Pain Relieving Effects of KHA-30 as a Herbal Medicine in an Animal Model of Arthritis

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= Abstract =

Background: Herbal medicine has traditionally been used in oriental medicine to relieve pain. Arthritis, accompanied with severe pain, became the most prominent disease as more people live to advanced ages. In the present study, the effect of KHA-30 was examined in an animal model of arthritic pain induced by the injection of carrageenan into the knee joints of the rat.

Methods: 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. The left saphenous artery was cannulated in order for to administer the KHA-30. Nerve fibers were characterized by their mechanical sensitivity to passive movement of the joint consisting of outward and inward movements.

Results: Intrarterially injected KHA-30 significantly reduced the duration of neural responses of the afferents in response to mechanical stimulation. And KHA-30 also significantly inhibited the number of neural responses to mechanical stimulation.

Conclusions: These results indicate that KHA-30 may be effective in relieving arthritic pain as a herbal medicine and useful as one of the analgesics.

Key Words: Arthritic pain, Electrophysiological recording, Herbal medicine, Rat

INTRODUTION

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 come 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 at a total disease expenses close to $65 billion. From the twentieth century, the disease 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 the discovery of curative medications, but also the increasing aging of the population. The elderly

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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 disorder of movement, and its major effects are on quality of life. Pain induced by arthritis can interfere with social life, and produce feelings of hopelessness and depression. Therefore, the development of the therapy of arthritic pain is the most important.

Traditionally, in oriental countries, herbal medicine has been shown to possess analgesic and anti-inflammation effects in arthritis.[1,2] KHA-30 is the methanol extract of Goreishi (the feces of Trogopterus xanthipes Milne-Edwards). According to our own unpublished study, KHA-30 has been shown to possess strong pain inhibition effects in formalin test using mice. KHA-30 may be expected to have analgesic effects in acute arthritic pain model of rats. Therefore, the present study was conducted to investigate pain relieving effects of KHA-30 by means of electrophysiological recording in the arthritic rats.

MATERIALS AND METHODS

Subjects

Experiments were performed on Thirty- seven 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°C) 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.

Induction of arthritis

A standardized model for the production of inflammatory arthritis was produced by injecting 2% carrageenan (50μl, 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/kg, i.p.) anesthesia. Electrophysiological recordings were performed from 4 h after the injection of carrageenan.

Electrophysiological recordings

The technique described by Just et al.[3] 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°C. 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 movements.[3,4] Joint passive movements are described as

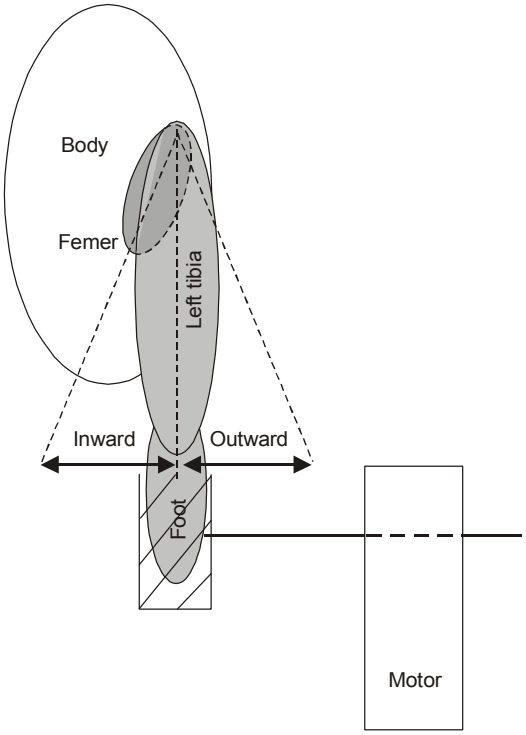

Fig. 1. Schematic drawing of the outward and inward movement stimulation applied to the knee joint.

