

원저

# Anti-nociceptive Effect of Bee Venom on Capsaicin or Bradykinin-induced Pain

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## 국문초록

### Capsaicin이나 Bradykinin으로 유발된 통증에 대한 봉독의 억제 효과

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**목적:** 봉독으로 유발된 통각수용의 강도와 봉독으로 나타나는 항통각수용(통각억제성)의 강도를 쥐의 포르말린 테스트를 통해 상호관련됨을 확인하고 capsaicin과 bradykinin으로 통증 유발된 쥐의 자발적인 통증행동(활동시간; LN), 꼬리경타시험(TFL)과 열판시험(HPL)을 통해 봉독의 항통각수용(통각억제)작용을 재확인 하고자 하였다.

**방법:** 쥐의 뒷다리에 통증유도 물질인 Capsaicin 또는 Bradykinin을 20 $\mu$ l를 주사하여 동통을 유발하고 자발적 통증행동인 활동시간(LN), 꼬리경타시간(TFL)과 열판 위에서의 온도 자극에 쥐가 반응하는 시간을 측정(HPL)하는 실험을 봉독을 주입하거나, 물핀을 주입하거나, 아무것도 주입하지 않고 통증유발만 시킨 이후에 각각 시행하였다.

**결과:** 1. Capsaicin 또는 Bradykinin으로 동통유발 후 LN은 두드러진 증가를 보임, HPL은 감소를 TFL은 두드러진 감소를 나타내었다.

2. 봉독이나 물핀 주입 30분후에 Capsaicin으로 동통유발이후 LN은 봉독과 물핀에서 모두 현격한 감소를, HPL은 봉침은 현격한 증가를, 물핀에서는 감소를, TFL은 봉침과 물핀에서 모두 현격한 증가를 나타내었다.

3. 봉독과 물핀 주입 30분 후에 Bradykinin으로 동통유발이후 LN은 봉독은 증가 물핀은 현격한 감소를,

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HPL은 봉침은 증가 물핀에서는 현격한 증가를, TFL은 봉침과 물핀에서 모두 증가를 나타내었다.

결론 : 봉독은 Capsaicin 또는 Bradykinin으로 동통유발된 통각수용 행동을 감소시키는 결과를 나타내었는데 이것은 기존의 연구결과들에서의 봉독의 항통각수용(통각억제성)의 효과를 입증하였고 봉약침은 염증의 개선이나 압과 관련된 동통에 유효한 방법임을 시사하는 것이다.

핵심단어 : Bee venom(BV), capsacin, bradykinin, formalin test, Spontaneous nociceptive behaviour test, LN, TFL, HPL, anti-nociception, nociception

## I. Introduction

Bee venom (BV) injection can produce both an initial nociceptive effect and a prolonged antinociceptive effect. BV contains a number of potential pain-producing substances including melittin, histamine, and phospholipase A2, and therefore it is not surprising that several reports described a nociceptive effect after intraplantar injection<sup>1-3)</sup>. Luo et al<sup>4)</sup> have also reported that intraplantar BV injection significantly increases Fos expression in the spinal cord dorsal horn of anesthetized rats. In contrast, subcutaneous injection of diluted BV into an acupoint, termed apupuncture, has been used clinically in Oriental medicine to produce a potent analgesic effect. In support of this alternative medicine approach recent experimental studies have demonstrated that subcutaneous injection of BV(0.01 to 1 mg/kg) into the Zusanli acupuncture point produces prominent antinociceptive and anti-hyperalgesic effects in animal models of acute and persistent pain, respectively<sup>2,5-7)</sup>. The above studies indicate that a dichotomy exists with respect to the physiologic response to subcutaneous injection of BV. On one hand, intraplantar injection of BV and its major constituent, melittin, produces robust nociceptive behaviors and hypersensitivity in rodents, whereas BV injection into the Zusanli acupoint, on the other hand, produces little nociceptive behaviors, but rather a significant antinociceptive effect in a variety of animal pain models. recently, Roh et al<sup>8)</sup> found a evidence

