

Inhibition of Articular Sensory Activities to Mechanical Stimulation by
Aqua-acupuncture in an Animal Model of Arthritic Pain*

관절통에 관한 동물모델에서 약침에 의한 기계적 자극에 대한
관절 감각신경 활동의 억제

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Abstract · The aim of this study was to examine the effects of aqua-acupuncture a mixture of bos taurus domesticus and selenarctos thiberanus, and bos taurus domesticus, selenarctos thiberanus and Moschus moschiferus on an animal model of arthritic pain Under halothane anesthesia, arthritic pain was induced by the injection of 2% carrageenan into the left knee joint cavity of male Sprague-Dawley rats. The responses of afferents to a movement cycle were recorded before and after aqua-acupuncture. The aqua-acupuncture at acupoints reduced neural responses to noxious movement stimulation. Aqua-acupuncture at Zusanli inhibited neural responses of articular afferents to noxious stimulation more than at Hegu. These results indicate that aqua-acupuncture of a mixture of bos taurus domesticus and selenarctos thiberanus, and bos taurus domesticus, selenarctos thiberanus and Moschus moschiferus may provide a potent strategy in relieving arthritic pain.

Key words · Aqua-acupuncture, arthritis, pain, articular afferent

요약 본 연구는 관절통 모델에서 응답 우황과 응답 우황 사향의 약침액의 효과를 검사하기 위해 수행되었다.

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할로탄 마취하에서 관절통은 수컷 쥐의 관절강내에 2% carrageenan을 주입하여 유발시켰다. 운동자극에 대한 감각신경의 반응은 약침을 시술하기 전과 후에 기록하였다. 경혈에 주입한 약침은 유해한 운동 자극에 대한 신경의 반응을 억제시켰다. 족삼리에 시술한 약침은 합곡에 비해 유해한 자극에 대한 관절 감각신경의 반응을 더 많이 억제시켰다. 이러한 결과는 응답 우황과 응답 우황 사향의약침이 관절통을 완화하는 데 효과적인 치료법을 제공할 수 있다는 것을 시사한다.

주제어 : 약침, 관절염, 통증, 관절 구심성 신경

1. Introduction

Acupuncture is the process of inserting needles into subcutaneous tissues in regions called 'meridians' or 'acupoints' [10]. Acupuncture is an important medical therapy technique in oriental medicine and is acquiring popularity in the West as an alternative and complementary therapy [2-4]. Acupuncture can aid in relieving acute pain, chronic pain, drug addiction, asthma, surgery and chemotherapy-induced nausea, vomiting, and is used in stroke rehabilitation [9]. Moreover, it has been demonstrated that injections of an aqua-acupuncture into a specific acupoint produced much more pronounced analgesic effects than those of acupuncture stimulation alone [12].

Arthritis is a chronic disease which is induced when the immune system is attacked and the body's joints start to degrade. Pain induced by arthritis can interfere with the social life of the afflicted and can produce feelings of hopelessness and depression. Therefore, the development of therapy for arthritic pain is of the utmost importance.

The aim of the present study was to investigate pain relieving effects of aqua-acupuncture of a mixture of *bos taurus domesticus* and *selenarctos thiberanus* (BU), and *bos taurus domesticus*, *selenarctos thiberanus* and *Moschus moschiferus*

(BUM) through behavioral and electrophysiological evaluation of rats with induced arthritic pain.

2. Materials and Methods

2.1 Subjects

Experiments were performed on adult male Sprague-Dawley rats (200-350 g, Daehan Biolink Co. LTD., Eumsung, Korea). The animals were housed in groups of four in plastic cages with soft bedding under a 12/12 h light-dark cycle (light cycle: 8:00 AM - 8:00 PM) in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Temperature ($22\pm 2^{\circ}\text{C}$) and humidity ($50\pm 10\%$) were controlled to be constant. 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 of Yonsei University.

2.2 Induction of arthritis

A standardized model for the production of inflammatory arthritis was produced by injecting a solution of 2% carrageenan ($50\ \mu\text{l}$, suspended in sterile saline; Type λ carrageenan, Sigma, St. Louis, MO, USA) into the knee joint cavity of

the left hind legs of the rat under halothane (1.0% in air) anesthesia in the behavioral study and urethane (1.25g/kg, i.p.) anesthesia in the electrophysiological study. Behavioral and electrophysiological experiments were performed from 4 h after the injection of carrageenan.

