial role (14). In some cases, multiple skin/serum positivity presented by many patients may be due to cross-reactivity among components of different Hymenoptera venoms (15, 16), so that VIT with one single venom could be effective. Discrimination between true double sensitization and the identification of primary and/or cross-sensitization has normally been studied by inhibition studies, which cannot be used in common laboratory practice because laborious, time-consuming and poorly automatable (17-20). Despite their different geographical distribution, the composition of allergenic proteins is similar in all Vespids venoms (21,22) with phospholipase A1, hyaluronidase and antigen 5, representing the most common allergens. Our preliminary data by shotgun proteomics analysis on the three venoms *Vespa crabro* (VC), *Vespula* spp (Vspp) and VVN, extracted with the technique "Entomon Capillary Extracted Venom®" and divided into aliquots corresponding to approximately 10.0 mg and 0.50 mg of protein, confirmed the presence of the major proteins phospholipase A1, hyaluronidase and antigen 5. SDS-PAGE comparison showed

differences between the relative abundance of the three proteins (23). Band digestion after SDS-PAGE of VVN phospholipase A1 venom showed a homology of 55% with VC phospholipase A1 and 36% with Vspp phospholipase A1 (23). Immunoblot analysis showed reactivity to dipeptidyl peptidase in VC but no reactivity in Vspp, higher reactivity to phospholipase A1 in VC and Vspp than in VVN venom extract (23). It is important to understand even if patients stung from VVN and showing allergic reactions could be treated with the *Hymenoptera* commercially available extracts VC and Vspp or need the specific VIT with VVN venom extract. Currently, there is no specific available VIT for VVN, so VVN stung patients with severe systemic reactions could be treated with *Vespula* venom (24).

Preliminary studies on VVN venom compared to the venoms of VC and Vspp have highlighted differences in the composition and structure of the venom of VVN suggesting the importance of a specific venom for VVN for a diagnostic and therapeutic use. The aim of this work was to assess the specific IgE response to VVN venom and to compare the degree of cross reactivity among the three venom extracts from VVN, VC, Vspp by means of CAP-inhibition assays in patients with severe systemic reactions and multiple sensitizations to *Hymenoptera.*

Materials and methods

Serum samples were obtained from four patients with a clinical history of systemic reactions after VVN sting, attending Allergology and Clinical Immunology Unit, San Giovanni di Dio Hospital, Florence, Italy and Allergology and Clinical Immunology Unit, Mexoeiro de Vigo, Pontevedra, Spain. In particular, three patients had a Mueller III grade and one patient a Mueller IV grade systemic reaction (25); all patients have had stings from unidentified vespids (certainly not VC or VVN) in the past, without systemic reactions. Serum specific IgE were assayed quantitatively with an automated fluoro-enzyme immunoassay ImmunoCAP™ Specific IgE by Phadia™ 1000 System (Thermo Fisher Scientific, Uppsala, Sweden) for VC, Vspp and VVN. The system uses as a solid phase a polimer of hydrophilic, highly branched cellulose derivative, enclosed in a capsule and extract or recombinant bound covalently to the solid phase. The inhibition assays were performed

with specific IgE level >5 kU/L. VVN, VC, Vspp venoms were supplied by Entomon S.R.L after capillary extraction. Serum samples were incubated separately for 12 h at 4 °C with 200 µl of each venom at increasing concentrations (0; 0.3; 3.0; 30; 300 µg/mL). Subsequently, specific IgE against each of the venoms were determined in the samples. The CAP-inhibition test was carried out with a specific program in Phadia™ 250 System (Thermo Fisher Scientific, Uppsala, Sweden). The extent of homologous (blockage of venom-specific IgE by the same venom) and heterologous (blockage of the venom specific IgE by other venoms) inhibition was computed with the following formula: % inhibition = 100 - [IgE inhibited sample (kU/L) *100/IgE anti-venom (kU/L) at zero concentration of venom]. A percentage of heterologous inhibition ≥75% was considered strongly suggestive of cross-reactivity (26).

Statistics

The differences in the mean inhibition in percentage between VC against VVN and Vspp against VVN were tested using a *t*-test for paired samples (27) at each dosage.

Similarly, at each dosage, the coherence between the values in the inhibition assay was assessed by a Pearson Linear Correlation (28).

