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Efficacy of Apitox (Bee Venom) for Osteoarthritis: A Randomized Active-Controlled Trail

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Part 1 Summary, Introduction, and Methods

This trial covered the period from December 1, 1995 to March 31, 1997. The study was an injectable bee venom (Apitox, IND) containing 10mg of dried honeybee venom in 10ml of saline solution. For the control drug, Nabumetone (Ralafen, Smith Kline Beecham, USA) 500mg, a non-steroidal anti-inflammatory drug (NSAID) was chosen because it is commonly used for osteoarthritic patients and has fewer gastrointestinal side-effects than other similar agents.

Introduction
Background

The venom of Apis mellifera (honeybee) has been used for arthritis for over 2,000 years, and many identified components of bee venom contain strong anti-inflammatory properties (Broadman, 1962; Kim, 1992, 1997; Yorish, 1977). Most recently China, Japan, Korea, Germany, Russia, South America and others have used bee venom to treat various chronic inflammatory diseases, but there are no scientific guidelines for using this substance. Therefore, this trial is an attempt to evaluate the efficacy and safety of honeybee venom in a well-designed clinical study.

Bee venom is composed of 30 different components, the main anti-inflammatory pharmacological components are peptides: melittin, apamin, peptide 401, adolapin, and protease inhibitors. Melittin stimulates the hypophyseal-adrenal system and produces cortisone. It is 100 times more potent than hydrocortisone (Couch, 1972; Knepel et al., 1987; Vick et al., 1972, 1975). Melittin also stabilizes the lysosome cell membrane to protect against inflammation (Shkenderov et al., 1986). Apamin works like melittin to produce cortisone (Vick and Shipman, 1972), and inhibits the complement system, C3, which is involved in inflammation (Gencheva et al., 1986). Peptide 401, or MDC peptide, blocks the arachidonic acid and inhibits prostaglandin synthesis (Hanson et al., 1974; Neubould, 1963; Surfer et al., 1973). Adolapin inhibits the microsomal cyclooxygenase. It is 70 times stronger than Indomethacin in animal models (Shkenderov et al., 1986). It also inhibits platelet lipoxygenase, which involves hydroperoxyeicotetranonic acid (HPETE) and leukotriens (Koburova et al., 1985), as well as inhibiting thromboxane (TXA2) and prostacycline (PGI2), which are activated during inflammation (Shkenderov et al, 1986). Protease inhibitors inhibit carrageenin, prostaglandin E1, bradykinin, and histamine induced inflammations; they also inhibit chymotrypsin and leucine-aminopeptidase (Shkenderov, 1986). Schmidt-Lange (1941), Ortel (1955), and Fennell et al. (1968) reported that bee venom has a strong anti-bacterial and anti-fungal effect as well as a radioprotection effect (Ginsberg et al., 1968; Kanno et al., 1970; Shipman et al, 1967, 1968).

It has been reported that bee venom has a strong anti-inflammatory effect, as mentioned above. It has also been proven that bee venom is a strong immunological agent and stimulates the body's protective mechanisms against disease, but there are only a few reports on this substance for clinical use. Therefore, the International Pain Institute produced a formula of pure bee venom solution (Apitox) and applied it to animals and to human volunteers to study it for toxicity and safety. Apitox contains 1.0mg pure dried bee venom in 1.0ml solute. The result of these studies very clearly show that bee venom is very safe to use in therapeutic doses (Hwang et al., 1994; Kang and Kim, 1993; Kim, 1989, 1992, 1994). The next step was to perform a clinical study in the efficacy and safety of Apitox in osteoarthritis patients.

Purpose of the Study

Apitox (honeybee venom) has a variety of strong anti-inflammatory actions and immunological effects leading to its wide use for degenerative disorders, as well as arthritis and related diseases. Presently, the safety of Apitox has been confirmed after completion of Pre-Clinical Animal Studies and Phase I Human Studies. Therefore, the main purpose of this trial was to investigate the efficacy and safety of Apitox in different dosing schedules. Thus, this clinical trial was a Phase II Study performed on degenerative osteoarthritis patients. The main objective goal was to investigate safety as it related to different dosing schedules and to determine the minimum effective dosage.

