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원 저

The Analgesic Effect of Bee Venom Acupuncture and Its Mechanism on the Type II Collagen-Induced Arthritis Rats

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Abstract

Objectives : to evaluate the analgesic effect of bee venom acupuncture on Choksamni (ST36) in the collagen-induced arthritis rats and investigate the role played by serotonergic receptor subtypes (5-HT1a, 5-HT2a) in the antinociceptive effect of bee venom acupuncture in a thermal hyperalgesia test

Methods : Experiments were performed on 5 week-aged 60 male Sprague-Dawley rats according to National Institute of Health guidelines and the ethical guidelines of the International Association for the Study of Pain (IASP). Arthritis was induced with arthrogenic collagen emulsion (Bovine type II collagen ㎍ with incomplete Freund's adjuvant 100 ㎍). The onset of arthritis was considered to be present when erythema and swelling were detected in at least one joint. The thermal hyperalgesia was evaluated weekly with tail flick test in the rats of severity grade 3 without any injury at tail and foot (including inflammation, ulceration, snap). In the fourth week after first immunization, the analgesic effect of bee venom acupuncture (Choksamni, ST36) was measured with consecutive tail flick latency after intraperitoneal injection of spiroxatrine (1mg/kg) and spiperone (1mg/kg).

Results : Chronic inflammatory pain was induced as time elapsed after the immunization of arthrogenic collagen and the maximum value was reached from third to fifth week. Chronic inflammatory pain induced by CIA was inhibited by bee venom acupuncture on the left ST36. The analgesic effect of bee venom acupuncture was inhibited by intraperitoneal injection of 5-HT1a antagonist spiroxatrine and 5-HT2a antagonist spiperone.

Conclusions : Therefore, a conclusion that the analgesic effect of bee venom acupuncture in the chronic inflammatory pain is partially mediated by 5-HT1a and 5-HT2a receptors can be made.

Key words : bee venom, Choksamni (ST36), serotonergic mechanism, spiroxatrine, spiperone

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Ⅰ. Introduction

Rheumatoid arthritis (RA) is a systemic, inflammatory autoimmune disorder that presents as a symmetric polyarthritis associated with swelling and pain in multiple joint, often initially occurring in the joints of the hands and feet[1]. Pain associated with RA can occur spontaneously or can be evoked by gentle stimulation of the joint when it is moved within its normal working range[2]. The ability to feel pain is essential for the survival and wellbeing of organisms. It alerts the organism to imminent danger. The experience of acute pain serves a crucial biological purpose of alerting a living organism against environmental dangers, inducing behavioral responses which protect the organism from further damage. In contrast, chronic pain arising from disease states and pathological responses of the nervous system offers no advantage and may be debilitating to those afflicted[3].

As a traditional therapy for rheumatoid arthritis, bee venom acupuncture has been applied to relieve inflammation and pain in clinical situation[4]. In spite of the wide clinical application as pain-relieving agents, the mechanistic aspects of the bee venom acupuncture remain unclear. In the recent studies, it has been revealed that the analgesic effect of bee venom acupuncture was mediated by adrenergic mechanism[5], not opioidergic mechanism[6].

Although numerous studies have shown that spinally projecting serotonergic cells mediate opioid analgesia and the serotonergic modulation produces inhibition on the transmission of nociceptive information in the spinal cord in chronic inflammatory pain[7], the conflict on the role of the specific subtypes of serotonin receptors in the pain modulation does exist. In spinal cord, 5-HT1a[8] and 5-HT2a[9] receptors were reported to mediate the central modulation in chronic painful situation. Intrathecal administration of 5-HT1a and 5-HT2a agonist were reported to prolong the tail flick latency[10] and 5-HT1a and 5-HT3 receptors were reported to mediate partially the analgesic effect of electroacupuncture[11]. On the contrary, activation of 5-HT1a and 5-HT2a receptors were reported to suppress the electroacupuncture-induced analgesia[12] and the inflammatory pain induced by bee sting was reported to be mediated by 5-HT1a receptors[13].

The objective of the present study is to evaluate the analgesic effect of bee venom acupuncture on Choksamni (ST36) in the collagen-induced arthritis rats and investigate the role played by serotonergic receptor subtypes (5-HT1a, 5-HT2a) in the antinociceptive effect of bee venom acupuncture in a thermal hyperalgesia test.