resolving the apparent conflicting data in the literatures through investigation of a possible relationship between BV's nociceptive and antinociceptive effects, particularly with respect to BV injection into an acupoint that BV stimulation of the Zusanli acupoint produces a significant anti-nociceptive effect in the second phase of the formalin behaviour test involving spinal neuronal transmission without detectable nociceptive behavior, and that peripheral capsaicin-insensitive primary afferents(CIPAs) are primarily involved in activating central catecholaminergic pathways associated with BV's anti-nociceptive effect. In the present study, inconsistent with the above study to confirm whether the intensity of the BV-induced nociception is correlated with the intensity of the BV-induced antinociception in the mouse formalin test(as measured by spontaneous pain behaviour).

To reconfirm the anti-nociceptive effect of BV, we evaluated anti-nociceptive effect of BV on capsaicin or bradykinin-induced pain behaviors of rats by spontaneous pain behaviour(lick), tail flick latency(TFL) and hot plate test.

## II. Materials and Methods

### 1. Chemicals

Dried BV was purchased from You-Miel Bee Venom Ltd. (Hwasoon, Jeonnam, Korea). The composition of the BV was as follow: 45~50%

melittin, 2.5~3% apamin, 2~3% MCD peptide, 12% PLA2, 1% lyso-PLA, 1~1.5% histidine, 4~5% 6pp lipids, 0.5% secarpin, 0.1% tertiapin, 0.1% procaine, 1.5~2% hyaluronidase, 2~3% amine, 4~5% carbohydrate, and 19~27% other, including protease inhibitor, glucosidase, invertase, acid phosphomonoesterase, dopamine, norepinephrine, and unknown amino acids, with >99.5% purity. Capsaicin and Bradykinin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). DAPTA was obtained from Tocris Bioscience (West woods Business Park Ellisville, Missouri, USA).

## 2. Animals

Male mice IcrTacSam: ICR(Samtako, Gyeonggi-do, Korea) were maintained in accordance with the National Institute of Toxicological Research of the Korea Food and Drug Administration guideline for the care and use of laboratory animals. All mice were housed in a room that was automatically maintained at 21~25°C and relative humidity (45~65%) with a controlled light-dark cycle. All experiments were conducted in accordance with the guidelines of the International Association for the Study of Pain (IASP)<sup>9</sup>.

## 3. Pain Induction

As Kwon et al.<sup>6</sup> had employed 1mg/Kg of BV in their study, we also tried to use 1mg/Kg of BV. but we encountered a problem that spontaneous pain behaviour such as licking was consistent over 30minutes. To resolve it, 0.5mg/Kg of BV was adopted and 20 $\mu$ l of BV was pre-treated into a paw 30minutes before pain induction, while same amount of saline and 30 $\mu$ l(10mg/Kg) of morphine was pre-treated in the control. Pain was induced by Capsaicin or Bradykinin. although our previous thought was to select 10 nmol of capsaicin or bradykinin as described by Mattos et al.<sup>10</sup>, in case of bradykinin, des Arg9-Bradykinin was chosen for the alternative to bradykinin acetate and the concentration was also changed

into 20 nmol due to lack of licking phenomenon (Fig. 1). as a pain inducer, 20 $\mu$ l of capsaicin or bradykinin was injected into a unilateral paw of rats respectively(Fig. 2).

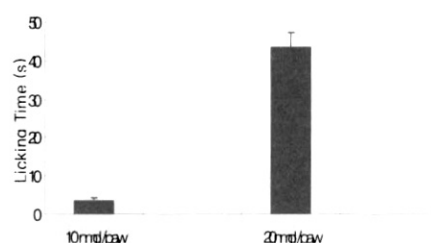


Fig. 1. Effect of Concentration of Bradykinin on the Licking Time

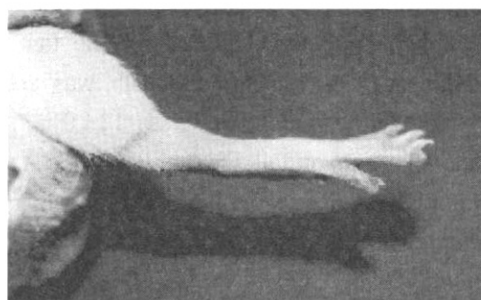


Fig. 2. Hind Paw of a Rat(Injection Site)

