The water-soluble fraction of bee venom produces antinociceptive and anti-inflammatory effects on rheumatoid arthritis in rats

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Abstract

We recently demonstrated that bee venom (BV) injection into the Zusanli acupoint produced a significantly more potent anti-inflammatory and antinociceptive effect than injection into a non-acupoint in a Freund’s adjuvant induced rheumatoid arthritis (RA) model. However, the precise BV constituents responsible for these antinociceptive and/or anti-inflammatory effects are not fully understood. In order to investigate the possible role of the soluble fraction of BV in producing the anti-arthritic actions of BV acupuncture, whole BV was extracted into two fractions according to solubility (a water soluble fraction, BVA and an ethylacetate soluble fraction, BVE) and the BVA fraction was further tested. 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 Freund’s adjuvant injection. BVA treatment also reduced the increase in serum interleukin-6 caused by RA induction to levels observed in non-arthritic animals. In addition, BVA therapy significantly reduced arthritis-induced nociceptive behaviors (i.e. nociceptive scores for mechanical hyperalgesia and thermal hyperalgesia). 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 antinociceptive effects on RA. The results of the present study demonstrate that BVA is the effective fraction of whole BV responsible for the antinociception and anti-inflammatory effects of BV acupuncture treatment. Thus it is recommended that this fraction of BV be used for long-term treatment of RA-
induced pain and inflammation. However, further study is necessary to clarify which constituents of the BVA fraction are directly responsible for these anti-arthritis effects. © 2002 Published by Elsevier Science Inc.

*Keywords:* Bee venom; Water-soluble fraction; Antinociception; Anti-inflammation; Arthritis; Acupuncture

**Introduction**

Bee venom (BV) therapy has been utilized to relieve pain and to treat inflammatory diseases such as rheumatoid arthritis (RA) in humans [1] and experimental animals [2,3]. BV contains a variety of different peptides including melittin, apamin, adolapin and mast cell degranulating (MCD) peptide [2]. In addition, it also contains enzyme (i.e. phospholipase A₂), biologically active amines (i.e. histamine, epinephrine) and non-peptide components (including lipids, carbohydrates and free amino acids) [4]. Only a few of these individual components of BV have been tested to date for their possible anti-inflammatory and/or antinociceptive effects. In two early studies, it was reported that adolapin and purified MCD peptide had anti-inflammatory and/or antinociceptive activity [5,6]. While these two components could contribute to the anti-arthritic effects of whole BV, it is important to note that these substances are present in very small quantities (1-2%) in dried whole BV.

It is well established that bee stings evoke pain. Further studies have shown that injection of whole BV produces a tonic pain response and causes the development of hyperalgesia to both mechanical and thermal stimuli [4,7]. It has also been recognized that the major components of BV, including melittin (50% of whole BV) and phospholipase A₂ (10% of whole BV), are responsible for the development of local inflammation and nociception [8–10]. While important studies have been made in our understanding of BV constituents that produce pain, further investigation is required to elucidate the major components of whole BV that are responsible for the anti-arthritic effect of BV treatment.

In experimental animals, the induction of arthritis is successfully suppressed by long-term BV treatment [2,3]. As an extension of these early studies, we have recently demonstrated that injection of whole BV into the Zusanli acupoint produces a significantly greater anti-arthritic effect than injection into a non-acupoint located on the back in a rodent model of RA [11]. The specificity of the injection site location (acupoint versus non-acupoint) suggests that the BV-induced anti-arthritic effect may be the result of specific acupoint stimulation rather than a systemic anti-arthritic effect induced by an analgesic and/or anti-inflammatory substance present in whole BV. However, it is unlikely that an individual, stimulating component of BV (i.e. melittin) was solely responsible for this site-specific anti-arthritic effect of BV. Rather, we hypothesize that the anti-arthritic effect of BV was due to a complex stimulation effect involving several individual components of BV that are water-soluble. Based on this hypothesis, we grossly extracted whole BV according to solubility to evaluate whether water-soluble constituents of BV were more potent anti-arthritic agents than organic soluble components.