Ⅱ. Materials and Methods

1. Animals

Experiments were performed on 5 week-aged 60 male Sprague-Dawley rats (Samtaco, Osan, Korea) weighing 150±10 g at the beginning of the experiment. They were kept in a 12:12 light-dark cycle (light on 6:00 AM to 6:00 PM) in specific pathogen free condition at a controlled temperature (22±1℃) and humidity (55±5%). Food and water were available ad libitum. The food was directly available on the sawdust in the cage to minimize the need for animals to make potentially painful movements to obtain food.

The experimental procedures were carried out according to National Institute of Health guidelines (NIH publication No. 86-23, revised 1985) and the ethical guidelines of the International Association for the Study of Pain (IASP) for investigating experimental pain in conscious animals.

2. Drugs

Type II collagen (BnCII, Chondrex Arthrogen-CIA Immunization Bovine Type II Collagen) and incomplete Freund's adjuvant (iCFA) were purchased from Chondrex (Washington, USA). 5-HT1a antagonist spiroxatrine (M.W.=379.44) and 5-HT2a antagonist spiperone (M.W.=431.94) were purchased from Tocris (Bristol, UK) and were dissolved in 10% of dimethyl sulfoxide (DMSO, SIGMA, St. Louis, MO, USA) just before use.

3. Preparation of bee venom

Bee venom was extracted from honey bee by electrical stimulation on the brain, Then it was dried on sterile plate and electrified to obtain homogenized bee venom powder. This was done at the Kyung Hee Medical Center (Seoul, Korea). The dried bee venom was dissolved in physiological saline (0.3%) and filtered by 0.22 ㎛ syringe filter.

4. Induction of collagen-induced arthritis model

Bovine type II collagen (Chondrex Inc., Washington, USA) extracted from bovine articular cartilage was dissolved overnight at 4℃ in 10 mM acetic acid at 4.0 mg/ml. The solution was emulsified in the equal volume of incomplete Freund's adjuvant (iCFA, Chondrex Inc., Washington, USA) and mixed for 30 minutes at 1,000 rpm with a homogenizer. Arthritis was induced by an intradermal injection of 50 ㎕ of the emulsion (BnCII 100 ㎍ with iCFA 100 ㎍) into the base of the tail. Rats were boosted subcutaneously into the plantar surface with the same volume of the emulsion (BnCII 100 ㎍ with iCFA 100 ㎍) at fourteenth day after first injection[14]. The onset of arthritis was considered to be present when erythema and swelling were detected in at least one joint and the thermal hyperalgesia was evaluated weekly with tail flick test.

5. Evaluation of severity of arthritis

The severity of inflammation was scored subjectively as grade 0, 1, 2, 3 or 4 in the fourth week after initial sensitization. The criteria for the severity of inflammation was made on the basis of erythema and edema of the periarticular tissue, such as; (0) normal appearance without any erythema and edema, (1) detectable erythema and edema restricted in the mid foot (restricted from ankle joint to tarsal area), (2) moderate erythema and edema in the mid foot, (3) marked erythema and edema at the entire foot including toe, and (4) severe erythema and edema at the entire foot including toe[15].

6. Grouping and experimental protocol

The subjects of severity grade 3 without any injury at tail and foot (including inflammation, ulceration, snap) were divided into six groups in the fourth week from first immunization:

(1) Arthritic animals without any treatment for the measurement of TFL every week (n=10)

(2) Arthritic animals without any treatment (Control, n=10)

(3) BV-treated arthritic animals without any i.p. injection (BV, n=10)

(4) DMSO-pretreated (i.p.)/BV-treated arthritic animals (DMSO-BV, n=10)

(5) Spiroxatrine-pretreated (i.p.)/BV-treated arthritic animals (SPROX-BV, n=10)

(6) Spiperone-pretreated (i.p.)/BV-treated arthritic animals (SPPRO-BV, n=10)

Tail flick latency to thermal stimulation was measured at baseline, 1, 2, 3, 4 and 5 week after first immunization with bovine type II collagen emulsified in iCFA to evaluate the time-related change of the pain-avoiding behavior in the collagen-induced arthritis rats. The algesiometric assays to evaluate the analgesic effect of bee venom acupuncture and influence of spiroxatrine and spiperone on the analgesic effect of bee venom acupuncture were performed in the fourth week after first immunization. Rats were received intraperitoneal injection of 1 mg/kg of spiroxatrine, spiperone, or 1 ml/kg of vehicle (10% of DMSO) with the same volume per body mass, followed 20 minutes later by first measurement of tail flick latency. Just after first measurement of tail flick latency, 1 mg/kg of bee venom dissolved in physiological saline was subcutaneously injected into left Choksamni (ST36). Consecutive tail flick latency was measured at 10, 20, 30, 45 and 60 minutes after bee venom acupuncture[6]. Preliminary test was done to find out the effective dosage with safety of bee venom acupuncture and all the antagonists used. The ST36 was located 5 mm lower and lateral to the anterior tubercle of tibia by Koh's method[16].

