Pain Relieving Effects of the Acupuncture and Electroacupuncture on the Arthritic Model of the Rat

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Pain Relieving Effects of the Acupuncture and Electroacupuncture on the Arthritic Model of the Rat

Directed by Professor Bae Hwan Lee

The Master's Thesis submitted to the Department of Medical Science, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medical Science

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June 2003

This certifies that the Master's Thesis
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June 2003

ACKNOWLEDGEMENTS

생명공학도가 되고자 부푼 꿈을 안고 시작한 길. 이제 어느덧 석사를 마치게 되었습니다. 열정과 혼신의 힘을 다해 ‘신경과학’이라는 학문에 푹 빠져보고 싶었지만, 못내 아쉬움이 많이 남습니다. 부족함이 많은 제가 이렇게 하나의 결실을 이룰 수 있었던 것은 모두 저를 아껴주시고, 사랑해주신 분들의 한없는 마음 덕분이라 생각합니다.

먼저, 부족한 저를 이끌어 주시고, 지도해 주신 이배환 선생님께 머리 숙여 감사드립니다. 그리고, 바쁘신 와중에도 제 논문을 바로 잡아주시고, 심사해주신 윤덕미 선생님과 장진우 선생님께 감사드립니다.

늘 곁에서 무한한 사랑과 격려로, 때론 호된 꾸지람으로 저의 원동력이 되어주시는, 저의 변함없는 후원자이신 아버지와 어머니께 깊은 감사를 드립니다. 그리고, 언제나 형을 믿고 잘 따라주는 동생 창환이에게도 감사의 말 전합니다.

힘들고 어려운 시간들 함께 해온 우리 실험실 가족들에게도 감사드립니다. 그리고, 언제나 마음속에서 잔잔히 피어오르는, 진한 향기를 지닌 나의 친구들에게도 심심한 감사의 말을 전합니다.

꿈은 계속 될 것입니다.

2003년 6월

꼬마 과학자!

오 진 환

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ABSTRACT

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Acupuncture has traditionally been used in oriental medicine to relieve arthritic pain. Arthritis accompanying with severe pain became the most popular disease as more persons live to advanced ages. In the present study, the effect of acupuncture and electroacupuncture was examined in an animal model of arthritic pain induced by the injection of carrageenan into the knee joints of the rat. Adult male Sprague-Dawley rats were used. Under urethane anesthesia, the arthritis was induced by the injection of 2% carrageenan into the left knee joint cavity of rats. To record neuronal activity of articular nerve afferents, the saphenous nerve was cut distally from the knee joint and centrally in the inguineal region. The left femur and tibia were fixed by a grip and a mineral oil pool was made. Nerve fibers were characterized by their mechanical sensitivity to passive movement of the joint consisting of outward and inward movement. Different acupuncture points were stimulated using conventional acupuncture technique and the neural responses of the afferents to mechanical stimulation were recorded. After electroacupuncture was applied onto the Zusanli and

Yinlingquan acupoints, the same procedure was repeated. Following acupuncture in Zusanli, Yinlingquan, and Hegu, neural activities of articular afferents tended to reduce. After electroacupuncture of low frequency (2 Hz) and high frequency (100 Hz) stimulations, neural activity of articular afferents tended to reduce. Acupuncture in Zusanli and Hegu were more efficient than Yinlingquan, Taichong. These results indicate that acupuncture and electroacupuncture may provide a potent strategy in relieving arthritic pain.

Key Words : pain, acupuncture, electroacupuncture, arthritis, articular afferent

Pain relieving effects of the acupuncture and electroacupuncture on the arthritic model of the rat

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(Directed by Professor Bae Hwan Lee)

I. INTRODUTION

Acupuncture derived from the Latin *acus* (needle) and *punctura* (pricking), is essentially the insertion of small, thin, solid needles into the skin, immediate subcutaneous, muscular tissue in regions called 'meridians'. Acupuncture, one of the oriental medical therapeutic techniques that can be traced back at least 2500 years, is gaining popularity in the West as an alternative and complementary therapeutic intervention.[1,2,3] Acupuncture has relieving effects in acute pain, chronic pain, drug addiction, asthma, surgery and chemotherapy-induced nausea, vomiting, and stroke

rehabilitation.[4,5] Acupuncture is also now available as a treatment option in most chronic pain clinics in the United Kingdom.[6] In oriental medicine, the human body is considered to be a complex network of intricately related processes played upon by opposing forces ('Yin' and 'Yang'). The two forces always combine to make up the whole. This polar system has an important role in the description of life processes in the human body and of their disturbance. Health is viewed as the maintenance of harmony between 'Yin' and 'Yang' similar to the autonomic nervous system with its duality of sympathetic versus parasympathetic nervous system, while illness is an expression of disharmony. If one system outweighs the other, abnormal symptoms are felt by the patient depending upon which side is excessive and which is deficient.[7,8] Acupuncture plays a role in manipulating and balancing 'Yin' and 'Yang' when the body's innate homeostatic potentialities are overwhelmed by acute or chronic stress conditions.[7] Acupuncture is essentially a technique for correcting reversible physiological malfunction of various parts of the body by physiological means. Acupuncture-initiated impulses may activate the autonomic centers and the hypophyseal system in the brain so as to improve the efficiency of homeostatic and self-defense mechanism of the body.[9]

Arthritis (from the Greek word for joint) is a chronic multifactorial disease induced when the immune system attacks and begins degrading the body's joints. The disease knows racial boundaries and comes in many forms, including chronic, gouty,

calcific periarthritis, enteropathic arthritis, and hand osteoarthritis, hip and knee osteoarthritis, thumb, Jaccoud's and juvenile osteoarthritis, oligoarthritis, polyarthritis, and peripheral, psoriatic, rheumatoid, and septic arthritis. In the US, arthritis and other rheumatic conditions affect about 43 million people, or about 15% of population, at a total disease burden close to $65 billion. One phenomenon of the twentieth century is the shift in major health problems from acute to chronic diseases. This is a function not only of the control of infectious diseases resulting from improved sanitation and the discovery of curative medications, but also the increasing aging of the population. The elderly are most vulnerable to chronic conditions, among which arthritis is a prime example. Thus there can be no doubt that as more persons live to advanced ages, there will be an accompanying increase in the numbers and proportions significantly affected by arthritis and by osteoarthritis in particular. Arthritis is not a primary cause of death. It is mainly a condition of morbidity, and its major effects are on quality of life. Pain and loss or reduction of mobility can interfere with family and social life, and produce feelings of hopelessness and depression. Therefore, the development of the therapy of arthritic pain is the most important. The aim of the present study is to investigate pain relieving effects of acupuncture and electroacupuncture by methods of electrophysiological recording in the arthritic rats.

