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Comparison of Antinociceptive Effect of Korean and American Bee Venoms on Pain in Rodent Models

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Abstract : Experiments were undertaken to assess the antinociceptive effect of bee venom (BV) in rodent animal models. Comparison of antinociceptive efficacy between Korean BV and commercially available American BV was the primary interest of the study. Korean BV was collected using BV collector devices in which an electrical impulse is used to stimulate the worker bee (Apis mellfera L.) to sting and release venom. After collection, whole BV was evaporated until dry using the BV collector. Commercially available dried American BV was purchased from Sigma Company in USA. Korean and American sourced BVs were diluted and amounts of 6 mg/kg body weight (BW), 0.6 mg/kg BW and 0.06 mg/kg BW were tested. BV was subcutaneously injected to produce an antinociceptive effect and the antinociceptive efficacy was evaluated using a writhing test in mice and a formalin test in rats. The antinociceptive effects of the two BVs tested were similar in mice for visceral pain and showed a dose-dependent response. The antinociceptive effect of Korean BV was not significantly different compare to American BV. These results suggest that Korean BV may be used to achieve an antinociceptive effect for use in medical therapies.

Key words **:** Bee venom, Korean, American, pain, rodent.

Introduction

Bee venom (BV) has been used in Eastern Asia as a therapeutic modality since the second century BC and, its use in clinical applications as a meridian therapy has been extensively researched and practiced in Korea. BV is used to treat a variety of conditions and evidence exists for its effectiveness in alleviating symptoms in pain syndrome, herniation nucleus pulpous, cervical disc protrusion and progressive muscle atrophy. The main activities of BV include anti-inflammatory action (13), antibacterial action (33,35), high-potency hemolytic action (32), enhancement of immunologic function (9,16), radio-protective effect (18,30), palliative effect in the nervous system, analgesic action, and neurotoxic action (14,20,26). These BV activities have been well documented in many cases; however, such studies have utilized BV sourced from non-local suppliers. The work presented here was undertaken to assess the analgesic action of locally sourced Korean BV.

Among the animal models used to study the mechanisms underlying persistent pain, the formalin test is one of the most useful for assessing prolonged pain after injury (12,23,34,36). Electrophysiological studies in rats demonstrated that subcutaneous formalin injection causes two phases of increased firing of dorsal horn-convergent neurons. An acute phase (early phase) lasts for 5-10 min and a tonic phase (late phase) follows 10-20 minutes later and lasts for 35-65 min, both of which correlated very well with behavioral responses observed previously (11,12). The administration of BV directly into an acupoint produces a significantly more potent antinociceptive effect when compare to injection into a non-acupoint, in a model of adjuvant-induced arthritis and in a model of tonic pain (21). Therefore, in this study BV was injected into specific acupoints.

The present study was conducted to determine whether Korean BV produces an antinociceptive effect in rodent animal models and to compare the antinociceptive efficacy of Korean BV and a commercially available American BV.

Materials and Methods

Animals

Experiments were performed on male ICR mice (weighing 30~35 g, 6 weeks old) and male Sprague-Dawley rats (260~270 g, 6 weeks old). Laboratory animals were obtained from Orient Bio (Seoul, Korea). The animal care protocol was approved by the Animal Care and Use Committee of Chungbuk National University and its methodology conforms to the published guidelines of the USA (NIH publication #85-23). In addition, the ethical guidelines of the International Association for the Study of Pain for investigating experimental pain in conscious animals were also followed. Animals were housed under the conditions of constant temperature (23 ± 2°C), relative humidity (55% ± 5%), and day/night cycle (12h light/12h dark: illumination beginning at 7:00 AM) until the day of the experi-

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ment (7 days acclimation period). Each animal was tested only once. Antinociceptive tests were performed between 12:00 and 18:00 in order to minimize potential variability in nociceptive sensitivity due to circadian rhythms.

Bee venom

Korean BV was collected from a farm of Choongju city in Chungbuk province, in September 2006. Whole Korean BV was obtained using BV collector devices that emit electrical impulses to stimulate the worker bee (Apis mellifera L.) to sting. Whole Korean BV was evaporated until dry in the BV collector. Commercially available dried American BV was purchased from Sigma Company (St. Louis, Missouri. USA).