The adjuvant-induced chronic RA model is widely used for evaluation of potential new therapeutic agents. In general, the therapeutic potencies of anti-arthritic agents are evaluated with respect to both anti-inflammatory and antinociceptive effects. A change in paw volume has classically been used for evaluating anti-inflammatory effects on RA [2]. Plasma concentrations of inflammatory cytokines (i.e. interleukin-6) have also been used as a valuable clinical index for adjuvant induced RA model in rats [12]. In addition, the measurement of bone changes that occur during the course of experimental
arthritis using quantitative image analysis has been shown to be useful for evaluating anti-inflammatory effects on RA [13]. Evaluation of potential antinociceptive effects of therapeutic agents on RA has employed measurements of the development of either thermal or mechanical hyperalgesia following the induction of chronic arthritis. All of these procedures were utilized in the present study to evaluate the effectiveness of BV fraction injection into an acupoint. In addition, the antinociceptive effect of BV fraction injection on adjuvant-induced spinal cord Fos expression was analyzed using a computerized image analysis system.

**Methods**

**Animals**

Experiments were performed on 60 male Sprague-Dawley rats (obtained from the Laboratory Animal Center of Seoul National University, South Korea) weighing 130–150 g at the beginning of the experiment. Animals were kept in a 12:12 light-dark cycle (7:00 AM onset) in a temperature controlled room (23 ± 0.5 °C). Food and water were available ad libitum. The food was placed on the sawdust in the cage to minimize the need for animals to make potentially painful movements to obtain food. All of the methods used in the present study were approved by the Animal Care and Use Committee at Seoul National University and conform to NIH guidelines (NIH publication No. 86-23, revised 1985). All algesiometric assays were conducted under the ethical guidelines set forth by the International Association for the Study of Pain (IASP) [14].

The induction of arthritis

Experimental animals were briefly anesthetized with 3% isoflurane in a mixed N₂O/O₂ gas. Arthritis was induced by a single subcutaneous injection (50 μl) into the plantar surface of the right hind paw of heat-killed *Mycobacterium butyricum* (Difco Laboratory, Detroit, MI, USA) suspended in sterile mineral oil (20 mg/ml). Control animals were similarly injected with sterile vehicle.

**Extract preparation and experimental groups**

Whole BV (*Apis mellifera*) was purchased from Sigma. The BV was dissolved in water and then partitioned with hexane (1:1 vol/vol). The hexane fraction was evaporated to dryness and the resulting water layer partitioned with ethylacetate to provide an ethylacetate soluble fraction and a water soluble fraction. Each fraction was completely dried and stored at refrigerator temperature. Whole BV contains 90% water-soluble (BVA), 5% ethylacetate-soluble (BVE) and 5% hexane-soluble substances. In the present study, we only evaluated the BVA and BVE extracts because the hexane soluble extract is generally known to be toxic. Experimental animals were divided into four groups: 1) a saline treated non-arthritic group (Sham, n = 10); 2) a saline treated arthritic group (RA-Sal, n = 10); 3) a BVA treated arthritic group (RA-BVA, n = 10); and 4) a BVE treated arthritic group (RA-BVE, n = 10). Each extract was dissolved in appropriate vehicle [BVA: 0.9% saline, BVE: ethanol and saline (1:10 vol/vol)] prior to injection.

In a previous study, we observed that whole BV produced a significant antinociceptive effect when injected into an acupoint at a concentration of 1 mg/kg [11]. Based on these results, the dose of each
extract in the present study was determined by considering the partial ratio of each extract in whole BV (1 mg/kg). Thus, BVA and BVE were used at doses of 0.9 mg/kg and 0.05 mg/kg, respectively, to determine whether the BVA or BVE extract was responsible for the anti-arthritic effect produced by whole BV. Each extract was administered subcutaneously and bilaterally into the Zusanli point as previously described [11]. The Zusanli point is anatomically located 5 mm below and lateral to the anterior tubercle of the tibia. Sham and RA-Sal animals were injected bilaterally into the Zusanli point with an equal volume of saline. BV extract treatment was started the day after adjuvant injection and animals were injected daily for a period of three weeks. All algesiometric assays were performed beginning 9 days after adjuvant injection at the time of induction of systemic arthritis.

