

Purified vs. nonpurified venom immunotherapy

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Purpose of review

Although highly effective, venom immunotherapy (VIT) may be responsible for local and systemic allergic reactions. There is a good theoretical basis for believing that purified aqueous and purified aluminium hydroxide adsorbed (so-called depot) extracts, commercially available in Europe, have the potential to reduce the incidence of VIT's side effects. The aim of this article is to review the literature on safety and effectiveness of purified preparations as well as compare them with nonpurified extracts.

Recent findings

Old and new noncomparative studies reveal good tolerance of purified aqueous and purified depot extracts. In comparative trials purified extracts appear to be better tolerated than nonpurified extracts, whereas depot extracts seem to be safer than the corresponding purified aqueous preparation, especially in the prevention of severe large local reactions. The efficacy of purified aqueous and depot extracts is supported by studies using both sting challenge and in-field stings and is comparable to that of nonpurified preparations.

Summary

The theoretical basis of the safer profile of purified extracts is supported by a number of clinical studies, making the use of purified depot preparations preferable for conventional treatment also by specialists with less experience in managing VIT. In specialized centres purified aqueous extracts may be preferred for faster build-up protocols. However, further prospective controlled studies are needed in order to evaluate the ability of purified extracts to reduce the frequency of severe systemic reactions over the corresponding nonpurified preparation.

Keywords

nonpurified aqueous extract, purified aluminium hydroxide adsorbed extract, purified aqueous extract, side effects, venom immunotherapy

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Introduction

Subcutaneous venom immunotherapy (VIT), which lessens the risk of a subsequent systemic reaction, prevents morbidity and mortality [1–5] and improves health-related quality of life [6], is probably the most effective treatment currently available to physicians. Its efficacy has been proven in prospective controlled trials, uncontrolled studies and in one meta-analysis by both sting challenge and in-field stings, demonstrating that the protection rate of vespid VIT is greater than that of honeybee VIT [1–4]. Subcutaneous VIT is also effective in mastocytosis patients, albeit to a lesser extent [7•].

Indications for VIT are based on the history of a systemic reaction, a positive venom skin and/or in vitro radioallergo-sorbent test (RAST) [8], knowledge of the natural history and established risk factors for severe outcome [9•,10•].

Protocols of various duration have been devised in an endeavour to maximize protection and optimize patient

convenience. The time required to reach a generally adequate maintenance dose of 100 µg with slow protocols ranges from several weeks to months, whereas rush, and ultra-rush protocols only require several days or a few hours, respectively. Rush VIT is routinely used as core treatment in Europe, but is not readily available at most American medical centres [5,11].

Compliance with VIT can be impaired by large local reactions (LLRs) and systemic reactions [1,3,4], the latter explaining why in some European countries only selected allergy centres usually opt for treatment.

Some studies, however, show that VIT's side effects are less frequent than those caused by subcutaneous immunotherapy for inhalant allergens [12,13].

Side effects of venom immunotherapy

Large local reactions to injections are commonplace, especially during the incremental phase, and occur in

Table 1 Systemic reactions during venom immunotherapy (original)

Author	No. of patients	% of patients with a SR	No. of SRs ^a	Severity of SR
Lockey <i>et al.</i> [14]; AAAAI Multicenter Study	1410	12	327	Severe: 9%; moderate: 32%; mild: 59%
Mosbech and Muller [15]; EAACI Multicenter Study	840	20	299	Mainly mild; 1/3 of patients required treatment: epinephrine in one patient
Niedoszytko <i>et al.</i> [7 ^{••}] ^b ; review article	3006	2–16	N.A.	Epinephrine used in 0.6–5% of patients

NA, not available; SR, systemic reaction.

^aSide effects were more frequent with honeybee venom extracts and during the incremental phase.

^bThis review included reference [15].

up to 50% of patients. Though LLRs during VIT are not a risk factor for systemic reaction, and do not usually necessitate a reduction in venom dose or prevent the protective dose from being reached, they can be particularly bothersome, sometimes requiring corticosteroids and antihistamines treatment for several days. However, anaphylaxis represents the biggest risk posed by VIT. The literature reports a large variation (0–46%) in the incidence of VIT's side effects [3,4], with the tolerance of vespid VIT faring better than honeybee VIT.