2.3 Aqua-acupuncture

A mixture of *bos taurus domesticus* and *selenarctos thiberanus* (50;50%, BU) and *bos taurus domesticus*, *selenarctos thiberanus* and *Moschus moschiferus* (85;10,5%, BUM) were manufactured and supplied by Korean Aqua-acupuncture Society (Seoul, Korea). BU or BUM were used as an aqua-acupuncture and compared with physiological saline. Each aqua-acupuncture or saline (100ul) was injected with 26G needle at two acupoints equivalent to specific human acupuncture points: Zusanli (ST36) and Hegu (LI4). ST36 and LI4 were named by the World Health Organization (WHO). Zusanli (ST36) is located near the knee joint, 5 mm lateral to the anterior tubercle of the tibia. Hegu (LI4) is located on the dorsal aspects of the hand near the midpoint between the first and second metacarpal bones.

2.4 Electrophysiological recordings

The technique described by Just et al. [7] was modified to record electrical activities of primary articular afferents innervating the knee joint via the medial articular nerve (MAN). The arthritic rat was placed on its left side and the skin overlying the medial face of the 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 centrally as possible to obviate any potential spinal reflexes. The left side femur and tibia were held by grips which were 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°C. In this pool, the whole medial aspect of the left leg, including the medial part of the knee joint and the patellar ligament, was exposed. Fine neurofilaments were dissected at the proximal end of the saphenous nerve using sharpened forceps and were subsequently placed over a platinum electrode for extracellular recordings. To visualize the fine filaments, a black platform was used. The 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 linear outward and inward movements. Passive joint movements, described as hyper-movements of the joint against tissue resistance without soft tissue injury [7, 8], were induced by a linear head motor (Oriental Motor Co. Ltd., Japan) as shown in Fig. 1. Each cycle of the outward and inward movement lasted 15 s, and the responses of afferents to a stimulation cycle consistent with linear outward

and inward movement were tested before (Pre) aqua-acupuncture and were repeated at 0 min, 5 min, 10 min, 30 min, 60 min, 90 min, 120 min, 150 min, and 180 min after manipulation.

2.5 Statistical analysis

Data are represented as percent(%) changes compared with responses before aqua-acupuncture. Statistical tests were done using the one-way repeated measures analysis of variance (ANOVA) followed by Dunnett's (2-sided) post-hoc multiple comparison tests at each time point. A P-value of less than 0.05 was considered statistically significant.

3. Results

In order to evaluate the effects of aqua-acupuncture on arthritic pain, electrophysiological recordings were conducted from 4 h after the injection of carrageenan. When arthritic pain was induced by the injection of carrageenan, the afferents responded vividly to passive movement. The responses of afferents to inward and outward movements were analysed in terms of the duration or total number of activities. Response duration was the period for which neural responses to passive movement lasted and the total number of activities was the sum of neural activities induced by a cycle of passive movement. The effects of aqua-acupuncture on neural responses were examined in three groups at each aqupoints (Fig. 1 and 2): Control group (n=15), BU (n=12), and BUM (n=13).

At Zusanli acupoint, the duration of neural activities tended to be increased in the saline-treated control group (Fig. 1A). In contrast,

the duration of activities in response to the stimulation of outward movement was significantly reduced after aqua-acupuncture of BU or BUM. BUM was more effective in reducing the responses of afferents to the stimulation than BU (Fig. 1A). However, the duration of responses to inward stimulation was not significantly different between groups (data not shown).