## 4. Pain Behaviour Assessment

### 1) Spontaneous nociceptive behaviour (Licking Number;LN)

A frequently used method of producing injury in the rat is the subcutaneous injection of a small volume of irritant such as formalin, capsaicin, bradykinin into its hind paw. Typically, after the injection, the rat displays a biphasic(phase I and phase II) incidence of flinching (rapid paw shaking) and licking of the injected paw<sup>11-13</sup>. The behavioral syndrome produced by the injection of formalin into the paw has been widely used to define the pharmacology of systems that regulate facilitated processing. The "formalin test" has evolved into a widely used tool in the screening of analgesic and anti-hyperalgesic drugs<sup>14</sup>. but in

the present study, we used capsaicin or bradykinin instead of formalin. Lick phenomenon was observed from baseline(right after pain induction) to 10 minutes after pain induction in the absence of BV or morphine pre-treatment, while it was observed from 30 to 40minutes after baseline in the presence of BV or morphine pre-treatment (Scheme 1, 2). Total number of licking was measured and recorded.

## 2) Tail Flick Latency(TFL)

For assessing the antinociceptive effect, a tail flick unit(한국리레이아, Seoul, KOREA) was used to evaluate the pain threshold by using the change of tail flick latency(TFL). The light of the tail flick unit was turned out as soon as the rat flicked its tail and the time lapse between the onset of irradiation and the flick of the tail was read. The intensity of the light bulb was set so the baseline reaction time was  $12 \pm 0.3s$ . For proper application of tail flick test and BVA, the rat was restrained in a plastic holder( $5.3 \times 15cm$  in diameter  $\times$  length), and the tail was laid on the light bulb. When TFL exceeded 15s during an experimental procedure, the light bulb was switched off to minimize tissue damage of the tail. The degree of analgesia was expressed as a percentile change in TFL and was determined as follows<sup>15,16</sup>.

$$\text{Acquired TFL change (\%)} = \frac{\text{Post treatment TFL} - \text{Baselime TFL}}{\text{Baselime TFL}} \times 100$$

The behavioral test was performed with the tail flick unit from 15minutes after pain induction to 30 minutes after pain induction in the absence of BV or morphine pre-treatment, while it was performed from 45 to 60minutes after baseline in the presence of BV or morphine pre-treatment. Experimental rats were properly fitted in plastic holders with their tails protruding outside and were allowed to adapt to the environment for 60 min/day for 7days. This procedure was also for

adaptation to the restraint that the animals were submitted to during the BVA treatment<sup>17</sup>.

## 3) Hot Plate Test(Hot Plate Latency; HPL)

Mice are brought to the testing room and allowed to acclimatize for 10minutes before the test begins. Pain reflexes in response to a thermal stimulus are measured using a Hot Plate Analgesia Meter from Panlab s.l.(Cornella, Spain). The surface of the hot plate is heated to a constant temperature of  $50^{\circ}C$ , as measured by a built-in digital thermometer with an accuracy of  $0.1^{\circ}C$  and verified by a surface thermometer. Mice are placed on the hot plate( $25.4cm \times 25.4cm$ ), which is surrounded by a clear acrylic cage (19 cm tall, open top), and the Start/Stop button on the timer is activated. The latency to respond with either a hindpaw lick, hindpaw flick, or jump (which ever comes first) is measured to the nearest 0.1seconds by deactivating the timer when the response is observed. The mouse is immediately removed from the hot plate and returned to its home cage. If a mouse does not respond within 30seconds, the test is terminated and the mouse is removed from the hot plate. Animals are tested one at a time and are not habituated to the apparatus prior to testing. Each animal is tested only once.

this test was performed with the hot plate unit from 10minutes after pain induction to 15 minutes after pain induction in the absence of BV or morphine pre-treatment, while it was performed from 40 to 45minutes after baseline in the presence of BV or morphine pre-treatment.