II. MATERIALS AND METHODS

1. Subjects

Experiments were performed on adult male Sprague-Dawley rats (200-350 g, Daehan Biolink Co. LTD., Eumsung, Korea). Animals were housed in groups of four in plastic cages with soft bedding under a 12/12 h reversed light-dark cycle (light cycle: 8:00 AM - 8:00 PM). Temperature (22±2℃) and humidity (50±10%) were controlled constantly. Food and water were available *ad libitum*.

The care and use of laboratory animals in this experiment were based on the Guidelines and Regulations for Use and Care of Animals in Yonsei University.

2. Induction of arthritis

A standardized model for the production of inflammatory arthritis was produced by injecting 2% carrageenan (50 ㎕, suspended in sterile saline; Type Ⅳ lambda-carrageenan, Sigma, St. Louis, MO, USA) into the knee joint cavity of the left hind leg of the rat under urethane (1.25 g/㎏, i.p.) anesthesia. Electrophysiological recordings were performed from 4 h after the injection of carrageenan.

3. Electrophysiological recordings

The technique described by Just, et al.[10] was used to recording electrical activity of primary articular afferents innervating the knee joint via medial articular nerve (MAN). The arthritic rat was placed on its left side and the skin overlying the medial face of thigh was incised from the abdominal region to the medial part of the knee joint. The saphenous nerve was isolated distal to the knee joint and transected to prevent afferent input from the foot and surrounding non-articular regions. The saphenous nerve was also exposed in the inguinal region and transected as central as possible to obviate any potential spinal reflexes. The left side of femur and tibia were hold by the grip, which fixed to a stereotaxic frame thereby immobilizing the proximal aspect of the hind limb. The skin flaps were sewn onto a Y-shaped frame to form a shallow pool, which was then filled with mineral oil to prevent tissue desiccation at 37℃. In this pool, the whole medial aspect of the left leg was exposed including the medial part of the knee joint and the patellar ligament. Fine neurofilaments were dissected at the proximal end of the saphenous nerve using sharpened forceps and subsequently placed over a platinum electrode for extracellular recordings. To visualize the fine filaments a black platform was used. Afferent nerve fibers originating from the knee joint were identified by recording neural discharges generated in response to probing of the joint and consequently their receptive field with a blunt glass rod.

Thereafter, nerve fibers were characterized by their mechanical sensitivity to passive movement of the joint consisting of noxious

outward as well as inward movements1.[10,11] Joint passive movements are described as hyper-movement of the joint against tissue resistance without imparting soft tissue injury and are induced by a Linear head Motor (ORIENTAL MOTOR Co. LTD., Japan). It was described in Figure 1.

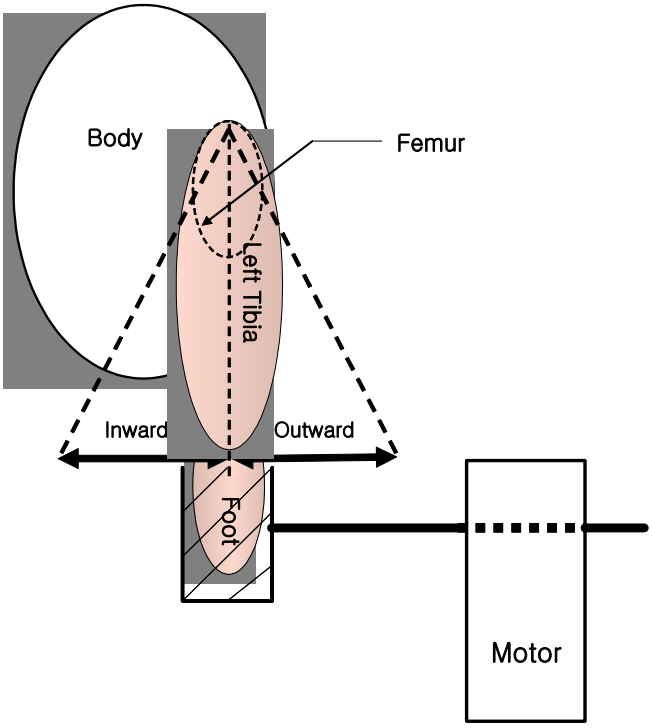


Figure 1. Schematic drawing of the outward and inward movement stimulation applied to the knee joint.

Each of the outward and inward movement lasted 15 s, and a movement cycle were tested before (Pre) and repeated the response of afferents to 0 min, 5 min, 10 min, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min after acupuncture and electroacupuncture.

4. Acupuncture and electroacupuncture

In acupuncture, the following four acupoints of various acupuncture points that were equivalent to specific human acupuncture points were used: Zusanli (ST36), Yinlingquan (SP9), Hegu (LI4), and Taichong (LR3). ST36, SP9, LI4, LR3 were named by the World Health Organization (WHO).

Zusanli (ST36) point is located near the knee joint and 5 mm lateral to the anterior tubercle of tibia. Yinlingquan (SP9) point is located near the knee joint. Hegu (LI4) point is located on the dosal aspects of the hand near the midpoint between the first and second metacarpal bones. Taichong (LR3) point is located on the dosal aspects of the foot near the midpoint between the big and second toe bones.

Stainless steel needle of 0.3 mm diameter and 30 mm in length was used at Zusanli, Yinlingquan and the needle of another size of 0.15 mm diameter and 5 mm in length was used at Hegu, Taichong. Acupuncture used manipulation. Rotation the depth of insertion of the needle was used each 30 min stimulation period. The stainless acupuncture needle was inserted vertically through the skin to a depth of about 5 mm, and then rotated manually right and left three times per second. Manipulation persisted for 1 min in every 10 min. As acupuncture control, no treatment acupuncture was performed.