To reduce the type I error, a Bonferroni correction was applied, and alfa levels were corrected ($\alpha' = \alpha/k$, with k=2) and set to 0.025 (for 0.05), 0.005 (for 0.01) and 0.0005 (for 0.001) for both tests.

All statistical analyses were carried out using the software package IBM SPSS 28.0.0.0 (Chicago, IL, USA).

Results

Data from inhibition studies are summarized in Table 1. No significant differences were detected between the mean inhibition by its own venom, versus other species venoms (for all t-tests $p > 0.025$). As for the correlations, regardless of the dosage, there was a very high concordance between the inhibition levels of VVN and VC (all comparisons $r > 0.98$, $p < 0.005$). Only at the last

concentration of 300 µg/mL there was a concordance of the inhibition of specific IgE for VVN by the venoms VC and Vspp, while at the lowest concentrations (3 and 30 µg/mL) there was a statistically significant difference (Fig. 1).

Pat	Inhibitor µg /mL	sIgE (kU/L) to VVN after inhibition with VVN venom (% of inhibition)	sIgE (kU/L) to VVN after inhibition with VC venom (% of inhibition)	sIgE (kU/L) to VVN after inhibition with Vspp venom (% of inhibition)
Italy 1	0	14.3 (0)	13.6 (0)	12.1 (0)
Italy 1	0.3	14 (2.1)	12.8 (5.88)	11.5 (4.96)
Italy 1	3	10.7 (25.17)	7.7 (43.38)	10.2 (15.7)
Italy 1	30	4.9 (65.73)	4.2 (69.12)	4.2 (65.29)
Italy 1	300	2.8 (80.42)	2.3 (83.09)	2.1 (82.64)
Italy 2	0	41.4 (0)	41.2 (0)	12.7 (0)
Italy 2	0.3	40.2 (2.9)	40.7 (1.21)	11.6 (8.66)
Italy 2	3	22.7 (45.17)	16.1 (60.92)	4.2 (66.93)
Italy 2	30	1.52 (96.33)	1.78 (95.68)	1 (92.13)
Italy 2	300	0.79 (98.09)	0.73 (98.23)	0.6 (95.28)
Spain 1	0	20.5 (0)	21.2 (0)	3.8 (0)
Spain 1	0.3	13 (36.59)	12.9 (39.15)	3.6 (5.26)
Spain 1	3	3.2 (84.39)	3 (85.85)	1.7 (55.26)
Spain 1	30	1.8 (91.22)	1.98 (90.66)	1 (73.68)
Spain 1	300	1 (95.12)	1.07 (94.95)	0.6 (84.21)
Spain 2	0	17.2 (0)	16.4 (0)	10.9 (0)
Spain 2	0.3	15.7 (8.72)	14.1 (14.02)	10 (8.26)
Spain 2	3	4.3 (75)	2.6 (84.15)	5.6 (48.62)
Spain 2	30	0.28 (98.37)	0.28 (98.29)	0.2 (98.17)
Spain 2	300	0.08 (99.53)	0.06 (99.63)	0.06 (99.45)

Tab.1 Inhibition studies: sIgE to VVN after inhibition with VVN venom; sIgE to VVN after inhibition with VC venom; sIgE to VVN after inhibition with Vspp venom .