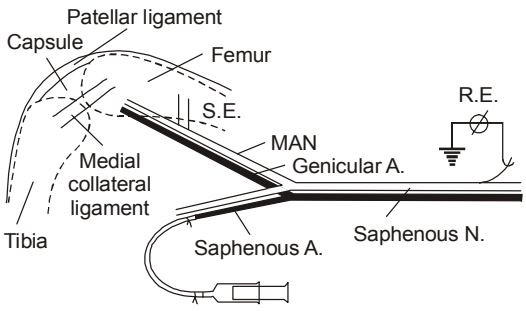

Fig. 2. Schematic diagram for experimental set-up. KHA-30 was injected through the cannula inserted into the saphenous artery. The neural activity was recorded from the proximal end of the median articular nerve fibers.

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 Fig. 1.

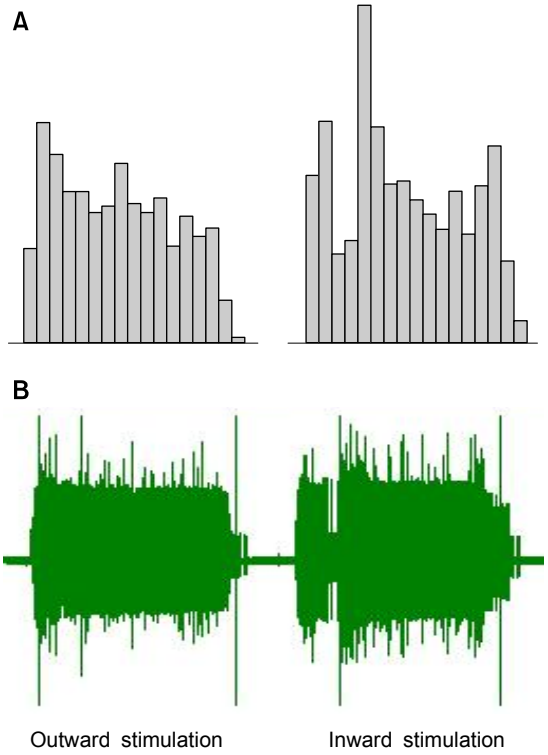

Fig. 3. Responses of a median articular nerve fiber to passive mechanical stimulation of outward or inward movement. A: Histogram constructed from digitized neural activities. B: Discharges produced by outward or inward movement.

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 the administration of KHA-30 or physiological saline.

Administration of KHA-30

KHA-30 was desolved in physiological saline. The concentration of KHA-30 was as follows: 10 mg/ml (× 1), 50 mg/ml (× 5), 100 mg/ml (× 10). KHA-30 was injected in a volume of 0.2 ml through the cannula inserted into the saphenous artery in order to be delivered around the inflamed joint. The control group rats were injected the same volume of physiological

saline (Fig. 2).

Statistical analysis

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

RESULTS

The neural activities were recorded from a total of 105 primary articular afferents in the inflamed knee joint. KHA-30 was applied to inflamed joint area through the saphenous artery and physiological saline was applied for comparison. All electrophysiological recording data were analysed into duration of activity, number of activity. Duration of activity consisted of lasting time of neural activities produced by passive movement of outward or inward. Number of activity was the sum of neural activities induced by the same passive movement. Electrophysiological recordings were performed from 4 h after the injection of carrageenan.

Effects of KHA-30 on the duration of neural activity

Four hrs after the induction of arthritis in the knee joint by the intra-articular injection of carrageenan, the articular afferents normally developed neural discharges responsive to passive movement of joint and the duration of neural responses increased (Fig. 3). When KHA-30 was applied into the inflamed knee joint, the duration of neural responses by the stimulation of outward movement was significantly reduced at 5 min and 10 min after KHA-30 injection (Fig. 4A). But the duration of responses by the stimulation of inward movement tended to reduce but the differences were not statistically significant at all time points (Fig. 4B).