## 5. Experimental groups

Experimental groups were divided into three groups:

- (1) Saline group; saline with Capsaicin(n=7) or saline with bradykinin(n=3)
- (2) BV group; BV pre-treated group with

- Capsaicin(n=7) or saline with bradykinin(n=3)  
 (3) Morphine group; Morphine pre-treated group  
 with Capsaicin(n=7) or saline with bradykinin  
 (n=3)

## 6. Statistical Analysis

Data were analyzed using one-way analysis of variance followed by Tuckey test as a post hoc test. Differences were considered significant at  $p < 0.05$ .

## III. Results

### 1. Effect of Capsaicin or Bradykinin on LN, HPL and TFL in the Saline treated Mice

$20\mu\text{l}$  of Saline was injected into a hind paw of mice intraplantarly with 10nmol of capsaicin or 20 nmol of bradykinin treated. Lick phenomenon was observed from baseline(right after pain induction) to 10minutes after pain induction, and then Hot Plate test was performed with the hot plate unit from 10minutes after pain induction to 15minutes after pain induction, and last Tail Flick test was followed from 15minutes after pain induction to 30minutes after pain induction. In spontaneous nociceptive behaviour test, LN significantly increased by Capsaicin or Bradykinin was  $20.1 \pm 7.6$  times or  $45.5 \pm 19.7$  times compared with  $2.9 \pm 3.0$  times of saline treated mice without pain inducers(Fig. 3-5). In Hot Plate test, HPL decreased by Capsaicin or Bradykinin was  $32.2 \pm 7.0$  s or  $38.8 \pm 0.4$ s compared with  $40 \pm 18.5$ s of saline treated mice without pain inducers(Fig. 3, 4, 5). In Tail Flick Test, TFL significantly decreased by Capsaicin or Bradykinin was  $10.6 \pm 3.2$ s or  $11.1 \pm 2.9$ s compared with  $38.4 \pm 4.5$ s of saline treated mice without pain inducers(Fig. 3-5).

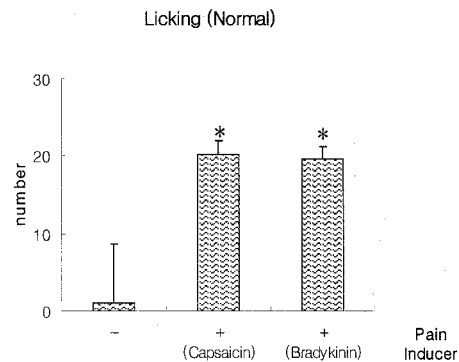


Fig. 3. Effect of Capsaicin or Bradykinin on LN in the Saline treated Mice

$20\mu\text{l}$  of Saline was injected into a hind paw of mice intraplantarly with 10nmol of capsaicin or 20 nmol of bradykinin treated. Lick phenomenon was observed from baseline(right after pain induction) to 10minutes after pain induction. Each value means  $\pm$  S.E. from three separated experiments. \* represents Significantly different from saline treated group without pain inducers( $p < 0.05$ ).

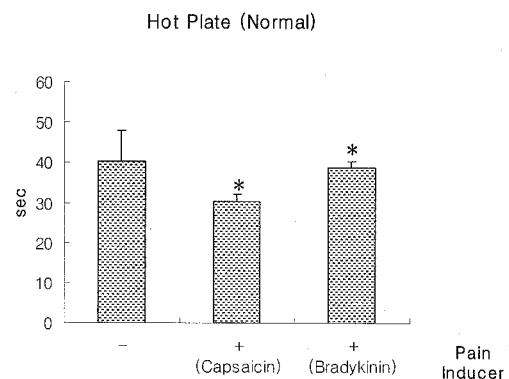


Fig. 4. Effect of Capsaicin or Bradykinin on HPL in the Saline pre-treated Mice

$20\mu\text{l}$  of Saline was injected into a hind paw of mice intraplantarly with 10nmol of capsaicin or 20 nmol of bradykinin treated. Hot Plate test was performed with the hot plate unit from 10minutes after pain induction to 15minutes after pain induction. Each value means  $\pm$  S.E. from three separated experiments.

