Endothelium-Independent Vasorelaxation by *Ligusticum wallichii* in Isolated Rat Aorta: Comparison of a Butanolic Fraction and Tetramethylpyrazine, the Main Active Component of *Ligusticum wallichii*

Eun-Young KIM, Jung-Hyun KIM, and Mee-Ra RHYU*

*Food Function Research Division, Korea Food Research Institute; Baekhyun-dong, Bundang-gu, Seongnam-si, Gyeonggi-do 463–746, Korea. Received March 23, 2010; accepted May 21, 2010; published online May 25, 2010*

*Ligusticum wallichii* is an herb widely used to treat vascular disorders in Asian countries, and tetramethylpyrazine (TMP) has been identified as one of its vasorelaxant active components. This study was performed to examine the endothelium-independent relaxation produced by the butanolic-soluble fraction of *L. wallichii* extract (LwBt) and its possible mechanisms of action in isolated rat aortic rings. The effects were compared with those of TMP. LwBt produced vasorelaxation that increased gradually after 2—3 min of LwBt administration and reached a maximum within 30 min. LwBt-induced relaxation was significantly attenuated by pretreatment with 4-aminopyridine and apamin. Additionally, LwBt attenuated CaCl₂-induced vasoconstriction in high-potassium depolarized medium. Thus, LwBt-induced vasorelaxation apparently involved inhibition of calcium influx, mediated by the opening of voltage-dependent and/or Ca²⁺-activated potassium channels. On the other hand, the effect of TMP was significantly attenuated by pretreatment with glibenclamide, and 4-aminopyridine had no effect. In conclusion, LwBt-induced endothelium-independent vasorelaxation was mediated by the opening of voltage-dependent potassium channels, while TMP-induced relaxation was mediated by the opening of ATP-dependent potassium channels. These effects of LwBt may be due to a substance other than TMP.

Key words *Ligusticum wallichii; calcium influx; potassium channel; tetramethylpyrazine*

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**Ligusticum wallichii** Franchat (or *Ligusticum chuanxiong* hort; *L. wallichii*), a member of the Umbelliferae family, is a Chinese medicinal herb that is a common constituent in polypharmaceutical herbal drugs. It has been widely used in Asian countries to increase coronary blood flow and systemic circulation, and to relieve stasis. Previous studies have indicated that *L. wallichii* improves blood fluidity and inhibits endothelial cell damage and vascular smooth muscle cell proliferation.

We demonstrated that an extract of *L. wallichii* showed endothelium-dependent acute relaxation in isolated rat thoracic aorta. The effect of *L. wallichii* was due to endothelial nitric oxide (NO). However, the tetramethylpyrazine (TMP)-induced vasorelaxant effect was not related to endothelial NO. TMP was isolated as a biologically active component from *L. wallichii*. Since 1970, TMP has been used in China for the treatment of patients with angina pectoris and cerebral ischemic syndromes. Earlier pharmacological studies demonstrated that aortic vasorelaxation induced by TMP involved the opening of ATP-sensitive K⁺ channels and/or small-conductance Ca²⁺-activated K⁺ channels.

Endothelium-derived relaxing factor (EDRF) and the membrane potential are major components in modulating vascular tone. After EDRF, the most important molecule is probably NO and K⁺ channels play an important role in the regulation of membrane potential. Five distinct types of K⁺ channel have been documented in arterial smooth muscle: voltage-dependent K⁺ (Kᵥ) channels, large-conductance Ca²⁺-activated K⁺ (LKCa) channels, small-conductance Ca²⁺-activated K⁺ (SKCa) channels, inward rectifier K⁺ (Kir) channels, and ATP-sensitive K⁺ (KATP) channels. Additionally, changes in K⁺ channel activity to produce a subsequent alteration in the activity of calcium channels may result in vasorelaxation. Thus, the present study was performed to separate the active fraction, to examine the active fraction-induced changes in membrane potential, the possible underlying mechanisms, and to perform comparisons with TMP using isolated endothelium-denuded rat aortic rings.

**MATERIALS AND METHODS**

**Extraction and Fractionation** Dried, sliced *L. wallichii* was obtained from a local market and ground with a commercial food mixer. The resulting powder was extracted under reflux with absolute ethanol for 1 h. The extract was evaporated under reduced pressure at low temperature (37—40 °C) and lyophilized (LwEx). Extracts for all of the experiments were prepared from four batches of *L. wallichii* to assess variation in individual extracts. LwEx was fractionated successively with water and n-butanol, and each phase was concentrated and lyophilized into water-soluble (LwDw) and butanol-soluble (LwBt) fractions. The solid was stored at −20 °C until use. LwEx and LwBt were dissolved in 30% ethanol and LwDw was dissolved in water to give final concentrations of 0.3 to 3.0 mg/ml in the bath. Voucher specimen nos. Lw-001 (LwEx), Lw-002 (LwDw), and Lw-003 (LwBt) have been deposited with the Korea Food Research Institute, Gyeonggi-do, Korea.

