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Analgesic and anti-inflammatory effect of the aqueous extract of root of *Angelica Dahurica*

In-Ho Choi[1], Hyung-Ho Lim[1], Yun-Kyung Song[1], Jin-Woo Lee[2], Young-Sick Kim[2], Il-Gyu Ko[2], Ki-Jeong Kim[2], Mal-Soon Shin[2], Khae-Hawn Kim[3] and Chang-Ju Kim[2,*]

[1]*Department of Rehabilitation Medicine, College of Oriental Medicine, Kyungwon University, 65 Bokjung-dong, Sujung-gu, Songnam 461-701, Republic of Korea;* [2]*Kohwang Medical Research Institute, Department of Physiology, College of Medicine, Kyung Hee University, 1 Hoegi-Dong, Dongdaemun-gu, Seoul, 130-701, Republic of Korea;* [3]*Department of Urology, Gil Medical Center, Gachon University of Medicine and Science, 1198 Goowhol-dong, Namdong-gu, Inchon 405-760, Republic of Korea*

SUMMARY

Angelica dahurica (Umbelliferae) grows in China, Japan, Russia, and Korea. The root of *Angelica dahurica* has been used as a traditional folk medicine to treat headache and toothache. In this study, the effects of the aqueous extract of *Angelica dahurica* on acetic acid-induced abdominal pain, carrageenan-induced edema, and thermal hyperalgesia were investigated using mice and rats. The present results showed that the aqueous extract of *Angelica dahurica* inhibited acetic acid-induced abdominal pain in mice and reduced carrageen-induced edema in rats. The present study showed that the aqueous extract of *Angelica dahurica* possesses anti-inflammatory and analgesic effects.

Key words: *Angelica dahurica*; Analgesic and anti-inflammatory effect; Edema; Thermal pain

INTRODUCTION

The aromatic medicinal plant *Angelica dahurica* (Umbelliferae) is a perennial herb growing to 2.5 m with large three-branched leaves and umbels bearing many white flower heads. It grows wild in thickets in China, Japan, Russia, and Korea. The roots of this species have been used as traditional folk medicine to treat headache, toothache, aching eyes, abdominal pain, hysteria, bleeding, menstrual disorder, and neuralgia. Several coumarins that are constituents of *Angelica*

dahurica have been extensively studied for their chemical structures (Saiki *et al.,* 1971; Wang *et al.,* 2001), and pharmacological effects (Kimura *et al.,* 1982; Kim *et al.,* 1992; Kwon *et al.,* 1997).

Pain is the most common symptom encountered in clinical practice. It is believed that current analgesic drugs such as opiates and non-steroidal anti-inflammatory drugs (NSAID) are not always useful in many painful conditions, because of their side effects and potency. As a result, the search for other alternatives seems necessary and beneficial.

The writhing test is a well-known animal model of inflammatory and visceral pain. It is useful in animal model studying anti-nociceptive effects. Carrageenan-induced local inflammation is a commonly used method to evaluate the

*Correspondence: Chang-Ju Kim, Department of Physiology, College of Medicine, Kyung Hee University, 1 Hoigi-dong, Dongdaemoon-gu, Seoul 130-701, Republic of Korea. Tel: +8229610407; Fax: +8229642195; E-mail: changju@khu.ac.kr

effects of NSAID. Therefore, carrageenan-induced paw edema has been used to asses the contribution of mediators involved in vascular changes associated with acute inflammation (Di Rosa *et al.,* 1971). Also, the plantar test using Hargreaves apparatus can determine thermal pain threshold by exposing the animals to the heat (Hargreaves *et al.,* 1988).

In the present study, we evaluated the anti-nociceptive and anti-inflammatory effects of the aqueous extract of *Angelica dahurica* using mice and rats. For this study, several experimental pain models such as acetic acid-induced writhing response, carrageenan-induced edema, and plantar test were performed.

MATERIALS AND METHODS

Preparation of the aqueous extract of *Angelica dahurica*

To obtain the aqueous extract of *Angelica dahurica,* 50 g of *Angelica dahurica* was added to distilled water, and extraction was performed by heating at 90°C for 2 h, concentrating with a rotary evaporator, and lyophilization (Eyela, Tokyo, Japan). The resulting powder, weighing 12.4 g, was dissolved in saline solution and filtered through a 0.45 μm syringe before use.