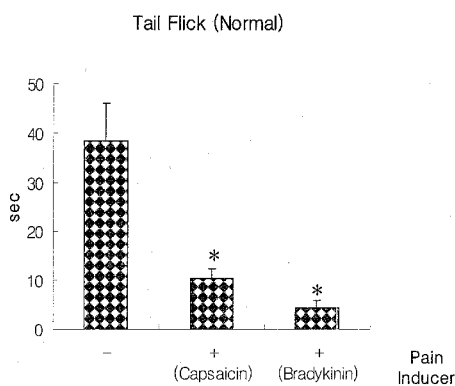


Fig. 5. Effect of Capsaicin or Bradykinin on TFL in the Saline pre-treated Mice

20 $\mu$ l of Saline was injected into a hind paw of mice intraplantarly with 10nmol of capsaicin or 20 nmol of bradykinin treated. Tail Flick test was followed from 15minutes after pain induction to 30 minutes after pain induction. Each value means  $\pm$  S.E. from three separated experiments. \* represents Significantly different from saline treated group without pain inducers(p<0.05).

## 2. Effect of Capsaicin on LN, HPL and TFL in the BV pre-treated Mice

20 $\mu$ l and 0.5mg/Kg of BV or 30 $\mu$ l and 10mg/Kg of morphine was injected into a hind paw of mice intraplantarly 30minutes before pain was induced by 10 nmol of capsaicin. Lick phenomenon was observed from 30 to 40minutes after baseline(from right after pain was induced to 10 minutes after pain induction), and then Hot Plate test was performed with the hot plate unit from 40 to 45minutes after baseline, and last Tail Flick test was followed from 45 to 60minutes after baseline. In spontaneous nociceptive behaviour test, LN of BV or Morphine significantly decreased by capsaicin was 9.2 $\pm$ 1.8 times or 6.7 $\pm$ 1.6 times compared with 20.1 $\pm$ 7.6 times of saline treated mice with capsaicin(Fig. 6-8). In Hot Plate test, HPL of BV significantly increased or that of Morphine decreased by Capsaicin was 48.9 $\pm$ 16.8s or 26.9 $\pm$ 8.9s compared with 28 $\pm$ 18.5s of saline treated mice with capsaicin(Fig. 6-8). In Tail

Flick Test, TFL of BV or Morphine significantly increased by capsaicin was 20.8 $\pm$ 5.1s or 50 $\pm$ 3.1 s compared with 10.6 $\pm$ 3.2s of saline treated mice with capsaicin(Fig. 6-8).

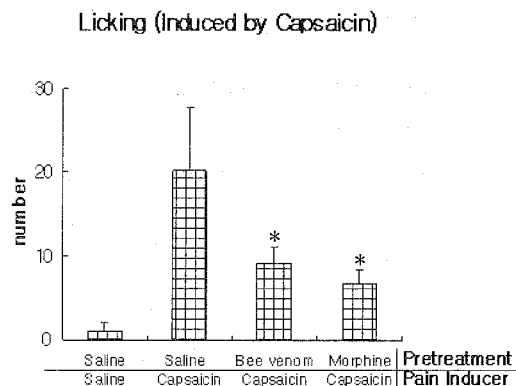


Fig. 6. Effect of Capsaicin on LN in the BV or Morphine pre-treated Mice

20 $\mu$ l and 0.5mg/Kg of BV or 30 $\mu$ l and 10mg/Kg of morphine was injected into a hind paw of mice intraplantarly 30minutes before pain was induced by 10nmol of capsaicin. Lick phenomenon was observed from 30 to 40minutes after baseline (from right after pain was induced to 10minutes after pain induction), and then Hot Plate test was performed with the hot plate unit from 40 to 45minutes after baseline, and last Tail Flick test was followed from 45 to 60minutes after baseline. Each value means  $\pm$  S.E. from three separated experiments. \* represents Significantly different from saline and capsaicin treated group without pain inducers(p<0.05).

