

Original article

Specific immunotherapy in honeybee venom allergy: a comparative study using aqueous and aluminium hydroxide adsorbed preparations

Background: For the immunotherapy of Hymenoptera venom allergy various preparations and treatment protocols are in use. However, controlled studies making direct comparisons of the efficacy and safety of different regimens are rare.

Objective: To assess prospectively different venom immunotherapy (VIT) protocols using an aqueous or an aluminium hydroxide adsorbed allergen preparation for the treatment of honeybee venom (HBV) allergy.

Methods: Sixty-five HBV allergic patients (42 males, 23 females; aged 17–75 years) with a history of systemic anaphylactic reactions (SARs) to honeybee stings were treated according to three different regimens. During the incremental phase, patients in group A ($n = 21$) or B ($n = 21$) received an aqueous preparation according to a rush protocol. Patients in group C ($n = 23$) were treated with conventional ('slow') VIT using an aluminium hydroxide adsorbed depot preparation. The maintenance dose was 100 µg venom in all groups. Maintenance treatment in group A was performed with the aqueous preparation administered every 4 weeks, whereas in groups B and C the depot preparation was administered every 8 weeks (group B) or every 4 weeks (group C). A sting challenge test with a living honeybee was performed in 49 patients, 6–12 months after reaching the maintenance dose. Another seven patients were stung accidentally by a honeybee ('field sting').

Results: Treatment with the aqueous preparation evoked large local reactions more frequently than the depot preparation in the dose increase phase [53/693 (7.6%) vs 8/206 (3.9%); $P = 0.059$] and also in the course of maintenance therapy [85/172 (49.4%) vs 58/478 (12.1%); $P < 0.001$]. During the dose increase phase, systemic side-effects seemed to occur more frequently in patients on rush VIT with the aqueous preparation compared to patients initially treated with the conventional schedule using the depot preparation [13/42 (31.0%) vs 3/23 (13.0%); not significant]. When re-stung by the culprit insect, SARs were observed in 3/20 patients (15.0%) in group A, 2/18 (11.1%) in group B and 3/18 (16.7%) in group C (not significant).

Conclusions: The aluminium hydroxide adsorbed HBV preparation caused fewer large local reactions than the aqueous preparation. The therapeutic efficacy of the three treatment protocols did not differ.

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Venom immunotherapy (VIT) is highly effective in protecting Hymenoptera venom allergic patients from further systemic anaphylactic reactions (SARs) to insect stings. Numerous protocols for VIT have been published (1). In principle, two different ways of arriving at the maintenance dose can be distinguished: 'Rush' VIT reaches the maintenance dose by giving injections at short intervals for one up to several days, whereas

'conventional' VIT involves injections given at longer intervals for several weeks or months. The usual maintenance dose is 100 µg venom protein, which is administered at intervals of several weeks. Aqueous as well as aluminium hydroxide adsorbed preparations are available for VIT.

Studies performed at various centres have revealed large differences in the safety and efficacy of VIT. The number of patients with systemic adverse reactions ranged from 0% (2) to more than 80% (3) and the protection rate at a sting challenge was between 80%

Abbreviations: HBV, honeybee venom; SAR, systemic anaphylactic reaction; VIT, venom immunotherapy.

(4, 5) and 100% (2, 6, 7). The reason for these differences is not clear. In part, they may be attributed to divergent treatment protocols and patient selection. Although VIT has been in use for more than 20 years, only a few studies have been published giving direct comparisons of various treatment protocols with regard to safety and efficacy.

Here, we report on the experience with various VIT protocols using an aqueous or a depot preparation for treatment of honeybee venom (HBV) allergic patients.

Patients and methods

Study design

At our clinic, consecutive patients with an indication for VIT with HBV were included in the study. According to three different treatment schedules patients received injections of an aqueous or an aluminium hydroxide adsorbed HBV preparation, both licensed for the German market. Routine follow-up investigations were done immediately after reaching the maintenance dose, 6 and 12 months later, then every 12 months up to 3 years. After 6–12 months a sting challenge test with a living honeybee was performed.