**Evaluation of paw volume**

Paw volume of the contralateral hind paws was dually measured using a water displacement plethysmometer (UGO BASIL, Italy) every three days for 21 days after adjuvant injection and the mean values were recorded. Paw volume measured just prior to adjuvant injection was used as the control volume (day 0). Data were plotted as the change of paw volume versus control volume at each time point. Measurement of paw volume and all behavioral tests were performed blindly.

**Evaluation of radiological change in the hind limb**

At the end of experiment, rats were sacrificed with an overdose of ether. The left hind limb was amputated and placed on a film carrier with the medial aspect of the limb down (lateromedial view). All radiographs were taken with a Westinghouse Rivera Instrument set at 12.5 mA/s, 40 kV with Kodak Ektascan M Film. The film-to-source distance was 40 inch.

Radiographs were digitized with a Hewlett-Packard scanner in a 200% magnification of the original image and save as TIFF files for later measurements. We evaluated two parameters: 1) new bone formation and 2) soft tissue swelling in the lower part of tibio-tarsal joint. Each parameter was analyzed with a computer-assisted image analysis system (Metamorph, Universal Imaging, West Chester, PA, USA) using a modification of Esser’s method [13]. Briefly, the mean area of the calcaneus bone was determined in normal (Sham) animals and this was designated ‘the standard bone area’ (SBA). New bone proliferation was measured by subtracting the value of SBA from the bone area of the test animal. Soft tissue swelling was calculated using the following equation: [soft tissue area (whole paw area - bone area) in test animal-mean soft tissue area in Sham animals].

**Evaluation of interleukin 6 level in serum**

Blood samples were collected in sterile tubes by cardiac puncture and centrifuged, and the serum was stored at -20 °C. The level of IL-6 was measured by enzyme-linked immunosorbent assay for rat IL-6 (kit from Biosource International, CA, USA).

**Thermal hyperalgesia test (Hargreaves’s Method)**

Rats were placed in a plastic chamber with a glass floor and allowed to acclimate to their environment for 5 min before testing. A radiant heat source was positioned under the glass floor
beneath the hindpaw to be tested. The withdrawal latency of both hind paws was measured to the nearest 0.1 sec using a photoelectric cell connected to a digital clock. The intensity of the light source was calibrated to produce withdrawal in 9–10 sec in normal animals. The test was duplicated at 5 min intervals in each hind paw.

**The mechanical hyperalgesia test**

A graded mechanical force (g) was delivered through an analgesy meter (LETICA, LE7356) onto the convex surface of the paw. Rats withdrew their hind paw or vocalized when the applied force reached the nociceptive threshold. The test was duplicated at 5 min intervals in each hind paw. The threshold force in normal animals ranged from 160 to 180 g.

**Fos expression in lumbar spinal cord**

One group of adjuvant-injected rats was not subjected to any of the above nociceptive behavioral tests (n = 5/group), but at the end of the experiments (21 days) were used for Fos immunohistochemistry. The animals were deeply anesthetized with 5% isoflurane, perfused transcardially with calcium-free Tyrode’s solution, followed by a fixative containing 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 6.9). All perfusions were performed between 9:00 AM and 12:00 AM. The spinal cord was then removed immediately after perfusion, post-fixed in the same fixative for 4 h and then cryoprotected in 30% sucrose in phosphate buffered saline (PBS, pH 7.4) for 48 h.

Frozen serial frontal sections (40 μm) were cut through the lumbar L3-L5 spinal cord using a cryostat (Microm, Germany). After elimination of endogenous peroxidase activity with 0.3% hydrogen peroxide in PBS and preblocking with 1% normal goat serum and 0.3% triton X-100 in PBS, the free-floating sections were incubated in polyclonal rabbit anti-Fos antibody (Calbiochem, 1:10,000) at 4°C overnight. Fos-like immunoreactive (FLI) neurons were visualized using a 3-3 diamino-benzidine reaction intensified with 0.2% nickel chloride.

