Possible mechanisms of action of cobra snake venom cardiotoxins and bee venom melittin

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Abstract

J. E. FLETCHER and M.-S. JIANG. Possible mechanisms of action of cobra snake venom cardiotoxins and bee venom melittin. Toxicon 31, 669–695, 1993.—Cobra snake venom cardiotoxins and bee venom melittin share a number of pharmacological properties in intact tissues including hemolysis, cytolysis, contractures of muscle, membrane depolarization and activation of tissue phospholipase C and, to a far lesser extent, an arachidonic acid-associated phospholipase A2. The toxins have also been demonstrated to open the Ca2+ release channel (ryanodine receptor) and alter the activity of the Ca2+ + Mg2+-ATPase in isolated sarcoplasmic reticulum preparations derived from cardiac or skeletal muscle. However, a relationship of these actions in isolated organelles to contracture induction has not yet been established. The toxins also bind to and, in some cases, alter the function of a number of other proteins in disrupted tissues. The most difficult tasks in understanding the mechanism of action of these toxins have been dissociating the primary from secondary effects and distinguishing between effects that only occur in disrupted tissues and those that occur in intact tissue. The use of cardiotoxin and melittin fractions contaminated with trace (‘undetectable’) amounts of venom-derived phospholipases A2 has continued to be common practice, despite the problems associated with the synergism between the toxins and enzymes and the availability of methods to overcome this problem. With adequate precautions taken with regard to methodology and interpretation of results, the cobra venom cardiotoxins and bee venom melittin may prove to be useful probes of a number of cell processes, including lipid metabolism and Ca2+ regulation in skeletal and cardiac muscle.

The nociceptive and anti-nociceptive effects of bee venom injection and therapy: A double-edged sword

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Abstract

Bee venom injection as a therapy, like many other complementary and alternative medicine approaches, has been used for thousands of years to attempt to alleviate a range of diseases including arthritis. More recently, additional therapeutic goals have been added to the list of diseases making this a critical time to evaluate the evidence for the beneficial and adverse effects of bee venom injection. Although reports of pain reduction (analgesic and antinociceptive) and anti-inflammatory effects of bee venom injection are accumulating in the literature, it is common knowledge that bee venom stings are painful and produce inflammation. In addition, a significant number of studies have been performed in the past decade highlighting that injection of bee venom and components of bee venom produce significant signs of pain or nociception, inflammation and many effects at multiple levels of immediate, acute and prolonged pain processes. This report reviews the extensive new data regarding the deleterious effects of bee
Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds

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Abstract
Bee venom (BV) therapy (BVT), the therapeutic application of BV, has been used in traditional medicine to treat diseases, such as arthritis, rheumatism, pain, cancerous tumors, and skin diseases. BV contains a variety of peptides, including melittin, apamin, adolapin, the mast-cell-degranulating (MCD) peptide, enzymes (i.e., phospholipase [PL] A2), biologically active amines (i.e., histamine and epinephrine), and nonpeptide components which have a variety of pharmaceutical properties. BV has been reported to have anti-arthritis effects in several arthritis models. Melittin, a major peptide component of BV, has anti-inflammatory and anti-arthritis properties, and its inhibitory activity on nuclear factor kappaB (NF-κB) may be essential for the effects of BV. The anti-nociceptive effects of BV have also been demonstrated in thermal, visceral, and inflammatory pain models. Acupoint stimulation (apipuncture) therapy into subcutaneous region may be important in the BV-induced anti-nociceptive effects. Multiple mechanisms, such as activation of the central and spinal opioid receptor, and α2-adrenergic activity, as well as activation of the descending serotonergic pathway have been suggested. The inhibition of c-Fos expression in the spinal cord by BV apipuncture in several nociceptive models is also reported to be a possible mechanism. BV also has anti-cancer activity. The cell cytotoxic effects through the activation of PLA2 by melittin have been suggested to be the critical mechanism for the anti-cancer activity of BV. The conjugation of cell lytic peptide (melittin) with hormone receptors and gene therapy carrying melittin can be useful as a novel targeted therapy for some types of cancer, such as prostate and breast cancer.

Keywords: Bee venom; Melittin; Apamin; Mast-cell-degranulating peptide; Adolapin; Procamines; Phospholipase A2; Hyaluronidase; Histamines; Anti-arthritis effect; Anti-inflammatory effect; Anti-nociceptive effect; Anti-cancer effect

Abbreviations: 5-HT, 5-hydroxytryptamine; AP5, 5-aminophenonylvaleric acid; BV, bee venom; BVT, bee venom therapy; c-AMP, cyclic adenosine monophosphate; c-GMP, cyclic guanosine monophosphate; CH, chelerythrine chloride; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; COX, cyclooxygenase; CSPA, capsaicin-sensitive primary afferent; DNQX, 6,7-dinitroquinoxaline-2,3-dione; ERK, extracellular signaling-regulated kinases; H89, N-(2-[P-bromocinnamylaminojethyl]-5-isoquinoline sulfonamide hydrochloride; HA, hyperalgesia; IL, interleukin; INOS, inducible NO synthase; i.i., intrathecal; MCD, mast cell degranulating; MIH, mirror image heat; MMP, matrix metalloproteinase; NO, nitric oxide; OA, osteoarthritis; ORL1 receptor, opioid receptor-like 1 receptor; PG, prostaglandins; PKC, protein kinase C; PL, phospholipase; PSN, persistent spontaneous nociception; RA, rheumatoid arthritis; SK, small conductance Ca2+-dependent K+ channels; SMU, single motor units; TNF, tumor necrosis factor; TPA, 12-O-tetradecanoyl phorbol-13-acetate

