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Antinociceptive Efficacy of Korean Bee Venom in the Abdominal Pain of the Mouse

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Abstract : This study was undertaken to assess the antinociceptive effect of Korean bee venom (BV) in mice. Korean BV was collected using BV collector devices in which an electrical impulse is used to stimulate the worker bee (*Apis mellifera* L.) to sting and release venom. After collection, whole BV was evaporated until dry using the BV collector. Experiments were performed on male ICR mice (weighing 30~35 g, 6 weeks old). Mice were divided into 4 groups, each comprising 8 mice. BV was 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. Intraperitoneal injection of acetic acid produced a tonic pain behavior, first observed at 3 to 9 min post-injection. This writhing response peaked at 20 min post-acetic acid injection, and then declined until it was undetectable at 60 min post-injection. The time elapse between the administration of acetic acid and the first observed pain behaviors indicated a dose-dependent suppressive effect. These results suggest that Korean BV may be used to achieve an antinociceptive effect for use in medical therapies.

Key words **:** Antinociceptive efficacy, Korean bee venom, mice.

Introduction

For several centuries, bee venom (BV) therapy has been utilized in Oriental medicine to relieve neouropathic pain and to treat inflammatory diseases such as rheumatoid arthritis, neuritis and fibromyositis (4). Today's proponents of BV therapy cite the benefits of BV for alleviating chronic pain and for treating many ailments including various rheumatic diseases involving inflammation and degeneration of connective tissues, neurological diseases, autoimmune diseases, and dermatological conditions. Medication therapy used in most arthritis utilizes various kinds of medicine such as nonsteroidal anti-inflammatory drugs, adrenal corticosteroid hormone, antirheumatic drugs and immunosuppressive agents, but they can cause severe adverse effects such as depression, peptic ulcers, enterohemorrhage, liver dysfunction and renal disorders (1). To resolve the demerits of these medicines, the medicines from natural substances which have the high pharmacologic efficacy and no side effect have been attempted to search continuously in Korea and advanced nations. As one of the results, BV therapy has been broadly used in inflammatory diseases and pain remedy. Most of all, BV(*Apis mellifera* L.) has been utilized and accomplished in the treatment of gout and rheumatic arthritis by means of the private remedy and BV acupuncture remedy for a long time (22). These BV activities have been well documented in many cases; however, such studies have utilized BV sourced from non-local suppliers. This work presented here was undertaken to assess the analgesic action of locally sourced Korean BV.

In Europe, the extraction of various ingredients and the examination of medicinal efficacy about BV have been progressed even now. In Korea, BV acupuncture has been utilized in lumbago and arthritis for the purpose of treatment for a long time(18) Recently, the BV acupuncture of animals is utilized in the treatment of arthritis, bacterial diarrhea, and agalactia syndrome (7,8,18). BV is what comes out of the stinging apparatus of the bee. Quantity of BV depends on the age and the race of the bee, and composition of BV depends on the pollen consumed and the age of the bee. BV keeps fresh and pure in the live bee only.

The present study was conducted to determine whether Korean BV produces an antinociceptive efficacy in the abdominal pain of the mouse.

Materials and Methods

Animals

Experiments were performed on male ICR mice (weighing 30~35 g, 6 weeks old). Laboratory animals were obtained from the Orient Bio (Seoul, Korea). The animal care protocol was approved by the Animal Care and Use Committee of Chungbuk National University. In addition, the ethical guide-

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lines of the International Association for the Study of Pain for investigating experimental pain in conscious animals were also followed. Animals were housed under 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 experiment (7 days acclimation period). Each animal was tested only once. Antinociceptive test was 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 the farm of Chungbuk province, in September 2006. Whole BV was obtained using BV collector devices that emit electrical impulses to stimulate bees (*Apis mellifera* L.) to sting. Whole Korean BV was evaporated until dry in the BV collector. Analysis of BV components was made by liquid chromatography (AKTA explorer, Amersham Pharmacia Biotech, USA).

Writhing test in mice

Mice were divided into 4 groups, each comprising 8 mice. Korean BV was reconstituted and serially diluted; amounts of 6 mg/kg BW, 0.6 mg/kg BW and 0.06 mg/kg BW were injected. A standard 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 chamber (23 ± 2°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 the umbilicus. 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 ± SE. One-way analysis of variance was applied to analyze the effect of BV treatment in comparison to the saline control group. A value of $P < 0.05$ was considered to be statistically significant.