Osteoarthritis is the most common disease affecting joints (Dieppe, 1994). Heine (1926) reported that the damage of the joint cartilage was quite common after the age of 65. There is more than 80 percent radiological evidence of osteoarthritis after age 75 (Cooper, 1994). The management of osteoarthritis depends mainly on pharmacological approaches. Recently, the development of a prosthesis has helped to improve the quality of life in victims of advanced disease, but it is an important medical need to develop a new drug with high efficacy and few side effects. Bee venom has the dual major properties of encouraging strong immune responses and anti-inflammatory effects with relatively few side reactions. Therefore, it is important to investigate the efficacy of Apitox.

Methods

Design of the Study

There were three Study Drug Groups (A, B, and C) divided according to injection dosages. This trial compared those three groups to a Control Group (D). A participant who fit the inclusion criteria was given a preset number and randomly assigned to a group. Each participant went through six weeks of treatment, with a follow-up visit four weeks after the last treatment. The complete trial period for each participant was 11 weeks including a one week wash-out period. Therefore, this trial was a randomized controlled study comparing four groups.

This trial was not a double blind study because there is no substance that provokes a similar skin reaction to that provoked by bee venom that is safe for humans. We tested the possibility of using Histamine phosphate on three volunteers, but the tests failed. We concluded that histamine cannot be used as a control drug for a double blind study, and we could not find any other substance to provoke a reaction similar to that of the study drug.

Another issue was one of ethical consideration, that of using a placebo as a control drug for 11 weeks for patients suffering from a painful disease. We therefore designed a comparison study using one of the nonsteroidal anti-inflammatory drugs, Nabumetone, as the control drug.

Subject Diseases

The diseases studied were degenerative osteoarthritis affecting the knee joint and spine, based on radiological findings and physical examinations. Participants were chosen according to the selection criteria found in Table 1-1.

Table 1-1 Patient Selection Criteria

Inclusion Criteria

a) Male or female age 40 to 80
b) Primary knee or spinal osteoarthritis with criteria of i and ii met
i) At least two of the following symptoms are present:
1) Pain in involved joints
2) Tenderness on involved joints
3) Limitation of motion
4) Swelling of joints
ii) Radiographic evaluation shows Grade 2 or higher according to the following Radiographic Grading System by KeUgren and Lawrence:

Grade 0: No features of OA
Grade 1: Minute osteophyte, doubtful significance
Grade 2: Definite osteophyte, unimpaired joint space
Grade 3: Moderate diminution of joint space
Grade 4: Joint space greatly impaired with sclerosis of subchondrat bone

Exclusion Criteria

a) History of allergy to bee sting
b) Pregnant or nursing women
c) Complicated diabetes mellitus
d) Advanced cardiovascular complications
e) Connective tissue diseases (scleroderma, lupus, periarthritis nodosa, etc.)
f) Hematologic abnormality (agammaglobulinemia, leukemia, etc.)
g) Ankylosing spondylitis
h) Gouty arthritis
i) Infectious arthritis
j) Active infection elsewhere in the body (TB, syphilis, gonorrhea, etc.)
k) Secondary osteoarthritis (due to trauma, congenital deformity, avascular necrosis etc.)
l) Rheumatoid Arthritis (at least four of the following seven criteria are met):
1. Morning stiffness lasting at least one hour
2. Arthritis in three or more joints in 14 possible sites (e.g. right or left PIP, MCP, wrist, elbow, knee, ankle, or MTP joints)
3. Arthritis in at least one site in the hands (wrist, MCP, or PIP joints)
4. Symmetrical arthritis, i.e., with simultaneous involvement of the same joints on both sides of the body (bilateral involvement of wrist, MCP, and PIP joints; does not have to be absolutely symmetrical)
5. Rheumatoid nodules
6. Rheumatoid factors present in the serum
7. Radiological changes (erosion or osteoporosis around the joints)

Study Groups and Control Group

The following therapeutic course has been used at the International Pain Institute, USA. Treatment is given twice weekly for 12 sessions (6 weeks). Initial dose is 0.3ml with the dose increasing gradually at each subsequent visit; the maximum dose is 2.0ml. The three groups were (A) minimum dose group, up to 0.7ml which was given in our Phase I Trial; (B) medium dose

group, up to 1.5ml in one session; and (C) maximum dose group, up to 2.0ml in one session. In the control group (D), the participants were given a 500mg Nabumetone tablet twice daily for 6 weeks (Table 1-2).