Anti-inflammatory effect of bee venom on antigen-induced arthritis in rabbits: Influence of endogenous glucocorticoids

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Abstract
Aim of the study
This study assessed the involvement of endogenous glucocorticoids (GCs) in the anti-arthritic properties of bee venom (BV) on antigen-induced arthritis (AIA) in rabbits.

Materials and methods
BV (1.5–6 μg/kg/day) was injected for 7 days before AIA induction, whereas the control group received sterile saline. The total and differential leukocyte count, PGE2 levels in synovial fluid and synovial membrane cell infiltrate were evaluated. The contribution of GCs to BV action was assessed in rabbits treated with BV plus metyrapone, an inhibitor of GC synthesis, or RU-38 486, a steroid antagonist.

Results
Treatment with BV (1.5 μg/kg/day) reduced the leukocyte count and PGE2 level (18571 ± 1909 cells/mm3 and 0.49 ± 0.05 ng/mL, respectively) as well as the cellular infiltrate compared with the control group (40968 ± 5248 cells/mm3 and 2.92 ± 0.68 ng/mL, p < 0.05). The addition of metyrapone to BV treatment completely reversed the inhibition of AIA, whereas RU-38 486 was ineffective.

Conclusion
Our data show that bee venom treatment prevents the development of antigen-induced arthritis in rabbits through the action of GCs.

Bee venom injection into an acupuncture point reduces arthritis associated edema and nociceptive responses
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Abstract
Bee venom (BV) has traditionally been used in Oriental medicine to relieve pain and to treat inflammatory diseases such as rheumatoid arthritis (RA). While several investigators have evaluated the anti-inflammatory effect of BV treatment, the anti-nociceptive effect of BV treatment on inflammatory pain has not been examined. Previous studies in experimental animals suggest that the therapeutic effect of BV on arthritis is dependent on the site of administration. Because of this potential site specificity, the present study was designed to evaluate the anti-nociceptive effect of BV injections into a specific acupoint (Zusanli) compared to a non-acupoint in an animal model of chronic arthritis. Subcutaneous BV treatment (1 mg/kg per day) was found to dramatically inhibit paw edema caused by Freund's adjuvant injection. Furthermore, BV therapy significantly reduced arthritis-induced nociceptive behaviors (i.e. the nociceptive scores for mechanical hyperalgesia and thermal hyperalgesia). These anti-nociceptive/anti-inflammatory effects of BV were observed from 12 days through 21 days post-BV treatment. In addition, BV treatment significantly suppressed adjuvant-induced Fos expression in the lumbar spinal cord at 3 weeks post-adjuvant injection. Finally, injection of BV into the Zusanli acupoint resulted in a significantly greater analgesic effect on arthritis pain as compared to BV injection in a more distant non-acupoint. The present study demonstrates that BV injection into the Zusanli acupoint has both anti-inflammatory and anti-nociceptive effects on Freund's adjuvant-induced arthritis in rats. These findings raise the possibility that BV acupuncture may be a promising alternative medicine therapy for the long-term treatment of rheumatoid arthritis.

Author Keywords: Bee venom; Anti-nociception; Anti-inflammation; Arthritis; Fos immunohistochemistry; Acupuncture

The anti-inflammatory effect of peripheral bee venom stimulation is mediated by central muscarinic type 2 receptors and activation of sympathetic preganglionic neurons
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The anti-inflammatory effect of peripheral bee venom stimulation is mediated by central muscarinic type 2 receptors and activation of sympathetic preganglionic neurons
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Antinociceptive effects of systemic paeoniflorin on bee venom-induced various ‘phenotypes’ of nociception and hypersensitivity

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Abstract

Paeoniflorin (PF), one of the active chemical compounds identified from the root of Paeonia lactiflora Pall, has been well-established to exhibit various neuroprotective actions in the central nervous system (CNS) after long-term daily administration. In the present study, by using the bee venom (BV) model of nociception and hypersensitivity, antinociceptive effects of PF were evaluated by intraperitoneal administration in conscious rats. When compared with saline control, systemic pre- and post-treatment with PF resulted in an apparent antinociception against both persistent spontaneous nociception and primary heat hypersensitivity, while for the primary mechanical hypersensitivity only pre-treatment was effective. Moreover, pre- and early post-treatment with PF (5 min after BV injection) could successfully suppress the occurrence and maintenance of the mirror-image heat hypersensitivity, whereas late post-treatment (3 h after BV) did not exert any significant impact. In the Rota-Rod treadmill test, PF administration did not affect the motor coordinating performance of rats. Furthermore, systemic PF application produced no significant influence upon BV-induced paw edema and swelling. Finally, the PF-produced antinociception was likely to be mediated by endogenous opioid receptors because of its naloxone-reversibility. Taken together, these results provide a new line of evidence showing that PF, besides its well-established neuroprotective actions in the CNS, is also able to produce analgesia against various ‘phenotypes’ of nociception and hypersensitivity via opioid receptor mediation.
Studies of the Apitox (Apitoxin)