Electroacupuncture was applied by stimulating an acupuncture point with electrical current through a pair of bipolar stimulating electrodes, which were modified acupuncture needles. Two stainless steel acupuncture needles (size: 0.3 mm in diameter and 30 mm in

length) were inserted into specific points at a depth of 5 mm and electrical stimulations with 2 Hz and 100 Hz frequency for 30 min were applied by a Stimulus Isolator A385 (World Precision Instruments, Sarasota, FL, USA) equipped with an Pulsemaster A300 (World Precision Instruments, Sarasota, FL, USA). For the electrical stimulation of 2 Hz, the pulse duration was 0.6 ms and the intensity of the electric current was 2 mA for 30 min as a bipolar square wave current output. For the electrical stimulation of 100 Hz, the pulse duration was 0.2 ms and the intensity was 2 mA for 30 min as a bipolar square wave current output. Electroacupuncture was applied to two different points on the hind limb. One was the Zusanli (ST36). The second point used was the Yinlingquan (SP9). As electroacupuncture control, it was performed that a pair of acupuncture needles inserted into the acupoints of Zusanli and Yinlingquan for 30 min without electrical stimulation

5. Experimental design

Experiments were designed to be the groups of acupuncture and electroacupuncture. As previously described, acupuncture experiment consisted of five groups according to each four acupoints (Zusanli, Yinlingquan, Hegu, and Taichong) and contol (no treatment of acupuncture). The acupuncture's control group was selected in method described by Ezzo, et al.[12] As control group, many acupuncture studies was widely used no treatment of acupuncture than non-acupuncture point.[12,13] So, it was used the control group.

Electrocupuncture experiment consisted of three groups according to each frequencies (2 Hz, 100 Hz) and control (a pair of acupuncture needles inserted into the acupuncture points of Zusanli and Yinlingquan without electrical stimulation). The electroacupuncture's control group was selected in method described by Koo, et al.[13]

6. Statistical analysis

Data were presented as mean ± standard error of the mean (SEM). Statistical tests were done using the one-way repeated measures analysis of variance (ANOVA) followed by the Dunnett's (2-sided) posthoc multiple comparison at each time point. A P value of less than 0.05 was considered to be statistically significant.

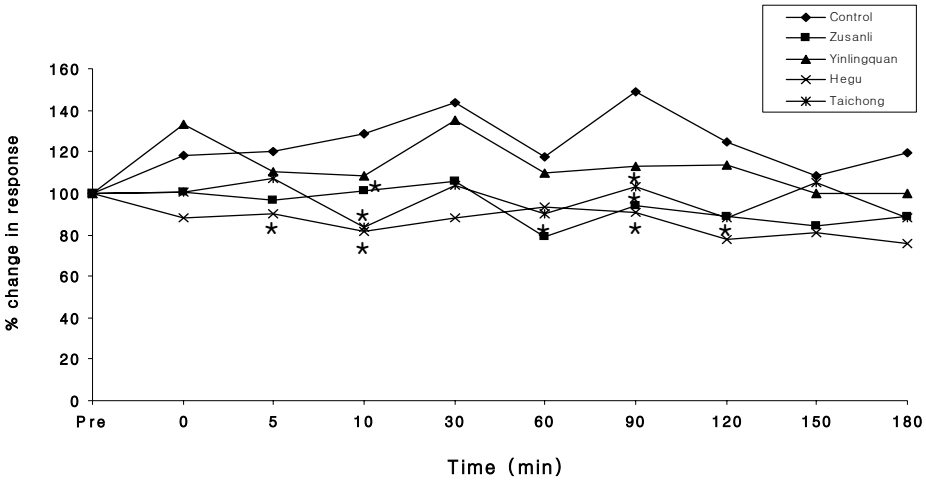
Ⅲ. RESULTS

The neural activities were recorded from a total of 105 primary articular afferents in the inflamed knee joint. Acupuncture was applied to each acupuncture point, Zusanli (n=15), Yinlingquan (n=15), Hegu (n=12), Taichong (n=13) and sham (n=15) for comparison. Electroacupuncture was applied to each frequency, 2 ㎐ (n=12), 100 ㎐ (n=11) and no electrical stimulation (n=12) for comparison. All electrophysiological recording data were analysed into duration of activity, number of activity, and firing rate. Duration of activity consisted of lasting time of neural activities time by passive movement. Number of activity was the sum of neural activities induced passive movement. Firing rate was impulses per second. Electrophysiological recordings were performed from 4 h after the injection of carrageenan.

1. Effects of the acupuncture on the duration of activity

There were effects on duration of activity by stimulation of outward and inward movement in acupuncture. After intra-articular injection of carrageenan, articular afferents developed an irregular discharges response to passive movement of joint, whose duration of neural activity increased, in control group (Figure 2A and B). In Zusanli, Hegu, and Taichong, the duration of activity by the stimulation of outward movement was significantly reduced at 10 min, 90 min after acupuncture. But in Yinlingquan, duration of activity was not reduced at all time points (Figure 2A). Duration of activity by the stimulation of inward movement was significantly reduced from 5 min after acupuncture in Zusanli, Yinlingquan, and Hegu (Figure 2B). At 5 min, 120 min, Hegu was significantly reduced duration of activity in comparison with control in outward and inward movement. At 10 min, 90 min, Zusanli and Hegu were significantly reduced duration of activity in comparison with control in outward and inward movement. At 30 min, 60 min, 150 min, 180 min, Zusanli, Yinlingquan, and Hegu were significantly reduced duration of activity in comparison with control in inward movement. The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) posthoc multiple comparison test. A P value of less than 0.05 was considered to be statistically significant.