7. Thermal hyperalgesia test (tail flick latency)

The examination of nociceptive response to thermal stimuli was made by the tail flick unit (Ugo Basile Model 7360, Comrio, Italy). The tail-flick unit was designed to perform rapid precise testing of analgesic compounds using the method described in the "Tail Flick Test", according to D'Amour & Smith[17]. The heat from a 50 watt I.R. projector bulb was directed onto the marked point of the tail and the power adjusted so that the rat flicked its tail away from the noxious stimuli in approximately 3-4 seconds. At this intensity of stimulation, three stable consecutive readings were obtained at different adjacent site of marked point of the tail of rats. The response at each time was calculated as a percentage of change of tail flick latency:

$$\text{Change of TFL (\%)} = \frac{\text{TFL acquired} - \text{baseline TFL}}{\text{baseline TFL}} \times 100$$

A cut-off time of 10 seconds was set to prevent thermal injury of the rat's tail. Rats were accommodated in the cylinder-shaped holder (6.0×18.0 cm) three times a week to reduce the stress of tail flick test[8]. All the process was performed in an air-conditioned noiseless closed laboratory room to minimize environmental stress.

8. Statistical analysis

All the data were described as percentage changed and compared with control group at each tested time point. Data were presented as the mean ± S.D. The Friedman's rank test (followed by Dunnett's post-hoc test) was used to compare the data distribution by time. The statistical difference at each time point between groups was evaluated by Mann Whitney U test. Throughout, $p<0.05$ was considered to be statistically significant. SPSS 11.5 for windows was used for statistical analysis.

Table 1. Changes of Tail Flick Latency in the Collagen-induced Arthritis Rats

No of animals	Change of tail flick latency (%)					
	baseline	1 week	2 week	3 week	4 week	5 week
n = 10	0	-17.41±1.97	-37.19±1.96	-44.41±2.05	-45.7±1.54	-46.6±0.99

Results were presented as mean ± S.D.

Table 2. Changes of Tail Flick Latency by Bee Venom Acupuncture in the collagen-induced Arthritis Rats

Group	No. of animals	Change of tail flick latency (%)					
		0 min	10 min	20 min	30 min	45 min	60 min
BV	10	0	10.14±2.17**	25.98±6.95**	36.74±5.86**	22.95±5.66**	7.99±3.69**
control	10	0	1.17±1.22	2.77±1.05	1.56±0.41	-0.63±1.13	0.40±1.43

Results were presented as mean ± S.D. The statistical difference between groups was evaluated by Mann Whitney U test. **p<0.01, compared with control group

BV: group of arthritic animals treated by 1 mg/kg of bee venom acupuncture on the ST36, control: group of arthritic animals without any treatment

III. Results

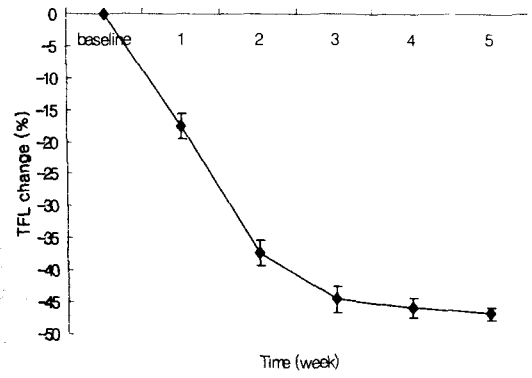

Fig. 1. Changes of tail flick latency in the collagen-induced arthritis rats.

1. Change of tail flick latency in the collagen-induced arthritis rats

Tail flick latency was measured at baseline, 1, 2, 3, 4 and 5 week after first immunization with bovine type II collagen emulsified in iCFA. The results were shown in Table I and Fig. 1. Tail flick latency began to decrease with time elapsed after first immunization. The gradient of downslope was blunted from third week, and began to draw plateau from third to fifth week.