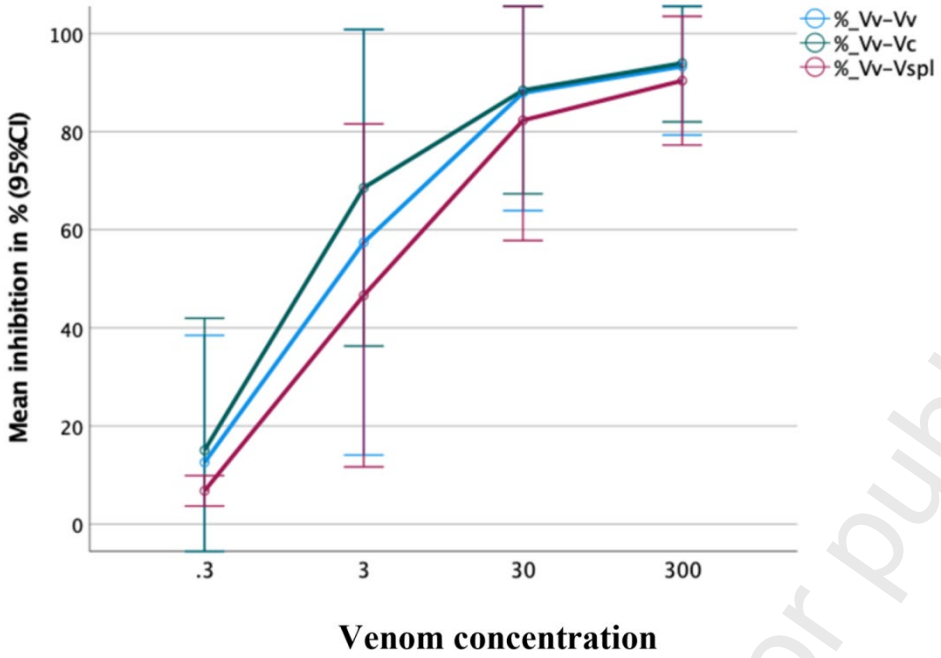

Fig. 1 Percentage mean inhibition (% 95CI) at different concentrations of venom inhibitor (0.3, 3, 30 and 300 µg/mL) from different species in 4 patients: blue line, VVN specific IgE inhibited by VVN venom; green line, VVN specific IgE inhibited by VC venom; red line, VVN specific IgE inhibited by Vspp venom.

Discussion

Vespa velutina nigrithorax an alien species in Europe, was accidentally introduced into France, probably in 2004 (2) and since then has rapidly colonized other European countries: Spain (3), Portugal (4), Belgium (7), Germany (8) Great Britain (5,6) and Italy (9). VVN, competitor of VC, both belong to the same genus *Vespa*, is considered invasive for its impact on apiculture; adult wasps prey on bees and contribute to the loss of honeybee colonies. Apart from this social aspect VVN may cause allergic reactions similar to those induced by other *Hymenoptera* species. Vidal et al, [13] studied 100 consecutive Spanish patients with anaphylaxis to Hymenoptera venom and showed that 77 patients had anaphylaxis to VVN. Of these, only 16 reported previous VVN stings, but were stung by other *Hymenoptera*, showing a sensitization pattern similar to that of patients with Vespid anaphylaxis. Different studies (13, 24, 29) on VVN have tried to identify the allergens present in the venom and the most suitable VIT to use. Antigen 5, phospholipase A1 and two

isoforms of hyaluronidase have been demonstrated to be the major components of the VVN venom. By comparing the VVN phospholipases and antigen 5, the authors demonstrated a high percentage of structural identity with the VC and Vspp phospholipases and antigen 5 compared to those present in *Polistes dominula* [29]. VVN antigen 5 showed a homology of 88 % and 66 % with VC e Vspp respectively, while phospholipase A1 a homology of 69 % and 61 %. Currently, there is no specific available VIT for VVN, so VVN stung patients with severe systemic reactions are treated with *Vespula* venom (24). Moreover, VVN ImmunoCAP™ allergen, Asian hornet, for the quantitative measurement of venom specific IgE, is available for Research Use Only" (RUO) and not for diagnostic procedures. It is important to understand even if patients stung from VVN and showing allergic reactions could be treated with the *Hymenoptera* commercially available extracts VC and Vspp or need the specific VIT with VVN venom extract. On the basis of the structural homology between the Vspp and VVN allergens, considering that in most of the subjects, there had been a previous Vspp sting, a VIT with Vspp was proposed in 46 patients with VVN reactions (24); in accordance with the literature data, the patients showed an increase in specific VVN and Vspp specific IgG and a decrease in specific VVN and Vspp specific IgE during the first year of therapy; 13 patients (28.2%) with anaphylaxis due to VVN and treated with Vspp venom were stung again by VVN during immunotherapy and none presented systemic reactions. VC venom is available in Italy, both for diagnosis and VIT. Immunotherapy with VC in patients with anaphylaxis to VC has proven to be effective and safe (30). VC and VVN belong to the same genus *Vespa*, while Vspp belongs to another genus, *Vespula*; therefore, from a phylogenetic point of view, VVN is closer to the VC than to the Vspp. The aim of our study was to verify by inhibition studies whether the venom of VC and Vspp were able to inhibit specific IgE for VVN. Our results suggested that both Vspp and VC venoms are able to inhibit the specific IgE for VVN, although the VC venom, compared to the venom of Vspp showed a higher inhibition. Moreover, the percentage of heterologous VC/VVN inhibition was equal to or greater than the percentage of homologous VVN/VVN inhibition, particularly from the first concentration of venom in the RAST inhibition,

emphasizing the efficacy of inhibition. Our study has the limitation of the case history limited to only 4 patients. More studies are needed for validation of our results.