Animals and treatments

Male ICR mice weighing 28 - 30 g and Male Sprague-Dawley rats weighing 150 - 160 g were used for the experiments. The experimental procedures were performed in accordance with the animal care guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. The animals were housed under laboratory conditions at a controlled temperature ($20 \pm 2^{\circ}$C) and maintained under light-dark cycles, each consisting of 12 h of light and 12 h of darkness (lighting from 07:00 to 19:00 h) with food and water made available *ad libitum*.

Acetic acid-induced writhing response in mice

The animals were divided into five groups: the control group, the acetic acid-injection group, the acetic acid-injection and 50 mg/kg *Angelica dahurica*-treated group, the acetic acid-injection and 100 mg/kg *Angelica dahurica*-treated group, and the acetic acid-injection and 200 mg/kg *Angelica dahurica*-treated group (n = 10 in each group). The aqueous extract of *Angelica dahurica* were orally administered to the mice at 1 h before acetic acid injection. Mice were injected intraperitoneally with 0.15 ml of 1.0% acetic acid as an irritant, and then placed in an individual plastic cage (20 × 30 × 12 cm high) for the observation of writhing response. The number of writhing reflex was counted for 30 min starting immediately after acetic acid injection.

Carrageenan-induced edema in rats

The animals were divided into five groups: the control group, the carrageenan-induced edema group, the carrageenan-induced edema and 100 mg/kg *Angelica dahurica*-treated group, the carrageenan-induced edema and 200 mg/kg *Angelica dahurica*-treated group, and the carrageenan-induced edema and 400 mg/kg *Angelica dahurica*-treated group (n = 10 in each group).

The volume of the paw edema in each animal was measured using a plethysmometer (Ugo Basile, Italy) with a precision of two decimal places. To induce edema in the experimental animals, a single subplantar injection of carrageenan (1%, 0.05 ml; Sigma Chemical Co., St. Louis, MO, USA) was given to each animal, and the animals in the control group received injections of equivalent dose of normal saline as a same method (Sluka and Chandran, 2002).

The animals in the *Angelica dahurica*-treated groups received orally with 1 ml of the aqueous extract of *Angelica dahurica* at the respective dose at 1 h before carrageenan injection, and those in the control group and in the carrageenan-induced edema group received equivalent amount of

drinking water at 1 h before carrageenan injection. The paw volume was measured at 1, 2, 3, 4 and 5 h after carrageenan injection. The percentage of edema was calculated as follows:

Percentage of edema (%) = (Vt-Vn)/Vn × 100

Vt = The paw volume of each time after the injection of carrageenan

Vn = The paw volume before the injection of carrageenan

The plantar test (Hargreaves's method) in rats

The animals were divided into four groups: the thermal stimulation-induced nociception (control) group, the thermal stimulation-induced nociception and 50 mg/kg *Angelica dahurica*-treated group, the thermal stimulation-induced nociception and 100 mg/kg *Angelica dahurica*-treated group, and the thermal stimulation-induced nociception and 200 mg/kg *Angelica dahurica*-treated group (n = 6 in each group).

To assess nociceptive response to thermal stimulus, paw withdrawal latency was measured by the method of Hargreaves *et al.* (1988) The
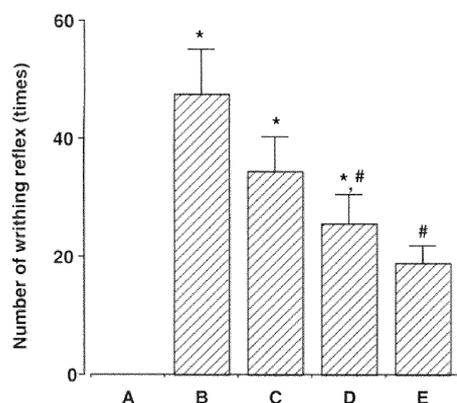