Results

Bee venom

Korean BV is a colorless clear liquid with a slightly bittersweet taste. Dried Korean BV contains melittin (45%), apamin (3%) and mast cell degranulating (MCD) peptide (2.3%).

Writhing test in mice

Observers counted the total number of abdominal stretches over the 60 min post-acetic acid injection period, and the data were used to evaluate the antinociceptive effect of BV treatment. Intraperitoneal injection of acetic acid produced a tonic pain behavior (abdominal stretch reflex), first observed at 3 to 9 min post-injection (Fig 1). Abdominal contraction was characterized by strong constrictions of the abdominal mus-

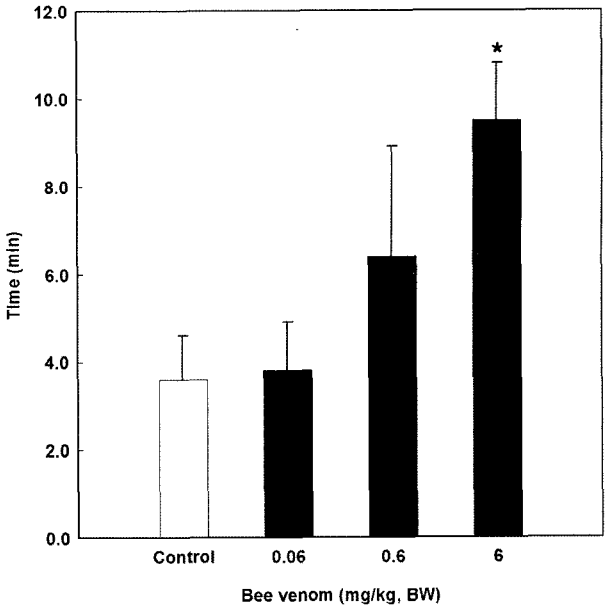

Fig 1. Effect of Korean bee venom treatment on the response initiation time for abdominal stretches in mice receiving acetic acid injection. The data were presented as M ± S.E (n = 8) and the testing was considered significant if $p < 0.05$.

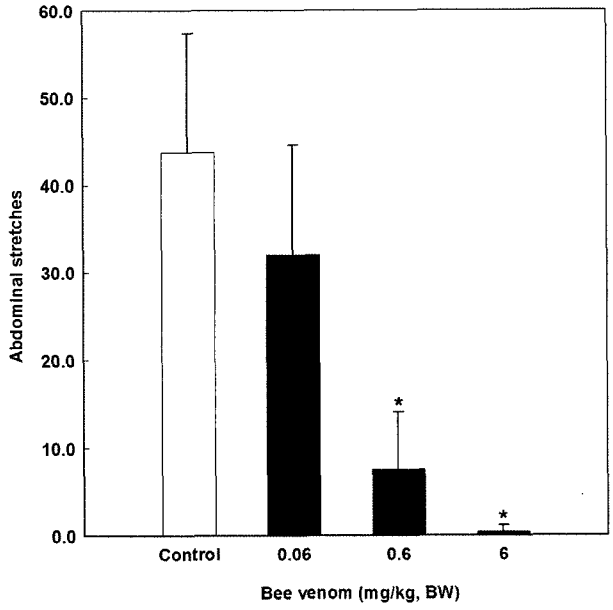

Fig 2. Effect of Korean bee venom treatment on the number of abdominal stretches in mice receiving acetic acid injection (n = 8). The data were presented as M± S.E (n = 8) and the testing was considered significant if $p < 0.05$.

culature 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.

Discussion

BV test satisfies many of the requirements for testing pain mechanisms in animals as proposed by Dubner (10) and Vyklicky (23). BV is a complex mixture of substances including melittin, apamin, mast cell degranulation (MCD) peptide and others (17). BV reproduces the antinociceptive effect in acetic acid-induced visceral pain model (15). While subcutaneous melittin injection produces an initial pain sensation, it subsequently produces an antinociceptive effect through stimulation of axon reflexes (6). For instance, subcutaneous injection of melittin reduced the number of abdominal stretches induced by acetic acid injection (Kwon *et al.*, 2004). It is known to block conductance of calcium activated potassium channels (9). 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 feasible that apamin could alter peripheral nerve firing induced by visceral sensation. MCD peptide causes MCD and histamine release, so MCD peptide clearly is a potential agent in allergy and inflammation (5).

