

Original article

Bee venom immunotherapy – how early is it effective?

Background: Although the effectiveness of venom immunotherapy (VIT) in bee venom (BV) allergy has been well established over the past 30 years, no previous study has demonstrated its efficacy immediately after reaching the maintenance dose (MD). We examined the effectiveness of bee VIT within a week after the MD was achieved.

Methods: Bee venom allergic patients underwent conventional or rush VIT. Within 1 week after reaching the 100 µg MD, patients were challenged with a live bee sting.

Results: Seventy-nine of 107 patients (73.8%) who reached the MD agreed to be challenged. Seventy patients (88.6%) tolerated the sting uneventfully. Four patients (5.1%) developed a very mild local transient rash and continued to receive the 100 µg MD. In five patients (6.3%), the sting resulted in a mild-moderate systemic reaction. In four of these, the MD was increased to 200–250 µg. All four patients uneventfully tolerated a repeated sting that was performed within 1 week after achieving the increased MD in three patients and after 14 months in the fourth patient.

Conclusions: Bee VIT is effective in most patients immediately after the conventional MD has been reached. In the minority of patients who are not protected with this dose, an increased MD will provide appropriate protection immediately after it is achieved. Thus, the dosage of the MD seems to be the major factor affecting protection from re-stings rather than the accumulated venom dose or the duration on the MD.

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The effectiveness of venom immunotherapy (VIT) has become well established over the past 30 years and it has become the treatment of choice for systemic allergic reactions induced by *Hymenoptera* stings (1). The first description of successful desensitization with BV was published in 1974 (2). Subsequent studies have further confirmed the effectiveness of VIT by demonstrating favorable patient responses to deliberate or field stings from the culprit insect after the maintenance dose (MD) had been achieved (3–29).

Although desensitization by immunotherapy has been practiced in several allergic situations for almost 100 years, its precise mechanism remains obscure. Consequently, immunotherapy guidelines are based primarily on accumulated clinical experience rather than on a genuine understanding of the mechanism of the desensitization process. The previous studies that described the superb efficacy of VIT drew their conclusions from

patient reactions to re-stings after the MD had been achieved. The dynamics of this process have never been investigated and it was probably intuitively assumed that the MD should be considered protective once it was achieved. As the mechanism of immunotherapy is not known, this assumption may not be accurate because, theoretically, an unknown duration on the MD may be required or an unknown number of MDs should be administered before the treatment becomes effective. Therefore, we sought to investigate if the postulation regarding the immediate protective effect of the MD in VIT is correct. To examine this, bee venom (BV) allergic patients receiving VIT were deliberately sting-challenged by a honeybee within 1 week after the first MD was administered.

Methods

Patients

All patients had a history of systemic reactions (SRs) inflicted by an insect sting and a positive skin test response to BV (wheal diameter ≥5 mm in the presence of erythema diameter ≥11 mm following intradermal injection of venom at concentration ≤1 µg/ml),

Abbreviations: BV, bee venom; CVIT, conventional venom immunotherapy; MD, maintenance dose; RVIT, rush venom immunotherapy; SR, systemic reaction; VIT, venom immunotherapy; W, wasp; YJ, yellow jacket.

elevated titers of serum BV-specific IgE (≥ 0.70 kU/l), or both. Patients underwent either conventional VIT (CVIT) once a week for 3–4 months or rush VIT (RVIT) for 3–5 days with BV until reaching a MD of 100 μ g. In patients who chose RVIT and in whom several venoms were to be administered, BV was administered first, whereas in patients choosing CVIT all venoms were administered simultaneously. In addition to the majority of patients who could freely choose their treatment modality ('regular' patients), there was a sub-group of patients who were obliged to undergo RVIT after several trials to reach the MD by CVIT failed because of recurrent SRs. Some of these patients have been described in detail (30).

Sting challenge

After receiving the first 100 μ g MD of BV, all patients were offered a live bee sting challenge, which was performed within 1 week after reaching the MD. All patients who reached the MD were offered the challenge regardless of the severity of the initial SR or any other variable. Patients who agreed to be challenged signed an informed consent approved by the Ethics Committee. The patients did not take antihistamines before the sting challenge. Honeybees were collected from a hive on the day of the challenge and were identified by an entomologist. As previously described (31), after the insertion of an intravenous catheter and continuous monitoring of cardiac rhythm and blood pressure, a cut-edge plastic syringe containing a single honeybee was applied to the volar aspect of the patient's arm and the syringe plunger was gently pushed, pressing the insect against the patient's skin until a sting was observed and sensed. Venoms used for diagnostic (skin tests) and treatment purposes were produced by ALK-Abello, Horsholm, Denmark and serum venom-specific IgE was measured with a commercial kit (AlaSTAT; DPC, Los Angeles, CA, USA). Definitions of positive skin-tests and elevated titers of serum BV-specific IgE were made in accordance with the manufacturers' recommendations.

