J Vet Clin 24(4) : 499-502 (2007)

Journal of Veterinary Clinics
www.ksvc.or.kr

Antinociceptive Efficacy of Korean Bee Venom in the Rat Formalin Test

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(Accepted: October 31. 2007)

Abstract : The study was performed to assess the antinociceptive efficacy of Korean bee venom (BV) in the rat formalin test. 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 Korean BV was evaporated until dry using BV collector. Experiments were performed on male Sprague-Dawley rats (weighing 260-270, 6 weeks old). Rats divided into 4 groups, each comprising 8 rats. 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 rat formalin test. BV was subcutaneously injected into an acupoint (Zusanli, ST36) at 15 min prior to 1% formalin (50 μl) injection. The antinociceptive effect observed during 60 min following formalin administration. BV produced antinociceptive efficacy from 10 to 60 min after formalin injection. The antinociceptive efficacy of Korean BV showed a dose-dependent response. These results suggest that Korean BV may be a suitable and preferred choice for antinociceptive efficacy in pain management.

Key words **:** Antinociceptive efficacy, Korean bee venom, formalin test, zusanli

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 (19). BV acupuncture is a kind of herbal acupuncture taking advantage of diluted BV instead of distilled herbal decoction. BV once extracted and processed is utilized on the relevant sites accor- ding to specific diseases or acupoints. BV simultaneously exerts pharmacological actions from the bioactive compounds isolated from BV and mechanical actions from the acupuncture stimulation. BV has been considered as a promising therapeutic method for various diseases, especially in Korean medicine. BV has long been used in a variety of conditions and good evidence for its effectiveness exists in pain syndrome, herniation nucleus pulpous, cervical disc protrusion and progressive muscle atrophy. For several centuries, BV has been utilized in Oriental medicine to treat inflammatory diseases such as tendonitis, bursitis and rheumatoid arthritis (1). Traditional acupuncture can be defined as a procedure that involves the insertion of a needle into an acupuncture point and subsequent application of mechanical stimulation to the acupoint using manual rotation and vertical movement of the needle to teat pain associated with a particular disease state.

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Among the animal models used to study the underlying persistent pain mechanisms, the formalin test is one of the most useful in assessment of prolonged pain after injury (7,8,16,18). Electrophysiological studies in rats demonstrated that subcutaneous formalin injection could result in two phases of increased firing of dorsal horn-convergent (wide dynamic range, WDR) neurons; including an acute phase (early phase) lasting for 5 to 10 min, followed 10-20 minutes later by a tonic phase (late phase) lasting for 35 to 65 min, which correlated very well with the behavioral responses previously observed (6,7). Formalin injection is often used as an experimental model of pain because it produces a vigorous response and permits the study of both acute and tonic pain (13).

The purpose of this study was to determine whether Korean BV produces an antinociceptive efficacy in the rat formalin test.

Materials and Methods

Animals

Experiments were performed on male Sprague-Dawley rats (weighing 260-270 g, 6 weeks old). All laboratory animals were obtained from the Orient Bio Co. (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 National Institutes of Health (NIH publication No. 86-23, revised 1985). In addition, the ethical guidelines of the Inter-

national 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 (12 h light/12 h dark: illumination beginning at 7 : 00 AM) until the day of the experiment (7 days acclimation period). Each rat was used only one. 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

Whole Korean BV was collected from a farm of Choongju city in Chungbuk province, in September 2006. Korean BV was obtained using BV collector devices that emit electrical impulses to stimulate the worker bee (*Apis mellifera* L.) to sting and release venom. Whole BV was evaporated until dry in the BV collector. Analysis of BV components was made by liquid chromatography (AKTA explorer, Amersham Pharmacia Biotech, USA).

Formalin test in rats

Rats were divided into 4 groups, each comprising 8 rats. Korean BV was reconstituted and serially diluted; amount of 6 mg/kg body weight (BW), 0.6 mg/kg BW and 0.06 mg/kg BW were injected. A 0.9% sterile 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 injected into the acupoint of Zusanli (ST36). This acupoint is located 5 mm lower and lateral to the anterior tubercle of the tibia. Control rats were 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 hindpaw with a 30 gauge needle. Following formalin injection each rat was observed for 60 min by three experienced investigators.

