Original Article

Antinociceptive Effect and the Mechanism of Bee Venom Pharmacopuncture on Inflammatory Pain in the Rat Model of Collagen-induced Arthritis: Mediation by 5HT-3 & Muscarinic Cholinergic Receptors

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국문초록

Collagen-induced Arthritis Rat Model에서 염증성 통증에 대한 봉독약침의 진통효과 및 기전연구: 5HT-3 & Muscarinic Cholinergic Mechanisms에 대한 연구

서병관 · 박동석 · 백용현

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배경 및 목적 : 봉독약침요법(bee venom pharmacopuncture, BVP)은 rheumatoid arthritis(RA)의 치료에 활용되고 있으나, RA로 인한 염증성 통증에 대한 봉독약침의 진통효과와 specific mechanism은 아직까지 명확하게 밝혀지지 않았다. 이에 본 연구에서는 RA animal model로서 collagen-induced arthritis(CIA) rat model에서 봉독약침의 a1-adrenergic, 5HT-3 그리고 muscarinic cholinergic mechanism을 확인하고자 한다.

방법 : CIA를 유도하기 위하여 male Sprague-Dawley rat에 freund's incomplete adjuvant에 乳化시킨 bovine type II collagen을 주입하고 14일 후 booster injection 시행하였다. 진통효과는 tail flick latency (TFL)로 평가하였다.

결과 : 관절염의 유도 이후 염증성 통증 역치는 시간이 지나면서 낮아지며, 5주 이후로는 통증 역치에 큰 변화가 없이 유지되었다. 첫 번째 immunization으로부터 5주 경과 후 족삼리(ST_{36})에 봉독약침처치(0.25 mg/kg)를 시행하여 유의한 진통효과를 관찰하였다. 또한 봉독약침의 진통효과는 ondansetron(5HT-3 receptor antagonist,

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0.5㎎/kg, i.p.), atropine(muscarinic cholinergic receptor antagonist, 1㎎/kg, i.p.)의 전처치에 의하여 억제되었으나, prazosin(α1-adrenergic receptor antagonist, 1㎎/kg, i.p.)의 전처치에 의해서는 억제되지 않았다.

결론 : 봉독약침은 CIA로 인한 염증성 통증에 유의한 진통효과를 나타내며 그 analgesic mechanism은 5HT-3와 muscarinic cholinergic receptor에 의하여 매개되며 α1-adrenergic receptor에 의하여 매개되지는 않았다.

핵심 단어 : 봉독약침(BVP), collagen-induced arthritis(CIA), ondansetron, atropine, prazosin, tail Flick latency(TFL)

Ⅰ. Introduction

Rheumatoid arthritis (RA) is a kind of autoimmune disease that is characterized by progressive joint destruction, deformity, disability with swelling and pain in multiple joints[1]. As a remedy of RA, non-steroidal anti-inflammatory drugs (NSAIDs) are recommended in medical field, but long term management with NSAIDs may result in serious side effects, such as gastrointestinal ulcer and renal morbidity[2]. Therefore, another treatment without side effects is needed in the management of inflammatory pain induced by RA.

Bee Venom Pharmacopuncture (BVP) has been used to relieve pain and to treat inflammatory diseases such as rheumatoid arthritis (RA) in humans[3] and experimental animals[4,5]. Related with the antinociceptive mechanisms of BVP, there were several studies showing that the analgesic effect of BVP was mediated by α2-adrenergic and 5HT-1 receptors in models of neuropathic pain, acetic acid-induced visceral pain, and formalin pain[6-9]. And, in the rat model of collagen-induced arthritis (CIA) as an animal model of RA[10], BVP showed antinociceptive effect which is mediated by α2-adrenergic receptor[11]. While Bee Venom Pharmacopuncture (BVP) shows the analgesic effect in RA, the antinociceptive mechanisms related with the inflammatory pain by using the bovine type Ⅱ collagen-induced RA model have not been fully studied.

The primary purpose of this study was to determine whether Bee Venom Pharmacopuncture (BVP) is able to show the antinociceptive effect on inflammatory pain in the rat model of collagen-induced arthritis (CIA). A second goal of this study was to clarify whether the antinociceptive effect of BVP is related to the activation of α1-adrenoceptor, 5HT-3 and muscarinic cholinergic receptors. These receptor types are thought to play important roles in spinal analgesic mechanisms associated with the descending pain modulatory system.