A. Acupuncture – Duration – Outward



B. Acupuncture – Duration – Inward

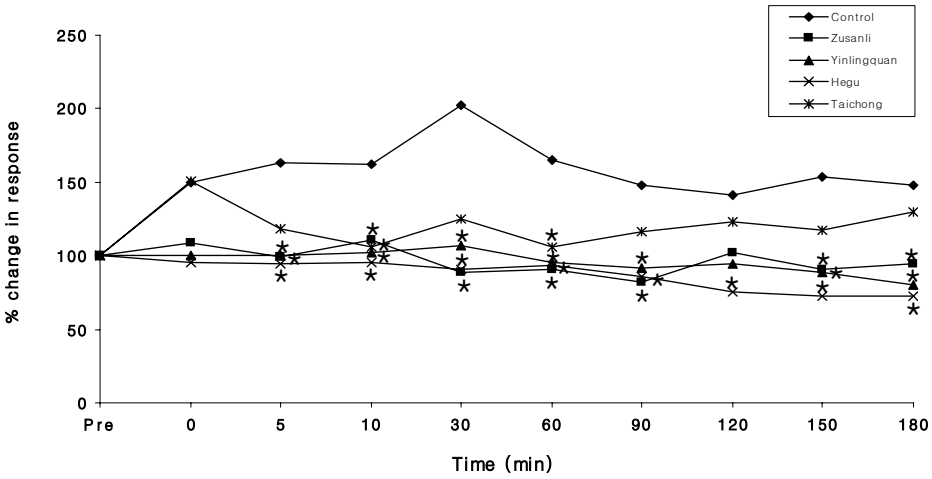


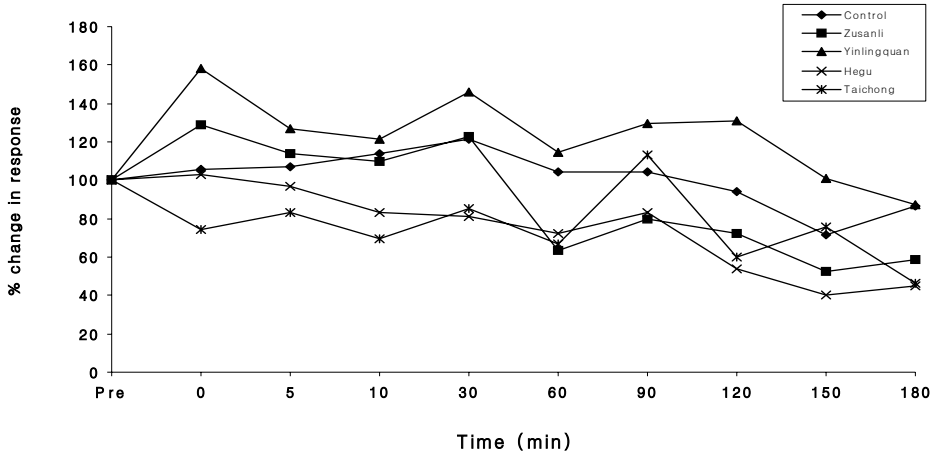
Figure 2. Effects of the acupuncture on the duration of activity. Changes in the duration of activities in the articular primary afferents induced by passive **(A)** outward and **(B)** inward movement after acupuncture. The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) posthoc multiple comparison test. *A P value of less than 0.05 was considered to be statistically significant.

2. Effects of the acupuncture on the number of activity

There were effects on number of activity by stimulation of outward and inward movement in acupuncture. The effect of the acupuncture in number of activity by stimulation of outward movement was not significant (Figure 3A).

At 5 min, Yinlingquan and Hegu were significantly reduced number of activity in comparison with control at inward movement. At 10 min, Yinlingquan, Hegu, and Taichong were significantly reduced number of activity in comparison with control in inward movement. At 30 min, 60 min, all acupuncture points were significantly reduced number of activity in comparison with control in inward movement. At 150 min, 180 min, Zusanli, Yinlingquan, and Hegu were significantly reduced number of activity in comparison with control in inward movement (Figure 3B). The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) posthoc multiple comparison test. A P value of less than 0.05 was considered to be statistically significant.

A. Acupuncture - Number - Outward



B. Acupuncture - Number - Inward

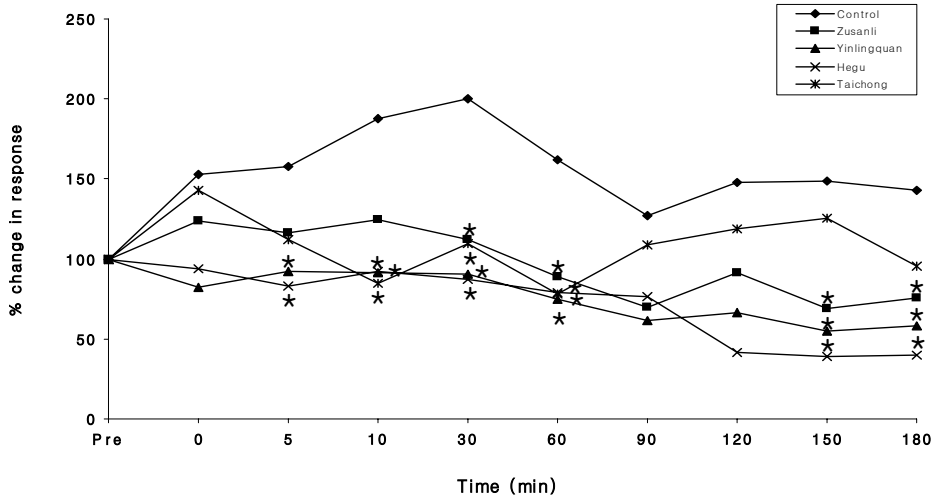
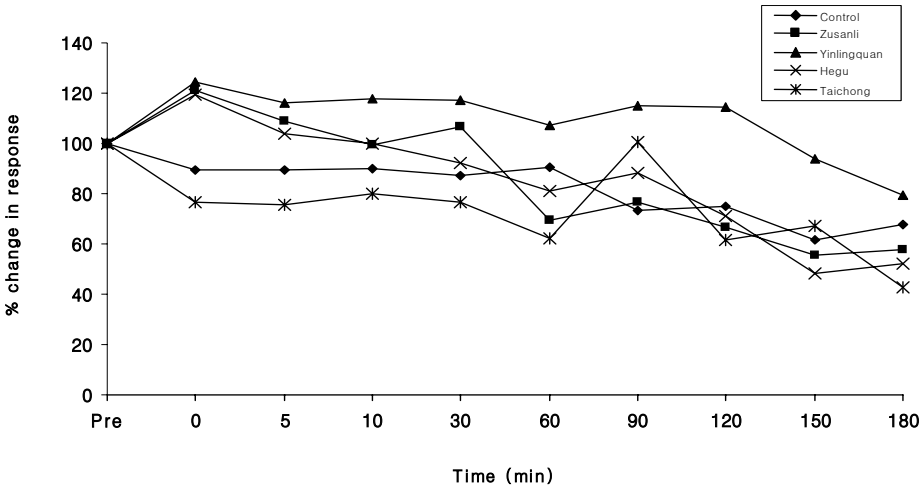


Figure 3. Effects of the acupuncture on the number of activity. Changes in the number of activities in the articular primary afferents induced by passive (A) outward and (B) inward movement after acupuncture. The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) posthoc multiple comparison test. *A P value of less than 0.05 was considered to be statistically significant.

3. Effects of the acupuncture on firing rate

There were effects on firing rate by stimulation of outward and inward movement in acupuncture. Except Yinlingquan, firing rate of all groups decreased, but all groups had no significant effects in outward stimulation (Figure 4A). Except Zusanli and Hegu, firing rate of Yinlingquan and Taichong decreased, but all groups had no significant effects in outward stimulation (Figure 4B). The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) posthoc multiple comparison test. A P value of less than 0.05 was considered to be statistically significant.