For quantitative analysis of FLI neurons, lumbar spinal cord sections were scanned and the five with the greatest number of labeled cells at the L3-5 level were selected from each animal. Individual sections were digitized with 4096 gray levels using a cooled CCD camera (Micromax Kodak 1317, Princeton Instruments, Tucson, AZ, USA) connected to a computer-assisted image analysis system (Metamorph, Universal Imaging, West Chester, PA, USA) as previously described [11,15].

To assess the effect of BV extract on spinal cord Fos expression, the following four gray matter regions were selected for analysis based on cytoarchitectonic criteria: 1) superficial dorsal horn (SDH, laminae I and II), 2) nucleus proprius (NP, laminae III and IV), 3) neck (NECK, laminae V and VI), and 4) the ventral horn (VENT, laminae VII-IX).

**Statistical analysis**

Thermal and mechanical hyperalgesia data were expressed as percent change and compared to that of the Sham group at each time point. Data were expressed as the mean ± SEM. Repeated measures ANOVAs were performed to determine the overall effect. Paired t-tests were then used to determine probability values when repeated measures ANOVAs indicated a significant drug effect. Throughout, p < 0.05 was considered to be statistically significant.
Results

Evaluation of paw volume

A latent systemic arthritic response, characterized by swelling of the non-injected contralateral hind paw and tail, was first evident at 12 days post-adjuvant injection into the right hind paw (Fig. 1). The paw volume of the left hind paw was measured and used for evaluating the possible anti-inflammatory effect of each BV fraction on RA. In the RA-BVA group, arthritis-induced paw edema was significantly decreased beginning 12 days post RA induction as compared to that of the RA-Sal group (Fig. 1). However, the anti-inflammatory effect in RA-BAE group was delayed, being first evident at 21 days post-RA induction. Moreover, the RA-BVA group showed a significant decrease in paw volume in comparison to that of the RA-BVE group (Fig. 1).

Evaluation of radiological change

Radiographs of the left hindpaw taken at 3 weeks after RA induction were evaluated by image analysis. Adjuvant-induced arthritis produced severe soft tissue swelling and new bone proliferation in the RA-Sal group (Fig. 2B, Fig. 3A and B) as compared with the Sham group (Fig. 2A). BVA treatment significantly inhibited these pathological changes (Fig. 2C, Fig. 3A and B) whereas BVE treatment was not significantly different from that of the RA-Sal group (Fig. 2D, Fig. 3A and B). This data indicated that BVA treatment successfully suppressed RA induction.

Fig. 1. The change of paw volume in saline-treated arthritic animals (RA-Sal, n = 10), the water-soluble fraction of BV treated arthritic animals (RA-BVA, n = 10) and the ethylacetate fraction of BV treated arthritic animals (RA-BVE, n = 10). Vehicle or fractions of BV was administrated into the Zusanli acupoint for 3 weeks after RA induction. Graph depicts the change in the paw volume of the contralateral left hind limb. **p < 0.01: significantly different from RA-Sal group.
Evaluation of serum interleukin-6 level

A basal, systemic IL-6 concentration of 53.5 ± 7.9 pg/ml was observed in normal (Sham) animals (Fig. 4). Although the systemic IL-6 concentration (161.4 ± 15.3 pg/ml) in the RA-Sal group was significantly increased at 3 weeks post-RA induction, BVA treatment (61.1 ± 9.1 pg/ml) was found to significantly inhibit the increase in IL-6 concentration down to Sham level (Fig. 4). The IL-6 concentration in BVE treated RA animals was not significantly different than that of the RA-Sal group (Fig. 4).

Thermal hyperalgesia test

The paw withdrawal latency (PWL) of animals in the Sham group did not change significantly throughout the 21 days of the experiment. In contrast both the ipsilateral and contralateral PWL of
animals in the RA-Sal group decreased significantly and remained lower throughout the experiment. There was a significant decrease in the PWL of the contralateral hind paw of all the arthritis-induced groups compared to the Sham group, beginning 9 days after adjuvant injection (Fig. 5). However, the

Fig. 3. The image analysis values at 3 weeks after arthritis (RA) induction in saline-treated arthritic animals (RA-Sal, n = 5), the water-soluble fraction of BV treated arthritic animals (RA-BVA, n = 5) and the ethylacetate fraction of BV treated arthritic animals (RA-BVE, n = 5). A: depicts the change of new bone proliferation B: depicts the change of soft tissue swelling. Data was evaluated from images taken at the level of tibio-tarsal joint in the contralateral left hind limb. **p < 0.01: significantly different from RA-Sal group.

