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Cirsium japonicum elicits endothelium-dependent relaxation via histamine H_1-receptor in rat thoracic aorta

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Abstract

Cirsium japonicum De Candole is widely used in traditional herbal medicine for the treatment of hemorrhage, hypertension or blood circulation in Korea. In this work, we investigated the vasorelaxant activity of an aqueous extract of *C. japonicum* whole plant (*CjEx*) and its possible mechanism in isolated rat thoracic aortic rings constricted with norepinephrine (NE; 300 nmol/l). *CjEx* elicited an acute relaxation in endothelium-intact rings in a concentration-dependent manner (0.1–1.0 mg/ml). This relaxation was eliminated by the removal of the endothelium and pretreatment with N^G-nitro-L-arginine (10 μmol/l), methylene blue (1 μmol/l) or diphenylhydramine (10 μmol/l), but indomethacin (10 μmol/l) atropine (100 nmol/l), [D-Pro^2, D-$Trp^{7,9}$] substance P (5 μmol/l) or HOE-140 (10 nmol/l) did not affect the relaxation. The results indicate that the response to *CjEx* involves enhancement of the nitric oxide-cyclic guanosine monophosphate system, and that it occurs via histamine H_1-receptor. Our findings may contribute to better understanding of the potential link between the clinical use and its beneficial effects on vascular health.

Keywords: *Cirsium japonicum*; Endothelium-dependent; Histamine H_1-receptor; Nitric oxide; Rat aorta; Vasorelaxation

1. Introduction

The endothelium participates in as an important regulator of vascular tone by releasing endothelium-derived relaxing factors (EDRF) (Török, 2000). Many EDRF, secreted from endothelium are involved in the control of vasomotor tone, the most important thing of these molecules is probably nitric oxide (NO) (Gryglewski et al., 1986; Lüscher, 1994; Barton et al., 1997). NO is released from the endothelial cell and it relaxes smooth muscle cells with stimulation cyclic guanosine monophosphate (cGMP) (Furchgott and Vanhoutte, 1989).

Cirsium japonicum De Candole (*C. japonicum*), a member of the family Compositae, is a wild perennial herb found in many areas of Korea, China, and Japan. It is listed in the Japanese and Chinese pharmacopoeias and has been used in Chinese medicine as an antihemorrhagic, antihypertensive, and antihepatitis agent, as well as a uretic (Liu et al., 2006). It is also used as a hemostatic agent in various herbal preparations to prevent epistaxis and metrorrhagia and to improve blood circulation (Donguibogam, 1999). The decoction should contain 8 g *C. japonicum* whole plant and should be boiled in 400 ml water over moderate heat until the final volume is reduced by half and is prescribed per day for one to three months to improve the blood circulation in Korea (Donguibogam, 1999). Epidemiological studies have suggested that a high intake of foods rich in flavonoids may reduce the risk of cardiovascular disease (Hertog et al., 1993, 1995). *C. japonicum* extracts contain various flavonoid compounds (Park et al., 1995; Ganzera et al., 2005), and Nazaruk and Jakoniuk (2005) reported that the apigenin and kaemperol derivatives were kinds of the *Cirsium*-derived flavonoids. The flavonoid apigenin and kaemperol have been proved to have vasorelaxant effect in aorta (Chan et al., 2000; Xu et al., 2006). Despite the wide clinical use

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and various flavonoid compounds of *C. japonicum*, knowledge of its mechanism of action or effect on blood circulation is limited. To elucidate these issues, we characterized the vasorelaxant effects and possible mechanism of aqueous extract of *Cirsium japonicum* (*CjEx*) on the vascular tone of isolated rat thoracic aortas.

2. Materials and methods

2.1. Plant extracts

C. japonicum was identified and provided by Prof. Dr. Byeong-Soo Kang, Department of Oriental Medicine, College of Oriental Medicine, Dongguk University (Gyeongju, Korea). Dried whole plant cut into small pieces and ground using a commercial food mixer. This powder (8 g) was extracted consecutively under reflux with water (200 ml) for 1 h. The resulting water extract was evaporated under reduced pressure at low temperature (37–40 °C) and lyophilized (*CjEx*, 1.4 g). Extracts for all of the experiments were prepared from 4 batches of *C. japonicum* to reflect variation in individual extracts. The solid was stored at −20 °C until use. A solution was prepared with physiological salt solution (PSS) at all concentration of 100–300 mg/ml on the day of the experiment. A voucher specimen No. CJ-001 has been deposited at the Korea Food Research Institute, Gyeonggi-Do, Korea.