Rats were immunized with bovine type II collagen emulsified in iCFA, followed by booster injection at day 14 with collagen emulsified in iCFA. After first immunization, TFL decreased as time elapsed and reached the minimum value from third to fifth week. Time = baseline: first immunization with bovine type II collagen emulsified in iCFA. Results were presented as mean ± S.D. in percentage of TFL change on each tested week

2. Analgesic effect of bee venom acupuncture

Tail flick latency in the control group and the BV group was measured in the fourth week after first immunization with bovine type II collagen emulsified in iCFA. The BV group

was treated with 1 mg/kg of bee venom acupuncture on the left ST36. The results were shown in Table II and Fig. 2. In the control group, tail flick latency was maintained almost the same. In the BV group, tail flick latency increased significantly ($p<0.01$) from 10 minutes after 1 mg/kg of bee venom acupuncture on the left ST36. It lasted for more than 60 minutes.

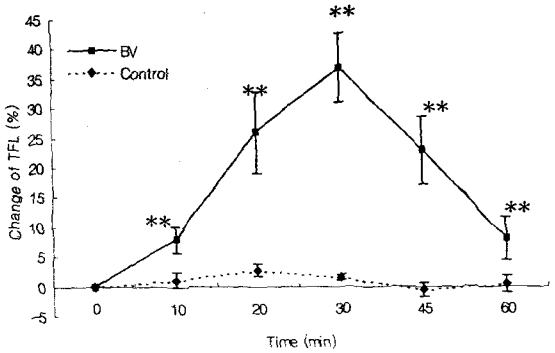

Fig. 2. Effect of bee venom acupuncture on the changes in tail flick latency

Control group was composed of arthritic animals without any treatment. BV group was composed of arthritic animals treated by 1 mg/kg of bee venom acupuncture on the left ST36. Time = 0 min: 1 mg/kg of bee venom acupuncture on the left ST36. Results were presented as mean ± S.D. The statistical difference between groups was evaluated by Mann Whitney U test. **p<0.01, compared with control group

3. Analgesic effect of bee venom acupuncture after spiroxatrine pretreatment

Tail flick latency in the DMSO-BV group and the SPROX-BV group was measured in the fourth week after first immunization with bovine type II collagen emulsified in iCFA. The SPROX-BV group was received intraperitoneal injection of 1 ml/kg of spiroxatrine dissolved in the 10% of DMSO

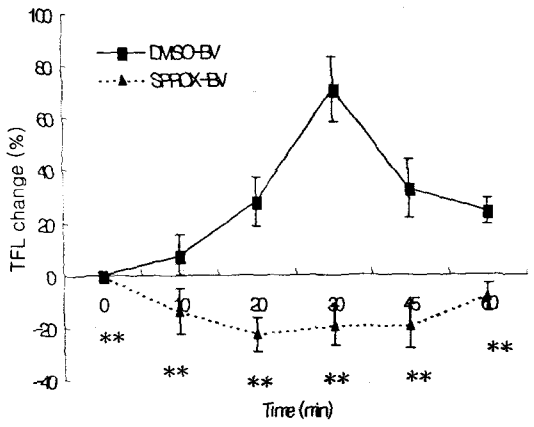

Fig. 3. Effect of bee venom acupuncture on the changes of tail flick latency

DMSO-BV group was composed of arthritic animals pretreated intraperitoneally with 1 ml/kg of 10% DMSO followed 20 minutes later by 1 mg/kg of bee venom acupuncture on the left ST36. SPROX-BV group was composed of arthritic animals pretreated intraperitoneally with 1 mg/kg of spiroxatrine dissolved in 10% DMSO followed 20 minutes later by 1 mg/kg of bee venom acupuncture on the left ST36. time = -20 min (arrow): intraperitoneal injection of 1 ml/kg of 10% DMSO or 1 mg/kg of spiroxatrine dissolved in 10% DMSO, time = 0 min: 1 mg/kg of bee venom acupuncture on the left ST36. Results were presented as mean ± S.D. The statistical difference between groups was evaluated by Mann Whitney U test. **p<0.01, compared with DMSO-BVgroup

Table 3. Changes of Tail Flick Latency by Bee Venom Acupuncture with Spiroxatrine Pretreatment in the Collagen-induced Arthritis Rats

Group	No. of animals	Change of tail flick latency (%)					
		0 min	10 min	20 min	30 min	45 min	60 min
SPROX-BV	10	0	-14.15±8.71**	-22.83±6.31**	-19.39±7.58**	-19.72±8.23**	-8.57±5.안전**
DMSO-BV	10	0	4.99±9.36	18.66±11.84	61.17±27.87	30.08±17.77	19.23±10.21

