Anti-inflammatory and Analgesic Effects of the Aqueous Extract of *Angelicae Tenuissimae* Radix

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Angelicae Tenuissimae Radix (ATR) has traditionally been used for flu-like symptoms, limb-ache and disability, and even for toothache. In the present study, the effect of ATR on carrageenan-induced edema, acetic acid-induced abdominal pain, and heat-induced hyperalgesia were investigated using rats and mice. In the present results, ATR reduced carrageenan-induced edema in rats and inhibited acetic acid-induced abdominal pain in mice. Here in this study, we have shown that ATR possesses anti-inflammatory and analgesic effects.

Key words : Angelicae Tenuissimae Radix, carrageenan, acetic acid, hyperalgesia

Introduction

Angelicae Tenuissimae Radix (ATR) belongs to Umbelliferae. ATR has traditionally been used in East Asian countries such as Korea, Japan, and China as medicinal herb for various pain especially headache, common cold, and arthralgia[1]. ATR is also effective to diarrhea and even ascariasis[2]. The essential oil of Angelicae Tenuissimae has been used to relieve pain and to treat gynecological diseases[3].

Many tests are used to assess nociception in laboratory animals[4]. These tests are different in stimuli modalities, application sites, and whether the response is mediated by spinal reflex or involves supraspinal structures[5].

The intraplantar injection of carrageenan is known to elicit inflammatory response characterized by time-dependent increase in paw edema. The early inflammation response of carrageenan-induced edema in rats results from the release of histamine and serotonin from mast cells[6,7]. On the other hand, the late phase of carrageenan-induced edema is dependent on cytokines production by resident cells and neutrophil migration[8].

Acetic acid is also known to cause pain by liberating many endogenous substances that excite pain nerve endings[9]. Nonsteroidal antiinflammatory drugs (NSAIDs) inhibit cyclooxygenase (COX) in peripheral tissues, thus interfere with transduction of pain in primary afferent nociceptors.

Thermal hyperalgesia can be explained by central convergence of afferents from deep tissues and the skin. Central sensitization and inhibition can be evaluated by administering an agonist such as morphine, which preferentially attenuates input to the spinal cord.

Hereby, the effects of the aqueous extract of ATR on carrageenan-induced edema in rats, and acetic acid-induced abdominal pain in mice, and heat-induced hyperalgesia in rats were investigated.

Materials and Methods

1. Animals

For the edema and thermal pain test, Male Sprague-Dawley rats (200 ± 20 g) were used. For the abdominal pain test, C57BL/6 mice (30 ± 2 g) were used. They were kept in controlled room temperature (20 ± 2℃) and humidity (50 ± 2%) under a 12:12 h light-dark cycle (lights on 07:00 h). The animals were allowed free access to food and water. All animal procedures were according to the rules of National Institutes of Health (NIH) and the guide of Korean Academic Science.

2. Carrageenan-induced paw edema in rats

Paw edema was induced by injecting 0.05 ml of 1.5% carrageenan into the subplantar region of the left hind paw of the rats as previously described method[10]. Fifty rats were

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divided into 5 groups: the control group, the carrageenan injection group, the carrageenan injection and 100 mg/kg ATR-treated group, the carrageenan injection and 200 mg/kg ATR-treated group, and the carrageenan injection and 400 mg/kg ATR-treated group. The rats in the control group received saline solution. The paw volume was measured using plethysmometer (Ugo Basile, Comerio, Italy) before and 1, 2, 3, 4, 5 h after administering carrageenan. The rats in the ATR-treated groups were received orally with 1 ml of 100, 200, 400 mg/kg of the aqueous extract of ATR, 1 h before the carrageenan injection. The rats in the control and carrageenan injection groups were received with distilled water in a same manner. Edema was calculated according to the following formula:

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

(Vt = The paw volume of each time after the injection of carrageenan, Vn = The paw volume before the injection of carrageenan)

3. Acetic acid-induced writhing reflex in mice

The effect of the aqueous extract of ATR on acetic acid-induced abdominal pain was evaluated by previously described method[11]. Fifty mice were divided into 5 groups: the control group, the acetic acid injection group, the acetic acid injection and 50 mg/kg ATR-treated group, the acetic acid injection and 100 mg/kg ATR-treated group, and the acetic acid injection and 200 mg/kg ATR-treated group. The aqueous extracts of ATR (50, 100, 200 mg/kg, p.o.) and saline solution (control, p.o.) were orally administered 1 h before the acetic acid injection. The mice were injected with 0.15 ml of 1% of acetic acid into the peritoneal cavity. The number of writhes was counted for 30 min.