Conclusion

VVN is an invasive and aggressive alien *Vespa* species in Europe, capable of provoking new serious health problems. Nowadays there is not specific available VIT for VVN. To prevent systemic sting allergic reactions we assessed the effectiveness of VIT from closely related species. Our inhibition studies suggested that VIT with VC venom may be more effective in patients with VVN sting reaction. When VIT with VC is not available, VIT with Vspp can be used. Further studies, particularly on specific IgE antibodies regarding the specific sensitization and specific IgG antibodies regarding the specific tolerance, are needed to confirm these data.

Conflict of interests

The authors declare that they have no conflict of interests

References

1. Perrard A, Arca M, Rome Q, Muller F, Tan J, Bista S, et al. Geographic variation of melanisation patterns in a hornet species: genetic differences, climatic pressures or aposematic constraints? PLoSONE 2014;9(4): e94162.doi: 10.1371/journal.pone.0094162. 2014;9: e94162
2. Haxaire J, Bouguet JP, Tamisier JP. *Vespa velutina* Lepeletier, 1836, une redoutable nouveauté pour la faune de France (Hym., Vespidae). Bulletin de la Société entomologique de France, 2006;194111:194
3. Castro L, Pagola-Carte S. *Vespa velutina* Lepeletier, 1836 (*Hymenoptera: Vespidae*), recolectada en la Península Ibérica. Heteropterus Revista de Entomología, 2010;10:193–6
4. Grosso-Silva JM, Maia M. *Vespa velutina* Lepeletier, 1836 (*Hymenoptera, Vespidae*), new species for Portugal, 2012; Arquivos Entomoloxicos 6:53–54
5. Budge GE, Hodgetts J, Jones EP, Ostoja-Starzewski JC, Hall J, Tomkies V, et al. The invasion, provenance and diversity of *Vespa velutina* Lepeletier (*Hymenoptera: Vespidae*) in Great Britain. PLoS ONE 2017;12(9): e0185172. doi: 10.1371/journal.pone.0185172
6. Keeling MJ, Franklin DN, Datta S, Brown MA, Budge GE. Predicting the spread of the Asian hornet (*Vespa velutina*) following its incursion into Great Britain. Sci Rep 2017;7(1):6240. doi: 10.1038/s41598-017-06212-0. 7: 6240
7. Bruneau E. Le frelon asiatique, déjà là. ActuApi, 2011;55, 1-6
8. Witt R. Erstfund eines Nestes der Asiatischen Hornisse *Vespa velutina* Lepeletier, 1838 in Deutschland und Details zum Nestbau (*Hymenoptera, Vespidae*). Ampulex, 2015; 7:42–5
9. Demichelis S, Manino A, Minuto G, Mariotti M, Porporato M. Social wasp trapping in north west Italy: comparison of different bait-traps and first detection of *Vespa velutina*. Bulletin of Insectology, 2014; 67:307–317
10. Porporato M, Manino A, Laurino D, Demichelis S. *Vespa velutina* Lepeletier (*Hymenoptera Vespidae*): a first assessment two years after its arrival in Italy. Redia, 2014; 97:189–194
11. Liu Z, Chen S, Zhou Y, Xie C, Zhu B, Zhu H, et al. Deciphering the venomic transcriptome of killer-wasp *Vespa velutina*. Sci Rep 2015; 5:9454. 12. doi: 10.1038/srep09454
12. Xie C, Xu S, Ding F, Xie M, Lv J, Yao J, et al. Clinical features of severe wasp sting patients with dominantly toxic reaction: analysis of 1091 cases. PLoS One 2013; 8: e83164. doi: 10.1371/journal.pone.0083164
13. Vidal C, Armisén M, Monsalve R, González-Vidal T, Lojo S, López-Freire S, et al. Anaphylaxis to *Vespa velutina nigrithorax*: Pattern of Sensitization for an Emerging

Problem in Western Countries. J Investig Allergol Clin Immunol 2021; Vol. 31(3): 228-235. doi: 10.18176/jiaci.0474