Effects of KHA-30 on the number of neural activity

After induction of acute arthritis in the knee joint by the intra-articular injection of carrageenan, the articular afferents normally developed neural discharges responsive to passive movement of joint and the number of neural activity increased. When KHA-30 was applied into the inflamed knee joint, the number of neural

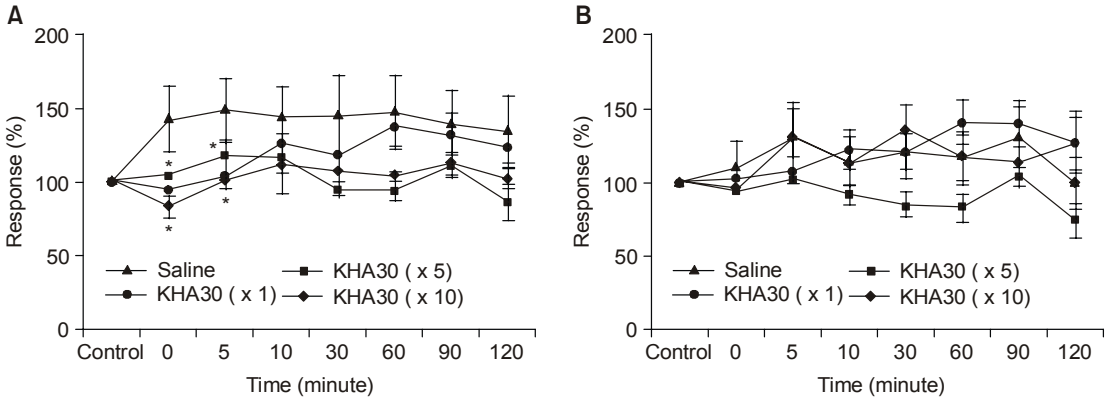

Fig. 4. Effects of KHA-30 on the duration of neural activities. Changes in the duration of activities in the articular primary afferents induced by passive outward (A) and inward movement (B) after KHA-30 injection. The data are expressed as mean ± SEM. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) post-hoc multiple comparison test to compare different doses of KHA-30 and saline-injected control group at each time point. *: A P value of less than 0.05 was considered to be statistically significant. KHA-30 significantly reduced the duration of neural activities produced by outward movement but not inward stimulation.

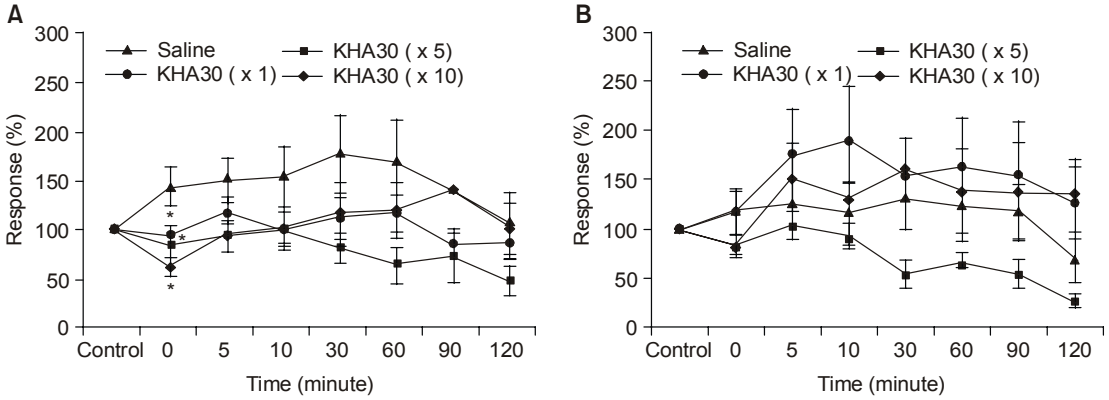

Fig. 5. Effects of KHA-30 on the number of neural activities. Changes in the number of activities in the articular primary afferents induced by passive outward (A) and inward movement (B) after KHA-30 injection. The data are expressed as mean ± SEM. Analysis was done by one way ANOVA followed by Dunnett's (2-sided) post-hoc multiple comparison test to compare different doses of KHA-30 and saline-injected control group at each time point. *: A P value of less than 0.05 was considered to be statistically significant. KHA-30 significantly reduced the number of neural activities produced by outward movement but not inward stimulation.

