

A Phase I Study of the Safety of Honeybee Venom Extract as a Possible Treatment for Patients with Progressive Forms of Multiple Sclerosis

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ABSTRACT

Although several reports suggest that bee venom may be an effective treatment for patients with multiple sclerosis (MS), patients may be subjected to real risks of serious allergic reactions as well as emotional and economic costs. This study was conducted to evaluate the safety of bee venom extract as a possible treatment for patients with progressive forms of MS. A total of nine bee venom nonallergic patients with progressive forms of MS, who were 21–55 years of age with no other illnesses, were entered into four groups (A, B, C, and D) on a structured 1-year immunization schedule. Hyperreactivity to bee venom was evaluated by ques-

tionnaire, physical examination, and a battery of hematologic, metabolic, and immunologic tests. Responses to therapy were evaluated by questionnaire, functional neurological tests, and changes in measurement of somatosensory-evoked potentials. Although no serious adverse allergic reactions were observed in any of the nine subjects, four experienced worsening of neurological symptoms, requiring termination in the study; this could not be ascribed to side effects of the therapy. Of the remaining five subjects, three felt that the therapy had subjective amelioration of symptoms and two showed objective improvement. Although this preliminary study suggests safety, because of the small numbers studied, there were no definite conclusions regarding efficacy and therefore there was little evidence to support the use of honeybee venom in the treatment of MS. Larger and more carefully conducted multicenter studies will be required to establish efficacy. (Allergy and Asthma Proc 26:470–476, 2005)

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Multiple sclerosis (MS) is a poorly understood demyelinating disease of the central nervous system (CNS) in which its pathogenesis generally is believed to involve an immune-mediated reaction to any of a number of suspected environmental agents (e.g., viruses, bacterial products, or toxins) and which occurs in genetically susceptible individuals.^{1,2} The disorder begins typically in the second or third decade of life with a high female prevalence and is characterized most commonly by a relapsing and often eventually progressive, unremitting course, often with

devastating clinical sequelae. Moreover, because of the considerable clinical, genetic, magnetic resonance imaging (MRI), and pathological heterogeneity known to occur with the disease, more than one pathogenetic mechanism has been suspected, which, in turn, is believed to have therapeutic implications, because more than one therapeutic approach may be required to treat this disease effectively.

In *relapsing–remitting MS*—the type present in 80% of patients during the early phase of their disease—new symptoms and signs typically evolve over a period of several days, stabilize, and then often improve within weeks, either spontaneously or in response to corticosteroids. This form of MS typically begins in the second or third decade of life and has a female predominance of ~2:1. Responsiveness to corticosteroids to speed recovery from relapses often diminishes with time. The majority of patients with relapsing–remitting MS eventually develop *secondary progressive MS*, characterized by progressive worsening of baseline CNS function, either instead of or in addition to relapses. *Primary progressive MS* comprises a third form, affecting ~10–20% of affected patients and is characterized by a gradually progressive clinical course without relapses and a similar incidence among men and women.

Recently, the introduction of a number of new therapeutic agents has led to reduced frequency of relapses and slowing of disease progression, but in most patients breakthrough disease activity eventually occurs.^{1,2} In addition to these established treatment regimens, a number of anecdotal reports have appeared suggesting that bee venom may be an effective treatment for MS. Recently, because of media exposure touting this treatment, there has been a large underground movement of patients to zealous lay practitioners who subject patients with MS to multiple and repeated bee stings. Because this practice entails a real risk of fatal allergic reactions as well as emotional and economic costs in chasing false hopes, properly conducted studies of safety and efficacy of bee venom therapy are needed. Furthermore, by failing to conduct such studies, bee venom enthusiasts could be depriving the majority of MS patients of an opportunity to benefit from a widespread use of the therapy, if it is indeed efficacious.