A. Acupuncture – Firing rate – Outward



B. Acupuncture – Firing rate – Inward

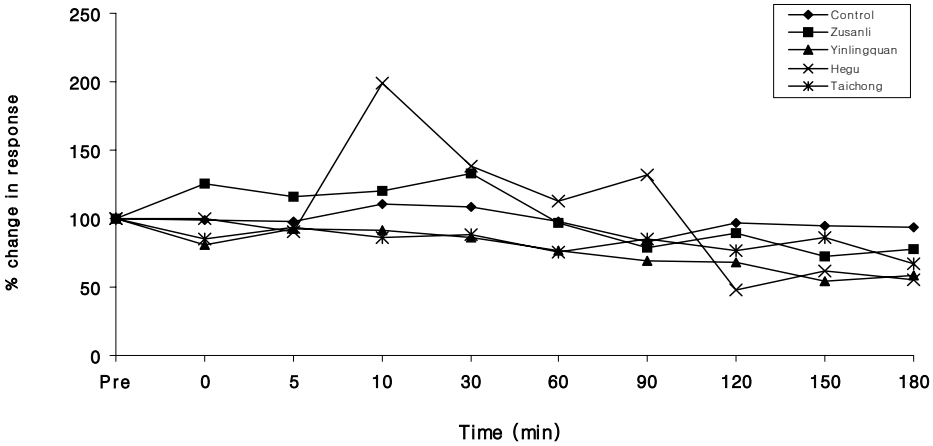
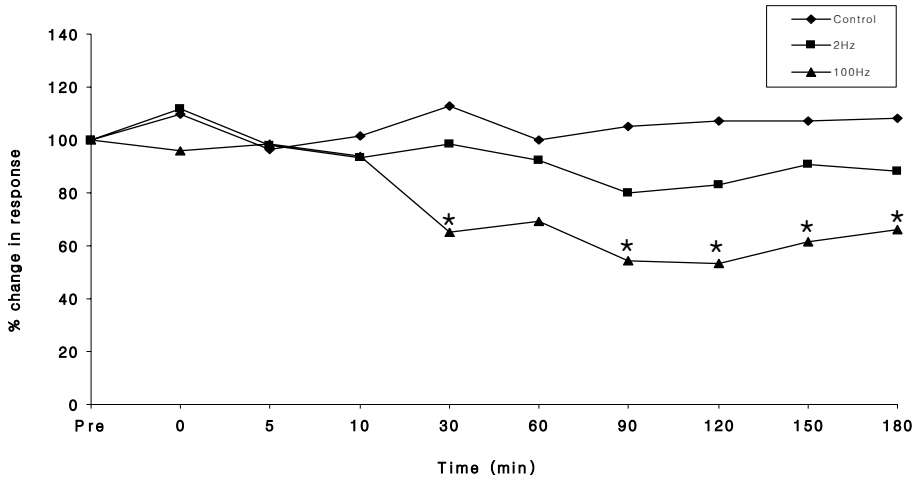


Figure 4. Effects of the acupuncture on firing rate. Changes in the firing rate in the articular primary afferents induced by passive **(A)** outward and **(B)** inward movement after acupuncture. The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) posthoc multiple comparison test. A P value of less than 0.05 was considered to be statistically significant.

4. Effects of the electroacupuncture on the duration of activity

There were effects on duration of activity by stimulation of outward and inward movement in electroacupuncture. The electroacupuncture of 100 ㎐ was significantly reduced duration of neural activity. But the stimulation of 2 ㎐ was not significantly reduced duration of activity (Figure 5A and B). At 5 min, 60 min, the stimulation of 100 Hz was significantly reduced duration of activity in comparison with control in inward movement. At 30 min, 90 min, 120 min, 150 min, the stimulation of 100 Hz was significantly reduced duration of activity in comparison with control in outward and inward movement. At 180 min, the stimulation of 100 Hz was significantly reduced duration of activity in comparison with control in outward movement. The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) posthoc multiple comparison test. A P value of less than 0.05 was considered to be statistically significant.

A. Electroacupuncture - Duration - Outward



B. Electroacupuncture - Duration - inward

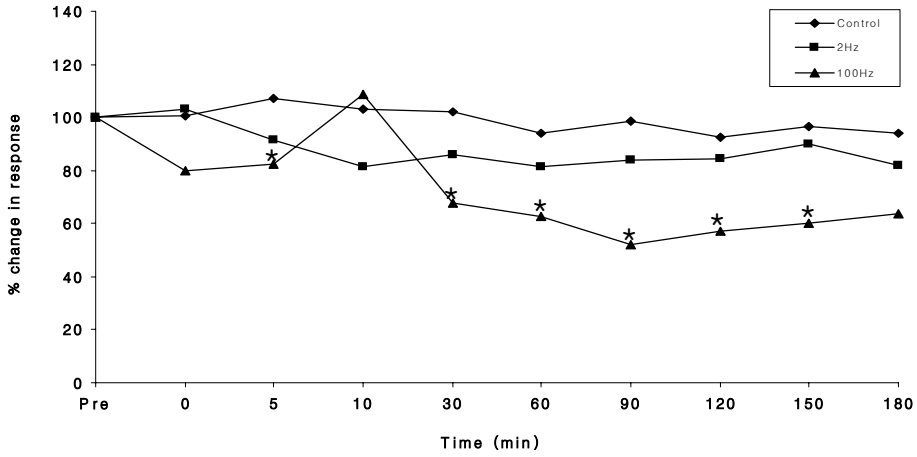


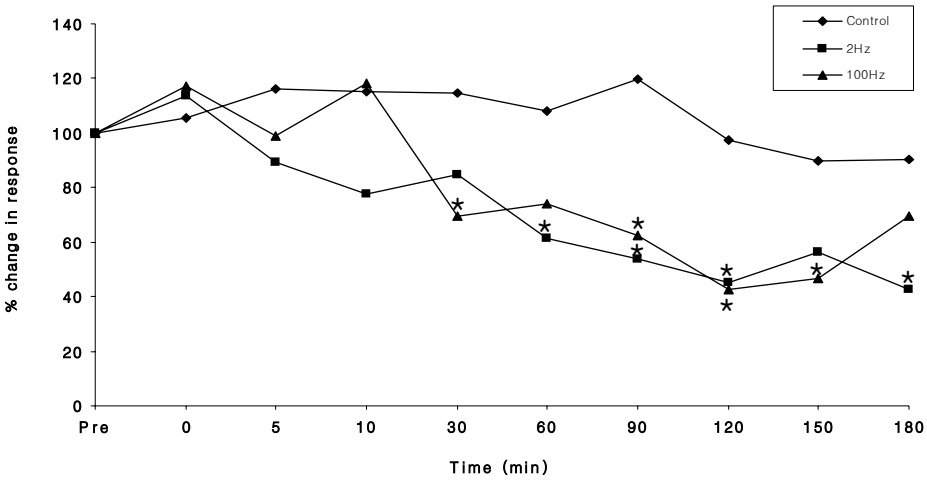
Figure 5. Effects of the electroacupuncture on the duration of activity. Changes in the duration of activities in the articular primary afferents induced by passive (A) outward and (B) inward movement after electroacupuncture. The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) posthoc multiple comparison test. *A P value of less than 0.05 was considered to be statistically significant.

