Vascular Relaxation by the Methanol Extract of Sorbus Cortex via NO-cGMP Pathway

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The methanol extract of Sorbus commixta cortex (MSC) induced relaxation of the phenylephrine-precontracted aorta in a dose-dependent manner, which was disappeared by removal of functional endothelium. Pretreatment of the aortic tissues with N^6^-nitro-L-arginine methyl ester (L-NAME), methylene blue, or 1H-[1,2,4]-oxadiazolo-[4,3-α]-quinoxalin-1-one (ODQ) inhibited the vascular relaxation induced by MSC. MSC-induced vascular relaxations were also markedly attenuated by addition of verapamil or diltiazem, while the relaxant effect of MSC was not blocked by pretreatment with indo- methacin, glibenclamide, tetraethylammonium (TEA), atropine, or propranolol, respectively. Incubation of endothelium-intact carotid arteries or of human umbilical vein endothelial cells (HUVECs) with MSC increased the production of guanosine 3’,5’-cyclic monophosphate (cGMP). Moreover, MSC-induced cGMP production was effect was blocked by pretreatment with L-NAME or ODQ. These results suggest that MSC dilates vascular smooth muscle via endothelium-dependent nitric oxide-cGMP signaling pathway, possible involvement of L-type Ca2+ channel.

Key words Sorbus commixta cortex; vascular relaxation; nitric oxide (NO); guanosine 3’,5’-cyclic monophosphate (cGMP)

Endothelial cells respond to various neurohumoral and physical stimuli by releasing endothelium-dependent vasodilators such as endothelium-derived relaxing factor (EDRF), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF). EDRF has been identified as nitric oxide (NO), which is produced from l-arginine by catalyzing of nitric oxide synthase (NOS). NO is released from endothelial cells under basal conditions and its release is further stimulated by various agents, such as acetylcholin, histamin, substance P, and isoproteinerol. NO activates soluble guanylyl cyclase and then increases the production of guano- sine 3’,5’-cyclic monophosphate (cGMP) leading to protein kinase G (PKG) activation which inhibits Ca2+ influx and decreases the sensitivity of contractile elements to Ca2+. Vascular tone plays an important role in the regulation of blood pressure. The development and maintenance of hypertension has been suggested to involve an inappropriately reduced endothelium-dependent vasodilator influence on the vascular tissue. Indeed, endothelium-dependent vascular relaxation is impaired in human and experimental hyperten- sion, and the ability of nitric oxide (NO) to maintain vascular tone has been shown to be deficient in this condition. Since NO is a potent vasodilator, a deficient production of endothelium-derived NO results in diminished vasodilator tone, allowing vascular resistance to increase, and this con- tributes to the elevated blood pressure. Therefore, many studies have been performed to directly measure vascular relaxation elicited by plant extracts and found that the vasorelaxant effect is related to stimulation of NO release from vascular tissues.

In the courses of screening for the vasorelaxant effect of the various extracts from the medicinal plants, a methanol extract of Sorbus commixta cortex (MSC) was found to exhibit distinctive vasorelaxant activity. The cortex of this species has been used as corroboration or antitussive in the Oriental medicine. From the cortex of S. commixta, triterpenoids such as lupenone and lupeol have been isolated. To our best knowledge, study on vascular relaxant mechanism of MSC has not been described previously. In the present study, therefore, we examined the vascular relaxant effect of MSC and investigated its mechanisms of vasorelaxation.

MATERIALS AND METHODS

Extraction of MSC The cortex of S. commixta was purchased from the herbal medicine co-operative association of Junbuk Province, Korea, in October 2003. A voucher specimen was deposited in the Herbarium of the Professional Graduate School of Oriental Medicine, Wonkwang University (Korea). The S. commixta (1.0 kg) was air-dried at room temperature and reduced to fine powder by milling. The powder was subjected to extraction with 1.2 l of methanol, three times, 24 h each. The MeOH extract was filtered through Whatman No. 3 filter paper and concentrated using rotary evaporator (61.2 g) and used in this study.