Current interest in bee venom therapy is largely based on anecdotal reports attesting to its efficacy in the treatment of chronic inflammatory diseases.^{3–9} The effects of bee venom on arthritis, particularly gout, have been described since the time of Aristotle, and bee venom therapy is widely practiced in both nonwestern and some western countries.^{3–6} Although several MS patients have reported symptomatic benefit after being stung by bees,^{6,7} a careful search of the literature has revealed no formal studies in which bee venom has been evaluated critically in the treatment of MS. The purpose of this study was to evaluate the safety of bee venom extract as a possible treatment for patients with progressive forms of MS.

METHODS

Study Design

The study was a phase I open label safety study, which was approved by the Georgetown University Institutional Review Board and was conducted under Food and Drug Administration–approved Investigational New Drug. All subjects provided statements of informed consent before entry into the study.

Subjects

Patients with primary or secondary progressive MS, who were between 21 and 55 years of age, with Kurtzke Expanded Disability Status Scale scores between 4.5 and 8.0 and who did not have other significant medical illnesses were eligible for the study. Subjects were required to show evidence of stable baseline neurological deficits over the prior year with no exacerbations over the prior 3 years. A total of 16 bee venom nonallergic patients were screened and found to be eligible for study entry. Before the start of the study, seven subjects dropped out due to various reasons, *i.e.*, inability to commit to the length of the study and worsening of MS symptoms. The study began with a total of nine eligible subjects. The patients were divided into four groups and each group was treated with different doses of honeybee venom over a period of 1 year according to the immunization schedule described in the following sections.

Patient Selection

On admission to the study, all subjects were asked to complete a questionnaire and were given thorough physical, neurological, and laboratory examinations. The following inclusion and exclusion criteria were observed.

Inclusion Criteria

The criteria for admission into the study included a clinically defined diagnosis of primary or secondary progressive MS as defined by Lublin and Reingold.¹ Because there was some concern that bee venom administration might exacerbate MS disease activity, the requirement for a neurologically stable state over the previous year was included in an effort to reduce the possibility of spontaneous disease worsening during the study. It was hoped that this would enhance the probability that any adverse effects of treatment on disease progression could be detected. Inclusion criteria also included magnetic resonance imaging findings typical of MS.

Exclusion Criteria

The exclusion criteria included the following: patients could not have received prior treatment with cyclophosphamide within the prior 8 years; azathioprine, methotrexate, or other immunomodulatory drugs within the prior 3 months; or steroids within the prior 2 months. Subjects could not have had a history of allergic reactions to honey-

bee venom products (*e.g.*, honeybee pollen) or any history of allergic reactions to any other stinging insects (*e.g.*, yellow jacket, yellow hornet, white-faced hornet, and wasp) and could not have received bee venom immunotherapy for stinging insect desensitization or for any other reason within the prior year. Additionally, they could not be currently receiving therapy for their multiple sclerosis with β -interferon (*i.e.*, Betaseron or Avonex, Biogen Idec, Cambridge, MA), glatiramer acetate (Copaxone, Teva Pharmaceutical Industries, Tel Aviv, Israel), or any other experimental medications or procedures. Patients were required to be seronegative for human immunodeficiency virus and to have no comorbid immunologic disorders, such as rheumatoid arthritis or systemic lupus erythematosus. There could be no history of comorbid renal heart or liver disease, cancer, or diabetes. Any patients with a history of sensitivity to local anesthetics were excluded also.

Honeybee Venom Extract

Honey bee venom extract containing 25 mg/mL of venom in buffer containing 50% glycerol was kindly provided by Allermid Laboratories, Inc. (San Diego, CA).