Fig. 4. The change of serum concentration of interleukin-6 (IL-6) in normal animals (SHAM, n = 5), saline-treated arthritic animals (RA-Sal, n = 5), the water-soluble fraction of BV treated arthritic animals (RA-BVA, n = 5) and the ethylacetate fraction of BV treated arthritic animals (RA-BVE, n = 5). Serums were obtained at 3 week after arthritis induction. **p < 0.01: significantly different from RA-Sal group.
Fig. 5. The changes of paw withdrawal latency (PWL) produced by noxious heat stimuli in saline-treated arthritic animals (RA-Sal, n = 10), the water-soluble fraction of BV treated arthritic animals (RA-BVA, n = 10) and the ethylacetate fraction of BV treated arthritic animals (RA-BVE, n = 10). Graph depicts the percent inhibition compared to the Sham PWL in the contralateral left hind paw. **p < 0.01: significantly different from RA-Sal group.

Fig. 6. The changes of mechanical threshold (Randall-Selitto test) in saline-treated arthritic animals (RA-Sal, n = 10), the water-soluble fraction of BV treated arthritic animals (RA-BVA, n = 10) and the ethylacetate fraction of BV treated arthritic animals (RA-BVE, n = 10). Graph depicts the percent inhibition compared to the mechanical threshold of the normal animal value in the contralateral left hind paw. **p < 0.01: significantly different from RA-Sal group.
PWL of the RA-BVA group showed a significantly higher thermal threshold from 15 days post RA induction as compared with those of the RA-Sal and RA-BVE groups (p < 0.01, Fig. 5).

**Mechanical hyperalgesia test**

There was a significant decrease in the mechanical pain threshold for the contralateral hindpaw of animals in the RA-Sal group as compared to those in the Sham group (Fig. 6). The pain threshold of the RA-BVE group was not statistically different from that of the RA-Sal group (Fig. 6). In contrast, the pain threshold was significantly increased in the RA-BVA group as compared to the RA-Sal group (p < 0.01, Fig. 6).

**Fos immunohistochemistry**

A significantly higher number of FLI neurons were observed in contralateral (left) side of the L3-L5 lumbar spinal cord segments in rats from the RA-Sal group at 21 days after unilateral Freund’s adjuvant injection as compared with the Sham group (Fig. 7). The number of FLI neurons in the RA-BVE group was not significantly different from that of animals in the RA-Sal group (Fig. 7). However, the number of FLI neurons was significantly reduced in both the SDH and NP regions of the spinal cord in the RA-BVA group as compared with those in the RA-Sal group (p < 0.01, Fig. 7).

![Image of graph showing the number of Fos positive neurons in different segments of the spinal cord](image)

**Fig. 7.** The number of Fos positive neurons in the contralateral (left) lumbar spinal cord in normal animals (Sham, n = 5), saline-treated arthritic animals (RA-Sal, n = 5), the water-soluble fraction of BV treated arthritic animals (RA-BVA, n = 5) and the ethylacetate fraction of BV treated arthritic animals (RA-BVE, n = 5) at 3 weeks after arthritis induction. The number of Fos expression was analyzed in each of four regions (SDH: lamina I-II, NP: lamina III-IV, NECK: lamina V-VI and VENT: lamina VII-IX). **p < 0.01: significantly different from RA-Sal group.
Discussion

Anti-inflammatory effect of the water soluble fraction of bee venom (BVA)

