Anti-inflammatory and anti-nociceptive effects of extract of *Astragalus membranaceous*

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SUMMARY

The root of *Astragalus membranaceous* (AM) has been used to treat edema and arthritis in the traditional Korean medicine. To elucidate the anti-inflammatory and anti-nociceptive effects of ethanol extract of AM, the carrageenan-induced paw edema using a plethysmometer and thermal hypersensitivity using the plantar test were measured. Ibuprofen was used as a control drug. Pretreatment with AM (400 mg/kg p.o.) significantly reduced paw edema, compared to the carrageenan-treated rats. In the plantar test, the thermal withdrawal latency in AM-treated group was significantly increased than the carrageenan-treated group. The results indicate that AM could have be the anti-inflammatory and anti-nociceptive properties.

Key words: *Astragalus membranaceous*; Inflammation; Nociception; Paw edema

INTRODUCTION

Recently, complementary and alternative medicine is increasingly used for the treatment of immune diseases and inflammation (Luo *et al.*, 2007). *Astragalus membranaceous* (AM) is a traditional medicinal plant, and it has been widely used as a tonic to enhance the body's natural defense mechanisms such as the immune reaction and circulation (Ryu *et al.*, 2008). AM may exhibit the immunomodulating and immunorestorative effects both *in vitro* and *in vivo* (Cho and Leung, 2007). Shen *et al.* (2008) also found that AM administration during 28 days significantly decreased inflammatory

infiltration and mucus secretion in the lung tissue of allergic mice. AM has been shown to have anti-allergic, anti-inflammatory, and anti-nociceptive properties (Kosuge *et al.*, 1985; Luo *et al.*, 2007). In addition, it seems to have an anti-oxidant property (Boucheny and Brum-Bousquet, 1990) and inhibit the aggregation of platelets (Yun-Choi *et al.*, 1985; Lu *et al.*, 2005). Luo *et al.* (2007) reported that AM can reduce the small intestine mucosal damage after hemorrhage shock reperfusion. However, the mechanism of action of AM is not fully defined.

The present study was aimed to investigate the anti-inflammatory and anti-nociceptive properties of AM in carrageenan-induced paw edema model. For the anti-inflammatory effect, the volume of edema was measured using a plethysmometer. To test the anti-nociceptive effect, the thermal withdrawal latency by the plantar test was estimated.

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MATERIALS AND METHODS

Plant material

The roots of AM were air-dried avoiding sun-light and then cut into small pieces for the experiment. The dried roots (200 g) were soaked in 70% ethanol (6 l) at room temperature for 1 day, and extracted for 1 h with 70% ethanol in an ultrasonic apparatus, and filtered with filter paper to remove the debris. The extract was evaporated under reduced pressure by rotary evaporator (R-205, Büchi, Germany) and lyophilized with freezing dryer (OPERON, Kimpo, Korea). The crude extract (62 g, yield 31%) was made.

Induction of paw edema

ICR mice of male were housed in pairs at standard temperature (22 ± 2°C) with standard 12 h light/ dark cycle (lights on at 7:00 h). Free access to food and water was allowed. Experimental procedures were carried out according to the animal care guidelines of the National Institute for Health (NIH) Guide and the Korean Academy of Medical Sciences. Acute inflammation was induced by intraplantar injection of 0.1 ml of 1% (w/v) λ-carrageenan into the right hind paw. The λ-carrageenan was purchased from Sigma-Aldrich (Milano, Italy).

Evaluation of paw edema

The volume of the injected paw was measured with a plethysmometer (Ugo Basile, Varese, Italy) at 0.5 h after carrageenan injection. The volume of edema was measured as difference in volume between right and left paws. AM (200 mg/kg) and ibuprofen (50 mg/kg) were administered orally 1 h before the subplantar injection of carrageenan. The animals of control group were received saline (1 ml/kg). The thickness (mm) of paw was measured at 0.5 h after the administration of carrageenan. Ibuprofen was used as a positive control (Eddy and Leimback, 1953).

Evaluation of thermal hypersensitivity

Thermal hypersensitivity was tested according to the Hargreaves' procedure (Hargreaves *et al.*, 1988) using the plantar test (Ugo Basile, Comerio, Italy). Briefly, animals were placed in a clear plexiglass box and allowed to acclimatize. A constant intensity, radiant heat source was aimed at the midplantar area of the hind paw. The time from initial heat source activation until paw withdrawal was recorded.

Statistical analysis

All results were expressed as mean ± S.E. Data were analyzed using one-way ANOVA followed by Duncan *t*-test. $P < 0.05$ was considered as statistically significant.

RESULTS

Anti-inflammatory activity

Subplantar injection of carrageenan resulted in an increase in ipsilateral hindpaw volume. In AM-treated group (0.47 ± 0.03 mm), the volume of edema was markedly reduced, compared to the carrageenan-treated group (1.05 ± 0.03 mm). The positive drug, ibuprofen also revealed reduced paw edema (0.94 ± 0.01 mm) (Fig. 1).