Sensitivity to Honeybee Venom

Patients were tested either by the skin-prick puncture method with honeybee venom extract using a 1:100 v/v dilution (0.025 mg/mL) of the concentrated product or by radioallergosorbent test RAST. This was done to ensure that the subjects would have little risk of anaphylaxis from receiving venom therapy. The tests were administered by a physician or research nurse on the research team. The skin response was measured after 15–20 minutes and the results were recorded as millimeters of edema/erythema. A histamine-glycerol-positive control and saline-glycerol-negative control were included as methodological controls. Patients who were skin-test negative by the skin-prick puncture test also were retested by the intradermal method using 0.05 mL of a 1:100 v/v dilution (0.025 mg/mL) of the concentrated product. The intradermal skin tests also were administered by a physician or research nurse and responses were measured after 15–20 minutes by measuring millimeters of edema/erythema. If the skin tests were negative, the patient was eligible for entry into the study. All adverse reactions to bee venom were recorded on an “Adverse Reaction Symptom Checklist.”

Procedures for Protection against Risks and Evaluation of Adverse Reactions

Techniques for skin testing and allergen immunotherapy as well as measures for prevention and management of any adverse allergic reactions associated with skin testing and immunotherapy were followed according to practice parameters described in publications dealing with stinging insect hypersensitivity¹⁰ and allergen immunotherapy.¹¹ All records were kept confidential. Blood

specimens were drawn four times during the study (at screening and 3, 6, and 12 months) for the following blood tests to monitor untoward effects: complete blood count, platelet count, comprehensive metabolic panel, sedimentation rate, circulating immune complexes, and immunoglobulin E (IgE) and IgG responses to honeybee venom by the RAST method. Urinalyses were obtained weekly and checked for the presence of cells and proteinuria. Subjects were tested by scratch and intradermal skin testing at four concentrations for sensitivity to honeybee venom extract at the baseline visit. Any patients who experienced a 1+ or greater reaction at 1 μ g/mL were excluded. During the course of the study, patients were tested for sensitivity to honeybee venom by RAST at four concentrations at 3, 6, and 12 months to monitor for sensitization to honeybee venom.

Patients were given a diary card and asked to record any symptoms associated with localized or systemic allergic reactions together with dates of onset and resolution and other pertinent information. Diary cards were reviewed by the research team on a weekly basis. Patients were instructed to contact immediately the senior investigators for any symptoms they considered serious. Additionally, patients were directed to call emergency services if they experienced any symptoms of a systemic allergic reaction.

A set of dosage-stoppage and dose-reduction guidelines were established, which were based on the severity and rapidity of any adverse symptoms that might have occurred during the course of the study. Because some degree of localized inflammation normally accompanies intradermal injection of honeybee venom because of its inflammatory constituents, no specific dose reduction was considered to be required for localized immediate or localized delayed reactions. The management of these localized reactions consisted of the usual procedures used in minimizing the inflammatory responses to injection therapy (*e.g.*, cooling site with ice, cold compresses, and use of antihistamines). Any subject who experienced a generalized immediate-type reaction (*e.g.*, angioedema, severe dyspnea, hypotension, and/or anaphylaxis) would be given appropriate medical treatment by the research team (*e.g.*, epinephrine, stabilized medically, and given any other necessary emergency treatment). After stabilization, such patients would be evaluated at the termination visit, cautioned to avoid honeybee venom injections in the future, instructed in appropriate precautionary measures, and would be excluded from further participation in the study.

Treatment Groups and Schedules

Study participants were randomly divided into four groups (A, B, C, and D) and treatment was instituted in each group at 2-week intervals (Table I). Subjects in group A were the first to be entered into the study and each was started on a dose of 0.025 mg/week for 2 weeks. As

TABLE I

Honeybee Venom Immunization Schedule				
Week	Group A (mg/wk)	Group B (mg/wk)	Group C (mg/wk)	Group D (mg/wk)
1	0.025	—	—	—
2	0.025	—	—	—
3	0.025	0.1	—	—
4	0.025	0.1	—	—
5	0.025	0.1	0.1	—
6	0.025	0.1	0.2	—
7	0.025	0.1	0.3	0.1
8	0.025	0.1	0.4	0.2
9	0.025	0.1	0.5	0.3
10	0.025	0.1	0.5	0.4
11	0.025	0.1	0.5	0.6
12	0.025	0.1	0.5	0.8
13	0.025	0.1	0.5	1.0
14	0.025	0.1	0.5	1.2
15	0.025	0.1	0.5	1.6
16	0.025	0.1	0.5	2.0
17 + subsequent weeks	0.025	0.1	0.5	2.0