5. Effects of the electroacupuncture on the number of activity

There were effects on number of activity by stimulation of outward and inward movement in electroacupuncture. At 30 min, 150 min, the stimulation of 100 Hz was significantly reduced number of activity in comparison with control in outward movement. At 60 min, 180 min, the stimulation of 2 Hz was significantly reduced number of activity in comparison with control in outward movement. At 90 min, 120 min, the stimulations of 2 Hz and 100 Hz were significantly reduced number of activity in comparison with control in outward movement (Figure 6A).

At 30 min, 180 min, the stimulation of 2 Hz was significantly reduced number of activity in comparison with control in inward movement. At 60 min, 90 min, 120 min, the stimulations of 2 Hz and 100 Hz were significantly reduced number of activity in comparison with control in inward movement. At 150 min, the stimulation of 100 Hz was significantly reduced number of activity in comparison with control in inward movement (Figure 6B). The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) posthoc multiple comparison test. A P value of less than 0.05 was considered to be statistically significant.

A. Electroacupuncture - Number - Outward



B. Electroacupuncture - Number - Inward

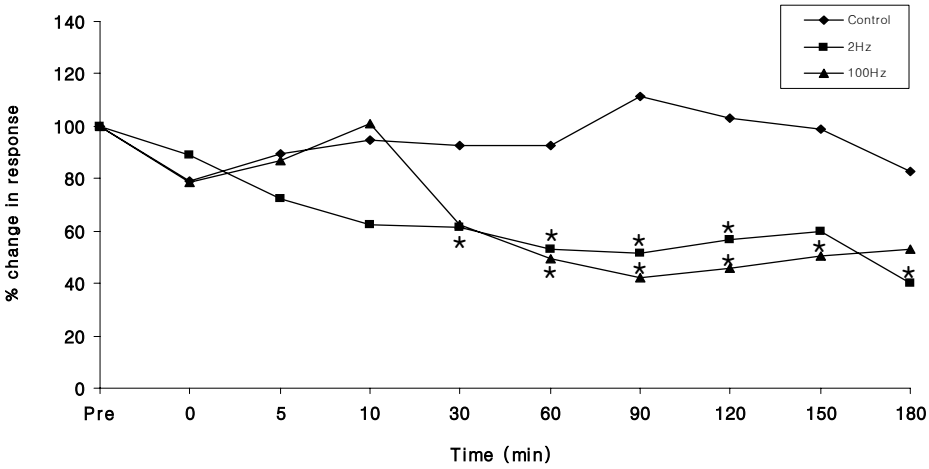
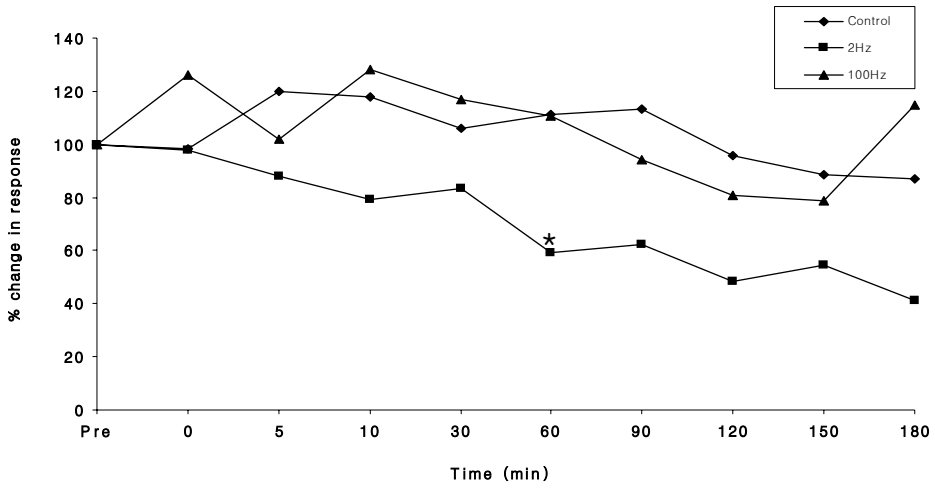


Figure 6. Effects of the electroacupuncture on the number of activity. Changes in the number of activities in the articular primary afferents induced by passive (A) outward and (B) inward movement after electroacupuncture. The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) posthoc multiple comparison test. *A P value of less than 0.05 was considered to be statistically significant.

6. Effects of the electroacupuncture on firing rate

There were effects on firing rate by stimulation of outward and inward movement in electroacupuncture. Firing rate after electroacupuncture significantly reduced at 60 min of 2 ㎐ in outward movement and at 90 min, 180 min of 2 ㎐ in inward movement (Figure 7A and B). The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) posthoc multiple comparison test. A P value of less than 0.05 was considered to be statistically significant.