Fig. 1. Effect of *Angelica dahurica* on the number of writhing reflex. (A) Control group, (B) acetic acid-injection group, (C) acetic acid-injection and 50 mg/kg *Angelica dahurica*-treated group, (D) acetic acid-injection and 100 mg/kg *Angelica dahurica*-treated group, (E) acetic acid-injection and 200 mg/kg *Angelica dahurica*-treated group. *represents *P* < 0.05 compared to the control group. #represents *P* < 0.05 compared to the acetic acid-injection group.

centre of a focused beam of radiant heat was applied to the plantar surface of the hind paw in rats, and then the withdrawal latency time was recorded. The intensity of the heat stimulus was adjusted so that the baseline latency was 6 s, and 20 s cut-off time was imposed to avoid tissue damage. Three min was allowed between each test. The animals in the *Angelica dahurica*-treated groups received orally with 1 ml of the aqueous extract of *Angelica dahurica* at the respective dose at 1 h before test, and those in the control group received equivalent amount of saline at 1 h before test. The withdrawal latency time was measured at 1 h and 2 h after drug administration.

Data analysis

Statistical analysis was performed using one-way ANOVA followed by Duncan *post-hoc* test. The results are presented as the mean ± S.E.M. Difference was considered significant at *P* < 0.05.

RESULTS

Effect of *Angelica dahurica* on acetic acid-induced writhing response in mice

The number of the writhing reflex in the control group was 00.00 ± 0.00. The number of writhing reflex in the acetic acid-injection group was 47.60 ± 7.47. The number of writhing reflex in the acetic acid-injection and *Angelica dahurica* (50 mg/kg, 100 mg/kg, and 200 mg/kg)-treated groups was 34.44 ± 5.74, 25.57 ± 4.83, and 18.80 ± 3.00, respectively.

The present results showed that acetic acid injection into the abdominal cavity induced writhing reflex. The pre-treatment with the aqueous extract of *Angelica dahurica* suppressed acetic acid-induced writhing response as a dose-dependently.

Effect of *Angelica dahurica* on the volume of carrageenan-induced paw edema in rat

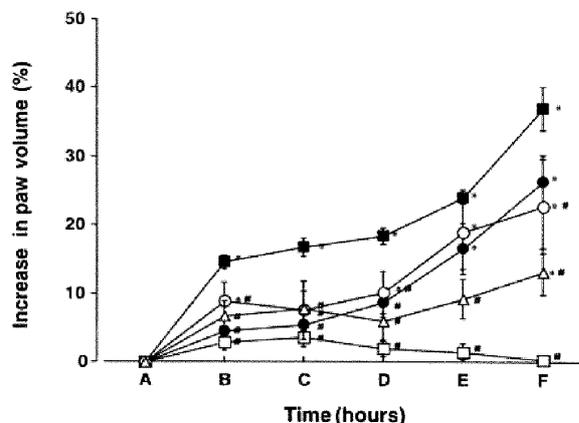After 1 h, paw volume in the control group was

Fig. 2. Effect of *Angelica dahurica* on carrageenan-induced paw edema. (□) Control group, (■) carrageenan-induced edema group, (●) carrageenan-induced edema and 100 mg/kg *Angelica dahurica*-treated group, (○) carrageenan-induced edema and 200 mg/kg *Angelica dahurica*-treated group, (△) carrageenan-induced edema and 400 mg/kg *Angelica dahurica*-treated group. *represents $P < 0.05$ compared to the control group. #represents $P < 0.05$ compared to the carrageenan-induced edema group.

Fig. 3. Effect of *Angelica dahurica* on thermal pain. (○) Thermal stimulation-induced nociception group (control), (●) thermal stimulation-induced nociception and 50 mg/kg *Angelica dahurica*-treated group, (□) thermal stimulation-induced nociception and 100 mg/kg *Angelica dahurica*-treated group, (■) thermal stimulation-induced nociception and 200 mg/kg *Angelica dahurica*-treated group.

$2.87 \pm 1.16\%$. Paw volume in the carrageenan-induced edema group was increased to $14.62 \pm 1.01\%$. Paw volume in the carrageenan-induced edema and *Angelica dahurica* (100 mg/kg, 200 mg/kg, and 400 mg/kg)-treated groups was decreased to $4.44 \pm 0.68\%$, $8.90 \pm 2.69\%$, and $6.71 \pm 2.17\%$, respectively.