Patients

Sixty-five consecutive patients (42 males, 23 females; aged 17–75 years) with a history of SAR to honeybee stings were included. Diagnosis of HBV allergy was established as described below. Patients were allocated consecutively to three treatment groups (A, $n = 21$; B, $n = 21$; C, $n = 23$).

Test procedures

Titration skin prick tests were performed with incremental concentrations (1.0, 10.0, 100, 300 µg/ml) of HBV (*Apis mellifera*) and *Vespa* spp. venom (ALK-lyophilisiert SQ, ALK-SCHERAX, Hamburg, Germany). Physiological saline and histamine dihydrochloride 0.1% served as controls. Reactions were evaluated after 20 min and interpreted as positive if there was a wheal ≥ 3 mm. Skin tests were not considered positive if there was a reaction to the negative control or no reaction to the positive control. HBV-specific IgE antibodies as well as HBV-specific IgG₄ antibodies in the serum were determined by CAP-FEIA (Pharmacia GmbH Deutschland, Freiburg, Germany) according to the instructions of the manufacturer.

SARs were graded as follows [according to ref. (8)]: severity grade I (mild), disseminated symptoms limited to the skin; grade II (moderate), minor to moderate respiratory, gastrointestinal and/or cardiovascular symptoms; grade III (severe), shock; grade IV, cardiac and/or respiratory arrest (resuscitation required). A diagnosis of HBV allergy was made if there was a definite history of SAR to a honeybee sting and HBV sensitization could be demonstrated by skin test reactions to HBV and/or by HBV-specific IgE antibodies in the serum.

Treatment regimens

Two preparations purified from low molecular weight irritants were used for VIT: an aqueous HBV preparation (ALK-lyophilisiert SQ, ALK-SCHERAX) and an aluminium-adsorbed HBV depot preparation (ALK-depot SQ, ALK-SCHERAX). With respect to allergen content both preparations were equal. Injections were

Table 1. Protocol of initial treatment

Dose (µg)	Groups A+B (aqueous preparation)		Group C (depot preparation)
	Day	Hour	Week
0.02	1	0	1
0.04		2	2
0.08		4	3
0.2		6	4
0.4	2	0	5
0.8		2	6
2		4	7
4		0	8
6	3	2	9
8		4	10
10	4	0	11
20		2	12
40	5	4	13
60		0	14
80		2	15
100		4	16

Table 2. Protocol of maintenance treatment

Dose (µg)	Group A (aqueous preparation)	Group B (depot preparation)	Group C (depot preparation)
	Week	Week	Week
100	2	2	18
100	4	4	20
100	Then every 4 weeks	8	Then every 4 weeks
100		14	
100		Then every 8 weeks	

administered subcutaneously at the distal lateral aspect of an upper arm. Initially, patients in groups A and B underwent a rush VIT with the aqueous preparation, whereas patients in group C were treated with the depot preparation given at weekly intervals (Table 1). For rush VIT, patients were hospitalized in our clinic. Outpatient injections were administered in our allergy unit by practising allergists or by general practitioners.

Maintenance VIT was performed in group A with the aqueous preparation given every 4 weeks, whereas the depot preparation was given at intervals of 8 weeks in group B and 4 weeks in group C (Table 2). Maintenance dose was 100 µg HBV in all groups.

Adverse reactions

Reactions to VIT injections were classified as large local reactions, i.e. erythema and/or swelling > 5 cm in diameter, or SAR according to the classification given above.

If a patient developed a SAR during dose increase, treatment was interrupted until complete recovery and then restarted with a dose reduced by two steps. In case of large local reactions with pronounced erythema and swelling (> 20 cm in diameter) of both upper arms, treatment was interrupted until recovery and then continued without dose reduction.