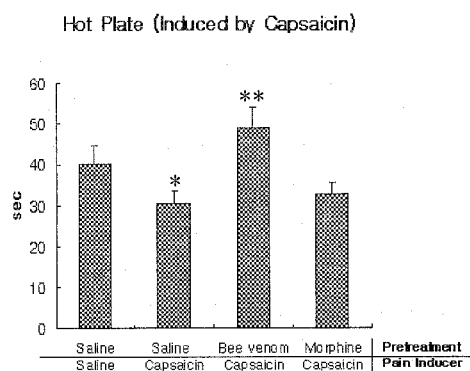


Fig. 7. Effect of Capsaicin on HPL in the BV or Morphine pre-treated Mice

20 $\mu$ l and 0.5mg/Kg of BV or 30 $\mu$ l and 10mg/Kg of morphine was injected into a hind paw of mice intraplantarly 30minutes before pain was induced by 10nmol of capsaicin. Lick phenomenon was observed from 30 to 40minutes after baseline(from right after pain was induced to 10minutes after pain induction), and then Hot Plate test was performed with the hot plate unit from 40 to 45minutes after baseline, and last Tail Flick test was followed from 45 to 60minutes after baseline. Each value means  $\pm$  S.E. from three separated experiments. \* represents Significantly different from saline and capsaicin treated group without pain inducers ( $p < 0.05$ ).

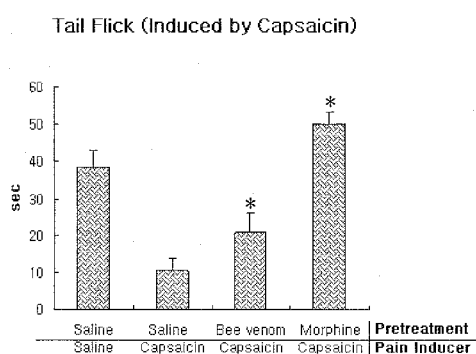


Fig. 8. Effect of Capsaicin on TFL in the BV or Morphine pre-treated Mice

20 $\mu$ l and 0.5mg/Kg of BV or 30 $\mu$ l and 10mg/Kg of morphine was injected into a hind paw of mice intraplantarly 30minutes before pain was induced by 10 nmol of capsaicin. Lick phenomenon was observed from 30 to 40minutes after baseline(from right after pain was induced to 10 minutes after pain induction), and then Hot Plate test was performed with the hot plate unit from 40 to 45minutes after baseline, and last Tail Flick test was followed from 45 to 60minutes after baseline. Each value means  $\pm$  S.E. from three separated experiments. \* represents Significantly different from saline and capsaicin treated group without pain inducers ( $p < 0.05$ ).

### 3. Effect of Bradykinin on LN, HPL and TFL in the BV or Morphine pre-treated Mice

20 $\mu$ l and 0.5mg/Kg of BV or 30 $\mu$ l and 10mg/Kg of morphine was injected into a hind paw of mice intraplantarly 30minutes before pain was induced by 20nmol of bradykinin. Lick phenomenon was observed from 30 to 40minutes after baseline(from right after pain was induced to 10 minutes after pain induction), and then Hot Plate test was performed with the hot plate unit from 40 to 45minutes after baseline, and last Tail Flick test was followed from 45 to 60minutes after baseline. In spontaneous nociceptive behaviour test, LN of BV increased or that of Morphine significantly decreased by Bradykinin was  $30.3 \pm 18.1$  times or 0 times compared with  $20 \pm 11.7$  times of saline treated mice with bradykinin(Fig. 9-11). In Hot Plate test, HPL of BV increased or that of Morphine significantly increased by Bradykinin was  $41.9 \pm 6.0$ s or  $88.8 \pm 1.1$ s compared with  $38.8 \pm 0.4$ s of saline treated mice with bradykinin(Fig. 9-11). In Tail Flick Test, TFL of BV or Morphine increased by Bradykinin was  $8.6 \pm 0.9$ s or  $10.8 \pm 15.5$ s compared with  $7.1 \pm 2.9$ s of saline treated mice without pain inducers(Fig. 9-11).