Ⅱ. Materials and Methods

A. Subjects

Young adult male Sprague-Dawley rats (Sam:acN (SD)BR, 180~200g, n=110) were housed in group cages (4~5per cage) with water and food available ad libitum. The room was light/dark (08 : 00~20 : 00h light, 20 : 00~08 : 00h dark) controlled and kept at 2124℃. All experiments were conducted in accordance with the guidelines of the International Association for the Study of Pain (IASP)[12].

B. The induction of collagen-induced arthritis (CIA)

CIA induction was performed as described by Trentham et al.[10]. 1.0ml of an emulsion containing 500mg of bovine type Ⅱ collagen (Chondrex Inc.,

Washington, USA) in a 0.3% acetic acid solution (Cosmo Bio., Tokyo, Japan) and 500mg of Freund's incomplete adjuvant (Chondrex Inc, Washington, USA) were intradermally injected into the base of the tail. Two weeks after the first injection, 0.5ml of the same emulsion was intracutaneously injected into the Lt. plantar surface of the rat. On the basis of the arthritis evaluation method by Trentham et al.[10], the rats with the sum total score over 10 were selected as experimental animals. The degree of arthritis severity was scored on a scale of 0~4 in each limb of the rat, where 0 = no inflammation, 1 = unequivocal inflammation of 1 joint, 2 = unequivocal inflammation of at least 2 joints of the limb or moderate inflammation of 1 joint, 3 = severe inflammation of ≥ 1 joint and 4 = maximum inflammation of ≥ 1 joint in the limb.

C. Behavior Assessments (Tail Flick Latency; TFL)

For assessing the antinociceptive effect, a tail flick unit (Ugo Basile Model 7360, Comrio, Italy) 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±0.3s. For proper application of tail flick test and BVP, the rat was restrained in a plastic holder (5.3×15cm in diameter×length) and the tail was laid on the light bulb. When TFL exceeded 20s 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[13,14].

$$\text{Acquired TFL change (\%)} = \frac{\text{post. acup. TFL} - \text{baseline TFL}}{\text{baseline TFL}} \times 100$$

5 weeks after the first administration of bovine type II collagen, the behavioral test was performed with the tail flick unit prior to (as baseline test) and 10, 20, 30, 45 and 60min after BVP treatment.

Experimental rats were properly fitted in plastic holders with their tails protruding outside, and were allowed to adapt to the environment for 60min/day for 7 days. This procedure was also for adaptation to the restraint that the animals were submitted to during the BVP treatment[15].

D. Bee venom treatment and agonists/ antagonists pretreatment

Whole Bee Venom (Sigma, St. Louis, MO) dissolved in saline was administrated subcutaneously and bilaterally, at a dose of 0.25mg/kg[9], into an acupoint (*Zusanli*, ST_{36}). The *Zusanli* acupoint was chosen because it is traditionally used for the relief of inflammatory pain. This acupoint is located at the anterior tibial muscle and about 10mm below the knee joint. Control animals were injected bilaterally into the *Zusanli* acupoint with an equal volume of dimethylsulfoxide (DMSO). Bee venom treatment was conducted 5 weeks after the first administration of bovine type II collagen.

In order to reveal the related analgesic mechanism, the selective α1-adrenoceptor agonist phenylephrine (ICN Biomedicals, Ohio, USA; 2mg/kg, i.p.) and antagonist prazosin (ICN Biomedicals, Ohio, USA; 1mg/kg, i.p.), 5HT-3 agonist m-chlorophenylbiguanide (ICN Biomedicals, Ohio, USA; 1mg/kg, i.p.) and antagonist ondansetron (ICN Biomedicals, Ohio, USA; 0.5mg/kg, i.p.), muscarinic cholinergic agonist neostigmine (ICN Biomedicals, Ohio, USA; 100μgg/kg, i.p.) and antagonist atropine (ICN Biomedicals, Ohio, USA; 1mg/kg, i.p.) were injected intraperitoneally 20 minutes before BVP treatment. Drugs were dissolved in DMSO.