**Artery Ring Preparation** Male Sprague–Dawley rats (200—250 g) were sacrificed by stunning and exsanguination. The thoracic aorta was dissected free from the surrounding connective tissues and cut into rings 2—3 mm in length. The rings were then transferred to 4-ml horizontal-type muscle chambers, and bathed in physiological salt solution (PSS) containing (mmol/l) NaCl (115), KCl (5), CaCl₂ (2.1), MgSO₄ (1.2), NaHCO₃ (25), glucose (11), and KH₂PO₄ (1.2) at 37 °C, in an atmosphere of 95% O₂ and 5% CO₂. The rings were mounted on stainless steel hooks, connected to a force-displacement transducer (FT 03; Grass, West Warwick, RI, U.S.A.), connected to a polygraph system.
Effects of LwEx, LwBt, LwDw and TMP on NE-Induced Contraction of the Rat Aorta  

Figure 1 shows a typical trace of the effects of LwEx (3.0 mg/ml), LwBt (3.0 mg/ml), LwDw (3.0 mg/ml), and TMP (3 mM) on endothelium-intact or endothelium-denuded rat aorta precontracted with NE (300 nm). LwEx caused relaxation, which increased gradually after 2—3 min of administration of the extract and reached a maximum within 30 min. Endothelium denudation did not influence the activity (Fig. 1A). LwBt, the successive butanol-soluble fraction of LwEx, caused relaxation in a manner qualitatively similar to that seen with LwEx in both endothelium-intact and endothelium-denuded aortas (Fig. 1B). On the other hand, LwDw (3.0 mg/ml) the other water-soluble fraction derived from LwEx, induced transient relaxation of the endothelium-intact aorta, which then gradually reverted to the original contraction induced by NE. Endothelium denudation almost completely abolished this relaxation (Fig. 1C). In contrast, TMP (3 mM) showed acute relaxation; a response within seconds of administering the extract to the endothelium-intact aortas in a manner qualitatively similar to that seen with LwBt in the endothelium-denuded aorta (Fig. 1D). In endothelium-denuded preparations, TMP caused gradual relaxation in a manner qualitatively similar to that seen with LwBt in the endothelium-denuded aorta (Fig. 1D). In inactive preparations, not all the extracts (0.03—10 mg/ml) or TMP (0.1—10 mM) evoked any change in tension in endothelium-intact or endothelium-denuded rings. The relaxation caused by LwEx, LwBt, LwDw, or TMP disappeared completely on washing with PSS. Subsequent stimulation with NE caused contraction to the same degree of tension as before exposure to LwEx, LwBt, LwDw, or TMP in endothelium-intact or endothelium-denuded rings (data not shown).

Influence of Potassium Channel Inhibitors on Endothelium-Independent Relaxation by LwBt or TMP  

As LwBt and TMP have direct effects on vascular smooth muscle, we investigated whether TMP could be responsible for LwBt-induced relaxation by using different potassium chan-
CaCl2-induced contraction in high-potassium (72 mM), depolarized, calcium-free medium in a concentration-dependent manner in the rat aorta (Fig. 3B).

**DISCUSSION**

To our knowledge, this is the first study to indicate that the vascular actions of *L. wallichii* in the rat aorta may involve opening of Kv and SKCA channels by a component other than TMP. LwEx caused gradual relaxation, and this effect was separated into endothelium-dependent and endothelium-independent relaxation in successive fractions, LwDw and LwBt. We reported *L. wallichii*-induced endothelium-dependent relaxation previously,\(^5\) and focused on LwBt-induced endothelium-independent relaxation in the present study. As LwBt and TMP have direct effects on vascular smooth muscle, we examined the differences in the effects of LwBt and TMP in endothelium-denuded rings.

In the present study, blockers specific for Kv and SKCA channels inhibited LwBt-induced vasorelaxation. *L. wallichii* is commonly used to treat cardiovascular diseases,\(^4,10,21\) and some organic acids, including ferulic acid, sedanonic acid, folic acid, vanillic acid, and caffeic acid, have been purified from *L. wallichii*.\(^22,23\) Additionally, Liang *et al.*\(^20\) reported that ligustilide and butylidenephthalide from *L. wallichii* significantly inhibited the vasoconstriction induced by NE and calcium chloride. Ko *et al.*\(^25\) also reported that butylidenephthalide exerted a significant antihypertensive effect in hypertensive rats. On the other hand, blockers specific for KvATP and SKCA channels inhibited TMP-induced vasorelaxation in endothelium-denuded rat aorta. Similarly, it has been reported that the TMP-induced relaxant effect in endothelium-denuded rings was related to opening of KvATP and/or SKCA channels.\(^10,12\) It is possible that the LwBt contains various compounds, and thus LwBt-induced endothelium-independent vasorelaxation could be caused by a component other than TMP in vascular K+ channel functions. Altered vascular K+ channels could be either a cause or an effect of the vascular disease, this study provided insight into the role of *L. wallichii*, which has long been used to promote the vascular health. Changes in potassium channel activity to produce a subsequent alteration in the activity of calcium channels may also result in vasorelaxation.\(^7\) It has been reported that TMP has a calcium antagonist-like action in vascular smooth muscle cells.\(^26,27\) In the present study, LwBt and TMP shifted the calcium-dependent contraction curve to the right in a high-potassium depolarization medium and reduced the maximal contraction. This required less than one-tenth the concentration of LwBt needed to elicit vascular relaxation. Thus, it is possible that regardless of whether LwBt or TMP elicited the vasorelaxant effect via opening of different potassium channels, LwBt and TMP caused endothelium-independent vasorelaxation by altering the activity of calcium channels. Similar vascular relaxation generated by calcium-inhibitive properties has been reported for *Angelica pubescens*,\(^28\) and we reported calcium influx-mediated relaxation by radix *Angelica gigas*.\(^29\)

In conclusion, the LwBt caused direct relaxation of smooth muscle. Inhibition of calcium influx in smooth muscle cells by opening of Kv and SKCA channels could contribute to endothelium-independent relaxation. The relaxation may be induced by a component other than TMP, which
elicits endothelium-independent relaxation via inhibition of calcium influx by opening of K_ATP and/or SKCa channels.

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