long as no adverse reactions were observed in group A, the succeeding groups began treatment in a staggered overlapping fashion as shown in Table I. Group B was the next to begin treatment at a dose of 0.1 mg/week. Provided no serious adverse effects were noted after 2 weeks, group B was continued at this dose and group C was begun on treatment at 0.1 mg/week for 1 week. Dosage was increased by 0.1 mg/week for 4 weeks in group C until a dose of 0.5 mg/week was reached, which was maintained throughout the remainder of the study. Provided no serious adverse effects were noted, group D began treatment at 0.1 mg/week for 1 week. Dosage was increased by 0.1 mg/week for 3 weeks, and then increased by 0.2 mg/week for 4 weeks, followed by an increase by 0.4 mg/week for 2 weeks and maintained at a dosage of 2.0 mg/week throughout the remainder of the study (Table I).

The doses of bee venom were administered by a physician or research nurse on the research team in two sessions per week delivering 10 (0.1 mL) intradermal injections per session calculated for the monthly dose. Each patient received two intradermal injections on the anterior aspect of both arms, both thighs and the lower back. Twenty minutes before the injections, EMLA (Astra Zenica, Wilmington, DE) cream (lidocaine 2.5% and prilocaine 2.5%) was applied to the injection site to minimize the pain of injections. All subjects were observed for 30 minutes after treatment for the development of any untoward reactions. The patients and the examining neurologist were blinded, but the other physicians and research nurse were not.

TABLE II

Expanded Disability Status Scale Score					
Subject	Group	Duration of Bee Venom Therapy (wk)	Expanded Disability Status Scale Score		
			Baseline	6 Mons	Final
1	A	17	7.5	—	—
2	A	40	7.5	8.0	8.0
3	B	72	4.5	4.0	4.0
4	B	4	6.0	—	—
5	C	28	8.0	—	8.0
6	C	43	8.0	8.0	8.0
7	D	11	5.5	—	5.0
8	D	43	8.0	8.5	8.5
9	D	30	6.0	—	6.0

Monthly Evaluation

Each study participant was evaluated monthly for any progression or diminution of signs and symptoms of MS. This information was recorded on a "Multiple Sclerosis Symptom Checklist" that was prepared for the study.

Neurological and Functional Evaluations

The neurological status of all subjects was monitored as follows: a comprehensive neurological examination was performed by the study neurologist on all subjects at baseline and at 6 and 12 months. The outcome measurements consisted of the combined Expanded Disability Status Scale (Table II) and the functional status scores; the nine-hole peg test, which measures upper extremity function and coordination; the Timed Ambulation Test, which measures the time it takes to walk 25 ft; and quality of life scales the (David Quality of Life scale and the Ferrans and Powers Quality of Life Index [*i.e.*, the MS version]).

CNS Conduction Velocities

Because CNS conduction velocities are slowed through areas of demyelination, somatosensory-evoked potentials were performed on all patients at the beginning of the study and at 10- to 12-month intervals into the study to obtain quantitative measurements of conduction velocity over CNS sensory pathways in the spinal cord and brain.

RESULTS

Subject Characteristics

Of the 16 subjects originally screened for entry, 7 subjects dropped out due to various reasons, *i.e.*, inability to commit to the length of the study and worsening of MS symptoms. Nine (six women and three men) subjects entered the study. The mean age was 44.7 years and the mean duration of the disease was 13.9 years.

TABLE III				
Outcome Results of the Study				
Subject	Group	Date of Initiation of Bee Venom Therapy	Status	Outcome
1	A	8/09/00	P	↓
2	A	9/11/00	C	↓
3	B	8/14/00	C	↑
4	B	9/07/00	P	—/↓
5	C	8/21/00	P	↓
6	C	8/21/00	C	—
7	D	8/28/00	P	—
8	D	8/28/00	C	↓
9	D	2/05/01	C	↑

C = Completed the study; P = dropped from study because of possible progression of disease; ↑ = improvement; ↓ = worsening; — = stable.