Table 1 shows the frequency of systemic reactions reported by two multicentre studies and one review of eight trials of at least 100 patients [7^{••},14,15].

Old and new potential risk factors for side effects during VIT are summarized below:

- (1) Honeybee venom > vespid venom
- (2) Build-up phase > maintenance phase
- (3) Dose increase schedule (?)
- (4) Treatment with beta-blockers/ACE inhibitors (?)
- (5) Cardiovascular disease (?)
- (6) Mastocytosis
- (7) Elevated baseline serum tryptase concentration

Apart from the well known risk factors of honeybee venom and the build-up phase, there are no definite conclusions regarding the role of the dose increase schedule [15–18]. Indeed, comparing the incidence of systemic reaction between different VIT protocols is problematic, as the classification system used for measuring the severity of adverse reactions [4] varies from investigator to investigator, who also administer different types of extracts. Moreover, there may be a difference between honeybee and vespid venoms in terms of tolerability of the different build-up protocols.

Even though there are good theoretical grounds for the contraindication of beta-blockers during immunotherapy, this does not seem to apply to VIT [19]. ACE inhibitors may possibly contribute to the onset of systemic reactions during VIT in some highly selected patients [20], but further data are required to confirm this [21].

Side effects during VIT are probably more frequent in mastocytosis patients, especially in those allergic to yellow jacket venom [7^{••}]. Preliminary data yielded by a multicentre European prospective study of approximately 800 venom-allergic patients demonstrate that an elevated baseline mast cell tryptase concentration also represents a risk factor for systemic reactions during vespid VIT (paper submitted).

How can the risk of venom immunotherapy's side effects be lessened?

Below some current and future strategies for reducing VIT's side effects are given:

- (1) Premedication
 - (a) Antihistamines
 - (b) Montelukast
 - (c) Omalizumab
- (2) Purified and depot extracts
- (3) Route of administration
 - (a) Sublingual
 - (b) Intralymphatic
- (4) Modified allergens

Since presently there is no reliable in-vivo or in-vitro test able to predict the risk of a systemic reaction to VIT, defining these risk factors would be helpful in reducing their occurrence [4,14,15].

H1-antihistamine pretreatment reduces the number and severity of LLRs and mild systemic reactions, such as urticaria and angioedema [4], though its effect on VIT efficacy is still uncertain [22].

Pretreatment with anti-IgE monoclonal antibodies in some cases permits more rapid and higher doses of allergen immunotherapy, whereas at the same time enhancing the safety of the treatment [23[•]].

On the contrary, it seems somewhat premature to hypothesize sublingual and intralymphatic administration as alternative routes for VIT [24,25^{••}].

T-cell epitope peptides of the major bee venom phospholipase A2 allergen caused reduced IgE binding, was

well tolerated during the build-up phase, but did not protect all patients from sting challenge [26].

A chimeric protein containing six pieces of the three BV phospholipase, hyaluronidase and melittin allergens, which reduces IgE binding while still preserving its T-cell stimulating activity, has only been studied in mouse models [27].

Purified extracts for venom immunotherapy

For more than 20 years in Europe now, nonpurified aqueous (NPA) and purified aqueous extracts of yellow jacket and honeybee venom have been commercially available for ultra-rush, rush, clustered and maintenance phases, and purified aluminium hydroxide adsorbed (PAHA) preparations (so-called 'depot') for the conventional build-up and maintenance schedule. Many European specialists switch to depot preparations after the up-dosing phase [11,15,28].

Polistes sp. and *Polistes dominulus* purified aqueous and PAHA extracts for diagnostic and therapeutic purposes are not commercially available in Europe at the moment.