E. Experimental groups

Experimental groups were divided into eleven groups:

a. CIA induction group (CIA, n=10);

b. non-treatment arthritic group (None-Tx, n=10);
c. DMSO-treated / *Zusanli* acupoint arthritic group (Z-DMSO, n=10);
d. BV-treated / *Zusanli* acupoint arthritic group (Z-BVP, n=10);
e. DMSO pretreatment / BV-treated / *Zusanli* acupoint arthritic group (BVP+DMSO, n=10);
f. phenylephrine pretreatment / BV-treated / *Zusanli* acupoint arthritic group (BVP+Phenyl, n=10);
g. prazosin pretreatment / BV-treated / *Zusanli* acupoint arthritic group (BVP+Prazo, n=10);
h. m-chlorophenyl-biguanide pretreatment / BV-treated / *Zusanli* acupoint arthritic group (BVP +m-chlo, n=10);
i. ondansetron pretreatment / BV-treated / *Zusanli* acupoint arthritic group (BVP+ondan, n=10);
j. neostigmine pretreatment / BV-treated / *Zusanli* acupoint arthritic group (BVP+Neosti, n=10);
k. atropine pretreatment / BV-treated / *Zusanli* acupoint arthritic group (BVP+Atrop, n=10);

F. Statistics

All data are represented as means ± standard error of mean. The significance of statistical differences were determined using non-parametric Friedman's rank test followed by Dunnett's post-hoc test in a group, non-parametric Mann-Whitney *U*-test between two groups and non-parametric Kruskal-Wallis ANOVA followed by Dunnett's post-hoc test among groups. $p<0.05$ was considered significant.

Ⅲ. Results

A. Induction of Inflammatory Pain by CIA

The changes of TFL after induction of CIA are shown in Fig. 1. After induction of CIA, there were statistically significant decreases of TFL until 5 weeks. After 5 weeks, there were no significant decreases in TFL. This means that inflammatory pain was induced by CIA and that the pain reached its maximum value starting from the 5th week. On the basis of this result, BVP treatment into *Zusanli* (ST_{36}) was conducted at 5 weeks after the induction of CIA (Fig. 1).

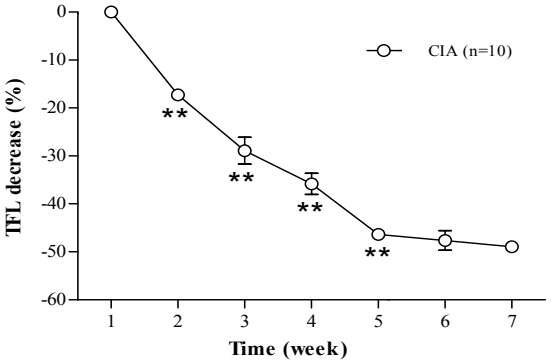

Fig. 1. Changes of tail flick latency (TFL) after induction of collagen-induced arthritis (CIA)
After induction of arthritis, TFL decreased as time passed and reached its minimum value starting from the 3rd week. Each datum is represented as mean±SE of TFL on each tested week. Asterisks indicate significantly different values from the previous week.
* : $p<.05$. **: $p<.01$.
Friedman's rank test followed by Dunnett's post-hoctest.

B. Antinociceptive Effect of Bee Venom Pharmacopuncture (BVP) Treatment

The antinociceptive effects of *Zusanli* BVP in CIA are shown in Fig. 2. In the *Zusanli* BVP treatment group (Z-BVP, n=10), there were marked increases in TFL. Z-BVP showed significant increases in TFL compare to the *Zusanli* DMSO treatment (Z-DMSO, n=10) and non-treatment group (None-Tx, n=10) at 10, 20, 30, 45 and 60min after the initiation of BVP. There were no significant differences between Z-DMSO and None-Tx. This means that *Zusanli* DMSO treatment did not show antinociceptive effect.

The maximal analgesic effect of BVP was seen at 30min after the initiation of BVP injection and this antinociceptive effect is maintained for at least 60minutes. These results indicate that the treatment of BVP can relieve the inflammatory pain in the CIA animal model.