Unilateral injection of Freund’s adjuvant into the hindpaw induces “primary” inflammatory signs and hyperalgesia at the site of inoculation within hours after injection. Subsequently, “secondary” inflammation and pro-nociceptive signs appear between the 10th and 15th day post-inoculation and are particularly evident in the contralateral paw [2,12]. This secondary inflammation is latently associated with the development of the systemic phase of adjuvant arthritis. In the present study this latent systemic arthritic response, which is characterized by swelling of the non-injected contralateral hind paw and tail, was first evident at 12 days post-adjuvant injection into the ipsilateral hind paw (see Fig. 1). In a recent study, we have further demonstrated that whole BV injection into the Zusanli acupoint has an anti-inflammatory effect on adjuvant-induced arthritis based on measurements of changes in paw volume [11]. One of the goals of the present study was to determine which BV fraction is responsible for this anti-inflammatory effect. In this regard we observed that only injection of the BVA fraction into Zusanli acupoint significantly inhibited arthritis induced paw edema beginning 12 days after RA induction. In addition to examining paw edema, we evaluated the anti-inflammatory effect of BV extracts on radiological changes induced by adjuvant injection. A number of changes in bone and periarticular soft tissue have been reported to occur during the course of adjuvant induced RA [13]. Using image analysis of radiographs, we measured new bone proliferation and soft tissue swelling in the tibio-tarsal joint. It has been previously demonstrated that these measurements are positively correlated with the results of conventional radiological and histological evaluation [13]. In addition, this method provides a more sensitive and quantitative approach for radiological image analysis as compared with conventional observation. The results obtained using this method clearly demonstrated that BVA injection inhibited the radiological changes (i.e. new bone proliferation and soft tissue swelling) induced by systemic RA.

In addition to evaluating radiographic changes and paw edema, we measured the serum concentration of IL-6 at 3 weeks post-RA induction as a way of evaluating the potential systemic anti-inflammatory effect of BVA injection. Proinflammatory cytokines including IL-6 have been implicated in the chondral degenerative process associated with decreased cartilage matrix synthesis in experimental arthritis [16]. It has been reported that the systemic concentration of IL-6 in adjuvant injected rats increases by 6 h post-injection, peaks at 12 h, and then returns to control concentration by 6 days [12]. In animals subjected to systemic RA induction, IL-6 levels persistently increased from day 12 post-adjuvant injection [12] and this pattern parallels the time-course of the changes observed in the present study with both paw edema and radiological analysis. In the present study, we observed a significant reduction in the elevated levels of IL-6 induced by RA following BVA treatment. Taken together these results suggest that the BVA fraction has an anti-inflammatory effect that may be partially mediated by a reduction in systemic IL-6 levels.

Possible mechanism of anti-inflammatory effect of water soluble fraction of bee venom (BVA)

In general, the water-soluble extract of BV contains peptides, proteins, carbohydrates and -OH attached small molecules. Of particular importance to the present results are the presence of several peptides (i.e. melittin, MCD peptide and adolapin) and enzymes (i.e. phospholipase A2) in BVA.
Melittin, a major component of whole BV and BVA, provokes local pain and edema in animals [8] and induces pain and axon reflexes in human subjects [9]. The vasodilatory effect induced by axon reflexes, even in deep tissues, may exert a favorable influence upon the chronic inflammatory process by increasing tissue metabolism and eliminating endogenous and exogenous irritants or toxic substances. It has previously been reported that counter-irritants, such as capsaicin, produce antinociceptive phenomenon that is associated with vasodilation induced by the axon reflex [17]. Further support for a role of peripheral nerves in joint inflammation comes from the recent work by Gouze-Decaris et al. [18] who show that unilateral subcutaneous or intra-articular injection of Freund’s adjuvant induces a significant decrease in prostaglandin synthesis in both patellae. These investigators further demonstrated that chronic administration of capsaicin, which blunts the normal response of C fiber stimulation, prevented the bilateral significant decrease in cartilage synthesis. Similarly, intrathecal injection of MK-801, which blocks glutamatergic synaptic transmission at the dorsal horn of signal originating in primary afferent C fibers, eliminated the Freund’s adjuvant induced prostaglandin synthesis decrease in both patellae. These findings implicate a role for C fibers in the arthritic changes occurring in the joint. If melittin induces a local axon reflex in nerves innervating the joint or serves as a counter-irritant that affects central spinal cord mechanisms, this could contribute to both its anti-inflammatory and antinociceptive effects on adjuvant-induced arthritis.