Statistical analysis

The number of the licking and flinching time in each group was expressed as the mean ± S.E. One-way analysis of variance (ANOVA) 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

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

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 decreased to nearly baseline levels during the subsequent 10 min period, typically called the intermediate period (Fig 1). Finally, licking and flinching behaviors increased again in the late phase, 15 min after the initial formalin injection, and was sustained for 60 min (Fig 2).

BV treatments induce an antinociceptive effect in 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

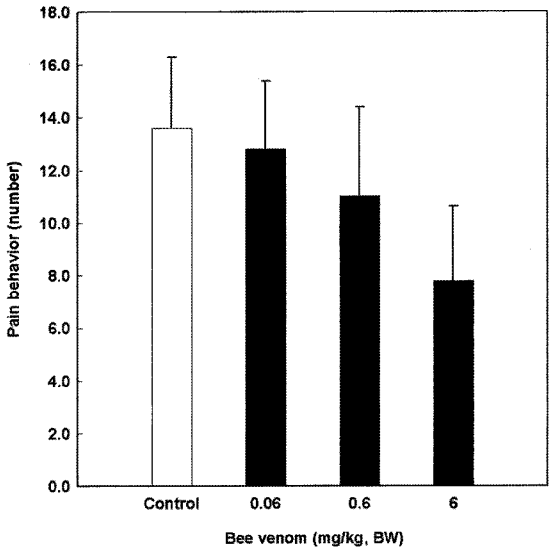

Fig 1. Effect of Korean bee venom treatment on acute phase in the rat formalin test.

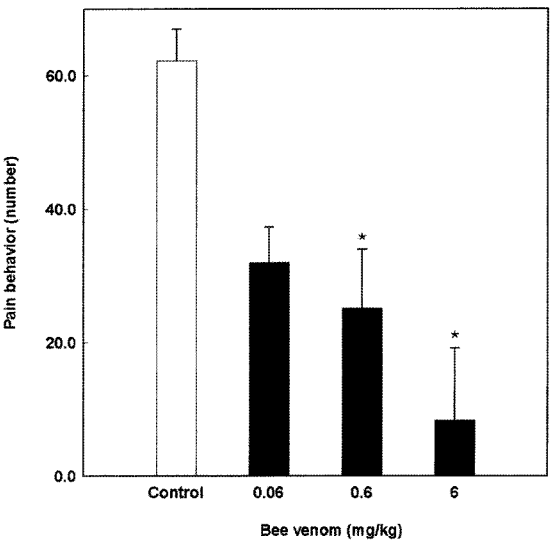

Fig 2. Effect of Korean bee venom treatment on tonic phase in the rat formalin test. The data were presented as mean ± SE (n = 8) and the testing was considered significant if $p < 0.05$.

phase ($p < 0.05$) in animals injected with BV, and the antiniciceptive effect demonstrated a dose-dependant pattern.

Discussion

The antinociceptive effect of BV was further verified using several animal models with acute and chronic nociception. The BV test satisfies many of the requirements for testing pain mechanisms in animals (8,17). 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 (13). In the current study, formalin test demonstrated that BV injected into a specific point prior to formalin injection evoked antinociceptive effects in rats. BV suppressed pain behaviors and spinal Fos expression in rats induced hindpaw formalin injection (12). Addition, subcutaneous treatment of BV produced a dramatic antinociceptive effect on Freund's adjuvant-induced arthritis in rats (13). But these products have been achieved about only foreign BV in Korea. So, we would like to were assessed the analgesic action of Korean BV. At this point, the present study was performed whether Korean BV would attenuate nociceptive behaviors induced in rodent animal models. BV is a natural substance that is relatively inexpensive and readily available from chemical retailers. It is also easily quantified and injected since it is soluble in saline. Although the exact concentration of components varies from bee to bee, the effect is minimized by the use of hundreds of bee stings in the preparation of injectable solutions.