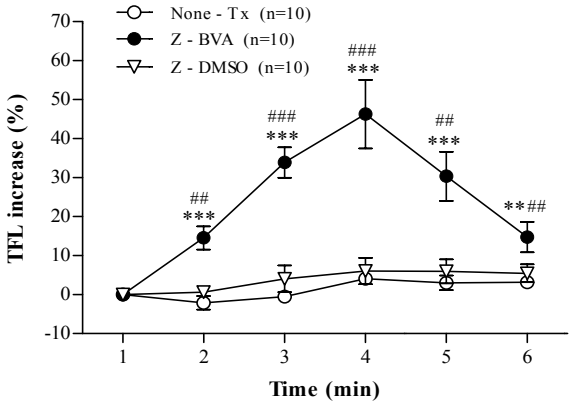

Fig. 2. Effects of Z-BVP on the antinociceptive effect in CIA

Z-BVP (●, n=10), group of BVP into a *Zusanli* (ST_{36}) acupuncture point. Z-DMSO (▽, n=10), group of DMSO injection into a *Zusanli* (ST_{36}) acupuncture point. None-Tx (○, n=10), group without any treatment. The Z-BVP group shows the significant increase of TFL after bee venom treatment (Mann-Whitney *U*-test).
** : $p<.01$. *** : $p<.001$.
Significantly different from Z-DMSO group.
: $p<.01$. ### : $p<.001$.
Significantly different from None-Tx group.

C. Involvement of α1-adrenoceptor on BVP Effect in CIA

The effects of α1-adrenoceptor agonist and antagonist on BVP-induced antinociception in CIA are shown in Fig. 3. There were no significant differences between the antagonist prazosin pretreatment *Zusanli* BVP treatment group (BVP+Prazo, n=10) and the DMSO pretreatment *Zusanli* BVP treatment group (BVP+DMSO, n=10). This result shows that the antinociception of BVP treatment was not blocked by α1-adrenoceptor antagonist pretreatment. In the phenylephrine and prazosin only treatment group, there were no statistically significant differences in TFL and likewise in the saline treatment group (data not shown).

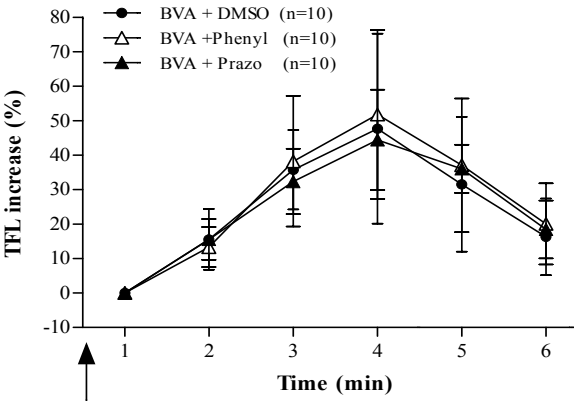

Fig. 3. Effect of α1-adrenoceptor agonist and antagonist pretreatment (arrow) on BVP-induced antinociceptive effect in CIA

BVP+DMSO(●, n=10), group of DMSO pretreatment BVP into a *Zusanli* (ST_{36}). BVP+Phenyl (△, n=10), group of agonist phenylephrine pretreatment BVP into a *Zusanli* (ST_{36}). BVP+Prazo (▲, n=10), group of antagonist prazosin pretreatment BVP into a *Zusanli* (ST_{36}). There are no significant differences between BVP+DMSO and BVP+Prazo.

D. Involvement of 5HT-3 receptor on BVP Effect in CIA

The effects of 5HT-3 receptor agonist and antagonist on BVP-induced antinociception in CIA are shown in Fig. 4. There were statistically significant differences in TFL between the antagonist ondansetron pretreatment *Zusanli* BVP treatment group (BVP+ondan, n=10) and the DMSO pretreatment

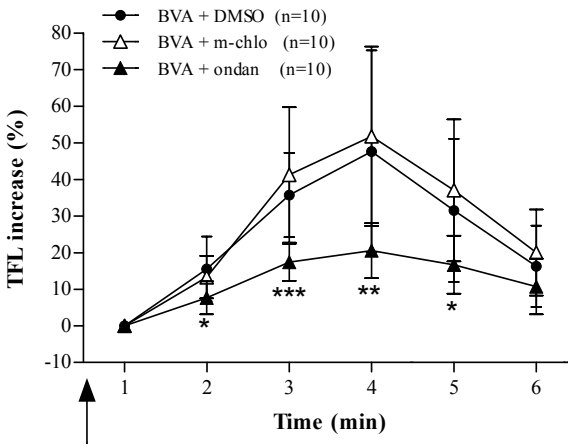

Fig. 4. Effects of 5HT-3 receptor agonist and antagonist pretreatment (arrow) on BVP-induced antinociceptive effect in CIA