In addition to potential effects on the nervous system, melittin has been shown to bind to secretory phospholipase A2 (PLA2) and inhibit its enzymatic activity. Because PLA2 is a major inflammatory enzyme involved in the release of arachidonic acid (whose activity is enhanced in RA), it is possible that the formation of a melittin-PLA2 complex is able to suppress some of the symptoms associated with the development of arthritis. Thus, melittin may play an important role in the anti-inflammatory and antinociceptive effect of BVA via two possible mechanisms: 1) induction of local axon reflexes via direct or indirect effects on peripheral nerves and 2) serving as a scavenger of PLA2. While melittin represents a likely candidate for the anti-inflammatory and antinociceptive effects observed in present study, it is currently unclear whether melittin is the major component of BVA that produced these effects. This question must be addressed in future studies involving direct injections of mellitin into the Zusanli point.

Antinociceptive effect of BVA and possible mechanisms

In the present study, arthritic-induced thermal and mechanical hyperalgesia was significantly reduced by long term BVA treatment. In order to evaluate the potential inhibitory effect of BVA on arthritic nociceptive input to the spinal cord, we examined changes in spinal cord Fos expression following BVA treatment. It has been previously reported that arthritis-induced nociception significantly increases Fos expression in the lumbar spinal cord three weeks after adjuvant injection [11,19]. This increased number of spinal cord neuronal Fos expression is significantly reduced by treatment with aspirin or with whole BV [11,15]. In the present study, we observed that BVA treatment also significantly suppressed the increased number of spinal cord Fos positive neurons induced by arthritic pain, indicating that BVA has a potent antinociceptive effect on RA.

From a historical perspective the curative properties of BV have been appreciated for a long time. In ancient Egypt, for instance, many diseases were treated with ointment made from bees. However, scientific research into the validity of employing BV therapy for the treatment of arthritic and other inflammatory diseases only began in the latter portion of the 20th century [2,3]. Traditionally, BV was administered using live bees by stimulating them to sting in the affected area or in specific acupuncture
points. More recently BV has been isolated and can now be administered in a cream, ointment or injection form, dependent on the nature of the disease. In the present study, an injectable form of BV extract was administered into the Zusanli acupoint. Clinically, acupoint stimulation is commonly used for pain reduction using one of several stimulation methods including mechanical (i.e. needle acupuncture, acupressure), electrical (i.e. electro-acupuncture) or thermal stimuli (moxibustion). The mechanism responsible for acupoint stimulation produced analgesia is not yet entirely clear. Whereas its action was widely explained by gate control theory in the past, acupuncture, like neurostimulation methods may also act by modulation of neurotransmitters in the central nervous system [20].

Whole BV injection has been shown previously to produce tonic pain responses [4,7] and a brief vocalization at the time of injection. In the present study BVE did not produce any vocalization at the time of injection whereas BVA was found to induce a brief vocalization. Based on this finding we hypothesize that BVA stimulation of the acupoint induced a brief nociceptive input to the spinal cord that in turn reduced RA nociceptive input according to the gate control theory and/or caused modulation of neurotransmitters. The data obtained in this study suggests that BVA potently activated the Zusanli acupoint and that acupoint stimulation by BV or BVA may be a valuable method for inducing the optimal analgesic effect of acupuncture. In this regard we have recently shown that BV acupuncture has a more potent anti-arthritic and antinociceptive effect than traditional needle acupuncture in humans [21].

In summary the present study showed that the previously demonstrated therapeutic effect of BV therapy can only be reproduced by the water-soluble fraction of BV (BVA). BVA injection into the Zusanli acupoint significantly reduced RA induced inflammatory symptoms including paw edema, radiological changes and the elevation of serum IL-6. BVA administration into the Zusanli acupoint was also found to produce a significant antinociceptive effect on arthritis induced inflammatory pain symptoms including thermal and mechanical hyperalgesia. Additional supporting data for an antinociceptive effect of BV was obtained using Fos immunohistochemistry on spinal cord sections. These data demonstrated that arthritis-induced Fos expression in the lumbar spinal cord was significantly decreased following BVA administration. Based on these results, we suggest that injection of the BVA fraction of whole BV into specific acupoints may be useful for the long-term treatment of RA-induced pain and inflammation.

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