Efficacy of VIT

An in-hospital sting challenge test with a living honeybee was offered to all patients for evaluation of efficacy of treatment. The

test was carried out as described in the Position Paper of the EAACI (9). If the patient did not undergo a sting challenge, but was stung accidentally by a honeybee, the outcome of this field sting was considered for evaluation. In patients with a systemic reaction to the sting the maintenance dose was increased to 200 µg and, in part, treatment was continued with another aqueous HBV preparation.

Statistics

Data were analysed using SAS software (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA). Comparisons were carried out with respect to treatment groups (A–C) and preparations (aqueous or depot). Categorical variables were compared by the chi-square test. For comparison of continuous variables the *U*-test or *t*-test and the Kruskal–Wallis test or *F*-test (ANOVA) were used. The parametric tests to analyse the concentration of HBV-specific IgG₄ and IgE were carried out after logarithmic data transformation by means of analysis of covariance with pretreatment values as covariate. Trends within a patient group were quantified by means of the Wilcoxon signed rank test. A probability value <0.05 was considered to be significant.

Results

History, test results

Five patients (7.7%) gave a history of a mild SAR, 44 patients (67.7%) had experienced moderate SAR and 16 patients (24.6%) severe SAR. Patients in the three treatment groups did not differ significantly with respect to sex, age, severity grade of SAR or parameters of HBV sensitization. Details of patient characteristics are shown in Table 3.

Dose increase phase

The maintenance dose was reached by rush VIT with the aqueous preparation after 4–10 days (median 6 days)

and by conventional dose increase with the depot preparation after 16–21 weeks (median 17 weeks). The cumulative doses during the incremental phase were 373.1 ± 130.3 µg for subjects treated with rush VIT and 344.1 ± 29.1 µg for patients with conventional dose increase.

During dose increase the frequency of large local reactions in relation to the number of injections was higher in patients from groups A and B who were treated with the aqueous preparation (53/693, 7.6%) than in patients from group C treated with the depot preparation [8/206 (3.9%); $P = 0.059$]. Patients from groups A and B treated with rush VIT seemed to experience systemic reactions more frequently (13/42, 31.0%) than patients from group C treated according to the conventional schedule [3/23 (13.0%); $P = 0.109$; not significant]. Details are shown in Table 4.

Maintenance phase

After reaching the maintenance dose, two patients stopped the treatment on their own due to minor side-effects: one patient from group C had repeatedly developed mild SAR (generalized urticaria) and one patient from group A complained of nausea and blackout. Another patient from group B discontinued VIT after a diagnosis of colon carcinoma.

During maintenance therapy, injections of the aqueous preparation evoked large local reactions more frequently (85/172 injections, 49.4%) than those of the depot preparation [58/478 injections (12.1%); $P < 0.001$]. Maintenance treatment with the aqueous preparation also caused mild or moderate SARs more frequently (12/172 injections, 7.0%) than treatment with the depot preparation [8/478 injections (1.7%); $P < 0.001$]. For details see Table 5.

Table 3. Characteristics of patients at the start of VIT

	Group A	Group B	Group C	Total	<i>P</i>
Number of patients	21	21	23	65	
Age (years): range (mean)	18–67 (36.5)	17–75 (37.6)	18–47 (29.3)	17–75 (34.3)	0.085*
Males (%)	11 (52.4)	14 (66.7)	17 (73.9)	42 (64.6)	0.319†
Most severe sting reaction					
Grade I (%)	1 (4.8)	1 (4.8)	3 (13.0)	5 (7.7)	
Grade II (%)	15 (71.4)	14 (66.6)	15 (65.2)	44 (67.7)	0.807†
Grade III (%)	5 (23.8)	6 (28.6)	5 (21.8)	16 (24.6)	
Skin test reaction to HBV					
Negative (%)	1 (4.8)	0	0	1 (1.5)	0.345†
Positive (%)	20 (95.2)	21 (100.0)	23 (100.0)	64 (98.5)	
HBV-specific serum IgE					
Negative (%)	2 (9.5)	2 (9.5)	0	4 (6.2)	0.311†
CAP class ≥1 (%)	19 (90.5)	19 (90.5)	23 (100.0)	61 (93.8)	
HBV-specific IgG ₄ (%): median	26.6	50.2	29.8	30.8	0.361‡

* *F*-test.