Components of BV, particularly melittin and phospholipase A2, are responsible for the local inflammation and nociceptive responses associated with bee stings (12, 16). 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 (11). 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 (3). Other less potent compounds, such as acetylcholine and noradrenaline, are found in smaller amounts and may also contribute to the production of pain responses (21).

Korean BV in this study produced a dose-dependent, pain-suppressing antinociceptive effect in the writhing test, an acetic acid-induced visceral pain model as described Kwon *et al.* (15). While subcutaneous melittin injection produces an initial pain sensation, it subsequently produced an antinociceptive effect through stimulation of axon reflexes (6). For example, subcutaneous injection of melittin reduced the number of abdominal stretches induced by acetic acid injection (15). Apamin is known to block conductance of calcium activated potassium channels to induce antinociception (9). Since these channels are present in 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 (15). BV significantly suppressed abdominal pain behavior characterized by abdominal stretches, as a result which corroborates previous report (14). Thus it is likely that BV treatment is affecting the sensory (nociceptive) component of the abdominal stretch reflex rather than the motor portion of the reflex (13).

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. Korean BV evoked an inhibitory effect on the nociceptive behaviors associated with the abdominal assay in mice. Korean BV induced noxious stimulation produces a dose-dependent suppressive effect on the abdominal stretch reflex induced by acetic acid injection which is in agreement with Kwon *et al.* (14).

The FDA approved the use of BV. By that time, a higher quality of venom was available for manufacturing of products. With the introduction of BV it became possible to prepare a standardized and superior product for BV therapy. Bees need pollen or protein rich nutrition to make good quality venom (19, 20). From spring to fall, this is easily archived in an area with continuous flowering plants. However, in the late fall and winter, bee keepers tend to feed their bees with sugar syrup and not with pollen; consequently, the quality of venom suffers in Korea. The BV is collected during the peak or just at the end of honey flow when the bees' venom sacs are full of quality venom (2), so BV is of high quality when it is reconstituted. This means 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 natural 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 solution. Furthermore, BV does not produce any obvious tissue damage after a single injection suggesting the possibility of repeated uses (17).

In this study, Korean BV injection evoked antinociceptive effects in mice. Korean BV produces antinociceptive effects without negative side effects associated with many of narcotic drugs. These results suggest that Korean BV may be a valuable choice for antinociceptive efficacy in pain.

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마우스 복통에 대한 한국산 봉독의 진통 효과

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요 약 : 본 연구에서는 마우스의 복부 신전시험을 통해 한국산 건조 봉독의 진통 효과를 평가하였다. 한국산 봉독은 특별히 고안된 봉독 추출기를 사용하여 일벌 (*Apis mellifera* L.)에 전기 충격을 가하여 생봉독을 수집하였으며, 수집된 생봉독은 봉독 건조기를 이용하여 봉독을 건조하였다. 실험 동물은 수컷 ICR 마우스 (체중 30-35 g, 6주령) 56마리를 각 군당 8마리씩 4개군으로 분류하였다. 건조 봉독은 6 mg/kg 투여군, 0.6 mg/kg 투여군, 0.06 mg/kg 을 투여군 및 생리식염수를 투여한 대조군으로 각각 분류하였다. 한국산 건조 봉독은 마우스에 피하로 투여하였고, 봉독투여 30분 후에는 0.9% 초산을 복강으로 투여하였다. 마우스의 복부의 신전 반사 횟수는 초산 투여 후 60분 동안 측정하였다. 초산 투여 후 3-9분에는 복부신전 반사를 보였고, 20분 후에는 가장 심한 복부신전 반사가 있었고 60분 후에는 반응이 거의 소실되었다. 한국산 건조 봉독의 복부 신전반응은 용량에 의존적으로 억제되었다. 이러한 결과에서 한국산 건조봉독은 통증치료에 있어 다른 약물을 대체하여 사용될 수 있을 것으로 생각된다.

주요어 : 통증신전반사, 봉독, 한국, 마우스