Preparation of Rat Aorta The animal procedures were in strict accordance with the National Institutes of Healthy Guidelines for the Care and Use of laboratory Animals and were approved by the Institutional Animal Care and Utilization Committee. Male Sprague-Dawley rats were purchased from Korean Experimental Animals Co. (Daejeon, Korea). The rats (weighing 250–300 g) were sacrificed by decapitation. The thoracic aortas were rapidly and carefully dissected and placed into ice-cold Krebs solution (pH 7.4) containing 118 mmol/l NaCl, 4.7 mmol/l KCl, 1.1 mmol/l MgSO4, 1.2 mmol/l KH2PO4, 1.5 mmol/l CaCl2, 25 mmol/l NaHCO3, and 10 mmol/l glucose. The aortae were removed free of connective tissue and fat, and then cut into rings of approximately 3 mm width. All dissecting procedures were done with extreme care to protect the endothelium from inadvertent damage. In some aortic rings, the endothelial layer was mechanically removed by gently rubbing the luminal surface of the aortic ring back and forth several times with plastic tubing. Endothelial integrity or functional removal was verified by the presence or absence, respectively, of the relaxant response to 3×10^{-6} M acetylcholine on phenylephrine (3×10^{-6} M) contracted vessels.
Record of Isometric Vascular Tone The aortic rings were suspended by means of two L-shape stainless-steel wires inserted into lumen in a tissue bath containing Krebs solution (pH 7.4) at 37°C, while being continuously bubbled with 95% O₂–5% CO₂. The baseline load placed on the aortic rings was 2.0 g, and the changes in isometric tension were recorded using a force-displacement transducer (Grass FT 03, Quincy, MA, U.S.A.) connected to a Grass polygraph recording system (Model 7E). In the first set of experiments, the aortic rings were contacted with phenylephrine (3×10⁻⁶ m) to obtain maximal response. After the maximal response to phenylephrine had been obtained, the aortic rings were washed every 20 min with Krebs solution until the tension returned to the basal level. The concentration-dependent response curve to acetylcholine (ACh) (10⁻⁹–10⁻³ m) was performed as a positive control in endothelium-intact aortic rings contracted by 3×10⁻⁶ m phenylephrine. The rings were then exposed to various drugs for 30 min, and then aortic relaxation was carried out by cumulative addition of MSC. The effect of vehicle, <0.2% dimethylsulfoxide (DMSO), was also tested. After each test, the aortic rings were washed three times with fresh Krebs solution and allowed for 30 min to equilibrate.

Measurement of cGMP Production in Carotid Arteries After equilibration of the carotid arteries isolated from healthy rats for 30 min in Krebs solution gassed with 95% O₂–5% CO₂, rings were incubated in 1 ml of fresh Krebs solution containing 3-isobutyl-1-methylxanthine (IBMX, 1×10⁻⁴ mol/l) at shaking constant temperature water bath (37°C). The vessels were then allowed to equilibrate for an additional 30 min before addition of phenylephrine (3×10⁻⁶ mol/l). After the aortic rings were subjected to MSC in the presence of inhibitors or not for 5 min, relaxation was carried out by cumulative addition of MSC. The relaxation returned to the basal level. The concentration-dependent relaxant effect of vehicle, or TEA (1×10⁻⁶ M) was completely blocked by vascular relaxation induced by concentration-dependent MSC, respectively. The relaxant effect of MSC was markedly inhibited by addition of diltiazem (1×10⁻⁵ m) or verapamil (1×10⁻⁶ m) (Fig. 2B). The relaxant effect of MSC was not altered by pretreatment with 1×10⁻⁶ m indomethacin, or 1×10⁻⁸ m atropine or propranolol (Figs. 3A, B).

RESULTS

Vasorelaxant Effect of MSC With endothelium-intact aortic preparation, MSC relaxed phenylephrine (3×10⁻⁶ m) precontracted aortic rings in a dose-dependent manner (Fig. 1A). The maximal relaxant effect of MSC was 95.3±2.1% (vs. phenylephrine contraction) under the concentration of 1×10⁻⁴ g/ml. This relaxant effect of MSC in the aortic tissues was completely abolished by denudation of endothelial layer. Pretreatment of the aortic tissues with L-NAME (1×10⁻⁵ m) completely inhibited the MSC-induced relaxation (Fig. 1A). As shown in Fig. 1B, methylene blue (1×10⁻⁵ m) or ODQ (1×10⁻⁶ m), inhibitors of soluble guanylyl cyclase, also completely blocked the vascular relaxation induced by concentration-dependent MSC, respectively. MSC-induced endothelium-dependent vascular relaxation was markedly inhibited by addition of diltiazem (1×10⁻⁵ m) or verapamil (1×10⁻⁶ m) (Fig. 2A), L-type Ca²⁺ channel blockers, but not by glibenclamide (1×10⁻⁶ m), an ATP-sensitive K⁺ channel blocker, or TEA (1×10⁻⁴ m), non-selective K⁺ channel blocker (Fig. 2B). The relaxant effect of MSC was not altered by pretreatment with 1×10⁻⁶ m indomethacin, or 1×10⁻⁸ m atropine or propranolol (Figs. 3A, B).
To determine whether vascular production of cGMP is involved in the MSC-induced endothelium-dependent relaxation, the effect of MSC on the production of cGMP in the carotid artery was determined. As shown in Fig. 4, incubation of carotid artery tissues with MSC significantly increased the accumulation of cGMP in a dose-dependent manner. The increased production of cGMP was also observed in HUVECs (Fig. 5A). However, pretreatment of HUVECs with L-NAME (1×10⁻⁶ M) or ODQ (1×10⁻⁶ M) also blocked the MSC-induced increase in cGMP accumulation (Fig. 5B).