Anti-nociceptive activity

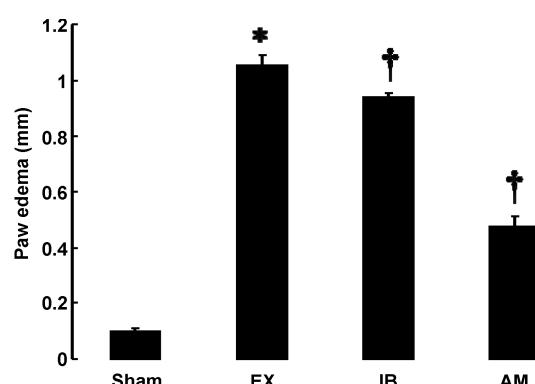In this study, anti-nociceptive effect against thermally

Fig. 1. Effect of AM on carrageenan-induced edema. Sham, control group; EX, carrageenan treatment group; AM, carrageenan with AM treatment group; IB, carrageenan with ibuprofen treatment group. Values represent the mean ± S.E. $^*P < 0.01$ vs. Sham; $^†P < 0.05$ vs. EX.
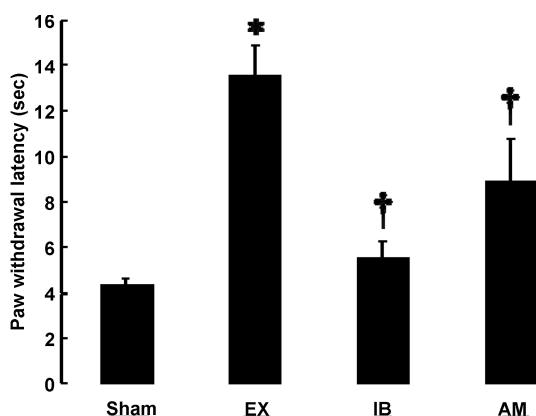
Fig. 2. Effect of AM by plantar test. Sham, control group; EX, carrageenan treatment group; AM, carrageenan with AM treatment group; IB, carrageenan with ibuprofen treatment group. Values represent the mean ± S.E. $^*P < 0.01$ vs. Sham; $^†P < 0.01$ vs. EX.

induced nociceptive pain stimuli was evaluated. As shown in Fig. 2, AM (8.91 ± 1.86 s) showed increasing inflamed paw thermal withdrawal latency, compared to carrageenan-treated group (13.5 ± 0.32). Ibuprofen (5.54 ± 0.74 s) showed more increased inflamed paw thermal withdrawal latency than AM (Fig. 2).

DISCUSSION

AM is one of the commonly used herbs in Oriental medicine. AM enhances immune response and activates circulation. Prieto *et al.* (2003) studied for influence of Chinese anti-inflammatory herbs on leukocyte and platelet functions. They selected fifteen extracts from traditional Chinese medicinal plants used to treat topical inflammations and screened for inhibitory effect on lipoxygenase, cyclooxygenase (COX), and elastase activities in intact leukocytes and platelets. AM, *Forsythia suspensa* and *Poria cocos* inhibited 5-lipoxygenase, with IC_{50} values of 141, 80 and 141 mg/ml, respectively (Prieto *et al.*, 2003). Zwickey *et al.* (2007) reported that subjects ingesting AM showed notable increases CD25 expression on T cells. The present study, AM

was found to exhibit remarkable anti-inflammatory and anti-nociceptive activities. In this study, the anti-nociceptive effect of the AM was evaluated by Hargreaves' procedure. The ability of the AM to reduce the thickness of the edematous hind paw (Di Meglio *et al.*, 2005; Joseph *et al.*, 2005) indicates the anti-inflammatory properties of the extract. The results of this experimental animal study suggest that AM possesses anti-nociceptive, anti-inflammatory properties, and thus lend pharmacological credence to the suggested folkloric uses of the herb in the management of inflammatory conditions. Ibuprofen inhibits COX in peripheral tissues, and interferes the pain transduction in primary afferent nociceptors. The analgesic action of AM may be attributed to the blockade of pain pathway or the release of endogenous substances that react pain nerve endings similarly to ibuprofen.

Carrageenan-induced paw edema experiment is regarded as one of the best methods for screening of anti-inflammatory properties of herb extract (Winter *et al.*, 1962). The carrageenan-induced paw edema production is related with the presence of kinins and prostaglandins (Damas *et al.*, 1986). Another study insisted the ability of AM stimulated immune cells as quantified by CD69 expression on CD4 and CD8 T cells (Brush *et al.*, 2006). AM has an anti-inflammatory effect that is mediated by the MKP-1-dependent inactivation of p38 and Erk1/2 and inhibition of NF kappa B-mediated transcription. These results imply that the AM herb has a potential anti-inflammatory activity (Ryu *et al.*, 2008). Astragaloside IV (AGS-IV), a new glycoside of cycloartane-type triterpene isolated from the root of AM, has been used experimentally for its potent immune-stimulating, anti-inflammatory, and antioxidative actions. A recent study has shown AGS-IV to be an aldose-reductase inhibitor and a free-radical scavenger (Yu *et al.*, 2006).

In conclusion, the present report revealed the anti-inflammatory and anti-nociceptive ability of AM, suggesting AM maybe used for the treatment of inflammation and pain.

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