After 2 h, paw volume in the control group was $3.58 \pm 1.37\%$. Paw volume in the carrageenan-induced edema group was increased to $16.72 \pm 1.30\%$. Paw volume in the carrageenan-induced edema and *Angelica dahurica* (at 100 mg/kg, 200 mg/kg, and 400 mg/kg)-treated groups was decreased to $5.34 \pm 0.87\%$, 7.53 ± 4.27, and $7.73 \pm 2.52\%$, respectively.

After 3 h, paw volume in the control group was $1.94 \pm 1.14\%$. Paw volume in the carrageenan-induced edema group was increased to $18.33 \pm 1.17\%$. Paw volume in the carrageenan-induced edema and *Angelica dahurica* (100 mg/kg, 200 mg/kg, and 400 mg/kg)-treated group was decreased to $8.61 \pm 2.05\%$, $10.07 \pm 3.07\%$, and $5.93 \pm 2.61\%$, respectively.
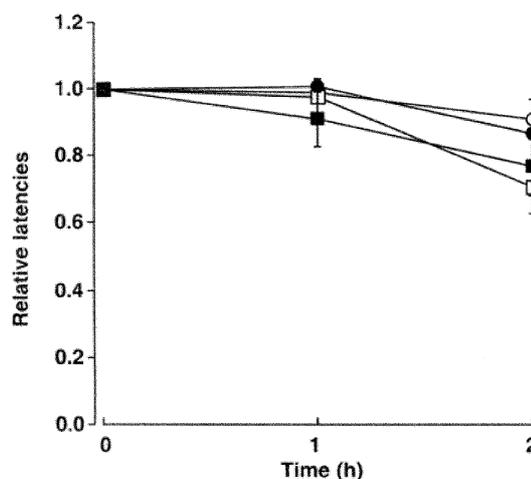
After 4 h, paw volume in the control group was $1.47 \pm 1.17\%$. Paw volume in the carrageenan-induced edema group was increased to $24.07 \pm 0.99\%$. Paw volume in the carrageenan-induced edema and *Angelica dahurica* (100 mg/kg, 200 mg/kg, and 400 mg/kg)-treated groups was decreased to $16.53 \pm 3.63\%$, $19.00 \pm 5.52\%$, and $9.25 \pm 2.88\%$, respectively.

After 5 h, paw volume in the control group was $0.36 \pm 0.42\%$. Paw volume in the carrageenan-induced edema group was increased to $36.97 \pm 3.17\%$. Paw volume in the carrageenan-induced edema and *Angelica dahurica* (100 mg/kg, 200 mg/kg, and 400 mg/kg)-treated groups was decreased to $26.29 \pm 3.85\%$, $22.68 \pm 6.84\%$, and $13.19 \pm 3.40\%$, respectively.

The present results showed that the paw volume in the control group maintained constant level, while carrageenan injection increased paw volume as time-dependently. Pre-treatment with the aqueous extract of *Angelica dahurica* suppressed carrageenan-induced paw edema.

Effect of *Angelica dahurica* on the plantar test (nociceptive thermal stimulation) in rats

After 1 h, paw withdrawal threshold of the pre-treated value was considered as 1.00. The withdrawal latency of thermal stimulation-induced nociception group was 0.99 ± 0.02. The withdrawal latency of thermal stimulation-induced nociception and *Angelica dahurica* (50 mg/kg, 100 mg/kg, and 200 mg/kg)-treated group was 1.01 ± 0.01, 0.98 ± 0.05, and 0.91 ± 0.08.

After 2 h, the withdrawal latency of thermal stimulation-induced nociception group was 0.91 ± 0.06. The withdrawal latency of thermal stimulation-induced nociception and *Angelica dahurica* (50 mg/kg, 100 mg/kg, and 200 mg/kg)-treated group was 0.87 ± 0.01, 0.71 ± 0.08, and 0.77 ± 0.09.

The present results showed that the aqueous extract of *Angelica dahurica* exerted no significant effect on the withdrawal latency of thermal stimulation-induced nociception.