Results were presented as mean ± S.D. The statistical difference between groups was evaluated by Mann Whitney U test. **p<0.01, compared with DMSO-BV group
SPROX-BV: group of arthritic animals pretreated intraperitoneally with 1 mg/kg of spiroxatrine dissolved in 10% DMSO followed 20 minutes later by 1 mg/kg of bee venom acupuncture on the left ST36, DMSO-BV: group of arthritic animals pretreated intraperitoneally with 1 ml/kg of 10% DMSO followed 20 minutes later by 1 mg/kg of bee venom acupuncture on the left ST36

followed 20 minutes later by 1 mg/kg of bee venom acupuncture on the left ST36. The results were shown in Table III and Fig. 3. In the SPROX-BV group, tail flick latency decreased significantly ($p<0.01$) compared with the DMSO-BV group at each measurement from 10 minutes after 1 mg/kg of bee venom acupuncture on the left ST36. The gradient of downslope was blunted from 10 minutes after 1 mg/kg of bee venom acupuncture on the left ST36. After 20 minutes, tail flick latency began to draw a up-gradiented curve to normalized level as time gone by.

4. Analgesic effect of bee venom acupuncture after spiperone pretreatment

Tail flick latency in the DMSO-BV group and the SPPRO-BV group was measured in the fourth week after first immunization with bovine type II collagen emulsified in iCFA. The SPPRO-BV group was received intraperitoneal injection of 1 ml/kg of spiperone dissolved in the 10% of DMSO followed 20 minutes later by 1 mg/kg of bee venom acupuncture on the left ST36. The results were shown in Table IV and Fig. 4. In the SPPRO-BV group, tail flick latency decreased significantly ($p<0.01$) compared with the DMSO-BV group at each measurement from 10 minutes after 1 mg/kg of bee venom acupuncture on the left ST36. The gradient of downslope was blunted from 10 minutes after 1 mg/kg of bee venom acupuncture on the left ST36. After 30 minutes, tail flick latency began to draw a up-gradiented curve to normalized level as time gone by.

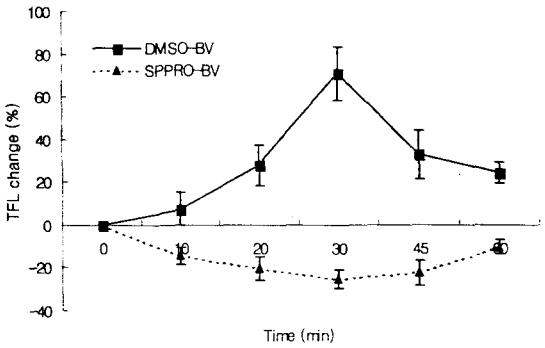

Fig. 4. Effect of bee venom acupuncture on the changes of tail flick latency

DMSO-BV group was composed of arthritic animals pretreated intraperitoneally with 1 ml/kg of 10% DMSO followed 20 minutes later by 1 mg/kg of bee venom acupuncture on the left ST36. SPPRO-BV group was composed of arthritic animals pretreated intraperitoneally with 1 mg/kg of spiperone dissolved in 10% DMSO followed 20 minutes later by 1 mg/kg of bee venom acupuncture on the left ST36. time = -20 min (arrow): intraperitoneal injection of 1 ml/kg of 10% DMSO or 1 mg/kg of spiperone dissolved in 10% DMSO, time = 0 min: 1 mg/kg of bee venom acupuncture on the left ST36. Results were presented as mean ± S.D. The statistical difference between groups was evaluated by Mann Whitney U test. **$p<0.01$, compared with DMSO-BV group

Table 4. Changes of Tail Flick Latency by Bee Venom Acupuncture with Spiperone Pretreatment in the Collagen-induced Arthritis Rats

Group	No. of animals	Change of tail flick latency (%)					
		0 min	10 min	20 min	30 min	45 min	60 min
SPPRO-BV	10	0	-14.34±4.02**	-20.10±5.49**	-25.03±4.36**	-22.39±5.92**	-10.23±3.33**
DMSO-BV	10	0	4.99±9.36	18.66±11.84	61.17±27.87	30.08±17.77	19.23±10.21

Results were presented as mean ± S.D. The statistical difference between groups was evaluated by Mann Whitney U test. **$p<0.01$, compared with DMSO-BV group