† Chi-square test.

‡ Kruskal–Wallis test.

Table 4. Side-effects during dose increase

	Patients			Injections		
	Group A + B (aqueous preparation)	Group C (depot preparation)	<i>P</i>	Group A + B (aqueous preparation)	Group C (depot preparation)	<i>P</i>
<i>n</i>	42 (%)	23 (%)		693 (%)	206 (%)	
Large local reaction	18 (42.9)	5 (21.7)	0.089*	53 (7.6)	8 (3.9)	0.059*
SAR†	13 (31.0)	3 (13.0)	0.109*	23 (3.3)	4 (1.9)	0.309*
Grade I	6 (14.3)	2 (8.7)		13 (1.9)	3 (1.5)	
Grade II	7 (16.7)	1 (4.3)		10 (1.4)	1 (0.5)	

* Chi-square test.

† SAR, systemic anaphylactic reaction.

Table 5. Side-effects during maintenance therapy

	Patients			Injections		
	Group A (aqueous preparation)	Group B + C (depot preparation)	<i>P</i>	Group A (aqueous preparation)	Group B + C (depot preparation)	<i>P</i>
<i>n</i>	14 (%)	32 (%)		172 (%)	478 (%)	
Large local reactions	10 (71.4)	15 (46.9)	0.124*	85 (49.4)	58 (12.1)	<0.001*
SAR†	3 (21.4)	5 (15.6)	0.633*	12 (7.0)	8 (1.7)	<0.001*
Grade I	2 (14.3)	3 (9.4)		6 (3.5)	6 (1.3)	
Grade II	1 (7.1)	2 (6.3)		6 (3.5)	2 (0.4)	

* Chi-square test.

† SAR, systemic anaphylactic reaction.

Specific immunoglobulins

Immediately after reaching the maintenance dose, HBV-specific IgG₄ increased more strongly in response to the depot preparation (from 36.7 to 70.8%; geometric means) than in patients treated with the aqueous preparation (from 39.4 to 57.2%). In 28 patients who could be assessed at all follow-up investigations the geometric mean increased from 34.6% at baseline to 86.6% ($P < 0.001$). Also during maintenance treatment patients on depot preparation showed a higher increase of HBV-specific IgG₄ (groups B and C; from 41.8 to 91.0%) than those treated with the aqueous preparation (group A; from 32.3 to 64.6%) (Fig. 1). However, these differences were not significant.

HBV-specific IgE changed in a similar pattern in patients treated with the aqueous preparation and in those treated with the depot preparation. In relation to the baseline concentration after the dose increase phase, no significant difference in the change of HBV-specific IgE concentration was found between 42 patients treated with the aqueous preparation and 21 patients on depot preparation. In patients who could also be assessed at all follow-up investigations during maintenance treatment after 12, 24 or 36 months no significant differences in the change in HBV-specific IgE were found between patients treated with depot preparation every 4 or 8 weeks and patients treated with the aqueous preparation (Fig. 2).

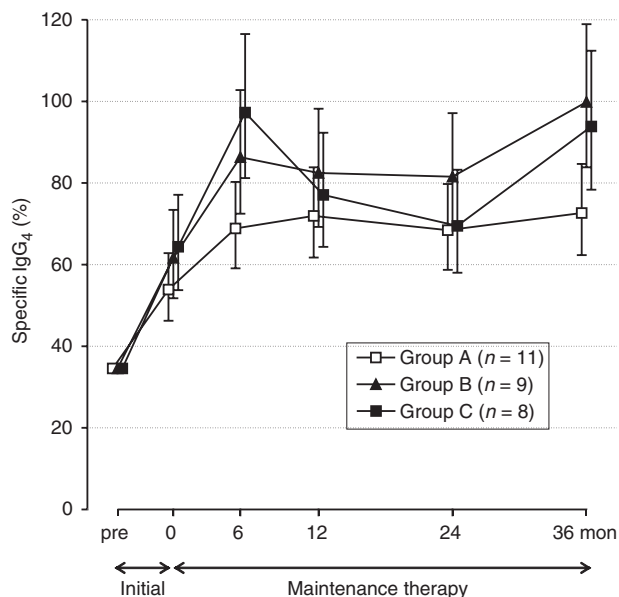


Figure 1. Development of HBV-specific IgG₄ (baseline adjusted mean) during VIT (geometric mean $\pm$ SEM).