**DISCUSSION**

The present study showed that MSC exerted a vasorelaxant effect on the phenylephrine-contracted aortic ring isolated from Sprague-Dawley rats. Removal of functional endothelium abolished this relaxant response to MSC, suggesting that the vasorelaxation caused by MSC was endothelium-dependent. To verify the involvement of endothelium-derived...
vasodilators, the effects of various inhibitors on MSC-induced vascular relaxation were examined. Pretreatment of aortic tissues with L-NAME, an inhibitor of nitric oxide synthase, abolished the MSC-induced vascular relaxation. The present study also showed that pretreatment with methylene blue or ODQ, which are soluble guanylyl cyclase inhibitors, completely blocked the relaxation induced by MSC. These results suggest that the MSC-induced relaxation of aorta is associated with the activation of NO-cGMP pathway. Relaxation of vascular smooth muscle by NO-cGMP signaling involves sequence of steps. Nitric oxide is formed in the endothelium with some entering the underlying vascular smooth muscle where it binds to and activates soluble guanylyl cyclase. This enzyme catalyzes the conversion of GTP to cGMP. cGMP-activated protein kinase G inhibits rho-associated kinase, adenosine triphosphate (ATP)-sensitive K⁺ (KATP) channel in the vascular smooth muscle. The present study showed that exposure of endothelium-intact aortic rings to MSC increased the tissue accumulation of cGMP. Moreover, cGMP production in the HUVECs was increased with the administration of inhibitor of NOS causes a vascular relaxation caused by MSC in aortic rings was not affected by indomethacin, indicating that vasoactive prostacyclin (PGI₂) may not contribute to the MSC-induced relaxation. To assess whether enhanced NO release by MSC was associated with the activation of muscarinic or adrenergic receptors, the effects of atropine or propranolol on the endothelium-dependent relaxation response to MSC were examined. Preincubation of the aortic rings with atropine or propranolol did not affect the relaxations induced by MSC. These findings indicated that MSC did not interact with muscarinic or β-adrenoceptors in MSC-induced vascular relaxation. NO-cGMP pathway plays an important role in the not only relaxation of vascular smooth muscle but also inhibition of VSMC proliferation, adhesion of platelets and leukocytes, endothelial permeability, and extracellular matrix collagen synthesis in the vascular system. A reduced production of NO by vascular endothelial cells is closely associated with the endothelial dysfunction or injury, which is proposed to be an important factor in severe pathologies such as atherosclerosis and hypertension. Chronic inhibition of NO synthesis with the administration of inhibitor of NOS causes a vascular inflammation as well as hypertension in animal experiments. Therefore, the development of vasodilators acting by restoring the level of NO-cGMP in the vascular system can be of great value for the treatment of these cardiovascular diseases. Recently, many studies have been performed to find more suitable anti-hypertensive or anti-atherosclerotic from natural resources. Among them, extracts of hawthorn, Gynostemma pentaphyllum, Cordyceps sinensis, Glycyrrhiza glabra, Uncaria rhynchophylla, and Cae-salpine sappan exhibited an anti-inflammatory effect by endothelium-dependent vasorelaxation or direct stimulation of NO-cGMP release. Our recent study also showed that Cuscuta tricuspidata and Fritillaria usturienis also have an anti-hypertensive effect through the activation of vascular NO-cGMP signaling. In the present study, the endothelium-dependent vasorelaxant effect of MSC may be mediated via the endothelial NO signaling in aortic tissues. These results could be useful for further study to MSC on animal models with cardiovascular diseases.

In conclusion, the present study demonstrated that MSC dilates vascular smooth muscle via endothelium-dependent nitric oxide-cGMP signaling, which may be related with the function of L-type Ca²⁺ channel.

Acknowledgements This study was supported by the Brain Korea 21 Project (2004) and the grant (PF03201-01-00) from Plant Diversity Research Center of 21st Century Frontier Research Program funded by Ministry of Science and Technology of Korean government.

REFERENCES