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(1) Pharmacology Written Summary of the Apitox

1. Brief Summary

The pharmacology profile of bee venom has been determined in in vitro systems and in various animal models. The results from some of these animal studies are summarized in the following sections and show that Apitoxin has biological activity in animal models that are suggestive of potential efficacy for the treatment of refractory osteoarthritic pain and inflammation in humans.

2. Primary Pharmacodynamics

Adjuvant-induced polyarthritis in the rat has been established as an experimental model for human rheumatoid arthritis. Arthritic symptoms are induced by administering a single SC injection of 1 mg *Mycobacterium butyricum* (Freund's adjuvant or CFA) suspended in mineral oil into a hind paw of the rat. Other studies, including those in rabbits, dogs, and monkeys, are also described.

The ability of bee venom (dose range: 0.01 to 1.0 mg/kg/day) to suppress adjuvant-induced arthritis was studied in rats following daily SC administration over a period of 17 days. Bee venom suppressed the development of adjuvant arthritis in a dose-related manner. A single SC administration of bee venom also suppressed the development of carrageenan-induced paw edema and, when administered SC the day before or on the day of injection of adjuvant, effectively suppressed the development of polyarthritis. This suppressive effect decreased progressively as dosing was delayed. Bee venom was found to be most effective when mixed and injected together with CFA, the disease-inducing agent. Similarly, antigens such as egg albumin, when incorporated into CFA and injected into the hind paw, prevented the development of arthritis. These results suggest that at least two mechanisms are involved in the antiarthritic action of bee venom: (1) alteration of the immune response, most likely via antigen competition, and (2) an anti-inflammatory action.

One of the earliest studies was conducted to evaluate the use of bee venom prophylactically (beginning 2 weeks prior to adjuvant injection) and therapeutically (beginning 1 week after adjuvant injection) to reverse adjuvant-induced arthritis in the rat. Bee venom (1 and 4 mg/kg injected SC three times a week for 4 weeks) was shown to prevent the arthritic syndromes (foot volume, secondary lesions, and reduction in inflammation units) both as a therapeutic and as a prophylactic (more pronounced effect).

The molecular mechanisms of the anti-inflammatory effects of bee venom were investigated in the rat model of carrageenan-induced acute edema in the paw and the rat model of chronic adjuvant-induced arthritis. Bee venom at 0.8 and 1.6 µg/kg administered daily for 14 days into the plantar surface of the right hind paw reduced hind paw edema. These animal results were consistent with in vitro data that showed an inhibitory effect of bee venom at 0.5, 1, and 5 µg/mL and melittin (a major component in bee venom) at 5 and 10 µg/mL on lipopolysaccharide-induced expression of cyclooxygenase 2, cytosolic phospholipase A₂, inducible nitric oxide (NO) synthase, generation of prostaglandin E₂ and NO, and the intracellular calcium level, providing information on the mechanism of action of anti-arthritic effects of bee venom.

The effect of chromatographic fractions (designated as Oa, Op, and the protease inhibitor) and pure proteins and peptides (melittin, apamin, and phospholipase A₂) from bee venom were tested in the rat models of arthritis and inflammatory edema. In rats with adjuvant-induced arthritis, the components of venom (100 µg/kg once daily for 20 days) caused a 15-25% inhibition of arthritic symptoms whereas phospholipase stimulated arthritic symptoms by 30%. Rats with hind paw edema induced by CFA were injected SC with various fractionated bee venom components (10 µg/kg) 24 hours and/or 0.5 hour (n = 6/group) prior to administration of the inflammatory agent. The inhibition of the carrageenan and prostaglandin E₁ inflammation under the effects of Oa and Op fractions and the protease inhibitor were the strongest.

Rats (n = 4/group) with induced adjuvant disease (which includes a severe and persistent polyarthritis) were treated for 21 days with SC administered bee venom (300 or 500 µg or 1.2 and 2 mg/kg for a 250 g rat) twice a day; saline (control); bee venom intraperitoneal (IP); or melittin, apamin, or phospholipase A₂ SC (at the amount contained in 1 mg of bee venom). Rats treated SC with bee venom developed little or no arthritis with the higher dose being more effective than the lower dose while rats injected IP with bee venom or with SC melittin, apamin, or phospholipase A₂ had no apparent benefit on the polyarthritis. When SC bee venom injections were initiated after moderate to severe polyarthritis had developed, rats did not have the course of the disease altered.