Reactions to re-stings

Forty-nine patients underwent a sting challenge with a living honeybee after 6–12 months of treatment, which was tolerated without SAR by 41 patients (83.7%); 12 of

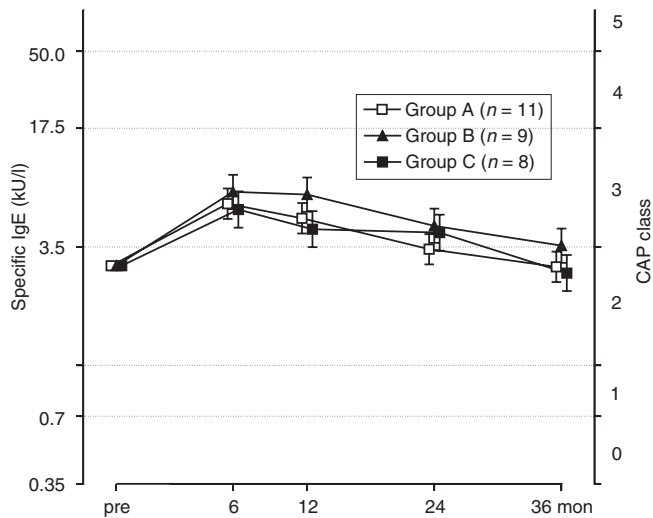


Figure 2. Development of HBV-specific IgE (baseline adjusted mean) during VIT (geometric mean of natural logarithm $\pm$ SEM).

these 49 patients additionally had field stings by a bee. Seven further patients were only stung accidentally by a honeybee, and none of them developed a SAR. Altogether, among the patients who had experienced a sting challenge, 3/19 (15.8%) in group A, 2/16 (12.5%) in group B and 3/14 (21.4%) in group C had mild to moderate SARs (not significant). Including the patients who had been stung only accidentally by a honeybee, 17/20 (85.0%) in group A, 16/18 (88.9%) in group B and 15/18 (83.3%) in group C, in total 48/56 (85.7%), tolerated a re-sting without SAR. Details are shown in Table 6.

Discussion

Treatment with the aqueous HBV preparation induced large local reactions more frequently than injections of the depot preparation. This is in good accordance with the results of another study showing that VIT with a depot preparation caused fewer large local reactions than treatment with an aqueous preparation (10). When depot

preparations were used exclusively for the treatment of honeybee or vespid venom allergy, large local reactions occurred only at a rather low rate of 2% of the injections (11).

Systemic side-effects were significantly more frequent during rush VIT with the aqueous preparation than during slow dose increase with the depot preparation. However, none of these reactions was severe: the majority of patients had only mild SAR, which was limited to the skin. In studies comparing VIT performed with depot or with aqueous preparations, the depot preparation caused urticaria in none of 15 (10) or one of 12 patients (6), whereas treatment with the aqueous preparation induced SAR in eight of 40 (10) or none of 20 patients (6) during dose increase. Also in the treatment of pollen, mite or animal dander allergy depot extracts were found to produce less SARs (12).

The lower frequency of side-effects following the injections of the depot preparation may be due to slower allergen release than from the aqueous preparation. However, the cumulative dose applied in a given period of time might also be important, i.e. the dose/time relation. With ultra-rush regimens a lower cumulative dose per time is administered than with rush protocols, and the former regimen seems to be tolerated at least as well as or even better than the latter despite rapid dose increase (13, 14). Also conventional schedules reaching the maintenance dose after several weeks appear to result in less SARs than rush schedules performed over a few days (15).