Evaluation of Safety and Outcome

No serious adverse effects were observed during the study. Minor localized reactions were observed frequently at the injection sites, which were readily controlled by the usual procedures described previously. Clinical outcomes are shown in Table III. By 9 months into the study, four of the nine subjects were dropped from the study because of worsening of symptoms of MS (Subjects 1, 4, 5, 7). These consisted of, *e.g.*, increased spasticity, decreased ambulation, increasing fatigue, new-onset nystagmus, new-onset gaze paresis, and worsening bowel/bladder dysfunction. Objective neurological measures showed improvement in two subjects (Subjects 3, 9), clear worsening in four subjects (Subjects 1, 2, 5, 8), equivocal worsening in one subject (Subject 4), and stability in two subjects (Subjects 6, 7). Subjective improvement was experienced in three subjects who felt that the therapy had a beneficial effect with amelioration of symptoms (Table III). There was no correlation between the worsening of symptoms and the dosage of bee venom administered. There was one dropout in each of the four treatment groups. There were no changes in the somatosensory-evoked potentials at 12 months compared with those measured at baseline.

Results of Immunologic Studies

During the course of the study there were no increases in specific IgE antibodies to bee venom or detection of circulating immune complexes measured by Raji cells or C1q binding assay. There were no specific IgE antibodies to bee venom in seven of the nine subjects. In two subjects there was detection of a 2+ reaction in serum obtained at 6 months of therapy. These findings were not associated with any clinical evidence of IgE-mediated disease or with detectable circulating immune complexes (Table IV). Exami-

nation of urine specimens did not reveal any abnormalities *i.e.*, cellular or protein.

DISCUSSION

Honeybee (*Apis mellifera*) venom for use in immunotherapy of stinging insect hypersensitivity has been obtained classically by venom sac extraction or more recently by electrical milking.¹² A variety of components are found in honeybee venom that have potent biological properties including mellitin¹³ phospholipase A2 and hyaluronidase.^{14,15} Some of these components, *e.g.*, mellitin,¹³ have been shown to have the capacity to alter pain threshold and others have been shown to alter cyclooxygenase 2 activity and proinflammatory cytokine (tumor necrosis factor α and interleukin-1 β) production.¹⁶ Honeybee venom is licensed exclusively for its use in immunotherapy for the prevention of allergic reactions seen in patients with severe localized or generalized reactions associated with stinging insect hypersensitivity to *Apis mellifera*.¹¹ There have been extensive studies of venom immunotherapy as the treatment of choice for the prevention of systemic allergic reactions to insect stings that have provided further evidence for the safety of bee venom exposure in nonmultiple sclerosis patients, which include patients with history of previous systemic allergic reactions to bee stings.^{10,11} Moreover, the use of bee venom for the amelioration of inflammatory or immunologically mediated diseases such as arthritis has been reported in both experimental animals and humans.^{3-9,17-22} This latter use, however, represents a nonlicensed use of the product and is not a generally accepted method of treatment for these conditions in Western medicine. Although there are anecdotal reports of patients with MS who subject themselves to multiple and repeated bee stings with alleged improvement in neurological symptoms, there are no reports of carefully conducted studies of the use of bee venom administered by injection in patients with this disease, except for a case report by Santilli of a single patient with multiple sclerosis treated for 10 years whose disease was stabilized by repeated intradermal injections of honeybee venom.¹⁷ Although it is recognized that the intradermal route may be more irritating than the subcutaneous or intramuscular route, the choice of the intradermal route was made on the previous study by Santilli *et al.*¹⁷ The present report represents the first controlled study evaluating the safety of bee venom therapy as a possible adjunctive treatment for MS. This was a phase I pilot study conducted under a Food and Drug Administration–approved Investigational New Drug primarily to establish safety but not efficacy. The results suggest that the administration of repeated injections of bee venom in a step-up dosage regimen in appropriately selected non-bee venom allergic patients with MS had no serious adverse allergic reactions. The difficulty in conducting such studies in patients with MS includes the great variability in disease symptomatology as well as the difficulty in determining whether any benefit or