Preparations contain 100 µg/ml of allergens. Purified aqueous venom extracts are products with no low molecular components present in the native venom extract (cut-off: 1000 D). Purification is mainly obtained through a Sephadex-gel filtration process, which allows separation of the protein fractions by means of their molecular weight. Purified aqueous venom extracts do not contain vasoactive amines like dopamine, histamine and serotonin. In addition a gel-filtration procedure reduces the presence of small peptides like apamine, kinins and mast cell degranulating peptide in the final product.

In the PAHA extract the raw venom underwent the same purification procedure with recovery of the allergen-containing fraction only and subsequently adsorbed onto aluminium hydroxide. PAHA extracts have the advantage of a slower release of allergens from the injection site, thus enhancing immune responses by prolonged antigen presentation. Furthermore, some studies describe an immunological effect of this adjuvant in down-regulating the allergen-driven Th2 cytokine profile in humans [29]. Therefore, there is a good theoretical basis for believing that both purified aqueous and depot extracts have the potential to reduce the incidence of VIT's side effects.

In the US neither purified aqueous nor PAHA preparations are commercially available, although European PAHA extracts do not exceed the FDA-approved aluminium dose (0.85 mg/dosage for aluminium and 1.14 mg/dosage for aluminium hydroxide) (FDA: CFR - Code of Federal Regulations Title 21).

What can clinical studies teach us?

In The European Academy of Allergology and Clinical Immunology multicentre study on the safety of VIT [15], among 840 venom-allergic patients 174 had been treated with depot preparations, whereas 720 had been administered aqueous extracts. Whereas systemic reactions were observed somewhat more frequently in patients treated with aqueous rather than depot preparations, the difference was not statistically significant during up-dosing or maintenance. It is not possible, however, to extrapolate which type of aqueous extracts were used (purified or nonpurified) from the data.

Purified aqueous and purified aluminium hydroxide adsorbed venom extracts

Strong evidence supporting the efficacy of VIT and its relative safety with NPA venom preparations exists [1,3,4,30].

As for PAHA extracts, in a prospective study [13] evaluating the frequency of side effects of allergen immunotherapy with aluminium hydroxide adsorbed extracts for airborne allergens and venoms, yellow jacket VIT yielded the highest frequency of patients reaching the maintenance dose without adverse reactions and the lowest frequency of side effects.

The safety, immunogenicity, and effectiveness of VIT with PAHA venom extracts were demonstrated in a prospective study over 3 years [31]. Among 35 yellow jacket and honeybee venom-treated patients, only three BV-allergic patients developed mild systemic reactions during the increase phase and one honeybee-allergic patient developed a mild systemic reaction after a third sting. A significant decrease in specific IgE antibodies after 1 year, and a significant increase in specific IgG and IgG4 antibodies on reaching the maintenance dose were observed [31].

In a retrospective study [32], no systemic reactions occurred during VIT in 36 yellow jacket venom-allergic adults with a history of severe systemic reaction who were administered a PAHA preparation of up to a 50 mcg as a monthly maintenance dose. Although the reliability of vespid in-field stings is poor, only mild local reactions occurred both during VIT and after its discontinuation [32].

Some studies reported the safety of purified aqueous extracts using rush and ultra-rush protocols [33–36].

A retrospective study [33] evaluated 1055 honeybee and yellow jacket venom patients treated with three different rapid protocols, among which 224 (21.2%) patients experienced adverse reactions, which were mainly mild.

Table 2 Purified aqueous and/or aluminium hydroxide adsorbed vs. nonpurified aqueous extracts: safety and efficacy (original)