The ability of a daily administration of bee venom (2 mg/kg/day) to suppress adjuvant-induced arthritis in rats was studied over a period of 24 days. The bee venom treatment suppressed but did not abolish the primary and secondary inflammatory responses to the adjuvant as monitored by decreases in the swelling of the left (where the
adjuvant was injected) and right hind paws, respectively. The response was delayed, with statistically significant suppressive effects being noted 2-3 weeks following the administration of the adjuvant. A sex difference in the suppressive effect of bee venom was noted, with female rats demonstrating a more pronounced beneficial effect than male rats. The dosage used in this study (2 mg/kg) was rather high, as compared to 50-70 µg of solids in the average honeybee sting.

In a study performed to assess the clinicotherapeutic effect of bee venom, 90 rats were administered bee venom (1 bee [about 0.1 mg bee venom or 0.4 mg/kg for a 250 g rat], SC), prednisolone (10 mg/kg, orally), or saline control (0.1 mL, SC) every other day over a period of 14 days. Clinical findings of lameness score, edema volume, hematological values, and histopathology (interphalangeal joint of the right hind paw) were observed during the treatment period. In the treatment groups, the development of inflammatory edema and polyarthritis was suppressed. No significant differences of hind paw edema volume and lameness score between the prednisolone and bee venom groups were observed during treatment. No differences in red blood cell count, hematocrit, or hemoglobin concentration were noted between the groups although significant leucocytosis was observed in the control group (p < 0.01). Erosions of articular cartilage and inflammatory cell infiltrations into the interphalangeal joint were effectively suppressed in treated groups. Thus, whole honeybee venom was found to suppress arthritic inflammation in the rat.

A study was conducted to determine whether some of the in vivo effects of bee venom may be mediated by alterations in lymphokine production and to observe the in vitro effect of lymphokines on reduced mitogenic responses of bee venom-treated rats. When 0.5 mg/kg/day bee venom was administered intramuscularly (IM) to rats over a period of 17 days, a reduction in interleukin (IL) production by splenocytes was observed. In vitro addition of IL-1 or IL-2 to the cultures resulted in an increase in responses to normal levels, suggesting that bee venom affects the production of IL-1 by macrophages. This finding suggests that the modifying effects by bee venom on inflammatory responses in local therapy may be due to interference with certain functions of inflammatory cells.

In an attempt to explain the mechanism of bee venom's anti-inflammatory action, the effect of bee venom on neutrophil O₂⁻ production was investigated. Neutrophil production of toxic oxygen radicals and metabolites are thought to play a role in chronic inflammation and tissue destruction in a wide variety of diseases. Using human peripheral blood leukocytes, the polymorphonuclear fraction was isolated and used in vitro assessments on the ability of melittin and other bee venom peptides to affect the production of O₂⁻. The results showed that melittin, but not other bee venom fractions, inhibited O₂⁻ production both pre- and post-stimulation, suggesting the melittin may have a role in the in vivo regulation of radical production and suggesting a possible mechanism of action for this major bee venom component.

The effect of bee venom on inflammatory cell function was studied following injection of immune complexes into rabbit knee-joints. Treatment with single SC injections of bee venom (1.2 to 20 µg/kg) significantly reduced the leukocyte counts in the immune complex challenged joints as compared to untreated rabbits (p < 0.02). Bee venom at a dose of 12 µg/kg decreased leukocyte counts 3 and 6 hours after challenge with immune complexes but this effect was not noted at 9 hours. Significant effects on leukocyte random migration, chemotactic responsiveness, or phagocytosis were not observed, indicating that bee venom did not interfere with normal phagocyte motility and ingestion. The modifying effects by bee venom on the inflammatory response to immune complexes in vivo was, therefore, thought to be most likely due to interference with other components of the inflammatory response.

In a study designed to investigate the possible role of the soluble fraction of bee venom in producing the anti-arthritic actions of bee venom acupuncture, whole bee venom was extracted into two fractions according to solubility: a water soluble fraction (BVA) and an ethylacetate soluble fraction (BVE). Subcutaneous BVA injection (0.9 mg/kg/day) into the Zusanli acupoint was found to dramatically inhibit paw edema and radiological change (i.e. new bone proliferation and soft tissue swelling) caused by CFA injection. The BVA treatment also reduced the serum IL-1 by macrophages. Finally, BVA treatment significantly suppressed adjuvant-induced Fos expression in the lumbar spinal cord at 3 weeks post-adjuvant injection. In contrast, BVE treatment (0.05 mg/kg/day) failed to show any anti-inflammatory or antiinociceptive effects on rheumatoid arthritis. These results demonstrate that BVA is the effective fraction of whole bee venom responsible for the antinociception and anti-inflammatory effects of bee venom acupuncture treatment. Further study is necessary to clarify which constituents of the BVA fraction are directly responsible for these anti-arthritic effects.