14. Sturm GJ, Varga EM, Roberts G, Mosbech H, Bilò MB, Akdis CA, et al. EAACI Guidelines on Allergen Immunotherapy: *Hymenoptera* venom allergy. Allergy 2018;73:744-64. doi: 10.1111/all.13262
15. Fernandez J. Distribution of vespid species in Europe. Curr Opin Allergy Clin Immunol 2004; 4:319–324. doi: 10.1097/01.all.0000136760.43571.f2
16. Golden DBK. Insect sting allergy and venom immunotherapy: a model and a mystery. J Allergy Clin Immunol 2005; 115:439–447. doi: 10.1016/j.jaci.2005.01.005
17. Muller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. *Hymenoptera* venom allergy: analysis of double positivity to honey bee and *Vespula* venom by estimation of IgE antibodies to species specific major allergens Api m1 and Ves v5. Allergy 2009; 64:543–548. doi: 10.1111/j.1398-9995.2008.01794.x
18. Caruso B, Bonadonna P, Severino MG, Manfredi M, Dama A, Schiappoli M et al. Evaluation of the IgE cross-reactions among *vespid* venoms. A possible approach for the choice of immunotherapy. Allergy 2007; 62:561–564. doi:10.1111/j.1398-9995.2007.01353.x
19. Hemmer W, Focke M, Kolarich D, Dalik I, Gotz M, Jarisch R. Identification by immunoblot of venom glycoproteins displaying immunoglobulin E-binding N-glycans as cross-reactive allergens in honeybee and yellow jacket venom. Clin Exp Allergy 2004; 34:460–469. doi: 10.1111/j.1365-2222.2004.01897.x
20. Jappe U, Raulf-Heimsoth M, Hoffmann M, Burow G, Hubsch-Muller C, Enk A. In vitro *hymenoptera* venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. Allergy 2006; 61:1220–1229. doi: 10.1111/j.1398-9995. 2006.01232.x
21. King TP, Sobotka AK, Alagon A, Kochoumian L, Lichtenstein LM. Protein allergens of white-faced hornet, yellow hornet, and yellow jacket venoms. Biochemistry 1978; 17:5165–5174. doi: 10.1021/bi00617a016
22. King TP, Spangfort MD. Structure and biology of stinging insect venom allergens. Int Arch Allergy Immunol 2000; 123:99–106. doi: 10.1159/000024440
23. Pantera B, Tinti L, Salvini L, Pepiciello I, Cervo R, Cappa F, Severino M, Manfredi M, Grossi V, Macchia D, Francescato E. Preliminary studies on the venom of the yellow-legged hornet *Vespa velutina nigrithorax*. Allergy 2019; 74(S106):139

24. Rodríguez-Vázquez V, Armisén M, Gómez-Rial J, Lamas-Vázquez B, Vidal C. Immunotherapy with *Vespula* venom for *Vespa velutina nigrithorax* anaphylaxis: Preliminary clinical and immunological results. Clin Exp Allergy 2022; 52(2):345-347. doi: 10.1111/cea.14039
25. Mueller H.L. Diagnosis and treatment of insect sensitivity. J Asthma Res 1966;3:331–3. 10.3109/02770906609106941
26. Straumann. F, Bucher C, Wüthrich B. Double sensitization to honeybee and wasp venom: immunotherapy with one or with both venoms? Value of FEIA inhibition for the identification of the cross-reacting ige antibodies in double-sensitized patients to honeybee and wasp venom. Int Arch Allergy Immunol 2000;123(3):268-74. doi 10.1159/000024453
27. Sokal, R. R. and F. J. Rohlf. Biometry: The Principles and Practices of Statistics in Biological Research. New York, W. H. Freeman, 1994
28. Fox, J. Applied regression analysis and generalized linear models. Thousand Oaks, CA, US, Sage Publications, Inc, 2016
29. Monsalve RI, Gutièrrez R, Hoof I, Lombardero M. Purification and molecular characterization of phospholipase, antigen 5 and hyaluronidases from the venom of the Asian hornet (*Vespa velutina*). PLoSONE 2020; 10;15(1): e0225672. doi: 10.1371/journal.pone.0225672.
30. Macchia D, Cortellini G, Mauro M, Meucci E, Quercia O, Manfredi 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(1):3. doi: 10.1186/s40413-018-0183-6.