4. Thermal nociception

Forty rats were divided into 4 groups: the control group, the 50 mg/kg ATR-treated group, the 100 mg/kg ATR-treated group, and the 200 mg/kg ATR-treated group. ATR was administered orally to each rats 1 h before the starting of test. In order to measure sensitivity to heat, paw withdrawal analgesiometer (Model 7360; Ugo Basile, Milan, Italy) was used. Each rat was placed into a Plexiglas chamber and allowed to habituate to the apparatus for 30 min. After this, various intensity of heat was applied to the hind paw, using infrared ray. Then the suitable intensity was determined. The time elapsed between onset of the stimulus and manifestation of the paw withdrawal response was measured. The maximal cut off of 20 seconds was used to prevent tissue damage. All animals were pre-tested to determine the index value. Each animal was tested 3 times and the average was used as the test value.

5. Statistical analysis

The results were presented as the mean standard error of the mean (S.E.M.). The data were analyzed by one-way ANOVA followed by Duncan's post-hoc test using SPSS. The differences were considered statistically significant at $P < 0.05$.

Results

1. Effect of ATR on carrageenan-induced paw edema in rats

One hour after the injection of carrageenan, the edema in the control group was increased 3.80 ± 1.86%. However, the edema in the carrageenan injection group was increased 29.72 ± 5.84%. The edema in the group administered with 100, 200, and 400 mg/kg of ATR was 20.22 ± 4.10, 18.25 ± 2.36, and 20.48 ± 3.37%, respectively. Two hours after the injection of carrageenan, the edema in the control group was 4.01 ± 0.97%. However, the edema in the carrageenan injection group was 34.87 ± 6.22. The edema in the group administered with 100, 200, and 400 mg/kg of ATR was 31.66 ± 6.22, 29.66 ± 2.75, and 28.95 ± 2.22%, respectively. Three hours after the injection of carrageenan, the edema in the control group was 3.69 ± 1.16%. However, the edema in the carrageenan injection group was increased 43.20 ± 7.39%. The edema in the group administered with 100, 200, and 400 mg/kg of ATR was 34.82 ± 6.26, 30.90 ± 4.28, and 33.61 ± 2.52%, respectively. Four hours after the injection of carrageenan, the edema in the control group was 3.44 ± 1.25%. However, the edema in the carrageenan injection group was increased 39.66 ± 5.64%. The edema in the group administered with 100, 200, and 400 mg/kg of ATR was 30.85 ± 5.33, 27.91± 4.53, and 29.84 ± 2.55%, respectively. Five hours after the injection of carrageenan, the edema in the control group was 1.77 ± 0.90%. However, the edema in the carrageenan injection group was increased 34.50 ± 6.00%. The edema in the group administered with 100, 200, and 400 mg/kg of ATR was 29.07 ± 4.35, 25.66 ± 4.75, and 24.75 ± 2.50%, respectively.

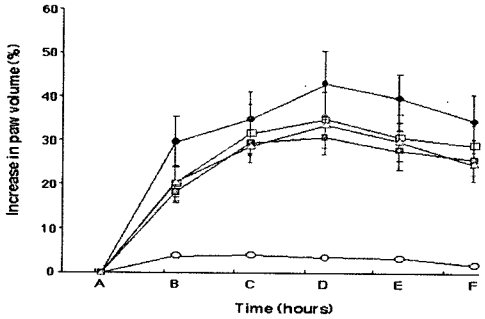

Fig. 1. Effect of Angelicae Tenuissimae Radix (ATR) in response to doses and time on carrageenan-induced plantar edema. (A) 0, (B) 1 h, (C) 2 h, (D) 3 h, (E) 4 h, (F) 5 h. (○) Control group, (●) carrageenan injection group, (□) carrageenan injection and 100 mg/kg ATR-treated group, (■) carrageenan injection and 200 mg/kg ATR-treated group, (△) carrageenan injection and 400 mg/kg ATR-treated group. Values are represented as mean ± S.E.M.

The present results showed that the rats in the control group maintained constant paw volume. Carrageenan injection increased paw volume as time-dependently, and its size reached maximum level at 3 h after carrageenan injection. ATR suppressed carrageenan-induced paw edema as dose-dependently.