TABLE IV				
Results of Immunological Studies				
Patient	IgE to Honeybee Venom Conventional Classification	IgE to Honeybee Venom Modified Classification	Circulating Immune Complexes	
			Raji Cell Method	C1q Binding Assay
1	(1) <0.35/0 (2) <0.35/0 (3) 1.08/ II	(1) 35/0 (2) 47/0 (3) 203/2	(1) 1 (2) 1	(1) <0.5
2	(1) <0.35/0 (2) 0.51/II (3) 0.92/I (4) 0.88/II (5) 0.55/I	(1) 58/1 (2) 121/2 (3) 175/2 (4) 87/2 (5) 145/2	(1) 2 (2) 1 (3) 1 (5) 1.3	NT
3	(1) <0.35/0 (2) <0.35/0 (3) <0.35/0 (4) <0.35/0 (5) <0.35/0	(1) 39/0 (2) 61/1 (3) 53/0 (4) 65/1 (5) 59/0	(1) 12 (2) 9	(1) 3.2 (3) 13.7 (4) 17.7
4	(1) <0.35/0	(1) 53/0	(1) 3	NT
5	(1) <0.35/0 (2) <0.35/0 (3) <0.35/0	(1) 51/0 (2) 54/0 (3) 61/0	(1) 16 (2) 5 (3) 2 (4) 7	(1) 4.3 (2) <0.5
6	(1) <0.35/0 (2) <0.35/0 (3) <0.35/0 (4) <0.35/0 (5) <0.35/0	(1) 37/0 (2) 44/0 (3) 54/0 (4) 49/0 (5) 43/0	(1) 12 (2) 2 (3) 2	(1) <13
7	(1) 7.94/III (2) 3.82/III	(1) 1301/4 (2) 671/4	(1) 11 (2) 1.9	(1) 6.8
8	(1) <0.35/0 (2) <0.35/0 (3) <0.35/0 (4) <0.35/0 (5) <0.35/0	(1) 45/0 (2) 51/0 (3) 72/0 (4) 65/1 (5) 89/1	(1) 10 (2) 5 (3) 1 (4) 3.1	(1) 2.7 (2) <0.5
9	(1) <0.35/0 (2) <0.35/0 (3) <1.85/II (4) 2.42/II (5) 0.83/II	(1) 67/1 (2) 65/1 (3) 316/3 (4) 386/3 (5) 170/2	(1) 4 (2) 7	NT

Serum samples were drawn before the start of therapy, i.e., (1) = baseline; (2) = after 1 month of therapy; (3) = 2 months of therapy; (4) = 6 months of therapy; (5) = after 12 months of therapy; NT = not tested.

neurological worsening occurring during the course of treatment represents a spontaneous occurrence of the natural disease or an event attributable to the treatment itself. Although a significant number of treated subjects experienced worsening of neurological symptoms during the course of bee venom therapy (*i.e.*, four of nine subjects), this response could not be definitively ascribed to side effects of the bee venom versus a spontaneous worsening of progression of the neurological disease independent of treatment. In sup-

port of its lack of a relationship to therapy was the lack of correlation of new disease symptoms with the dosage of bee venom. Although two of the remaining five subjects experienced some neurological improvement during therapy, the numbers are too small to draw any definite conclusions. Although this preliminary safety study suggests that the administration of repeated injections of bee venom in a step-up dosage regimen in appropriately selected non-bee venom allergic patients with MS had no

serious adverse allergic reactions, much larger carefully conducted multicenter studies are required to establish efficacy or unequivocally detect adverse effects on disease progression.

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