References	No. of patients/ venom	Incremental phase/extract (no. of patients)	Maintenance phase: extract	VIT duration	Safety	Efficacy
Mosbech <i>et al.</i> [37]	32/YJ	Conventional/NPA (n.a.); conventional/PA (n.a.); conventional/PAHA (n.a.)	NPA (4–8 weeks) ^a PA (4–8 weeks) ^a PAHA (4–8 weeks) ^a	≥2 years	PA > NPA (LLRs and SRs)	Comparable (sting challenge)
Quercia <i>et al.</i> [38]	55/HB	Rush/NPA (20); cluster/NPA (20); cluster/PAHA (15)	NPA (4 weeks) ^a NPA (4 weeks) ^a PAHA (4 weeks) ^a	≥5 years	PAHA > NPA (LLRs and SRs)	Not studied
Cadario <i>et al.</i> [39]	45/YJ and HB	Cluster/NPA r (18); conventional/PAHA (27)	NPA (4 weeks) ^a PAHA (4 weeks) ^a	3 years	PAHA > NPA (LLRs)	Comparable (in-field sting)
Bilò <i>et al.</i> [40•]	94/YJ and HB	Rush/PA (50); rush/NPA (44)	PAHA (4 weeks) ^a NPA (4 weeks) ^a	11 weeks	PA > NPA (LLRs)	Not studied

HB, honeybee; LLRs, large local reactions; n.a., not available; NPA extract, nonpurified aqueous extract; PA extract, purified aqueous extract; PAHA extract, purified aluminium hydroxide adsorbed extract; SRs, systemic reactions; VIT, venom immunotherapy; YJ, yellow jacket.

^aInterval between injections.

Patients who underwent VIT with yellow jacket purified aqueous extract had significantly fewer systemic reactions than those treated with honeybee venom [33].

Sturm *et al.* [34] described their 8-year experience in 101 predominantly high-risk patients using a purified aqueous in a 4-day rush protocol. They observed systemic reactions in 6.9% of patients (2% of yellow jacket venom and 12% honeybee venom-treated patients), highlighting the lower percentage of systemic reactions compared with that reported in the literature [34]. However, antihistamine premedication may in part account for this. Patients were then treated with PAHA extracts during the maintenance phase over at least 3 years, but reporting neither the frequency of side effects nor the VIT efficacy was within the scope of the study.

An overall systemic reaction rate of 17.9% in 178 mainly vespid-allergic patients treated with a purified aqueous extract without premedicating was reported in Wenzel *et al.*'s [35] study.

Venom immunotherapy with a purified aqueous bumblebee venom extract was well tolerated in a small group of allergic patients during both a modified semi-rush protocol and the maintenance phase, the protection rate being estimated at 96% from in-field stings and challenges [36].

In summary, these noncomparative studies revealed a good tolerance of VIT with PAHA and purified aqueous extracts, evident especially for yellow jacket venom, and its efficacy by mainly in-field stings.

Purified (aqueous and/or aluminium hydroxide-adsorbed) vs. nonpurified extracts

Some studies have compared safety and efficacy of purified extracts (purified aqueous and PAHA) with NPA extracts in yellow jacket and honeybee venom-allergic patients [37–39,40•] (Table 2).

In 1986 for the first time Mosbech *et al.* [37] demonstrated the same effectiveness of three different extracts (purified aqueous, NPA and PAHA extracts) in 32 yellow jacket venom-allergic adults, with no substantial differences pertaining to safety or immunogenicity. Purified aqueous extracts induced both milder systemic reactions and LLRs than did the NPAs. Though not severe or statistically significant, systemic reactions were more frequent in the dose-increase phase with the PAHA extract. None of the 19 patients challenged in-hospital with yellow jacket stings experienced a systemic reaction [37].

Forty Italian honeybee venom patients were treated with NPA extracts (20 following a cluster schedule and 20 a rush protocol), whereas an additional 15 were given a PAHA preparation via a cluster schedule, demonstrating that depot VIT was better tolerated than NPA VIT [38].

In an open uncontrolled trial [39] of 45 yellow jacket and honeybee venom-allergic patients, patients assigned to a conventional PAHA extract regimen experienced significantly less frequent side effects than those treated via a NPA-clustered protocol, the better tolerance being mainly due to the lower frequency of early local side effects during the induction phase. In the in-field re-stung patients, VIT efficacy was comparable in the two cohorts [39].