In total, SAR occurred during dose increase in 24.6% of our patients. This rather high frequency of side-effects of VIT is very similar to that found in a study on HBV: 20% of the patients on VIT using depot or aqueous extracts developed SAR (10). VIT with vespid venoms is evidently better tolerated than treatment with HBV (5, 13, 15–17).

Regarding efficacy of VIT, there were no significant differences between the three groups in our study. An investigation comparing treatment of yellow jacket allergic patients with a depot preparation or an aqueous preparation also showed equal efficacy for both preparations: all 19 patients treated with the depot preparation or the aqueous preparation were challenged by a wasp sting

Table 6. Systemic anaphylactic reactions to a sting challenge or to a field sting

	Group A (aqueous preparation)	Group B (depot preparation)	Group C (depot preparation)	Total
Interval of maintenance injections	4 weeks	8 weeks	4 weeks	
Patients re-stung	20	18	18	56
Sting challenge	19	16	14	49
Only field sting	1	2	4	7
Systemic anaphylactic reaction*	3 (15.0)	2 (11.1)	3 (16.7)	8 (14.3)
Grade I (%)	2 (10.0)	2 (11.1)	1 (5.6)	5 (8.9)
Grade II (%)	1 (5.0)	–	2 (11.1)	3 (5.4)

* No systemic reaction occurred to any field sting.

and turned out to be protected from a further SAR (6). Among 11 patients treated with HBV and/or vespid venom depot preparation only one developed a mild SAR after a field sting (11).

The overall protection rate of only about 85% in our study can be attributed to the fact that in HBV allergy a maintenance dose of 100 µg is not sufficient for a substantial number of patients (5). Patients who are not protected by VIT with 100 µg of venom can be treated effectively with higher doses of 150–200 µg or even more (18).

After the dose increase phase and also during maintenance treatment, HBV-specific IgE changed similarly in all treatment groups. Aluminium adjuvants have been shown to enhance the production of IgE in various models for sensitization of animals (19, 20). Interestingly, this effect depends on the allergen dose: only if lower doses of the allergen were used, an aluminium adjuvant enhanced the allergen-specific IgE-production (19, 20); if higher doses of the allergen were used, an IgE-stimulating property of the aluminium adjuvant was no longer demonstrable. It is not clear whether this observation can be extrapolated to human models. Specific immunotherapy with allergen preparations in general is performed with increasing amounts of allergens aiming the administration of a preferably high dose. Previous studies of VIT (6, 21) or of ragweed pollen extracts (22) in humans found no difference in the course of IgE during the treatment comparing immunotherapy with or without aluminium adjuvant.

During the course of the treatment, an increase in the concentration of HBV-specific IgG₄ was observed in all

groups. The protective function of an increase in the venom-specific IgG₄ or a decrease in IgE with respect to the efficacy of VIT has been controversially discussed by various authors (23–25). In either case the change in these parameters during the course of specific immunotherapy is related to immunologic effects of treatment, and thus it is interesting that different treatment schedules and preparations, which were used in our study, have a similar immunogenic action.

Patients who were given the depot preparation every 4 or 8 weeks during the maintenance phase were equally protected when re-stung. Prolonged injection intervals of more than 8 weeks have so far only been investigated in a few studies with a small numbers of patients. The results have remained inconclusive indicating that VIT at prolonged injection intervals may provide protection in most patients (26, 27), but may be insufficient in some (28).

Taken together, the therapeutic efficacy of the aqueous preparation is similar to that of the depot HBV preparation. As the depot preparation caused fewer adverse reactions, it has some advantage compared with the aqueous preparations. However, rapid induction of protection is needed in many patients. Moreover, compliance of patients who were initially treated as inpatients according to a rush protocol is better than that of those patients who were outpatients throughout the whole treatment (29). Thus, the initial use of the aqueous preparation for rush VIT, followed by maintenance treatment with the depot preparation, seems an appropriate regimen.

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