Formalin test in rats

Rats were divided into 7 groups, each comprising seven animals. Korean and American BVs were reconstituted and serially diluted; amounts of 6 mg/kg BW, 0.6 mg/kg BW and 0.06 mg/kg BW were injected. A 0.9% physiological saline solution was used as the vehicle for all experiments. For the formalin test rats were placed individually in observation cylinders, and allowed to adapt to the environment for 30 min prior to the start of the experiment. BV was subcutaneously injected into the acupoint of Zusanli (ST-36). This acupoint is located 5 mm lower and lateral to the anterior tubercle of the tibia. Control rats were subcutaneously injected at the same acupoint with the same volume of physiological saline. At 15 min after BV injection, 1% formalin (50 μl) was subcutaneously injected into the plantar surface of the right hind paw with a 30 gauge needle. Following formalin injection each rat was observed for 60 min by three experienced investigators.

Writhing test in mice

Mice were divided into 7 groups, each compromising seven animals. Korean and American BVs were reconstituted and serially diluted; amount of 6 mg/kg BW, 0.6 mg/kg BW and 0.06 mg/kg BW were injected. A 0.9% physiological saline solution was used as the vehicle for all experiments. Control animals were injected with the same volume of saline at the same site as that used for BV injection. The writhing test (abdominal stretches) was performed to further assess antinociceptive effects of BV. Mice were placed in a plexiglas temperature controlled ($23 \pm 2^{o}C$) observation chamber, and were allowed to adapt to the environment for 30 min before the experiment. BV was subcutaneously injected into the acupoint of Zhongwan (CV-12). This acupoint is located on the mid-line of the abdomen between the xiphoid process and umbilicus. Thirty minutes after administration of BV or vehicle, a 0.9% solution (20 μl/g BW) of acetic acid was intraperitoneally injected. Acetic acid was subsequently injected intraperitoneally to produce abdominal stretches. For 60 min following acetic acid injection, the number of abdominal stretches per animal was counted by three experienced investigators.

Statistical analysis

All data were expressed as the mean ± standard error. One-way analysis of variance (ANOVA) was applied to analyze the effect of BV treatment in comparison to the saline control group. Paired t-tests were used to determine probability values when repeated measures ANOVAs indicated a significant difference between Korean BV and American BV. A value of $P < 0.05$ was considered to be statistically significant.

Results

Formalin test in rats

Subcutaneous injection of 1% formalin produced biphasic licking and flinching behaviors at the injected hind paw in the saline treatment group. Licking and flinching time increased during the initial 5 min period following formalin injection, referred to as the early phase, then it decreased to nearly baseline levels during the subsequent 10 min period, typically called the intermediate period. Finally, licking and flinching behaviors increased again in the late phase, 15 min after the initial formalin injection and was sustained for 60 min.

BV treatment did not show a significant difference or induce

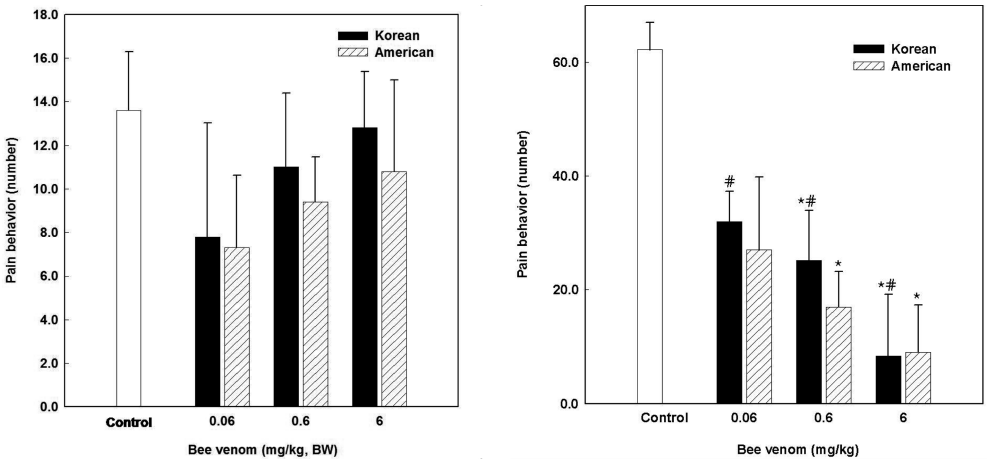

Fig 1. Effect of bee venom treatment on formalin test biphasic pain behavior in rats (left panel, early phase; right panel, late phase) (n = 7). *$p < 0.05$: as compared with saline control group. #$p < 0.05$: as compared with bee venom groups.

an antinociceptive effect in the early phase response to administration of formalin when compared to treatment with saline control. However, pain behavior such as licking and flinching was significantly reduced in the late phase ($p < 0.05$) in animals injected with BV, and the antinociceptive effect demonstrated a dose-dependant pattern (Fig 1).