Statistical analysis

Data are presented as the mean $\pm$ SD. Statistical analysis was performed with the statistical package spss-15 (SPSS Inc, Chicago, IL, USA) using the two-tailed Student *t*-test or the chi-squared test. *P*-values of < 0.05 were considered significant.

Results

Patients

From June 2002 to February 2009, sting challenge was offered to 107 consecutive patients who had reached the 100 μ g MD. Of these, 79 (73.8%) agreed whereas 28 patients (26.2%) refused. Patient characteristics are presented in Table 1. The most frequent reasons for challenge refusal included fear of the sting, considering it unnecessary and inability to comply with the study schedule. There were no statistically significant differences between patients who were or were not stung in regard to any patient characteristic. Forty-eight (60.8%) of the stung patients were allergic only to BV and 31 patients (39.2%) were allergic to BV and to other vespid venoms, as well. Nine patients belonged to the sub-group who underwent RVIT deliberately after recurrent failures of CVIT as a result of multiple SRs. Eight of these agreed to be challenged.

Sting challenges

In 70 of 79 challenges (88.6%), no objective symptoms were observed. Three patients reported mild localized itching without objective findings. The symptoms in four of nine patients who developed objective symptoms were limited to a localized mild rash that faded spontaneously within 10 min. These patients continued with the regular 100 μ g MD. Five patients (6.3%) developed grade 1 or grade 2 SR (Mueller's criteria) after the sting challenge. Their mean age was 28.2 years, male/female ratio: 3/2 and the venoms administered to these patients were BV in four patients and BV + YJ venom in one patient. None of them had cutaneous evidence of urticaria pigmentosa. The severity of the initial SR before commencement of VIT was grade 1–2 in four of these patients and grade 3 in one patient. Four of them received RVIT whereas a single patient received CVIT. Two patients belonged to the sub-group of patients who had previously suffered from

Table 1. Patients characteristics

	All stung patients (<i>n</i> = 79)	Stung patients allergic only to BV (<i>n</i> = 48)	Patients refusing sting challenge (<i>n</i> = 28)
Male/Female (%)	64/15 (81/19)	39/9 (81/19)	20/8 (71/29)
Age, mean $\pm$ SD (range)	30.1 $\pm$ 16.2 (4–69)	30.2 $\pm$ 16.7 (4–69)	24.3 $\pm$ 14.4 (3–59)
Severity of initial SR (Mueller's criteria), <i>n</i> (%)	Grade 1–13 (16)	Grade 1–10 (21)	Grade 1–4 (14)
	Grade 2–41 (52)	Grade 2–24 (50)	Grade 2–14 (50)
	Grade 3–16 (20)	Grade 3–10 (21)	Grade 3–8 (29)
	Grade 4–9 (11)	Grade 4–4 (8)	Grade 4–2 (7)
Time elapsed since initial SR (months), mean $\pm$ SD (range)	19.8 $\pm$ 36.4 (1–180)	20.7 $\pm$ 32.8 (1–120)	32.9 $\pm$ 51.6 (2–144)
Venoms to which patients were allergic, <i>n</i> (%)	BV = 48 (61)	BV = 48 (100)	BV = 16 (57)
	BV + YJ = 17 (22)		BV + YJ = 6 (21)
	BV + W = 2 (3)		BV + YJ + W = 6 (21)
	BV + YJ + W = 12 (15)		
Type of VIT RVIT/CVIT, <i>n</i> (%)	65/14 (82/18)	42/6 (87/13)	23/5 (82/18)

recurrent SRs to CVIT. Thus, three of 71 'regular' patients (4.2%), who had not experienced recurrent SRs to previous CVIT, developed grade 1–2 SRs after the challenge. In four of five patients with a grade 1–2 SR to the challenge, the MD was increased to 250 µg (three patients) or 200 µg (one patient (using RVIT in three patients and CVIT in one patient)). The fifth patient who had a grade 2 SR to the challenge also developed a large local reaction at the site of the sting. This patient declined continuation of VIT and was lost to follow-up. Repeated sting challenge was performed within 1 week after achieving the increased MD in the three patients who received RVIT and 14 months after it was achieved in the patient who received CVIT. All challenges were tolerated uneventfully and the patients continued to receive the increased MD regularly, thereafter. The study protocol together with the detailed follow-up of the various subgroups is depicted in Fig. 1.