SPPRO-BV: group of arthritic animals pretreated intraperitoneally with 1 mg/kg of spiperone dissolved in 10% DMSO followed 20 minutes later by 1 mg/kg of bee venom acupuncture on the left ST36, DMSO-BV: group of arthritic animals pretreated intraperitoneally with 1 ml/kg of 10% DMSO followed 20 minutes later by 1 mg/kg of bee venom acupuncture on the left ST36

Ⅳ. Discussions

Recent advances in pain research have begun to identify the mechanistic basis of chronic inflammatory pain. In RA, it appears that peripheral nociceptors become sensitized due to an altered cytokine milieu or changes in the expression of ion channels, receptors, neurotransmitters, and neurotrophins. Central sensitization is initiated in part by activation of key intracellular signal cascades bridging synaptic events to nuclear responses[3]. For example, the peripheral terminals of Aδ and C fibers, which are activated only by noxious stimuli under non-inflamed conditions, express many receptors and ion channels that recognize the various inflammatory mediators in the vicinity[2]. Thus, the local inflammation related with rheumatoid arthritis results in the release of multiple factors that activate local nerve terminals involved in pain preception. Chemical messengers such as histamine, bradykinin, serotonin, prostaglandin E2 (PGE2), adenosine triphosphate (ATP) and protons, can directly activate nociceptive peripheral afferent neurons leading to propagation of action potentials to the spinal cord, while others lower the threshold of activation of these neurons known as hypersensitization[18]. In addition, during chronic inflammation, Aβ fibers, which normally respond only to tactile rather than noxious stimuli, can mediate a long lasting tactile allodynic state. The heightened state of sensory/noxious processing mechanisms at the site of inflammation is referred to as peripheral sensitization[3]. During inflammation, the peripheral nerve endings of nociceptive fibers release various neuromediators, namely substance P, calcitonin gene related peptide (CGRP), or somatostatin, into the microenvironment. These in turn can modulate the inflammatory process (neurogenic inflammation) as well as autoactivate the sensory neurons via cognate receptors expressed on the nerve terminals[2]. The central terminals of peripheral sensory afferents transmit noxious information to secondary nociceptive neurons of the spinal dorsal horn, via the synaptic release of excitatory amino acids (EAAs)[18].

The oldest documental record ever on the bee venom acupuncture in oriental medicine was found in the tomb of Mawangdui of BC 168[19]. The bee venom acupuncture on certain acupoints tonifies and balances Yin-Yang by stimulating meridian system both chemically and mechanically[20], and exerts anti-inflammatory, analgesic, immuno-regulatory, circulatory, anti-bacterial, radioresistant, allergen, algesic and cytotoxic effect[20]. It has been revealed that bee venom contains a variety of different peptides including melittin, apamin, adolapin and mast cell degranulating (MCD) peptide[20]. It also contains enzymes (including phospholipase A2, hyaluronidase, acid phosphomonoesterase, etc.), biologically active amines (including histamine, dopamine, noradrenalin, etc.) and non-peptide components (including lipids, carbohydrates and free amino acids, etc.)[20] and bee venom acupuncture has been widely applied for relieving inflammation and pain in many disease such as rheumatoid arthritis[4]. In the previous studies, long-term bee venom acupuncture suppressed the induction of experimental arthritis[4], reduced severity of arthritis[21] and inhibited the production of inflammatory cytokines such as TNF-α[15]. Better anti-inflammatory effect was achieved by selecting the specific acupoint[22] and by selecting the water soluble fraction of whole bee venom[23]. In addition, bee venom acupuncture in a localized inflammatory state exerted a potent antinociceptive effect[21], and the analgesic effect

was possibly mediated by the effect of bee venom itself or possible other mechanism such as counter-irritation[21], and more potent effect was achieved by appropriately selection of acupoint (ST36)[22]. On the contrary, some studies reported the algesic property of bee venom. Experimental honey bee sting produced long-term spinal neuronal changes as well as persistent spontaneous nociception, heat /mechanical hypersensitivity and inflammatory responses[24] and chemical injury[25]. And Wang et al.[13] described that the inflammatory pain in such situation was mediated by 5-HT1a receptor.