A. Electroacupuncture - Firing rate - Outward



B. Electroacupuncture - Firing rate - Inward

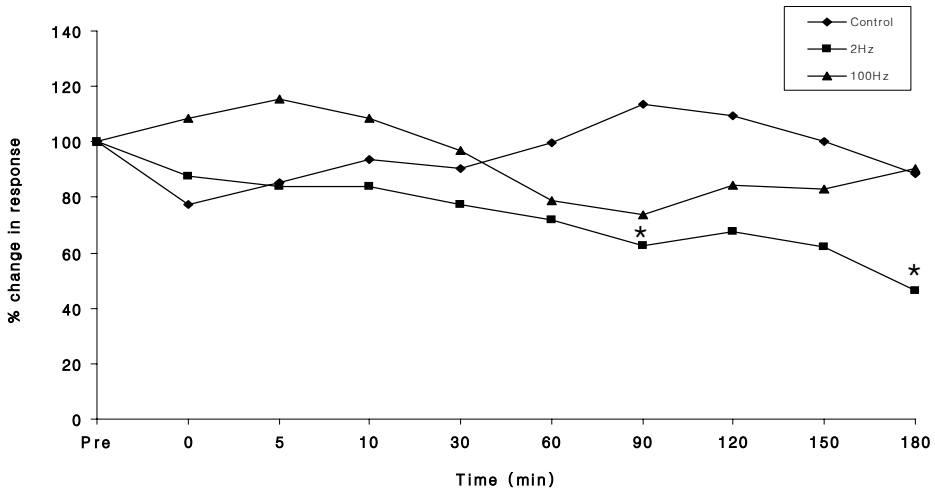


Figure 7. Effects of the electroacupuncture on firing rate. Changes in the firing rate in the articular primary afferents induced by passive (A) outward and (B) inward movement after electroacupuncture. The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) posthoc multiple comparison test. *A P value of less than 0.05 was considered to be statistically significant.

Ⅳ. DISCUSSION

Derived from ancient Oriental medicine, acupuncture has evolved in current Western medicine for the treatment or prevention of pain. The modality of acupuncture treatment is essentially the insertion of small, thin, solid needles into the skin, immediate subcutaneous, muscular tissue in regions called 'meridians'.[14]

Oriental medicine considered human health as the result of conflicting forces of nature, termed 'Yin' and 'Yang'. When these forces were out of harmony, 'disease' resulted. Vascular and neurologic energy flowed through meridians. These meridians allegedly followed a circadian rhythm, and each was directly associated with an organ system.[15]

The meridians were interconnected within the vital life energy 'Qi'. Allegedly, a deficiency of 'Qi' caused pain. Pain could thus be modified by inserting needles into the meridians that rebalanced energy flows. More recent concepts associated the meridian process with the autonomic nervous system.[16,17]

There are numerous techniques exit such as 'manipulation', twisting the inserted needle, pressing down on the needle, or applying a ball of herbs at the base and igniting the ball.

The site of insertion appears to be the major basis for success. The site of 'trigger points' or motor points has been postulated as the optimum site for acupuncture.[18]

Although there is increasing usage of acupuncture for pain management and a widespread availability of acupuncture at pain clinics, there is inconclusive evidence that acupuncture is more effective than placebo, sham acupuncture, or standard care.[12] The

main reasons for such inconclusive effectiveness of acupuncture despite its wide usage are both to lack of sufficient number of objective studies demonstrating acupuncture analgesic effect and a poor understanding of the physiological mechanisms underlying acupuncture analgesia. Unveiling the physiological mechanisms underlying acupuncture analgesia will not only help us to appreciate its mechanism of action but will also guide us toward improving the effectiveness of acupuncture.

The underlying mechanisms involved in acupuncture and electroacupuncture-induced analgesic effect are not clear. One of the most established hypothesis concerning the mechanisms of electroacupuncture analgesia is the so-called 'endogenous opioid' hypothesis. This hypothesis proposes that electroacupuncture stimulation activates the endogenous opioid system to raise the circulating endorphin level. Supporting evidence includes the observation that an injection of naloxone, an opiate antagonist, reverses electroacupuncture analgesia in experimental animals[19,20] as well as in humans.[21] Furthermore, naloxone blockade of electroacupuncture was found to be frequency dependent[22,23] and the effect of electroacupuncture at different frequencies was mediated by different subtype of opiate receptors.[24,25,26] It was later found that serotoninergic and noradrenergic pain inhibitory systems play additional roles in acupuncture analgesia.[27,28,29,30,31] On the other hand, other studies failed to reverse electroacupuncture induced analgesia by opiate antagoinsts, such as naloxone or naltrexone, in rabbits,[32] in rats,[33] or in humans.[34,35] Furthermore, opiate antagonists sometimes potentiate electroacupuncture analgesia in rats.[33,36] Therefore, the issue of whether or not electroacupuncture-induced

analgesia is mediated by endogenous opioid is not completely settled. It is possible that both endogenous opioid mediated and non-opioid mediated mechanisms are involved in electroacupuncture and a specific condition may bring out one component as a dominant form.[28,30,37,38]

In the present study, to examine pain relieving effects of acupuncture and electroacupuncture, the activities of articular afferent innervating knee joint were recorded. Mammalian joints and surrounding structure have a rich array of receptors potentially able to signal nociception.[39,40] Nociceptors homologous with C polymodal cutaneous nociectors are found in a diffuse lattice throughout the joint capsule. Nociceptors can be defined as primary afferent neurons that have the capacity to distinguished between noxious and innocuous events.[41] The axons from these neurons are generally lightly myelinated (A-delta fibers) or unmyelinated (C fibers). Those nociceptors innervating skin have been best characterized. A-delta nociceptors have been identified in numerous species, including man. The best studied units, which have been termed high-threshold mechanoreceptors by some investigators[42,43] usually respond to intense heat as well as noxious mechanical stimuli and, consequently, are better classified as mechano-heat nociceptors.[44] Silent in the absence of stimulation, some of these units discharge only when noxious stimulation is applied, and others respond to innocuous stimuli but increase their firing rates with a tissue-damaging stimulus.[43] Sensitization following exposure to noxious stimuli may occur.[42] There is ample evidence that these nociceptors are involved both in the perception of pain following noxious mechanical and heat stimuli and in the development of

hyperalgesia following tissue injury. Unmyelinated primary afferent neurons that respond to noxious thermal, mechanical, and chemical stimuli (so-called C polymodal nociceptors or C fiber mechano-heat nociceptors) have also been identified in humans and are clearly involved in pain perception.[45,46,47] Some of these polymodal nociceptors discharge with nonnoxious stimuli and increase their firing rate as stimulus intensity increase; others discharge only with potentially tissue-damaging stimulation.[48,49] Sensitization after a noxious stimulus has been demonstrated.[50] Other type of cutaneous nociceptors have been described. Electrophysiologic studies have identified A-delta fibers with response characteristics identical to that of the C polymodal nociceptors, unmyelinated units responsive only to high-threshold mechanical stimulation, and both A-delta and C fiber units responsive to intense cold.[48,49,51]