Previous studies in experimental animals suggested that the therapeutic effect of bee venom on arthritis is dependent on the site of administration. In particular, local injection of bee venom near the site of inflammation, such as the hind limb in the rat model, is more effective in inhibiting the development of adjuvant-induced arthritis than injections into a more distant site (e.g. on the back). Because of this potential site specificity, a study was designed to evaluate the anti-nociceptive effect of bee venom injections into a specific acupoint (Zusanli) compared to a non-acupoint in the rat model of chronic arthritis. Subcutaneous bee venom treatment (1 mg/kg per day) was found to dramatically inhibit paw edema caused by CFA injection and significantly reduced arthritis-induced nociceptive behaviors (i.e. the nociceptive scores for mechanical hyperalgesia and thermal hyperalgesia). These anti-nociceptive/anti-inflammatory actions of bee venom acupuncture, whole bee venom was extracted into two fractions according to solubility: a water soluble fraction (BVA) and an ethylacetate soluble fraction (BVE). Subcutaneous BVA injection (0.9 mg/kg/day) into the Zusanli acupoint was found to dramatically inhibit paw edema and radiological change (i.e. new bone proliferation and soft tissue swelling) caused by CFA injection. The BVA treatment also reduced the serum IL-1 by macrophages. Finally, BVA treatment significantly suppressed adjuvant-induced Fos expression in the lumbar spinal cord at 3 weeks post-adjuvant injection. In contrast, BVE treatment (0.05 mg/kg/day) failed to show any anti-inflammatory or antiinociceptive effects on rheumatoid arthritis. These results demonstrate that BVA is the effective fraction of whole bee venom responsible for the antinociception and anti-inflammatory effects of bee venom acupuncture treatment. Further study is necessary to clarify which constituents of the BVA fraction are directly responsible for these anti-arthritic effects.
effects of bee venom were observed from 12 days through 21 days post-bee venom treatment. In addition, bee venom treatment significantly suppressed adjuvant-induced Fos expression in the lumbar spinal cord at 3 weeks post-adjuvant injection. Finally, injection of bee venom into the Zusanli acupoint resulted in a significantly greater analgesic effect on arthritic pain as compared to bee venom injection in to a more distant non-acupoint. The study demonstrated that bee venom injection into the Zusanli acupoint has both anti-inflammatory and anti-nociceptive effects on CFA-induced arthritis in rats. These findings suggest that bee venom acupuncture may be an effective therapy for the treatment of rheumatoid arthritis.

The antinociceptive and anti-inflammatory effects of bee venom pretreatment on carrageenan-induced inflammation in the rat was studied in order to determine if bee venom, which is nociceptive under normal conditions, serves as an anti-inflammatory agent under conditions of inflammation. Rats were injected SC with bee venom at 0.8 mg/kg (n = 8) or 0.08 mg/kg ( n = 6) 30 minutes prior to carrageenan-induced acute paw and thermal hyperalgesia. At 0.8 mg/kg, bee venom significantly suppressed carrageenan-induced edematous paw volume (p < 0.001) and thermal hyperalgesia (p < 0.01) and induced expression of Fos positive neurons in the spinal cord (p < 0.01). These results suggested that bee venom may be useful in the treatment of pain and edema associated with chronic inflammatory diseases.

The effect of bee venom on cortisol levels and increase in activity in dogs with hip dysplasia was investigated. The 24 dogs, 8 of which had hip dysplasia and 16 of which were normal, were divided into 4 treatment groups. Groups I and II included 8 normal dogs each and Groups III and IV each had 4 dogs with hip dysplasia. Groups II and IV received 1 mg (about 0.067 mg/kg for a 15 kg dog) of bee venom SC on Days 30, 37, 50, and 60 and then were crossed over to received saline control treatment on Days 90, 97, 110, and 120. Groups I and III began with saline control treatment and then were crossed over to the bee venom treatment. Dogs receiving bee venom injections had increased plasma cortisol levels and arthritic dogs had increased daily cage activity. On day 90, the treatments were crossed-over and again dogs receiving bee venom had increased plasma cortisol levels and arthritic dogs had increased daily cage activity. The results suggest that bee venom stimulates the production of cortisol and enhances the cage activity of arthritic dogs.

The effect of whole bee venom on plasma cortisol levels was investigated in the unanesthetized monkey following single SC injections of bee venom (1.0 to 100 mg) or melittin (1.0 to 10 mg). Both bee venom and melittin injections produced marked and sustained elevations in plasma cortisol levels. These increases occurred at approximately 1 hour following SC injection and lasted for 2-4 days. The effect appeared to be dose-related with the higher doses of bee venom or melittin producing the earliest and most pronounced plasma cortisol elevations. When a second dose of bee venom (1 mg) and melittin (0.1 mg) was administered to 1 monkey each at 72 or 96 hours, respectively, there was an immediate and sustained rise in cortisol, which lasted for 20 to 30 days. Melittin appeared to be 10 times more potent than bee venom. Necropsy results from 4 monkeys receiving the highest doses of bee venom or melittin indicated no significant gross or microscopic tissue changes. Surgical removal of the pituitary gland from 4 monkeys prevented the effect, indicating that bee venom and melittin may be stimulating the production of cortisol from the adrenal gland, which may provide an explanation for the beneficial effects of bee venom therapy in a variety of disease conditions that respond to adrenal steroid therapy.