2. Effect of ATR on acetic acid-induced abdominal pain in mice

The number of the writhing reflex in the control group was 0.00 ± 0.00. The number of writhing reflex in the acetic acid injection group was 35.20 ± 4.10. The number of the writhing reflex in the group administered with ATR of 50, 100, 200 mg/kg was 28.55 ± 3.04, 23.33 ± 4.76, and 20.66 ± 3.71, respectively. The present results showed that acetic acid injection into the abdominal cavity induced writhing reflex. ATR suppressed acetic acid-induced abdominal pain as dose-dependently (Fig. 2).

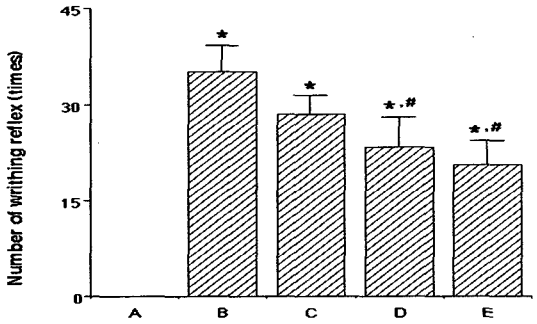

Fig. 2. Effect of Angelicae Tenuissimae Radix (ATR) on the number of writhing reflex. (A) Control group, (B) acetic acid injection group, (C) acetic acid injection and 50 mg/kg ATR-treated group, (D) acetic acid injection and 100 mg/kg ATR-treated group, (E) acetic acid injection and 200 mg/kg ATR-treated group. Values are represented as mean ± S.E.M. * represents P < 0.05 compared to the control group. # represents P < 0.05 compared to the acetic acid injection group.

3. Effect of ATR on heat-induced hyperalgesia in rats

The response time in the pre-test was set as 1. One hour after oral administration of ATR, the response time in the control group was 1.154 ± 0.149. The response time in the group administered with ATR of 50, 100, and 200 mg/kg was 1.089 ± 0.078, 1.019 ± 0.046, and 1.008 ± 0.0792, respectively.

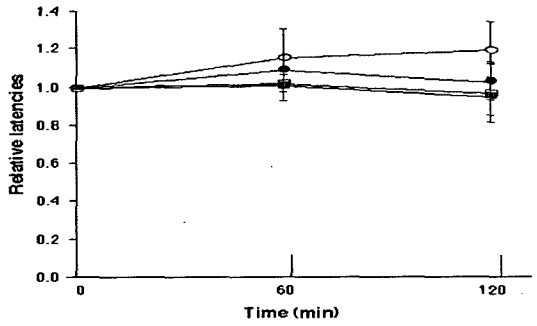

Fig. 3. The effect of Angelicae Tenuissimae Radix (ATR) in response to doses and time on thermal pain. (○) Control group, (●) 50 mg/kg ATR-treated group, (□) 100 mg/kg ATR-treated group, (■) 200 mg/kg ATR-treated group. Values are represented as mean ± S.E.M.

Two hours after oral administration of ATR, the response time in the control group was 1.192 ± 0.149. The response time in the group administered with ATR of 50, 100, and 200 mg/kg was 1.027 ± 0.096, 0.971 ± 0.159 and 0.950 ± 0.099, respectively. The present results showed that ATR exerted no significant effect on heat-induced hyperalgesia (Fig. 3)

Discussion

Carrageenan-induced inflammation is correlated with production of proinflammatory cytokines such as interleukin (IL)-1, IL-6, and TNF-α. These cytokines causes hyperalgesia and edema when injected into rat footpads[12,13]. In the present results, pre-treatment with ATR reduced carrageenan-induced footpad edema. Our in vivo findings that ATR attenuates the carrageenan-induced edema are probably associated with blockage and/or decreased production of interleukine (IL) and tumor necrosis factor (TNF)-α.

The writhing response of the mice to an intraperitoneal injection of noxious chemical is used to screen for both peripherally and centrally acting analgesic effect. In the present results, the mice pre-treated with ATR revealed a dose-dependent analgesic effect on acetic acid-induced writhing response.

Thermal hyperalgesia can be explained by central convergence of afferents from deep tissues and the skin[14]. Central sensitization and inhibition can be evaluated by administering an agonist such as morphine, which preferentially attenuates input to the spinal cord from C nociceptors[15], because slow temporal summation of pain depends upon NMDA receptor activation by C nociceptor input[16]. However, the present results showed that ATR does not preferentially attenuate pain sensitivity.

In the present results, ATR reduced carrageenan-induced edema in rats and inhibited acetic acid-induced abdominal pain in mice. Here in this study, we have shown that ATR possesses anti-inflammatory and analgesic effects.

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