Discussion

With the usual MD of 100 µg, the clinical protection rate provided by vespid VIT is approximately 98%. The protection rate provided by bee VIT is considered to be lower, reaching approximately 80% (32), although better results have also been described (33).

Upon achieving the MD at the end of the build-up phase of VIT, most allergists advise their patients that they can consider themselves protected from future stings. This is contrary to other allergic situations that are usually treated with immunotherapy. Thus, although

clinical improvement in allergic rhino-conjunctivitis or in asthma can be demonstrated in some patients very shortly after reaching the MD with aeroallergens, this improvement is still to be anticipated within 1 year on the MD. Discontinuation of immunotherapy is considered only after this period has passed without clinical improvement (34). Waiting that long for clinical efficacy in venom allergy, which is a potentially life-threatening situation, is unacceptable. Although theoretically logical, the early effectiveness of VIT has never been supported by objective data and has become common knowledge based solely on intuition. This study is the first to confirm the validity of this theory for most BV allergic patients.

As its introduction in the early 1970s, many studies have repeatedly documented the remarkable efficacy of VIT in preventing SR after re-stings. Many of these studies provided no data regarding the timing of the re-sting in relation to attaining the MD (3–17). Some studies that did include this information drew their conclusions from field stings (18–20), where accurate identification of the insect is sometimes doubtful and therefore, may lead to biased results. By performing deliberate sting challenges with the culprit insects, the issue of insect identification can be overcome. However, in most studies that used deliberate challenges, the challenges were performed more than 1 month (21–29) or even more than 6 months (25–28) after the MD had been achieved. Only in one study (29) comprised of 18 patients and in a single case report (2), the sting challenges were performed within a month after reaching the MD.

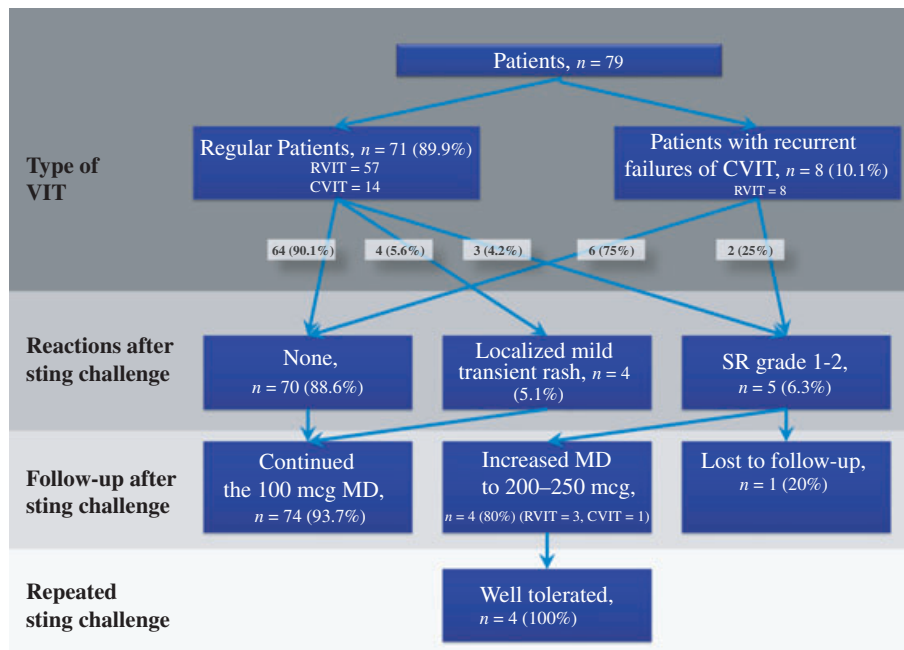


Figure 1. Study protocol and characteristics of the stung patients.