Recent studies further suggest that some classes of nociceptors are responsive to noxious stimuli only after they become conditioned by prolonged injury or inflammation.[52,53] Primary afferent fibers responsive to noxious stimulation also have been identified in the viscera[54] and other somatic tissues, including muscle,[55] joint,[56,53] cornea,[57] and tooth pulp and periodontium.[58] Free nerve endings in internal and external joint ligaments correspond to the high-threshold A-delta nociceptor in the skin. Joint cartilage does not contain such receptors. The synovium itself has been considered largely insensitive, but neuropeptide-containing, small-diameter nerve fibers were recently identified immunohistochemically in healthy human synovium.[59,60] These fibers may be relevant in the process of neurogenic inflammation in signaling nociception. The predominant populations of afferent fibers

in the articular nerves of the cat knee are A-delta and C, which may imply that nociceptors dominate within the joint.[61]These receptors also may respond to innocuous mechanical and chemical stimuli.[62] Mechanoreceptors potentially relevant to joint pain have also been described. These receptors are subserved by large-diameter, fast-conducting afferents. Corpuscular structures within the outer (type I) or subsynovial (type II) layers of the fibrous capsule function as static or low-threshold dynamic mechanoreceptors, and others (type III), which function as dynamic mechanoreceptors, are situated on the surface of joint ligaments.

Carrageenan is a sulphated mucopolysaccharide extracted from the seaweeds *Chondrus* spp. and *Gigartina* spp., commonly known as Irish Moss or carrageen moss. It has been used in the rat for inflammation models: footpad inflammation or paw edema model,[63] air pouch model,[64] and to induce acute arthritis[65] as well as in conjunction with other agents[66] and in the enhancement of inflammatory arthritis in other models.[67] The responses of ascending tract cells in the cat spinal cord is enhanced by carrageenan-induced inflammation of the knee joint.[68] Intra-articular injection of lamda carrageenan into the knee joint results in a localized inflammation, and decrease weight bearing, guarding of the affected limb, and hyperalgesia.[69]

The acupuncture's control group was selected in no treatment of acupuncture. Many studies evidenced the difference of the effect of non-acupuncture point from the effect of acupuncture,[70,71] and the difference of the effect of no treatment of acupuncture from the effect of acupuncture.[72,73,74] So, we did not need to prove the effect of the non-acupuncture point. As control group, many acupuncture

studies was also widely used no treatment of acupuncture than non-acupuncture point.[12,13]

In electroacupuncture, we used the electrical stimulation of 2 Hz and 100 Hz. It referred to Ulett, et al.[38] Also, we used that for the electrical stimulation of 2 Hz, the pulse duration was 0.6 ms and the intensity of the electric current was 2 mA for 30 min as a bipolar square wave current output, and for the electrical stimulation of 100 Hz, the pulse duration was 0.2 ms and the intensity was 2 mA for 30 min as a bipolar square wave current output. It referred to Han, et al[75] and Wan, et al.[76]

Arthritis is not a primary cause of death. It is mainly a condition of morbidity, and its major effects are on quality of life. Pain and loss or reduction of mobility can interfere with family and social life, and produce feelings of hopelessness and depression. Therefore, the deveploment of the therapy for arthritic pain is the most important. The previous studies were evidenced the pain relieving effects of acupuncture and electroacupuncture on the arthritis.[12,13,72,73] Therefore, the effect of acupuncture and electroacupuncture was examined in an animal model of arthritic pain induced by the injection of carrageenan into the knee joints of the rat.

The results of the present study indicate that acupuncture and electroacupuncture may provide a potent strategy in relieving arthritic pain.

Ⅴ. CONCLUSION

Acupuncture is utilized as a clinical treatment for various diseaes in Oriental medicine and through many studies its efficacy for producing analgesia is now well-accepted in the West. Although there are many reports that acupuncture has an effect for treatment or prevention of various pain, there are no reports about electrophysiological studies of acupuncture in arthritic pain yet. Therefore, the effect of acupuncture and electroacupuncture was examined in an animal model of arthritic pain induced by the injection of carrageenan into the knee joints of the rat.

In acupuncture, Zusanli, Yinlingquan, and Hegu were shown that neural activities of articular afferents tended to reduce. In electroacupuncture, low frequency (2 Hz) and high frequency (100 Hz) stimulations were shown that neural activities of articular afferents tended to reduce after 30 min after electrical stimulation. Also in acupuncture, Zusanli and Hegu are more efficient than Yinlingquan or Taichong. These results indicate that acupuncture and electroacupuncture may provide a potent strategy in relieving arthritic pain.

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국문 요약

백서의 관절염 모델에서 침과 전기침의 통증 억제 효과

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고대 동양 의학에서부터 유래된 침은 현재 통증의 치료 또는 억제를 위해 서양 의학에서도 널리 사용되고 있다. 통증을 수반하는 질병 중에 가장 대중적인 질병인 관절염은 사회가 점차 고령화 되면서 더욱 늘어나고 있는 실정이다. 이에 이 실험에서는 관절염 모델의 백서를 통하여 통증을 완화한다고 알려져 있는 혈자리인 족삼리, 음릉천, 합곡, 태충에서의 통증 완화 효과를 살펴보고, 아울러 침보다는 다양한 연구가 진행되고 있는 전기침의 통증 완화 효과도 알아보고자 하였다. 흰쥐의 좌측 뒷다리 무릎 관절강 내에 2% carrageenan을 주입하여 관절통을 유발하고, 무릎 관절에 물리적인 운동 자극을 주어 무릎 관절에서 나오는 구심성 신경의 신경 활동을 측정하기 위하여 복재신경을

분리한 후 경골과 대퇴골을 고정하고 mineral oil pool을 만들었다. 침과 전기침을 실시한 후 일정 시간 간격으로 외향 자극과 내향 운동 자극을 주어 신경 활동의 변화를 측정하였다. 그 결과, carrageenan을 주입하여 염증을 유발, 관절통이 일어난 쥐에서 침을 실시한 후 5분 이후에서부터 180분까지 족삼리, 음릉천, 합곡에서 통증을 완화 시키는 경향이 나타났고, 전기침의 경우에는 전기침을 실시한 후 30분 이후에서부터 180분까지 2 Hz 와 100 Hz 모두 통증 완화 효과를 나타내었다. 또한, 침에 있어서 통증 완화 효과가 족삼리와 합곡이 음릉천과 태충보다는 좀 더 나은 것을 알 수 있었다. 이러한 결과를 근거로 침과 전기침은 관절통 모델에서도 통증을 완화 시키는 효과를 나타냄을 알 수 있었다.

핵심되는 말 : 통증, 침, 전기침, 관절염, 관절의 구심성 신경