Writhing test in mice

Intraperitoneal injection of acetic acid produced a tonic pain behavior (abdominal stretch reflexes) first observed at 3~9 min post-injection. Abdominal contraction was characterized by strong constrictions of the abdominal musculature accompanied by dorsiflexion of the back and extension of the hind limbs. The time elapse between the administration of acetic acid and the first observed pain behaviors indicated a dose-dependent suppressive effect (Fig 2). This writhing response peaked at 20 min post-acetic acid injection, and then declined until it was undetectable at 60 min post-injection. The antinociceptive efficacy and time course response of Korean BV was similar to that of American BV (Fig 3).

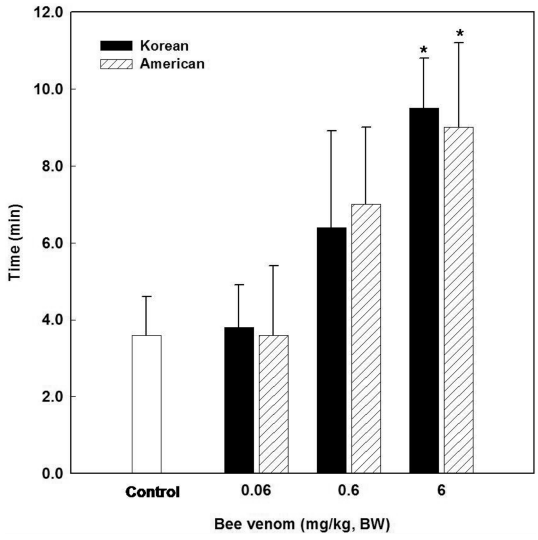

Fig 2. Effect of bee venom treatment on the response initiation time for abdominal stretches in mice receiving acetic acid injection (n = 7). *$p < 0.05$: as compared with saline control group.

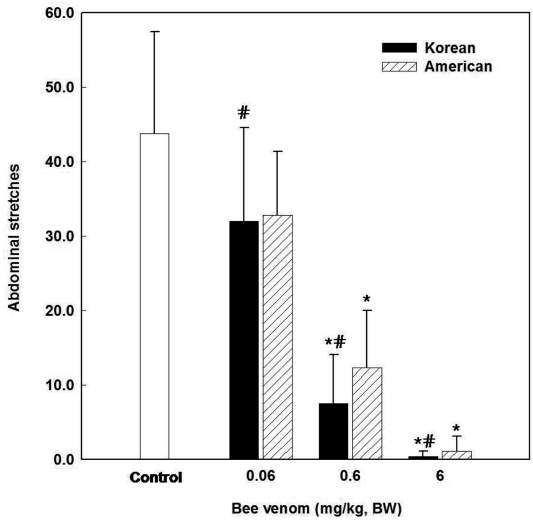

Fig 3. Effect of bee venom treatment on the number of abdominal stretches in mice receiving acetic acid injection (n = 7). *$p < 0.05$: as compared with saline control group. #$p < 0.05$: as compared with bee venom groups.

Discussion

Rodent models have been used extensively for experiment in pain studies (12,22,34,36), to clinically establish analgesic compounds that work to either suppress or prevent the sensation of pain. BV is one such compound that has been used widely among scientists and medical practitioners.

BV is a complex mixture of substances, including melittin, apamin, MCD peptide and others (25), which are known to produce a variety of physiological and pharmalogical changes in experimental animals (6,7). Components of BV, particularly melittin and phospholipase A2, are responsible for the local inflammation and nociceptive responses associated with bee stings (15,24). Moreover, BV injection produces persistent nociceptive responses and subsequent neuronal activation within the spinal cord (4,5,25). Melittin causes the release of histamine and serotonin from mast cells, erythrocytes, and thrombocytes. MCD peptide causes the release of histamine from destroyed mast cells. Apamin has neurotoxic effects, especially in the spinal cord, where it produces prolonged hyperexcitability and augments polysynaptic reflexes. These findings are supported by a report that the antinociceptive effect induced by diclofenac is blocked by apamin in the formalin test (27). Histamine and serotonin likely contribute in producing pain, since it has been demonstrated that intradermal injection of histamine and application of serotonin at the blister base produces transient pain in humans (2) and intraplantar injection of serotonin in rats produces pain behavior (17). Other less potent components, such as acetylcholine and noradrenaline, are found in smaller amounts and may also contribute to the production of pain responses (31).