BVP+DMSO (●, n=10), group of DMSO pretreatment BVP into a *Zusanli* (ST_{36}). BVP+m-chlo (△, n=10), group of agonist m-chlorophenyl-biguanide pretreatment BVP into a *Zusanli* (ST_{36}). BVP+ondan (▲, n=10), group of antagonist ondansetron pretreatment BVP into a *Zusanli* (ST_{36}). Asterisks indicate significantly different values between BVP+DMSO and BVP+ondan.
* : $p<.05$. ** : $p<.01$. *** : $p<.001$.
Significantly different from BVP+DMSO group.

Zusanli BVP treatment group (BVP+DMSO, n=10) at 10, 20, 30 and 45min after the initiation of BVP. This results show that the 5HT-3 receptor antagonist ondansetron pretreatment significantly blocked the BVP antinociceptive effects. There was no synergistic effect in the m-cholorophenyl-biguanide pretreatment *Zusanli* BVP treatment group (BVP+m-chlo, n=10). In the m-cholorophenyl-biguanide and ondansetron only treatment group, there were no statistically significant differences in TFL and likewise in the saline treatment group (data not shown).

E. Involvement of muscarinic cholinergic receptor on BVP Effect in CIA

The effects of muscarinic cholinergic receptor agonist and antagonist on BVP-induced antinociception in CIA are shown in Fig. 5. There were statistically significant differences in TFL between the antagonist atropine pretreatment *Zusanli* BVP treatment group (BVP+Atrop, n=10) and the DMSO pretreatment *Zusanli* BVP treatment group (BVP+DMSO, n=10) at 10, 20, 30 and 45min after the initiation of BVP.

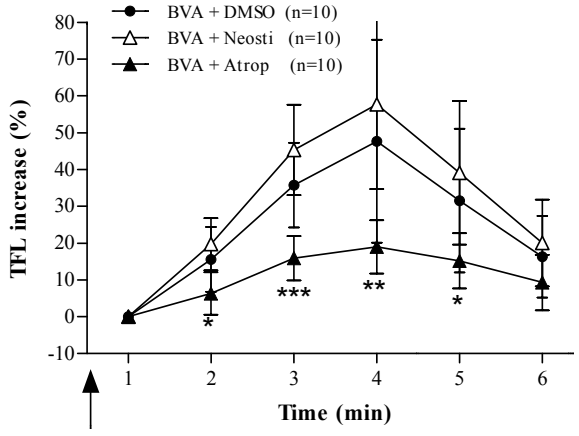

Fig. 5. Effects of muscarinic cholinergic receptor agonist and antagonist pretreatment (arrow) on BVP-induced antinociceptive effect in CIA

BVP+DMSO (●, n=10), group of DMSO pretreatment BVP into a *Zusanli* (ST_{36}). BVP+Neosti (△, n=10), group of agonist neostigmine pretreatment BVP into a *Zusanli* (ST_{36}). BVP+Atrop (▲, n=10), group of antagonist atropine pretreatment BVP into a *Zusanli* (ST_{36}). Asterisks indicate significantly different values between BVP+DMSO and BVP+Atro.

* : $p<.05$. ** : $p<.01$. *** : $p<.001$.

Significantly different from BVP+DMSO group.

This results show that the muscarinic cholinergic receptor antagonist atropine pretreatment significantly blocked the BVP antinociceptive effects. There was no synergistic effect in the neostigmine pretreatment *Zusanli* BVP treatment group (BVP+Neosti, n=10). In the neostigmine and atropine only treatment group, there were no statistically significant differences in TFL and likewise in the saline treatment group (data not shown).

Ⅳ. Discussion

In clinical area, Bee Venom Pharmacopuncture (BVP) has been widely used to treat rheumatoid arthritis (RA) and osteoarthritis (OA). And, in experimental study, BVP has shown the antinociceptive effect in RA and OA model[16,17]. Our study also demonstrated that *Zusanli* (ST_{36}) BVP markedly relieved inflammatory hyperalgesia produced by the induction of collagen-induced arthritis (CIA). Several studies have revealed the antinociceptive effect on inflammatory pain by using Freund's adjuvant arthritis model[16,17], but the antinociceptive mechanisms related with the inflammatory pain by using the bovine type II collagen-induced arthritis model have not been fully studied. Especially, collagen-induced arthritis (CIA) rats have been used as a rheumatoid arthritis model since this model was reported by Trentham et al[10,18].