DISCUSSION

Angelica dahurica (Umbelliferae) is a perennial herb distributed in the whole area of Korea, and its root has been used as an anti-pyretic and analgesic agent. This study evaluated the scientific basis for the use of *Angelica dahurica* on inflammation and pain. The anti-nociceptive and anti-edematogenic effects were analysed using different stimuli, such as chemical agents (acetic acid and carrageenan) and heat (plantar test). *Angelica dahurica* contains several coumarins and furanocoumarins. The coumarins are known to inhibit multiplication of bacteria, fungi, and viruses (Hudson *et al.*, 1993; Kofinas *et al.*, 1998) and they demonstrated anti-allergic (Kimura and Okuda, 1997), anti-inflammatory (Chen *et al.*, 1995), and immune suppressive activities (Kuzel *et al.*, 1992). The furanocoumarins also have a variety of biological properties such as inhibitory effects on prostaglandin E production (Ban *et al.*, 2003), acetylcholinesterase (Kim *et al.*, 2002), and nitric oxide generation (Ryu *et al.*, 2001).

These evidences indicate that coumarins and furanocoumarins can modulate the prostanoid biosynthetic pathway. Arachdonic acid, which is accumulated in the membrane lipid, is selectively released from the phospholipids pool by chemical or mechanical stimulation, it subsequently converted to prostaglandins (PGs) by two enzymes: COX-1 and COX-2 (Griffiths, 1999). COX-2 is primarily responsible for PGs produced in inflammation while COX-1 is involved in normal homeostasis. In this regard, COX-2 is up-regulated in the air pouch and catalyzes the production of large amounts of PGE_2 (Masferrer *et al.*, 1994). The up-regulation of COX-2 inducing PGE_2 synthesis is a major event in acetic acid-induced writhing response and in carrageenan-induced inflammation. Alternatively, the decrease of PGE_2 is induced by inhibition of the release of tumor necrosis factor-α (TNF-α), because stimulation of macrophages/ monocytes, fibroblasts, and epithelial cells with cytokines such as interleukin-1 (IL-1) and TNF-α increases PGE_2 production (Griffiths, 1999).

In the present study, the analgesic effect of *Angelica dahurica* was evaluated by acetic acid-induced writhing response. The acetic acid-induced abdominal writhing which is the visceral pain model. Releases of arachidonic acid via cyclooxygenase induces prostaglandin biosynthesis, and it plays a role in the nociceptive mechanism (Franzotti *et al.*, 2000). The results of the present study showed that the mice pre-treated with the aqueous extract of *Angelica dahurica* revealed a dose-dependent analgesic effect on acetic acid-induced writhing response and this effect may be due to inhibition of the synthesis of the arachidonic acid metabolites.

The carrageenan test was selected in this study, because of its sensitivity in detecting acute phase of inflammatory response (Di Rosa *et al.*, 1971). The intraplantar injection of carrageenan in rats induces paw edema. Its first phase (0 - 2.5 h after injection of carrageenan) was resulted from the increase of vascular permeability and concomitant release of mediators such as histamine, serotonin,

and kinins. The second phase is correlated with the production of PGs, oxygen-derived free radicals, and enhancing of COX-2 activity (Panthong *et al.,* 2004). In the present results, administration of the aqueous extract of *Angelica dahurica* suppressed the edematous response at 2 h after carrgeenan injection and this effect continued up to 5 h.

Thermal hyperalgesia can be explained by central convergence of afferents from the deep tissues and skin (Light *et al.,* 2003). Central sensitization and inhibition can be evaluated by administration of agonists such as morphine, which preferentially attenuates in put to the spinal cord from C nociceptors (Cooper *et al.,* 1986). Slow temporal summation of pain depends upon *N*-methyl-D-aspartate receptor activation by C nociceptor input (Vierck *et al.,* 1997). In the present results, however, the aqueous extract of *Angelica dahurica* did not preferentially attenuate pain sensitivity.

Here in this study, we demonstrated that the aqueous extract of *Angelica dahurica* has anti-nociceptive and anti-inflammatory activities, and it is possible that this herb can be wide used to treat inflammation and pain. Further studies are needed to elucidate the mechanisms behind these effects of this herb.

ACKNOWLEDGEMENTS

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