2.2. Artery ring preparation

Male Sprague–Dawley rats (200–250 g each) were obtained from Han-Lym Laboratory Animal Co. (Gyeonggi-Do, Korea). Animals were fed a standard laboratory rat chow (PICO-LAB Rodent Diet 20-5053, PMI Feeds, Richmond, IN, USA) for a 24 h–4 day period of acclimation with tap water. Diet and water were allowed *ad libitum*. The air-conditioned animal room was maintained at 22 ± 2 °C, with relative humidity of 59 ± 1 % and a 12 h light/dark cycle (light period, 07:00–19:00 h). After acclimation, rats were killed by stunning and bleeding. The descending thoracic aorta was dissected free from surrounding connective tissues and cut into rings (2–3 mm wide). These rings were then transferred into 4 ml horizontal-type muscle chambers, and were bathed in physiological salt solution (PSS) at 37 °C containing (mmol/l): NaCl, 136.9; KCl, 5.4; $CaCl_2$, 1.5; $MgCl_2$, 1.0; $NaHCO_3$, 23.8; glucose, 5.5; and EDTA 0.01 (pH 7.4); and gassed with 95% O_2 and 5% CO_2. Rings were mounted on stainless-steel hooks connected to a force-displacement transducer (FT03, Grass, Rhode Island, USA) connected to a polygraph system (RPS212, Grass, Rhode Island, USA) and a computer analyzer (Power Lab 400, MacLab System, Castle Hill, Australia) were used. A basal tension of 1 g was applied. Some segments were mechanically denuded of endothelium by gentle rubbing with a moistened cotton swab. The functional activity of vascular endothelium was assessed by measuring whether 1 μmol/l carbachol induce almost complete relaxation (>90%) in aorta stimulated with 300 nmol/l norepinephrine (NE) (Sudjarwo et al., 1992). Each experiment was performed on rings prepared from different rats. All studies were performed according to the Guiding Principles for the Care and Use of Laboratory Animals of The Ethics Committee of Korea Food Research Institute.

2.3. Experimental protocols

All rings were equilibrated for 60 min under a resting tension of 1 g and then exposed repeatedly to 72 mmol/l KCl PSS until responses became stable. Control contraction was produced using 300 nmol/l NE. After sustained tension (60%∼ or 80%∼ of the maximal contraction to 72 mmol/l KCl PSS in endothelium-intact or -denuded rings) was obtained, *CjEx* was added sequentially to the bath solution. The high-potassium solution was prepared by replacing NaCl of PSS with equimolar KCl.

In experiments where specific inhibitors were used, they were added 20 min before precontraction with NE. The inhibitors tested were N^G-nitro-L-arginine (L-NNA, 10 μmol/l) as an inhibitor of NO synthesis, methylene blue (1 μmol/l) as a guanylate cyclase inhibitor, or indomethacin (10 μmol/l) as a cyclooxygenase inhibitor, on *CjEx*-induced enthothelium-dependent relaxation. The different cellular receptors antagonists used were atropine (100 nmol/l) as a selective muscarinic receptor antagonist, diphenylhydramine (10 μmol/l) as a selective histamine H_1-receptor antagonist, [D-Pro^2, D-Trp^{7,9}] substance P (5 μmol/l) as a substance P receptor antagonist and HOE-140 (10 nmol/l) as a bradykinin/B_2-receptor antagonist (Kim et al., 1999; Vanhoutte, 2004; Fukada et al., 2005).

2.4. Reagents

Carbachol, methylene blue, NE, L-NNA, indomethacin, atropine, [D-Pro^2, D-Trp^{7,9}] substance P, HOE-140, and diphenylhydramine were purchased from Sigma (St. Louis, MO, USA). All drugs were dissolved in PSS, except for indomethacin (was dissolved in 100% ethanol at a concentration of 10 mmol/l).

2.5. Statistical analysis

Relaxation was expressed in terms of percentage decrease of the maximal contraction caused by NE (300 nmol/l). The EC_{50} (the concentration to produce a 50% of the maximal contraction in response to NE in endothelium-intact rings) value was determined from the concentration-response curve by linear interpolation. All results are expressed as mean ± S.E.M. The number of rings obtained from different rats was represented by *n*. The Student's *t*-test and one-way ANOVA with Student Newman Keul's test were used to evaluate between groups. The obtained *p*-values less than 0.05 were regarded as significant.

3. Results

3.1. Relaxant effects of CjEx

Fig. 1A shows a typical trace of the effect of *CjEx* on muscle tension stimulated with 300 nM NE in endothelium-intact rat aorta. *CjEx* (0.3 mg/ml) caused acute relaxation within sec-