It has been proposed that antinociceptive effect of bee venom acupuncture is mediated by different neuronal mechanisms depending on the type of stimulation applied to an acupoint[22]. Choksamni (ST36) is a he-sea and earth point of the stomach channel, gao wu command point, point of the sea of water and grain, located below the knee, 3 cun inferior to Tokpi (ST35), one finger breadth lateral to the anterior crest of the tibia and harmonizes the stomach, fortifies the spleen, resolves dampness, supports the correct Qi, fosters the original Qi, tonifies Qi, nourishes blood and Yin, clears fire, calms the spirit, activates the channel, revives the Yang and restores consciousness[26], and alleviates pain, such as stomachache, chest pain, abdominal pain, headache, flank pain and pain on the lower extremity including knee[27].

The biological responses induced by stimulation at certain acupoints occur both locally and distantly through the activation of pathways in the peripheral and central nervous systems[28] and the analgesic effect of acupoints stimulation is mediated by specific descending modulatory systems[28]. In general, multiple supraspinal sites of the descending pain modulatory system exert powerful effect on the inhibitory response of the nociceptive messages at the spinal level[29]. The central pathway may involve the ventrolateral spinal cord column, dorsal periaqueductal gray matter, lateral hypothalamus, lateral septum, cingulate bundle, dorsal hippocampus, habenulo-interpeduncular tract, and anterior hypothalamus[30]. In these supraspinal structures, the rostral ventromedial medulla (RVM), including the nucleus raphe magnus (NRM), the adjacent gigantocellularis pars alpha (NGC α) and ventral nucleus reticularis gigantocellularis (NGC), plays a crucial role in descending pain modulation. The NRM is a major source of the descending brainstem serotonergic pathways and the pontine locus coeruleus/subcoeruleus (LC/SC) sends the descending noradrenergic projections to the spinal dorsal horn in rats[31].

In spite of the numerous studies showing the importance of the central serotonergic pathways in pain regulation, the exact nature of the receptors involved in the serotonin-related modulation of pain in the spinal cord remains to be elucidated[30]. 5-HT produces its effect through a variety of membrane-bound receptors. 5-HT receptors are divided into seven distinct classes (5-HT1 to 5-HT7) largely on the basis of their structural and operational characteristics[32]. There is some conflict about the role of 5-HT receptor subtype in analgesic effect and the marked inconsistencies between these results may reflect the complexity of spinal serotonergic mechanisms but also raise questions regarding the influence of methodological aspects[33]. While 5-HT is known as a potent proinflammatory and noxious agent, which plays a important role in hyperalgesia and inflammation in periphery, a growing body of evidence indicates that descending serotonergic pathways in the CNS

are involved in the central regulation on pain transmission[9]. 5-HT has antinociceptive effect in several pain models including persistent neuropathic pain model (CCl model, according to Bennett and Xie, 1988), chronic diabetic neuropathic pain model (streptozocin, Courteix et al., 1993) and localized, less persistent inflammatory pain model (carageenan, Winter et al., 1962)[34]. In the tail flick test, intrathecally administered 5-HT1a antagonists do not alter or produce a dose-related blockade of 5-HT-induced antinociception[35]. Intrathecal administration of 5-HT1a agonists has been reported to facilitate, to inhibit or to not modify pain reactions[7,8]. Activation of the 5-HT2 receptors has been reported to facilitate or to inhibit the transmission of the nociceptive impulse[9].

Since collagen-induced arthritis (CIA) in rats and mice is well known to have both clinical and histological similarities to human RA, these models have been widely used to evaluate the anti-inflammatory and anti-nociceptive effect of anti-arthritic drugs[15]. CIA is a chronic inflammatory disease model bearing all the hallmarks of rheumatoid arthritis, e.g. polyarthritis, synovitis, and subsequent cartilage/bone erosions. Animals immunized with type II collagen develop an autoimmune polyarthritis that shares several clinical and histological features with rheumatoid arthritis. One feature of the disease contributing to joint damage is synovial hyperplasia[36]. Concomitant with the inflammatory process and hyperalgesia, there is an increased peripheral barrage of nerve impulses into the spinal cord leading to hyperexcitability of dorsal horn nociceptive neurons or central sensitization. All of these changes lead to increased neuronal activity at supraspinal sites and presumably contribute to increases in pain sensation and related responses[37]. In the present study, tail flick latency began to decreased as time elapsed after immunization with arthrogenic type II collagen and the decrement of tail flick latency reached the minimum value from 3rd to 5th week. The disposition obtained from this study correspond to other recent reports.