responses by the stimulation of outward movement was significantly reduced at 5 min and 10 min after KHA-30 injection (Fig. 5A). But the number of responses by the stimulation of inward movement tended to reduce but the differences were not statistically significant at all time points (Fig. 5B).

DISCUSSION

Carrageenan is a sulphated mucopolysaccharide extracted from the seaweeds *Chondrus* spp. and *Gigartina* spp. It is commonly known as Irish Moss or carrageen moss. It has been used in the rat for inflammation models: footpad inflammation or paw edema model,[5] air pouch model,[6] and to induce acute arthritis[7] as well as in conjunction with other agents[8] and in the enhancement of inflammatory arthritis in other models.[9] The responses of ascending tract cells in the cat spinal cord is enhanced by carrageenan-induced inflammation of the knee joint.[10] 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.[11]

Traditionally, in oriental countries, herbal medicine has been shown to possess analgesic and anti-inflammation effects in arthritis. For example, 'eng-Khia-U' is a folk medicine from Taiwan. According to Tsai and Lin,[1] pretreatment with 'Teng-Khia-U' significantly inhibited the carrageenan-induced acute arthritis and also suppressed the development of chronic arthritis induced by complete Freund's adjuvant. According to Ira Thabrew et al.,[2] 'Maharasnadhi Quathar' which is a poly herbal preparation in Sri Lanka exert the anti-inflammatory effects in carrageenan-induced arthritis. In human rheumatoid arthritis patients, after 3 months of 'Maharasnadhi Quathar' treatment, there was a marked improvement in the pain and inflammation experienced by the patients as well as in the mobility of the affected joints.

Arthritis is not a primary cause of death. It is mainly a disorder of movement, and its major effects are on quality of life. Pain induced by arthritis can interfere with social life, and produce feelings of hopelessness and depression. Therefore, the deveplopment of the therapy for arthritic pain is the most important. The previous studies were focussed on the pain relieving effects

of acupuncture and electroacupuncture on the arthritis.[12-15)]

In our own unpublished study, KHA-30 showed strong pain inhibition effects in formalin test using mice. However, the anti-arthritic properties KHA-30 have not been subject to any scientifically controlled investigations so far. In the present study, in order to examine pain relieving effects of herbal medicine, the activities of articular afferent innervating knee joint were recorded and the duration and total number of neural responses to owtward mechanical stimulation were analysed. According to the present data, intra-arterially injected KHA-30 was effective in reducing the duration or number of neural responses to mechanical stimulation. However, we could not observe any significant reduction of neural responses to inward stimulation in either the duration or number of activities. The difference between the effects of outward and inward stimulations can not be explained in detail but we infer that outward stimulation may produce a certain component of arthritic pain more affective to herbal medicine than inward stimulation.

In the present study, KHA-30 was administered intra-arterially through the cannula inserted into a branch of the saphenous artery instead of the direct injection into the joint cavity. This method was chosen because it was convenient to control the volume of KHA-30 to be spread around the inflamed site. If KHA-30 was injected directly into the cavity, it is possible that the needle tip containing KHA-30 might protrude the wall of the cavity or other tissues except for the cavity itself. Thus intra-arterial injection of KHA-30 was more convenient and found to be effective in reducing the arthric pain in the present study.

In conclusion, the effect of herbal medicine in the present study 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 the administration of KHA-30 may provide an alternative strategy to relieve arthritic pain. However, the detailed mechanisms that KHA-30 acts on the inflamed knee joint was not understood. Further studies on identification and isolation of active components in KHA-30 as well as mechanisms of action are needed.

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