Using HTB-94 human chondrosarcoma cells, gene expression profiles were determined following treatment with bee venom, lipopolysaccharide (LPS), or both. Of 344 genes profiled, 35 were down regulated by bee venom, 16 were up regulated and 7 down regulated by LPS, and 32 were down regulated by bee venom and LPS combined. Bee venom reversed the upregulation caused by LPS for some genes, such as the IL-6 receptor, matrix metalloproteinase-15 (MMP-15), tumor necrosis factor, superfamily-10, caspase-6, and tissue inhibitor of metalloproteinase-1 (TIMP-1). These results provided information for understanding the pharmacologic activity of bee venom for the treatment of arthritis.

3. Secondary Pharmacodynamics

1) General Pain

In a study designed to evaluate the potential antinoceptive effect of bee venom pretreatment on formalin-induced pain behavior and its associated spinal cord Fos expression, rats were administered a single SC injection of bee venom (dose range: 0.0016 mg/kg to 0.08 mg/kg) or saline control into the Zusanli acupoint (5 mm lower and lateral to the anterior tubercle of the tibia). Pretreatment with bee venom significantly decreased paw-licking time in the late phase of the formalin test. In contrast, bee venom injected into a non-acupoint in the back region did not suppress the paw-licking time. Bee venom pretreatment into the Zusanli acupoint markedly inhibited spinal cord Fos expression induced by formalin injection. These findings indicate that bee venom pretreatment into the Zusanli acupoint has an antinoceptive effect on formalin-induced pain behavior.

In a follow-up study to the one describe above, an abdominal stretch assay in mice and rats and formalin test in rats were used to further investigate bee venom’s antinoception effect. Bee venom was administered SC to the mice at a 1 to 100 or 1 to 1000 dilution and to rats at a 1 to 1000 dilution in 20 µL of saline. Bee venom at the 1 to 100 dilution into an acupoint or non-acupoint produced antinoceptive effects while bee venom at the 1 to 1000 dilution was effective only when dosed into an acupoint, indicating that bee venom may be a promising method for the relief of
2) X-Irradiation Protection

The response of animals to whole-body X-irradiation in the lethal range can be modified by certain changes in their physiological state if induced prior to exposure. The ability of bee venom to produce a degree of physiological stress in animals, thereby eliciting a neuroendocrine response (pituitary-adrenal stimulation) that would increase radiation resistance, was studied in mice. In a series of experiments, pre-treatment with bee venom (IP dose range: 1.1 to 1.24 µg; SC dose range: 4.3 to 5.6 µg) resulted in greater 30-day survival rates as compared to a saline control. Bee venom administered SC afforded the greatest protection (70-80% 30-day survival rate). When melittin, a major component of bee venom, was separated and injected SC at 5.4 µg, the 30-day survival rate was 7%. Based on these results it was proposed that at least 3 mechanisms of action may account for the radioprotective effect of bee venom in mice: 1) it has a stressor-like action that elicits an “adaptation syndrome,” 2) it produces changes in the hematopoietic system, or 3) it has antibacterial properties.

In a separate study, melittin provided statistically significant x-irradiation protection to mice that received an SC injection doses up to 60 mg/kg 24 hours prior to the irradiation. Whole bee venom did not show conclusive evidence for the same protection.


(2) PREVIOUS HUMAN EXPERIENCES OF APITOX
Several studies have been conducted with Apitox(Apitoxin), including a Phase 3 clinical trial that formed the basis of the marketing approval of Apitox in Korea. These studies are summarized below.

1. Studies in Healthy Normal Human Volunteers
Fourteen healthy male (10) and female (4) subjects between the ages of 26 and 52 years of age were enrolled in and completed this study. Among the male subjects, 3 were beekeepers who were included in order to obtain data from the maximum end of the spectrum without having to administered venom in high dosages (they received 10-350 stings per week during the course of their normal work). Injections were administered subcutaneously twice weekly, with each dose being dependent upon tolerance to the previous dose. All subjects were administered a maximum dose of 0.07 mg of Apitox and maintained on that dose for a week before continuing with an arthritic injection schedule. Each subject received 13 doses of Apitox according to the following schedule:

<table>
<thead>
<tr>
<th>Injection #</th>
<th>Concentration per Injection</th>
<th>Total Dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 × 0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>2</td>
<td>1 × 0.07 + 0.05</td>
<td>0.12</td>
</tr>
<tr>
<td>3</td>
<td>2 × 0.07</td>
<td>0.14</td>
</tr>
<tr>
<td>4</td>
<td>2 × 0.07 + 0.05</td>
<td>0.19</td>
</tr>
<tr>
<td>5</td>
<td>3 × 0.07</td>
<td>0.21</td>
</tr>
<tr>
<td>6</td>
<td>3 × 0.07 + 0.05</td>
<td>0.26</td>
</tr>
<tr>
<td>7</td>
<td>4 × 0.07</td>
<td>0.28</td>
</tr>
<tr>
<td>8</td>
<td>4 × 0.07 + 0.05</td>
<td>0.33</td>
</tr>
<tr>
<td>9</td>
<td>5 × 0.07</td>
<td>0.35</td>
</tr>
<tr>
<td>10</td>
<td>5 × 0.07</td>
<td>0.35</td>
</tr>
<tr>
<td>11</td>
<td>5 × 0.07</td>
<td>0.35</td>
</tr>
<tr>
<td>12</td>
<td>5 × 0.07</td>
<td>0.35</td>
</tr>
<tr>
<td>13</td>
<td>5 × 0.07</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Skin tests, vital sign measurements, and blood and urine laboratory evaluations were performed. One subject who became ill and was subsequently hospitalized was shown to have contracted an adenovirus infection. A female subject had a delayed local reaction: 18 hours after injection, she developed a 4+ wheal with a very large flare. A repeat injection of the same concentration and all subsequent injections were normal. None of the subjects had a systemic reaction and there were no changes noted in vital sign measurements. None of the subjects reported significant pain.