From our study, we knew that the antinociceptive effect of BVP lasted for 60min after the initiation of bee venom injection; this result is in line with the findings in which BVP showed the antinociceptive effect at 10~55min post-formalin injection on inflammatory pain induced by formalin injection[6]. In other studies using the neuropathic pain model which is induced by constriction injury of sciatic nerve, the antinociception effect of BVP also lasted for 50min after the initiation of bee venom injection[9].

In general, it has known that two major components of endogenous descending antinociceptive

system are composed of intrinsic opioidergic and descending monoaminergic (i.e., adrenalin, serotonin and acetylcholine) systems in the brainstem[19]. Several types of acupoint stimulation techniques including electroacupuncture, Bee venom pharmacopuncture, moxibustion and acupressure have been used in order to produce antinociceptive effects that are selectively mediated by the endogenous descending modulatory systems[20-22]. In this study, we intended to examine the relation with monoaminergic systems including adrenalin, serotonin and acetylcholine. So, to reveal the antinociceptive effect of BVP on the inflammatory pain induced by CIA, agonists and antagonists of α1-adrenoceptor, 5HT-3 and muscarinic cholinergic receptors were pretreated before the initiation of BVP.

In result, we observed that the antinociceptive effect of *Zusanli* (ST_{36}) BVP was totally reversed by intraperitoneal pretreatment with the 5HT-3 receptor antagonist ondansetron and muscarinic cholinergic receptor antagonist atropine. In contrast, the BVP-induced antinociception was not affected by intraperitoneal pretreatment with α1-adrenoceptor antagonist prazosin. These results demonstrate that the antinociceptive effect of BVP on inflammatory pain is mediated by specific 5HT-3 and muscarinic cholinergic pathways rather than by the α1-adrenergic system. In other CIA studies, BVP showed antinociceptive effect by the activation of α2-adrenoceptor, 5HT-1 and 5HT-2 receptors, but not by μ-opioid receptor[11,23]. In electroacupunctural antinociceptive effect in CIA, several studies revealed that opioid receptors (μ-, δ-, κ-), α2-adrenoceptor, serotonergic receptors (5HT-1, 5HT-3) and muscarinic cholinergic receptor are related with the analgesic effect of electroacupucture (EA). So, there are some differences in between bee venom acupunctural antinociception and electroacupunctural antinociception in CIA rheumatoid arthritis animal model study.

To examine the whole mechanism related with BVP antinociception in chronic inflammatory pain induced by RA, further study should be done in relation with other types of opioid receptors (δ-, κ-), serotonergic receptor (5HT-4) and nicotinic cholinergic receptor. And, in relation with the interactive effects among receptors on BVP-induced antinociception, we also think the further study is needed to confirm this fact.

Ⅴ. Conclusion

Taken together, the present study demonstrated that *Zusanli* (ST_{36}) Bee venom pharmacopuncture showed the antinociception in a collagen-induced arthritis rat model of inflammatory pain, so Bee venom pharmacopuncture can be an effective therapy on inflammatory pain related with rheumatoid arthritis. Further, this antinociceptive effect of Bee venom pharmacopuncture was found to be related with the activation of 5HT-3 and muscarinic cholinergic receptors, but not spinal α1-adrenoceptor activity, especially on inflammatory in the rat model of collagen-induced arthritis.

Ⅵ. References

1. Dror M, Stephen AP. Rheumatoid arthritis. Philadelphia : Lippincott Williams & Wilkins. 2000 : 192-229.
2. Pincus T, Marcum SB, Callahan LF. Longterm drug therapy for rheumatoid arthritis in seven rheumatology private practices. J Rheumatol. 1992 ; 19 : 1885-94,
3. Billingham ME, Morley J, Hanson JM, Shipolini RA, Vernon CA. Letter. An anti-inflammatory peptide from bee venom. Nature. 1973 ; 245 : 163-4.
4. Eiseman JL, vonBredow J, Alvares AP. Effect of honeybee (Apis mellifera) venom on the course of adjuvant-induced arthritis and depression of drug metabolism in the rat. Biochem Pharmacol. 1982 ; 31 : 1139-46.
5. Hadjipetrou-Kourounakis L, Yiangou M. Bee