Formalin injection is often used as an experimental model for pain because it produces a vigorous response and permits the study of both acute and tonic pain (23). In the current study, the formalin test demonstrated that BV injected into a specific acupoint prior to formalin injection evoked antinociceptive effects in rats. In the saline-treated rats, subcutaneous injection of formalin resulted in a highly repeatable biphasic behavior display of licking and flinching of the injected paw. Abundant evidence suggests that N-methyl-D-aspartate (NMDA) receptors are involved in the nociceptive responses to formalin, and NMDA receptor antagonists primarily affect the late phase behaviors of the formalin response (8) which appear to reflect central sensitization.

In the writhing test, Korean BV produced a dose-dependent pain-suppressing antinociceptive effect in the writhing test, an acetic acid-induced visceral pain model as described by Kwon

et al. (21). In addition, the antinociceptive effect was similar for Korean BV and commercially supplied BV. While subcutaneous melittin injection produces an initial pain sensation, it subsequently produces an antinociceptive effect through stimulation of axon reflexes (3). For example, subcutaneous injection of melittin reduced the number of abdominal stretches induced by acetic acid injection (19). Apamin is known to block conductance of calcium activated potassium channels to induce antinociception (10). Since these channels are present in dorsal root ganglion (DRG) cells and blocking them with apamin increases ectopic spontaneous discharges in injured DRGs, it is possible that apamin could alter peripheral nerve firing induced by visceral pain sensation. MCD peptide may participate in the initial short-lasting pain behaviors of the early phase, observed following BV injection. More importantly, the interaction of these constituents may be responsible for eliciting central mechanisms of stimulation-induced analgesia (19). BV significantly suppressed abdominal pain behavior characterized by abdominal stretches, a result which corroborates previous reports (21). Thus, it is likely that BV treatment affects the sensory (nociceptive) component of the abdominal stretch reflex rather than the motor portion of the reflex (19). In our study, BV treatment produces a significant antinocicpetive effect and did not affect motor activity. BV offers a unique advantage in pain management options, because it produces potent antinociception without negative side affects associated with many narcotic drugs.

Bees require pollen or protein rich nutrition to produce high quality venom (28,29). From spring to fall, this is easily achieved in areas with continuous flowering plants. However, in the late fall and winter when flowers and pollen are scarce, bee keepers often feed their bees with sugar syrup (carbohydrate) not with pollen (protein); consequently, the quality of venom produced in these seasons suffers in Korea. BV collected for drying and later reconstitution is best obtained during the peak or just at the end of honey flow when bees' venom sacs are full of quality venom (1). This ensures that the product always contains the same quality and quantity of venom and is suitable for use in both scientific studies and in treatments.

BV is a naturally occurring substance that is relatively inexpensive and readily available from chemical retailers. It is also easily manipulated for quantification and injection since it is soluble in saline. Although the exact concentration of active components varies from one bee to another, the variability is minimized by using hundreds of bee stings in a single preparation of injectable solutions. Furthermore, unlike formalin, BV does not produce any obvious tissue damage after a single injection, suggesting the possibility of repeated uses (25). BV produces a potent antinociception without the side affects associated with many narcotic drugs.

In this study, Korean BV injection evoked antinociceptive effects in rodent models. Korean BV produced antinociceptive effects similar to those of commercially available American BV. These results suggest that Korean BV may be a suitable and preferred choice for antinociceptive efficacy in pain management.

Acknowledgements

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설치동물에서 통증에 대한 한국산 및 미국산 봉독의 진통효과의 비교

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요 약 : 본 연구는 설치모델 동물에서 봉독의 항통각 효과를 평가하고, 한국산 봉독과 미국산 봉독의 항통각 효과를 비교하는 것이 주된 관심이다. 한국산 봉독은 특별히 고안된 봉독 추출기를 사용하여 일벌 (*Apis mellifera* L.)에 전기충격을 가하여 생봉독을 수집하였으며, 수집된 생봉독은 봉독 건조기를 이용하여 봉독을 건조하였다. 미국산 봉독은 미국 시그마회사에서 상업적으로 판매되는 건조 봉독을 이용하였다. 한국산 봉독과 미국산 건조봉독을 생리식염수에 희석하여 체중 kg당 6 mg과 0.6 mg, 0.06 mg을 마우스와 랫드에 피하로 투여하여 항진통 효과를 조사하였다. 항통각 효과는 한국산 봉독과 미국산 봉독은 서로 비슷하였으며, 봉독의 용량이 많을수록 항통각 효과가 크게 나타났다. 이상의 결과에서 한국산 건조 봉독은 통증 치료에 사용될 수 있을 것으로 생각된다.

주요어 : 봉독, 한국, 미국, 통증, 설치동물