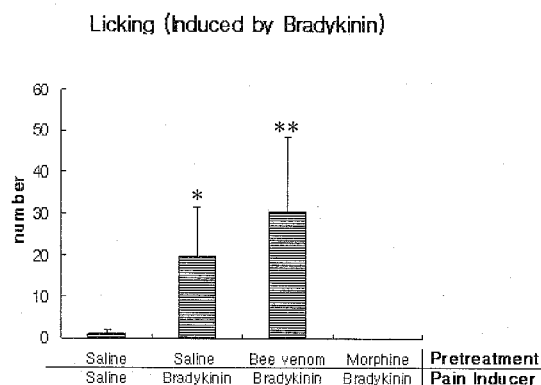


Fig. 9. Effect of Bradykinin on LN in the BV or Morphine pre-treated Mice

20 $\mu$ l and 0.5mg/Kg of BV or 30 $\mu$ l and 10mg/Kg of morphine was injected into a hind paw of mice intraplantarly 30minutes before pain was induced by 20nmol of bradykinin. Lick phenomenon was observed from 30 to 40minutes after baseline(from right after pain was induced to 10minutes after pain induction), and then Hot Plate test was performed with the hot plate unit from 40 to 45minutes after baseline, and last Tail Flick test was followed from 45 to 60minutes after baseline. Each value means  $\pm$  S.E. from three separated experiments. \* represents Significantly different from saline and bradykinin treated group without pain inducers(p<0.05).

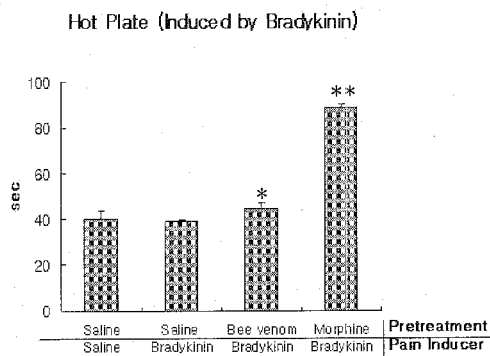


Fig. 10. Effect of Bradykinin on LN in the BV or Morphine pre-treated Mice

20 $\mu$ l and 0.5mg/Kg of BV or 30 $\mu$ l and 10mg/Kg of morphine was injected into a hind paw of mice intraplantarly 30minutes before pain was induced by 20nmol of bradykinin. Lick phenomenon was observed from 30 to 40minutes after baseline(from right after pain was induced to 10minutes after pain induction), and then Hot Plate test was performed with the hot plate unit from 40 to 45minutes after baseline, and last Tail Flick test was followed from 45 to 60minutes after baseline. Each value means  $\pm$  S.E. from three separated experiments. \* represents Significantly different from saline and bradykinin treated group without pain inducers(p<0.05).

Tail Flick (Induced by Bradykinin)

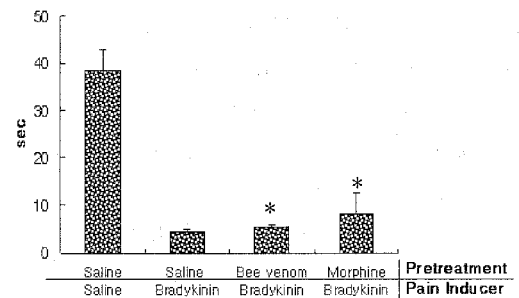


Fig. 11. Effect of Bradykinin on LN in the BV or Morphine pre-treated Mice

20 $\mu$ l and 0.5mg/Kg of BV or 30 $\mu$ l and 10mg/Kg of morphine was injected into a hind paw of mice intraplantarly 30minutes before pain was induced by 20nmol of bradykinin. Lick phenomenon was observed from 30 to 40minutes after baseline(from right after pain was induced to 10minutes after pain induction), and then Hot Plate test was performed with the hot plate unit from 40 to 45minutes after baseline, and last Tail Flick test was followed from 45 to 60minutes after baseline. \* represents Significantly different from saline and bradykinin treated group without pain inducers(p<0.05).