To the best of our knowledge, no controlled trial has examined the efficacy of the MD within 1 week after it was achieved. In addition, in the previously mentioned studies that described either field stings (17–19) or deliberate sting challenges (23, 25, 27–29), most patients were stung by yellow jackets or other vespids. Only three studies (21, 22, 26) and two case reports (2, 24) concentrated on patients allergic to BV and only in one case report (2) was the honeybee sting-challenge performed within a month after reaching the MD. Taking together these data and considering the lower efficacy of VIT with BV compared with YJ venom (22, 32), we found the issue of the immediate efficacy of the MD in BV allergic patients to be very intriguing. Indeed, in most of our patients (93.7%), the effectiveness of VIT, which was ascertained by the absence of SRs to the sting challenges, was evident within 1 week after reaching the MD.

The rate of treatment failures observed in our patients shortly after achieving the MD was 6.3%. This is in agreement with the acceptable protection rate provided by VIT which varies from 80% to 98% (32) and similar to our previous experience with a different group of BV allergic patients (33). The incidence of SRs to the sting challenge in the 'regular' group which did not include patients with previous recurrent SRs to CVIT was even lower and reached 4.3%. Treatment failures were not associated with the severity of the initial SR before commencement of VIT, with the specific type of the administered VIT or with any other characteristics of the patients. There may be several possible explanations for the lack of early effectiveness of VIT. Either the duration on the MD or the number of MD injections or the cumulative dose were not sufficient to provide complete protection or, alternatively, some individuals, for unknown reasons need a larger MD to gain this protection, regardless of the cumulative dose or the duration of MD administration.

Several immunological changes have been associated with successful VIT. These include increased specific IgG antibodies, particularly of the IgG4 class; reduction in the numbers and activation state of basophils; shift from Th2 to Th1 cytokine profile; a decrease in T cell responsiveness against the allergen; increased levels of IL-10 in the supernatants of lymphocytes cultures stimulated with venom extracts; expansion of IL-10 producing CD4⁺ T cells, and expansion of circulating regulatory T cells. In some studies, these changes were observed immediately after the total MD was administered as a single injection (35). In another study, the change in IFN- γ level was observed a week after the administration of several doses that equaled the total MD, whereas changes in IL-4 and IL-5 levels were observed only 2–3 weeks later (36). Another study which focused on the expansion of circulating regulatory T cells, found this change only 6–12 months after repeated administration of the MD (37).

Thus, there is great variability in the immunological factors that were evaluated, the specific methodology, and the timing of measurements after MD was achieved. Therefore, the currently available information, which is based on *in vitro* studies cannot provide even a theoretical indication about the duration on MD required for successful VIT. In the absence of *in vitro* as well as *in vivo* confirmed data on the kinetics of VIT, this study is the first to verify that in the majority of BV allergic patients, VIT is efficient within a week after the MD is achieved. The question whether multiple or double or single sting challenge is the proper method to evaluate the efficacy of VIT has been debated extensively in the past and so far, no consensus has been reached on this issue. Therefore, we elected to adhere to the single challenge which is the most frequently used method.

The two therapeutic alternatives for the few patients who were not protected by the 100 μ g MD were either to continue with the same MD in anticipation of gaining future protection or to increase the MD. As there was no guarantee that the first alternative would eventually become effective, while keeping these patients on an inadequate MD could jeopardize their lives, we chose the second option. Indeed, all these patients gained the desired protection with the increased dose. As already described previously (26), selected patients receiving 100 μ g MD failed to gain protection, whereas 150–200 μ g MD effectively protected them from a reaction to another sting challenge. As the first sting challenges were performed 7–38 months after these patients had been on the 100 μ g MD (26), it seems that the dosage, rather than the duration, was not sufficient. As for unselected patients like ours, the question of whether the protection achieved by increased MD was because of the larger dose, the longer duration, or the continual administration of the MD, cannot be answered at the moment. The lack of SRs to re-sting performed within 1 week after achieving the increased MD in three of our patients supports the first assumption, but the small number of patients prohibits this conclusion. A study comparing the results of deliberate challenges performed either 1 week or several months after the conventional 100 μ g MD has been administered to a large number of patients may shed light on this subject. Until then, we can assure most patients receiving VIT that they will be protected from re-stings within days after the MD is achieved.

A quarter of our patients refused the sting challenge by a live bee. Performing this challenge is not straightforward because it involves an in-hospital setting and requires a steady supply of the stinging insects. Therefore, the routine use of this procedure to assess the effectiveness of VIT is impractical for most patients. Thus, considering that some patients are not entirely protected immediately after achieving the MD, patients at higher risk, especially those who are allergic to BV, should be advised to continue carrying their epinephrine syringe at all times, even after MD had been reached.

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