venom and adjuvant induced disease. J Rheumatol. 1984 ; 11 : 720.
6. Kim HW, Kwon YB, Han HJ, Yang IS, Alvin JB, Lee JH. Antinociceptive mechanisms associated with diluted bee venom acupuncture (apipuncture) in the rat formalin test: involvement of descending adrenergic and serotonergic pathways. Pharmacological Research. 2005 ; 51 : 183-8.
7. Kwon YB, Ham TW, Kim HW, Roh DH, Yoon SY, Han HJ, Yang IS, Kim KW, Alvin JB, Lee JH. Water soluble fraction(<10 kDa) from bee venom reduces visceral pain behavior through spinal alpha2-adrenergic activity in mice. Pharmacology Biochemistry & Behavior. 2005 ; 80 : 181-7.
8. Kwon YB, Kang MS, Han HJ, Alvin JB, Lee JH. Visceral antinociception produced by bee venom stimulation of the Zhongwan acupuncture point in mice: role of Alpha2-adrenoceptors. Neuroscience Letters. 2001 ; 308 : 133-7.
9. Roh DH, Kwon YB, Kim HW, Ham TW, Yoon SY, Kang SY, Han HJ, Lee HJ, Alvin JB. Acupoint stimulation with diluted bee venom (Apipuncture) alleviates thermal hyperalgesia in a rodent neuropathic pain model: involvement of spinal a2-Adrenoceptors. The Journal of Pain. 2004 ; 5(6) : 297-303.
10. Trentham DE, Townes AS, Kang AH. Autoimmunity to type II collagen; An experimental model of arthritis. J Exp Med. 1977. ; 146 : 857-68.
11. Baek YH, Huh JE, Lee JD, Choi DY, Park DS. Antinociceptive effect and the mechanism of bee venom acupuncture (Apipuncture) on inflammatory pain in the rat model of collagen-induced arthritis: mediation by a2-adrenoceptors. Brain Research. 2006 ; 1073-1074 : 305-10.
12. Zimmerman M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983 ; 16 : 109-10.
13. Grossman ML, Basbaum AI, Fields HL. Afferent and efferent connections of the rat tail flick reflex: A model used to analyse pain control mechanism. J Comp Neurol. 1982 ; 206 : 9-16.
14. Takeshige C. Differentiation between acupuncture and non-acupuncture points by association with analgesia inhibitory system. Acupunct Electro-Therap Res. 1985 ; 10 ; 195-203.
15. Medeiros MA, Canteras NS, Suchecki D, Mello LE. Analgesia and C-Fos expression in the periaqueductal gray induced by electroacupuncture at the Zusanli point in rats. Brain Res. 2003 ; 973 : 196-204.
16. Kwon YB, Lee HJ, Han HJ, Mar WC, Kang SK, Yoon OB, Alvin JB, Lee JH. The water-soluble fraction of bee venom produces antinociceptive and anti-inflammatory effects on rheumatoid arthritis in rats. Life sciences. 2002 ; 71 : 191-204.
17. Kwon YB, Lee JD, Lee HJ, Han HJ, Mar WC, Kang SK, Alvin JB, Lee JH. Bee venom injection into an acupuncture point reduces arthritis associated edema and nociceptive responses. Pain. 2001 ; 90 : 271-80.
18. Trentham DE, McCune WJ, Susman P, David JR. Autoimmunity to collagen in adjuvant arthritis of rats. J Clin Invest. 1980 ; 66 : 1109-17.
19. Ernst E, White A. Acupuncture: a specific appraisal. Oxford : Butterworth-Heinemann. 1999 : 1.
20. Han JS. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. Trends Neurosci. 2003 ; 26 : 17-22.
21. Takagi J, Yonehara N. Serotonin receptor subtypes involved in modulation of electrical acupuncture. Jpn J Pharmacol. 1998 ; 78 : 511-4.
22. Ulett GA, Han S, Han JS: Electroacupuncture: mechanisms and clinical application. Biol Psychiatry. 1998 ; 44 : 129-138.
23. Seo BK, Baek YH, Choi DY, Park DS. The analgesic effect of bee venom acupuncture and its mechanism on the type 2 collagen-induced arthritis rats. Korean Acupuncture and Moxibustion. 2005 ; 22(2) : 19-32.