In a second study, 20 healthy (10 male, 10 female) subjects between the ages of 23 and 45 years of age were enrolled and 15 completed the study. Five subjects dropped out of study because of failure to keep study appointments, or due to consumption of alcohol during the study. Each subject received an initial test dose of 0.05 mL and then 12 doses of Apitox, starting with 0.1 mL with the first intradermal injection and increasing to 0.2 mL (second injection), 0.25 mL (third injection), 0.3 to 0.7 mL (fourth through twelfth injections). Injections were administered 2 to 3 times per week over a period of 4 to 6 weeks. Physical examination, blood and urine laboratory evaluations, and vital sign measurements were performed.

There were no significant changes from baseline noted. Localized itching was the most common adverse experience (11/15). Edema (5/15), pain at injection site (2/15), and blister at injection site (1/15) were also reported, but no serious adverse experiences were reported. Thus, it was concluded that Apitox can be safely administered to humans when applied in therapeutic doses.

2. Studies in Patient Populations
Studies evaluating the safety and efficacy of Apitox have been conducted in patients with chronic pain and inflammation (due to rheumatoid arthritis, osteoarthritis, fibromyositis, or peripheral neuritis) and those with osteoarthritis.

1) Chronic Pain and Inflammation Study (United States)
In the chronic pain and inflammation study, 180 subjects were randomized to receive, over a period of 6 weeks, twice weekly injections of either Apitox (1 mg/mL) or histamine phosphate (0.275 mg/mL). The number of injections increased with each subsequent visit (e.g. 3, 6, 9, 12, 15, 18, 20, 20, 20, 20, 20, and 20 injections). The injections were administered to the area of pain first and then beginning with the fifth session, as the number of injections increased, to the corresponding dermatomal area of the spine. The visual analogue scale (VAS) and McGill Pain Questionnaire (MPQ) were used to assess the level of pain. Thermographic evaluations and physical examinations...
Both groups had reductions in pain scores following treatment, with the Apitoxin treatment group demonstrating a greater improvement (pain scores: control 57 vs. Apitox 18). In addition, there were significant differences between control and the Apitox treatment group at the 6-month follow-up visit (pain scores: control 83 vs. Apitox 29). The Apitoxin treatment group also demonstrated greater improvement in the physical examination and thermographic findings.

2) Pain and Inflammation of the Osteoarthritis Study (Korea)
This was a randomized, active-controlled (nabumetone) study in which 101 subjects with osteoarthritis of the knee or spine were given twice weekly injections of Apitox (maximum doses of 0.7 mg [Group A], 1.5 mg [Group B], or 2.0 mg [Group C]) or an oral once daily dose of the control (1000 mg nabumetone, Group D) over a period of 6 weeks. A 4-point Likert-like symptom severity rating scale was used to assess pain, disability, and physical signs. A 5-point scale was used for subject self-evaluation. Safety was assessed through observation of adverse experiences and blood and urine laboratory measurements.

A total of 81 subjects completed the study. Those subjects assigned to an Apitox treatment group demonstrated a statistically significant greater improvement than those in the nabumetone group (p < 0.01). Within the Apitox groups, Groups B and C demonstrated greater improvements than Group A (p < 0.01). The most common adverse experiences reported were injection site itching and generalized body aches.

3) Other Experience (Korea)
Following approval of Apitox in Korea, a post-marketing survey was conducted in November 2005. Included in this survey were 1,596 patients who received Apitox therapy.

Patients, without any compensation, voluntarily filled out the survey forms. The information collected included personal information, present illness, past history, and present medications. The physicians recorded the treatment records, including the diagnosis, treatment dates for 12+ sessions, doses, and adverse experiences (including a detailed description). A complete blood count was performed before treatment and after the last treatment. The physician immediately notified the pharmaceutical company and Korean FDA in the event of a serious adverse experience.

According to the survey, no major adverse experiences were reported.

In addition to this survey, The Pain Center, PC University Medical Center located in Korea has documented the use of Apitox in 3, 679 intractable medical condition and autoimmune disease patients between September 2003 and November 2005. No major adverse experiences were reported. Minor adverse experiences included itching (injection site), swelling (injection site), pain, low-grade fever, flushing, headache, and diarrhea.

**Antibacterial Properties of Whole Body Extracts and Haemolymph of Maggots of Lucilia sericata**

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The aim of this study was to partially characterize maggot-secreted antibacterial substances and determine their range of activity against different bacteria. Sterile and non-sterile maggots maintained in the laboratory and taken from wounds of treated patients were used. Whole body extracts and haemolymph was fractionated and their range of activity against bacteria was tested by the zone inhibition assay. The mode of action of bacterial destruction was examined by viable counts, influx of K⁺, changes in the membrane potential by scanning electron microscope (SEM).