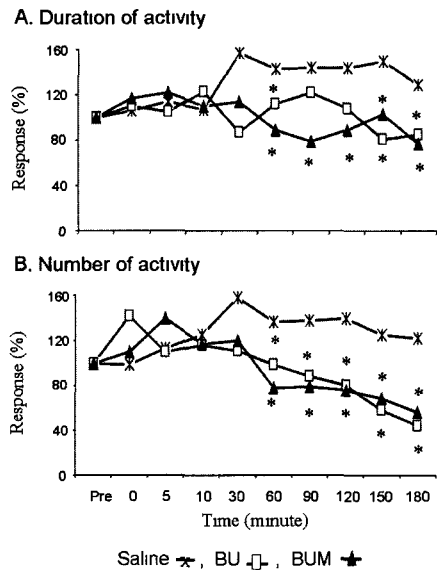


Figure 1. Effects of aqua-acupuncture at Zusanli on neural responses to noxious stimulation, A. Duration of activities, B Total number of activities, (*P<0.05)

The effects of aqua-acupuncture at Zusanli on the total number of responses of afferents are shown in Fig. 1B. The total number of neural activities tended to be increased in the saline-treated control group (Fig. 1B). The total number of activities in response to the stimulation of outward movement was significantly reduced after aqua-acupuncture of BU or BUM. BU and BUM were similar in effectiveness to reduce the responses of afferents to the outward stimulation (Fig. 1B). However, the total number of responses

to inward stimulation was not significantly different between groups (data not shown).

At Hegu acupoint, the duration of neural activities tended to be increased in the saline-treated control group as shown at Zusanli (Fig. 2A). The duration of activities in response to the stimulation of outward movement was significantly reduced after aqua-acupuncture of BU or BUM. BU was more effective in reducing the responses of afferents to the stimulation than BUM (Fig. 1A). However, the duration of responses to inward stimulation was not significantly different between groups (data not shown).

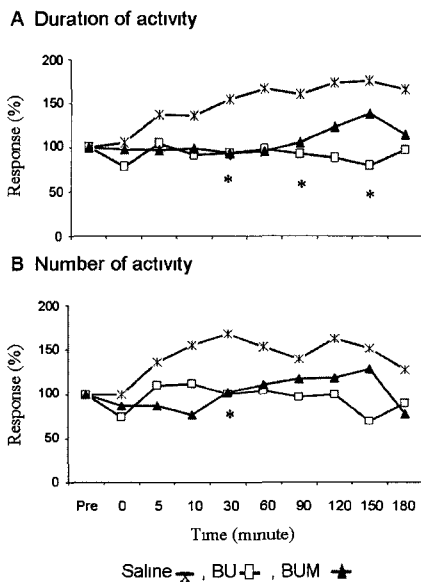


Figure 2. Effects of aqua-acupuncture at Hegu on neural responses to noxious stimulation A' Duration of activities, B' Total number of activities. (* $P < 0.05$)

The effects of aqua-acupuncture at Hegu on the total number of responses of afferents are shown in Fig. 2B. The total number of neural activities tended to be increased in the saline-treated control group (Fig. 2B). The total number of activities in response to the stimulation of

outward movement was significantly reduced after aqua-acupuncture of BU or BUM. BU and BUM were similar in effectiveness to reduce the responses of afferents to the outward stimulation (Fig. 1B). However, the total number of responses to inward stimulation was not significantly different between groups (data not shown).

4. Discussion

Acupuncture has been derived from ancient Oriental medicine and used for the treatment of pain. In the ancient understanding of acupuncture, vascular and neurologic energy flowed through meridians, which were directly associated with an organ system [11]. Meridians were interconnected within the vital life energy 'Qi'. Allegedly, a deficiency of 'Qi' caused pain [1]. Pain could thus be modified through acupuncture, whereby the insertion of needles into the meridians rebalanced energy flows. Recent concepts associated meridians with the autonomic nervous system [5, 6]. Numerous 'manipulation' techniques exist, including twisting the inserted needle, pressing down on the needle, or applying a ball of herbs at the base of the needle and igniting the ball. The site of insertion appears to be the major basis for success.

In the present study, the effect of aqua-acupuncture was examined in an animal model of arthritic pain induced by the injection of carrageenan into the knee joints of the rat. In order to determine the pain relieving effects of aqua-acupuncture, the activities of articular afferents innervating the knee joint were recorded. According to these electrophysiological data, aqua-acupuncture at

Zusanli and Hegu was very effective in relieving arthritic pain. These indicate that aqua-acupuncture of BU and BUM may be very useful for treating arthritic pain.

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