More recently, in a prospective randomized, controlled, multicentre study 97 yellow jacket and honeybee venom-allergic patients were randomly allocated to purified extracts VIT or to NPA VIT [40•]. The induction phase was carried out using the same rush protocol, whereas the maintenance phase lasted 11 weeks. Side effects were significantly more frequent in NPA extract-treated patients than in those treated with a purified extract, owing to the higher frequency of extensive LLRs. Six systemic reactions were observed in four honeybee venom-allergic patients, mostly in the NPA extract-treated group, without being statistically significant [40•]. This study provides

evidence of the safer profile of purified preparations, even with rush protocols, with respect to severe LLRs.

In conclusion, from these comparative studies, purified (aqueous and aluminium hydroxide-adsorbed) venom extracts appear to be safer than nonpurified preparations (Table 2).

Purified aluminium hydroxide-adsorbed vs. purified aqueous extracts

The safety and efficacy of PAHA extracts have also been compared with the corresponding purified aqueous preparations in different protocols [41–43].

Forty-two honeybee venom-allergic patients were assigned to purified aqueous rush VIT followed by a 4-week maintenance interval using the same extract in 21 patients and an 8-week maintenance interval using the PAHA preparation in the others; a third group of 23 patients used the same extract (PAHA) for both conventional build-up and then a 4-week maintenance phase [41]. Treatment with the purified aqueous extract caused significantly more frequent LLRs than those caused by the depot extract during both the rush and maintenance phases, whereas only a tendency of more frequent systemic reactions during the incremental phase was observed between the groups. Although HBV-specific IgG4 increased more significantly in response to the depot preparation, the therapeutic efficacy of the three treatment protocols by sting challenge did not differ [41], suggesting the possibility of extending the intervals between injections to up to 8 weeks for depot preparations too.

In a 5-year open uncontrolled study [42] of 70 honeybee venom-allergic patients comparison of the clinical results of the purified aqueous and PAHA extracts and the use of different induction protocols demonstrated that the depot VIT was better tolerated than purified aqueous VIT during rush protocol, which was in turn less tolerated than the cluster protocol with the purified aqueous extract. Systemic reactions during the build-up and maintenance phases were significantly less frequent in both

the purified aqueous cluster and depot cluster groups when compared with the purified aqueous rush group. No systemic reactions were recorded in approximately one half of the in-field re-stung patients [42].

In another open trial, a flexible approach to VIT was taken in yellow jacket venom-allergic patients [43]. Forty-three patients were treated with the purified aqueous extract for cluster induction and maintenance, 34 with the PAHA preparation for cluster induction and maintenance and 29 with the purified aqueous extract and the PAHA preparation for cluster induction and maintenance, respectively. PAHA *Vespula* VIT induced fewer early local adverse reactions, although there was no difference in terms of systemic reactions. As for efficacy, there was no difference between the three VIT schedules [43].

These studies show (Table 3) that the frequency of side effects was lower using a PAHA extract for conventional or cluster dose-increase protocol than with a purified aqueous extract rush protocol, which also may support the influence of the build-up schedule.

Switching from a purified aqueous to a depot extract

Patients undergoing the induction phase with a purified aqueous or NPA extract can benefit from switching to a depot extract during maintenance [41,43,44]. Some European specialists are also accustomed to switching to a PAHA extract at any time during treatment because of side effects from the aqueous preparations. In our case, among 11 honeybee venom-allergic patients who developed a systemic reaction during a 5-day rush protocol (nine treated with a NPA extract and two with a purified aqueous extract), all of them resumed the equivalent dosage of the PAHA preparation, achieving the maintenance dose of 100 mcg without any side effects (unpublished data). Moreover, two honeybee venom-allergic patients who tolerated an NPA extract rush phase but developed anaphylaxis during the maintenance phase using the same extract safely switched to the depot without having to reduce the dose. Later on, both of them were diagnosed as suffering from indolent systemic

Table 3 Purified aluminium hydroxide adsorbed vs. purified aqueous extracts: safety and efficacy (original)