Extracts of sterile maggots also demonstrated an activity of 200 arbitrary units (AU)/ml and 400AU/ml respectively. Maggots removed from chronic wounds had an activity of 1200AU/ml. Injuring sterile maggots with a sterile needle doubled the antibacterial activity within 24 hours, while the antibacterial activity of haemolymph increased fourfold after injuring with a sterile needle and sixteenfold with an infected needle. The fractions with a molecular weight of <1kDa and 3–10kDa showed antibacterial activity against Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa, Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from wounds. The fraction with a MW <1kDa lysed over 90% of the bacteria within 15 minutes by causing an influx of K⁺ and changing the membrane potential of bacteria. The nature of the antibacterial materials extracted from maggots indicates their potential significance in wound healing in addition to the actual ingestion of the necrotic tissue on the wound.
Treatment by Injection-Acupuncture with Bee-Venom(Apitoxin) and Apitoxin Combined by Chinese Herbal Medicine in Patients with Canine Hind Limb Paralysis

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*** Yeon Chang Veterinary Clinic, Miaoli City, Miaoli Province, Taiwan
# Shan Qun Animal Hospital, Shang Cheng City, Taipei Province, Taiwan
## Zoo Animal Hospital, Taipei City, Taiwan
### Sino Union Animal Hospital, Young He, Taipei, Taiwan

** Purpose: **The therapy by injection-acupuncture(AP) with bee venom (Aptoxin) and injection-AP with Apitoxin combined by administration of Chinese herbal medicine was applied in 2 cases with canine intervertebral disc disease(IVDD).

** Method: **Case 1 was diagnosed as thoraco-lumbar IVDD (T11-T12, T12-T13, L3-L4 and L4-L5) and case 2 was diagnosed as IVDD at T 10- T11 and T 12- T 13, respectively. Injection-AP with Apitoxin(total 200 ㎍ of Apitoxin, 0.1 ml /acupoint) plus physical exercise(walking with gocart, TID/day) and aquatherapy(swimming treatment, BID/week) were given to each patient. The used acupoints were GV20(Bai Hui), GB30(Huan Tiao), ST36(Zu San Li), GB34(Yang Ling Quan), ST40(Feng Long), ST41(Jie Xi) and BL40(Wei Zhong), the lesions, and trigger points. In addition, Chinese herbal medicine(Koda Pharmaceutical Co., Taiwan) including Zheng Gu Zi Jin Dan(正骨紫金丹: 1 g), Shiuh Duann(續断: 0.2 g), Du Zhong(杜仲: 0.2 g), Mo Yao(沒藥: 0.2 g), Ru Xiang(乳香: 0.2 g) and Pyrite(自然銅: 0.2 g) were orally medicated BID for 9 days in case 2.

** Result: **Walking was possible after session 11 for 4 weeks in case 1 and after session 6 for 2 weeks in case 2, respectively.

** Conclusion: **The present cases were canine IVDD which showed favorable therapeutic responses to injection-AP with Apitoxin only and injection-AP with Apitoxin combined by Chinese herbal medicine.

** Bee venom (Aptoxin) therapy on canine facial nerve paralysis

Hyung-Kyou Jun*, Hyun-Uk Oh*, Hyun-Hwa Lee*, Ji-Won Han*, Cristopher Moon-Ho Kim** and Duck-Hwan Kim*

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** Anapunsesang Clinic, Seoul, Korea

** Purpose: **To elucidate the therapeutic effect by Apitoxin therapy for canine facial nerve paralysis(FNP), the present study was performed.

** Method: **The 12 mongrel dogs (2-3 years old, 2.0-5.6 kg, BW) were used in this experiment. The experimental dogs were divided into control(4 dogs), experimental group I(4 dogs) and experimental group II(4 dogs). FNP was induced by clamping of facial nerve with hemostatic forceps for 20 minutes under zoletil anesthesia. As for the treatment, control group was intramuscularly injected with saline into head muscle, and experimental group I was treated by injection-acupuncture(AP) with dexamethasone (Huons Co., Korea, 1 mg/ml/head) and experimental group II was treated with injection-AP with Apitoxin(Guju Pharmacological Co. and Apimeds, Inc., Korea, 1mg/bottle, 200ug of Apitoxin: 2 % Iodocaine =1:1) after induction of FNP, twice per week for 2 weeks, respectively. The used acupoints were LI04, LI20, ST02, ST07, GB03, SI18, TH17 and GB34. The degrees of FNP improvement were evaluated as clinical scores(0: normal, 1: mild, 2: moderate and 3: severe) and serum creatine kinase(CK) activities were evaluated. Statistical significance was analyzed by student’s t-test.

** Result: **The FNP symptoms were not improved at all until day 14 in control group, however, the FNP symptoms were much improved until day 14 in experimental group I and II, respectively. The significant differences of clinical scores were detected on day 14 in experimental groups, compared by those of control group (p<0.05).