#### IV. Discussion

Bee venom (BV) is known to be a very complex mixture of active peptides, including melittin(a major component of BV), phospholipase A2, apamin, adolapin, and mast cell-degranulating peptide(MCDP)<sup>18)</sup>. As a traditional alternative medicine approach, bee venom(BV) therapy has been utilized to relieve pain and to cure inflammatory diseases such as RA in humans<sup>19)</sup>, and Increasing studies have demonstrated that BV and melittin inhibits cancer growth via induction of apoptotic cell death as well as inflammation<sup>2,18,20)</sup>.

Park et al elucidated anti-inflammatory and



anti-cancer mechanism of BV that target inactivation of nuclear factor  $\kappa$ B(NF- $\kappa$ B) by directly binding to the p50 subunit was an important mechanism of the anti-arthritic effect of BV<sup>21)</sup>, and that melittin inhibited human prostate cancer cell growth through induction of apoptotic cell death via down regulation of NF- $\kappa$ B and alteration of expression of apoptosis regulatory proteins<sup>22)</sup>. In pain control of BV, Chen et al.<sup>23)</sup> reported that subcutaneous BV injection into the peripheral cutaneous receptive

field resulted in a protracted, tonic monophasic increase in spike responses of wide-dynamic-range neurons for more than 1 hour. Kwon et al.<sup>2)</sup> demonstrated that BV administration into the Zusanli acupoint produced a significant anti-nociceptive effect on arthritis-induced inflammatory pain symptoms including thermal and mechanical hyperalgesia, and that BV may be a promising candidate for long-term treatment of RA-induced pain and inflammation and its administration directly into an acupoint provides a potent strategy for reducing the pain and inflammation associated with arthritis. Roh et al.<sup>8)</sup> found that BV stimulation of the Zusanli acupoint produces a significant antinociceptive effect in the second phase of the formalin behaviour test that involves spinal neuronal transmission without detectable nociceptive behavior, and peripheral capsaicin-insensitive primary afferents(CIPAs) are primarily involved in activating central catecholaminergic pathways associated with BV's anti-nociceptive effect.

In the current study, inconsistent with the above study to confirm whether the intensity of the BV-induced nociception is correlated with the intensity of the BV-induced antinociception in the mouse formalin test(as measured by spontaneous pain behaviour).

To reconfirm the anti-nociceptive effect of BV, we evaluated anti-nociceptive effect of BV on capsaicin or bradykinin-induced pain behaviors of rats by spontaneous pain behaviour(lick), tail flick latency(TFL) and hot plate test.

20 $\mu$ l and 0.5mg/Kg of BV or 30 $\mu$ l and 10mg/Kg of morphine was injected into a hind paw of mice intraplantarly 30minutes before pain was induced by 10nmol of capsaicin or 20nmol of bradykinin. Lick phenomenon was observed from 30 to 40minutes after baseline(from right after pain was induced to 10minutes after pain induction), and then Hot Plate test was performed with the hot plate unit from 40 to 45minutes after baseline, and last Tail Flick test was followed from 45 to 60minutes after baseline. In spontaneous nociceptive behaviour test, LN of BV as well as Morphine was significantly decreased by capsaicin, while that of morphine was significantly decreased by bradykinin. In Hot Plate test, HPL of BV was significantly increased by Capsaicin inconsistent with the result that of morphine was significantly increased by bradykinin. In Tail Flick Test, TFL of BV or Morphine demonstrated increasing tendency in both capsaicin and bradykinin induced nociceptive mice. but TFL of BV or Morphine induced by bradykinin didn't actually show significant difference. thus The above result that BV reduced nociceptive behaviour induced by capsaicin or bradykinin support novel anti-nociceptive ability of BV in the previous findings and suggested that BV acupuncture should be useful and available in ameliorating inflammation or cancer related pain.

## V. References

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