References	No. of patients/ venom	Incremental phase/ extract (no. of patients)	Maintenance phase: extract	VIT duration	Safety	Efficacy
Rueff et al. [41]	65/HB	Rush/PA (21); rush/PA (21); Conventional/PAHA (23)	PA (4 weeks) ^a ; PAHA (8 weeks) ^a ; PAHA (4 weeks) ^a	1 year	PAHA > PA (LLRs)	Comparable (sting challenge)
Quercia et al. [42]	70/HB	Rush/PA (20); cluster/PA (20); cluster/PAHA (30)	PA (4 weeks) ^a ; PA (4 weeks) ^a ; PAHA (4 weeks) ^a	5 years	PAHA > PA (SRs in build-up phase)	Comparable (in-field sting)
Alessandrini et al. [43]	106/YJ	Cluster/PA (43); cluster/PA (29); cluster/PAHA (34)	PA (3-8 weeks) ^a ; PAHA (3-8 weeks) ^a ; PAHA (3-8 weeks) ^a	3 years	PAHA > PA (LLRs)	Comparable (in-field sting)

HB, boneybee; LLRs, large local reactions; PA extract, purified aqueous extract; PAHA extract, purified aluminium hydroxide adsorbed extract; SRs, systemic reactions; YJ, yellow jacket.

^aInterval between injections.

mastocytosis (unpublished data). Indeed, looking at VIT side effects in 117 mastocytosis patients [7**], less than half were treated with purified aqueous extracts. Therefore, it might be interesting to compare in the future the safety of purified aqueous and PAHA extracts vs. the NPA extracts in these patients.

Possible disadvantages of aluminium hydroxide adsorbed venom preparations

Certain disadvantages of PAHA venom extracts have been pointed out [28], as well as for the inhalant depot extracts which are widely used in Europe [45,46].

Delayed side effects have been reported by a few VIT studies [39,43], but not noted in many others [37,40*, 41,42].

Subcutaneous granuloma formation is extremely rare with VIT, and probably related to inaccurate intradermal shots rather than subcutaneous injections [47].

Lastly, aluminium adjuvants can enhance IgE production in animal models if low doses of allergen are used. However, some studies of VIT [37,41] found no difference in the course of IgE during the treatment comparing VIT with and without aluminium adjuvant, perhaps due to the high dose of allergen that was administered.

Purified venom extracts for diagnosis

There are no European studies which compare purified aqueous and NPA extract for venom allergy diagnosis, even though purified aqueous extracts have been used for diagnosis in the same studies in which they were used for VIT [37].

A recent American study [48**] showed that dialysed venom skin test results were positive at 10 µg/ml or less in 79% of yellow jacket venom-allergic patients with a positive history but negative skin test using the undialysed extract, three of whom experienced a systemic reaction to a sting challenge. The authors concluded that purification of the US venom extract by dialysis provides greater diagnostic potency and accuracy [48**].

Conclusion

The efficacy of purified aqueous and PAHA extracts is supported by studies using both sting challenge and in-field stings and is comparable to that of NPA preparations. If a depot extract is used in patients without significant risk factors, extending the injection interval to 8 weeks is possible without compromising efficacy.

At present, VIT with the PAHA extract shows a safer profile than the therapy with the purified aqueous and

NPA extracts with respect to the occurrence of LLRs and systemic reactions. Purified extracts appear to be safer than nonpurified extracts, especially in terms of the frequency of LLRs. Further comparative studies are needed in order to evaluate their ability to reduce the frequency of systemic reactions over the corresponding NPA preparation using the same build-up protocol.

Therefore, while awaiting future strategies the use of the depot extracts for the conventional incremental and maintenance phases could be a workable solution for specialists who only routinely perform immunotherapy against inhalant allergens. If VIT protection is rapidly required, the use of purified aqueous preparations may be preferred for rush treatment in specialized centres with depot preparations for VIT continuation by specialists with less experience in managing VIT.

As in several countries VIT maintenance treatment is performed by general practitioners, this could be a practical solution to the organization a treatment programme in which specialistic resources are limited or are not easily reached from the patient's place of residence. These approaches may represent the means of improving both patient safety and compliance to VIT.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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