Table 1-2 Dosing Schedule of the Study Group and the Control Group

Study Group (Apitox)* Control Group

Inj #	Group A	Group B	Group C	Group D
1	.01ml	0.2ml	0.3ml	Nabumetone 500 mg
2	.02ml	.04ml	0.6ml	B.I.D.
3	.03ml	.06ml	.09ml	
4	.04ml	.08ml	1.2ml	
5	.05ml	1.0ml	1.5ml	
6	.06ml	1.2ml	1.8ml	
7	.07ml	1.5ml	2.0ml	
8	.07ml	1.5ml	2.0ml	
9	.07ml	1.5ml	2.0ml	
10	.07ml	1.5ml	2.0ml	
11	.07ml	1.5ml	2.0ml	
12	.07ml	1.5ml	2.0ml	

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* Treatment was given twice a week for 6 weeks (12 sessions).
* A single injection dose is 0.1ml.
* 2.ml means that the injection was given at 20 different sites.

Reducing Dose Criteria

The total doses in the study drug could be reduced only under the following conditions; injection site reaction is more than ++; the participant complains that the treatment is too irritable to tolerate; the investigator sees that a reduced dosage will be of more benefit to the participant. In these instances, the dosing schedule could be reduced one level, ie., C to B, B to A, A to Drop-out. In this study, no participant's dose was reduced.

Agreement of the Participants

All participants included in this study were volunteers who signed consent forms. All of them fully understood and were well informed about all conditions related to this study.

Randomization

There were 100 preset numbered tags written, 25 each for A, B, C, and D. These tags were kept in a black bag to maintain blindness. When a participant entered the trial, one random tag was removed from the bag, and the participant was assigned to a group according to the tag. 60 more preset tags were kept in reserve. The total number of participants was 101: A, 25; B, 26; C, 25; D, 25. The total assigned number was 104 because the investigator mistakenly skipped three slots.

Skin Test

We initially excluded five volunteers because of a history of hypersensitivity to bee venom. Subjects of all injection groups (A, B, and C) were given skin tests before treatment was initiated. Injections were given intradermally.

Skin tests were performed in two steps. First, Apitox was diluted 1:1,000 with injectable normal saline (1μg/ml) and 0.05ml was injected intradermally in the flexor surface of the forearm. Local reactions were observed for 15 minutes. Then Apitox (1mg/ml) 0.05ml was injected. All participants were observed carefully for systemic reactions for 20 minutes to check for local reactions such as the size of the weal, the size and shape of erythematous spreading, development of pseudopod, etc. Systemic reactions were generalized itching, rashes, dizziness, shortness of breath, chills, fever and possible anaphylactic response. None of the participants of this trial developed allergic reactions.
Criteria for local reactions is described in Table 1-3. If any of the systemic signs
developed during the observation, we declared the subject as positive, i.e. sensitive to bee venom, and excluded the subject from participation in the study. None developed allergic reactions.

Table 1-3 Criteria for Local Reaction

Grade	Erythema	Weal
0	<0.5cm	<0.5cm
+	0.5 to 1.0cm	0.5 to 1.0cm
1+	1.1 to 2.0cm	0.5 to 1.0cm
2+	2.1 to 3.0cm	0.5 to 1.0cm
3+	3.1 to 4.0cm	1.0 to 1.5cm / pseudopodia
4+	>4.0cm	>1.5cm / pseudopodia

Check the injection site 15 to 20 minutes after injection of AP001 inj 0.05ml with a concentration of 1µg/ml or less. A reaction of 2+ or greater is considered POSITIVE.

Collection of the Analysis Data

Once a participant was selected, we confirmed the wash-out period of seven days and the patient was randomly assigned as described above. All participants were treated for six weeks and laboratory tests were performed on three separate occasions: before treatment, after two weeks and after completion of treatment (six weeks). Efficacy analyses were conducted on five separate occasions: before treatment, after two weeks after six weeks, after eight weeks, and after ten weeks (four weeks after completion of treatment).

(The presentation of this study will continue in the Winter 2000 issue of Bee Informed.)

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