In the various behavioral tests to assess analgesic effect of drugs in short period, we chose the tail-flick test for it's convenience and reproducibility[8,17]. Like other behavioral tests, tail flick test has some drawbacks. There is evidence that the results obtained using the tail-flick test can be seriously misinterpreted if the tail skin temperature is not monitored properly, especially in the studies using the 5-HT or 5-HT agonist, because 5-HT has strong vascular effect and can modify temperature of tail[38]. Another problem is that spinal 5-HT is involved not only in nociception but also in non-nociceptive functions, such as motor control, which may in turn affect the response in nociceptive testing[38]. In the present study, to avoid these problems related with tail flick test, all the experiments was carried out in closed laboratory room with standardized temperature and minimized noise level. And rats with injured tail (inflammation, ulcer, snap) were eliminated from experimental group before evaluation.

While it is recently reported that acupoint stimulation activates central serotonergic systems and central serotonergic system mediates analgesic effect of acupuncture in parts, the conflict over the pain modulatory effect of acupuncture still exists. Takeshige et al.[39] demonstrated that acupuncture anesthesia descending through PAG is eventually serotonergic based on their experiment in which acupuncture effect were antagonized by methysergide. Kim et al.[40] reported that bee

venom acupuncture on the ST36 in rats increased the number of Fos immunoreactive neurons and the activity of serotonergic neurons, and the therapeutic effect of bee venom acupuncture may be associated with endogenous modulatory system such as serotonin according to the result. Chang et al.[11] reported that 5-HT1a and 5-HT3 receptors partially mediate the analgesic effect of electroacupuncture, but 5-HT2a receptor is controversly involved in the nociceptive response. Takagi et al.[12] suggested that 5-HT1, except 5-HT1a; 5-HT2, except 5-HT2a; and 5-HT3 receptors are positively involved in electroacupuncture-induced analgesia, whereas the activation of 5-HT1a and 5-HT2a receptors suppressively act on electroacupuncture-induced analgesia. In the present study, thermal hyperalgesia evaluated by tail flick latency was inhibited by bee venom acupuncture on the left ST36 and the analgesic effect lasted for more than 60 minutes from bee venom acupuncture. As Eide et al. previously reported that the tail flick reflex was markedly depressed 5-20 min after administration of 5-HT1a & 5-HT2a agonist and more markedly inhibited by stimulation of 5-HT1a than 5-HT2a receptors[10], in the present study, the tail flick latency increased as time gone by and reached to the maximum value from 30 min after bee venom acupuncture on the left ST36. And the increased tail flick latency inhibited by 5-HT1a antagonist spiroxatrine and 5-HT2a antagonist spiperone. Therefore we can conclude that bee venom acupuncture on the left ST36 alleviates chronic inflammatory pain in rheumatoid arthritis, and the analgesic effect of bee venom acupuncture is related with serotonergic modulatory system on pain transmission and mediated by 5-HT1a and 5-HT2a receptors.

Our findings conclusively revealed the analgesic effect of bee venom acupuncture in chronic inflammatory pain in the collagen-induced arthritis rats. And It is noteworthy that the analgesic effect of bee venom acupuncture on the chronic inflammatory pain was inhibited by 5-HT1a antagonist and 5-HT2a antagonist because it is suggested that the effect of bee venom acupuncture was mediated by serotonergic mechanism. Thus, further investigation on the correlation between the effect of bee venom acupuncture with other various subtypes of serotonin receptors must be made in different animal models and in other algesiometric methods.

V. Conclusions

Based on the present study concerning the analgesic effect of bee venom acupuncture applied on the left ST36 in the chronic inflammatory pain model induced by immunization with bovine type II collagen emulsified incomplete Freund's adjuvant evaluated through the thermal hyperalgesia test (tail flick test), it reveals that:

1. Chronic inflammatory pain was induced as time elapsed after the immunization of arthrogenic collagen and the maximum value was reached from third to fifth week.
2. Chronic inflammatory pain induced by CIA was inhibited by bee venom acupuncture on the left ST36. The analgesic effect reached the maximum value after about 30 minutes and lasted for more than 60 minutes from bee venom acupuncture.
3. The analgesic effect of bee venom acupuncture in the chronic inflammatory pain was inhibited by intraperitoneal injection of

5-HT1a antagonist spiroxatrine and 5-HT2a antagonist spiperone.

4. Therefore, a conclusion that the analgesic effect of bee venom acupuncture in the chronic inflammatory pain is partially mediated by 5-HT1a and 5-HT2a receptors can be made.

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