Whole BV is composed of many chemical agents: polypeptides (melittin, apamin, MCD peptide, etc.), enzymes (hyaluronidase, phospholipase A2, etc.), amines (histamine, dopamine, etc.) and others (14). These ingredients of BV, particularly melittin and phospholipase A2, are responsible for the local inflammation and nociceptive responses associated with bee stings (10). Moreover, BV injection produces persistent nociceptive responses and subsequent neuronal activation within the spinal cord (3,4,14). 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 (9). 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 (2) produced transit pain in humans and intraplantar injection of serotonin in rats produced pain behavior (11). Other less potent compounds, such as acetylcholine and noradrenaline, are found in smaller amounts and may also contribute to the production of pain responses (15).

In this study, subcutaneous injection of formalin in the saline treated rat results 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 which appear to reflect central sensitization (5). The physiological mechanism of central sensitization is complex and occurs within both the spinal cord and brain. Glutamate, acting at the NMDA receptor, is probably the main transmitter involved. The NMDA receptor is initially blocked by a magnesium ion; when glutamate causes depolarization of a neuron by an action at the amino methylisoxazole proprionic acid receptor (the main process involved in normal fast transmission across a synapse), this magnesium ion is displaced so the next packet of glutamate causes NMDA receptors to open and the cell is more likely to fire. Just like the results of Kim *et al.* (12), BV treatment produces a significant antinocicpetive effect and did not affect motor activity. Thus, it is likely that BV treatment affects the sensory component of the abdominal stretch reflex rather than the motor portion of the reflex.

In the present study, Korean BV injection into the acupoint evokes antinociceptive effects in the rat formalin test. The antinociceptive efficacy of Korean BV shows a dose-dependent response. BV offers a unique advantage in pain management options, because it produces potent antinociception without negative side affects associated with many narcotic drugs. These results suggest that Korean BV may be a suitable and preferred choice for antinociceptive efficacy in pain management.

Acknowledgements

This work was supported by a research grant of the Chungbuk National University, Republic of Korea, in 2007. We are grateful to Choul Goo Kim of Chung Jin Tech Co. for kindly providing with bee venom (*Apis mellifera* L.).

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랫드의 포르말린 시험에서 한국산 봉독의 항통각 효과

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요 약 : 본 연구는 전기충격 요법으로 얻은 한국산 건조봉독을 랫드의 포르말린 시험으로 항통각 효능을 관찰하였다. 실험동물로 수컷 Spraque-Dawley 랫드(평균 체중 265.38 g, 6주령) 56마리를 각 군당 8마리씩 4개군으로 분류하였다. 봉독 투여군은 6 mg/kg 투여군과 0.6 mg/kg 투여군, 0.06 mg/kg 투여군으로 분류하였고, 대조군에는 생리식염수를 투여하였다. 건조 봉독은 포르말린을 투여하기 15분 전에 족삼리(ST-36)에 피하로 투여하였다. 통증은 1% 포르말린 50 μl을 랫드의 우측 뒷발바닥의 피하에 투여하여 유발하였다. 랫드가 포르말린을 투여한 후 우측 후지를 입으로 핥거나 깨무는 등의 행동을 포르말린 유발 통증행동으로 평가하였으며, 포르말린 투여 후 60분간 랫드의 통증 반응을 관찰하였다. 랫드의 통증반응은 첫 10분간은 5분 간격으로 관찰하였고, 10분 후부터 60분까지 10분 간격으로 관찰하였다. 한국산 건조 봉독은 봉독 투여 후 10분 이내에는 통증 억제 반응을 나타내지 않았으나, 봉독 투여 10분 후에 용량 의존적인 통증 억제 반응을 보였다. 이상의 결과에서 랫드의 포르말린 시험에서 한국산 건조봉독의 통증 억제 반응은 봉독량에 의존성을 보였고, 통증치료에 있어 다른 약물을 대체하여 사용될 수 있을 것으로 생각된다.

주요어 : 항통각 효과, 한국산 봉독, 포르말린 시험, 족삼리, 랫드