Endothelium-dependent vasodilator effect of tannin extract from Cinnamonomi Cortex on isolated rat aorta

Kiyoaki TANIKAWA,*[a] Hirozo GOTO,[a] Norio NAKAMURA,[b] Nobumitsu TANAKA,[a] Masao HATTORI,[b] Takashi ITOH[a] and Katsutoshi TERASAWA[a]

[a] *Department of Japanese Oriental Medicine, Toyama Medical and Pharmaceutical University,*
[b] *Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University*

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Abstract

Cinnamonomi Cortex (the bark of *Cinnamomum cassia* BLUME) is a crude drug that is widely used in spices and medical products. Although improvement of blood flow by this plant component has long been known, there have been no reports concerning the mechanism involved. We studied the vasodilator actions of this drug especially focusing on the role of endothelium in the isolated vascular bed. Tannin from Cinnamonomi Cortex (TCC) relaxed prostaglandin $F_{2\alpha}$-precontracted ring preparations of rat aorta with intact endothelium. TCC did not cause relaxation of specimens without endothelium, and TCC-induced relaxation was inhibited by pretreatment with 10^{-4}M N^G-nitro-l-arginine methyl ester. Dimer, trimer, tetramer, and pentamer components of TCC also produced endothelium-dependent vasodilatation. Stronger relaxation was caused by higher molecular weight tannins, and endothelium-dependent vasodilation even appeared at low concentrations. In conclusion, we found that TCC exhibits an endothelium-dependent vasodilatation in the isolated rat aorta mainly via endothelium derived NO. NO mediated endothelium-dependent relaxation seems to be more potent for TCC with higher molecular weight than that with lower molecular weight.

Key words tannin from Cinnamonomi Cortex (TCC), endothelium-dependent vasodilation, nitric oxide (NO), rat aorta.

Abbreviations ACE, angiotensin-converting enzyme ; API-MS, atomospheric pressure ionization mass spectra ; L-NAME, N^G-nitro-l-arginine methyl ester ; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; TCC, tannin from Cinnamonomi Cortex.

Introduction

With regard to the biological effects of Cinnamonomi Cortex (the bark of *Cinnamomum cassia* BLUME), many studies on the essential oils that form a major component of this plant product have been performed.[1-5] On the other hand, there have been few investigations of tannin isolated from Cinnamonomi Cortex (TCC). Although the pharmacological actions of TCC are reported to include inhibition of angiotensin-converting enzyme (ACE),[6] there seem to be no reports concerning a direct vasodilatory effect. Accordingly, we investigated the vasodilatory effect of TCC and evaluated the mechanism of its influence on vasomotion *in vitro*.

Materials and Methods

Test materials and chemicals : Cinnamonomi Cortex (the bark of *Cinnamomum cassia* BLUME "Guang-nan Gui-pi" from Guang-Dong Province in China) was purchased from Tochimoto Tenkaido (Osaka, Japan). (−)-Epicatechin, N^G-nitro-l-arginine methyl ester (L-NAME), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), acetylcholine chloride (Ach), and indometh-

*〒930-0194 富山市杉谷2630
富山医科薬科大学医学部和漢診療学講座 谷川聖明
Sugitani 2630, Toyama 930-0194, Japan

acin were purchased from Wako Pure Chemical Industries (Osaka, Japan). (−)-Epicatechin was dissolved in 40 % dimethyl sulfoxide. The others were dissolved in water. Procyanidin B-2 (dimer of TCC), procyanidin C-1 (trimer), cinnamtannin A2 (tetramer), and cinnamtannin A3 (pentamer) were prepared from Cinnamonomi Cortex according to the method of Morimoto *et al.*[7] These were also dissolved in water.

Fractionation of the aqueous extract of Cinnamonomi Cortex : Powdered Cinnamonomi Cortex (100 g) was extracted with water (500 ml) at room temperature. The aqueous extract was combined and concentrated under reduced pressure to give a brown residue, fr.1 (3.0 g). The residue was suspended in water and chromatograghed on a Diaion HP-20 column (4.2×40 cm). Elution was started with water to give fr.2 (1.6 g), and then continued with MeOH to give fr.3 (1.3 g). The MeOH eluate was loaded onto to a Sephadex LH-20 column (3.0×32 cm), and elution was started with EtOH to give fr.4 (0.45 g), followed by MeOH to give the tannin fraction, fr.5 (0.79 g) (Fig. 1). fr.5 was loaded onto MCI-gel CHP 20P and with 30 % aqueous MeOH and then with MeOH to give the proanthocyanidin fraction. Table I shows the proanthocyanidin which we separated from fr.5. We measured the molecular weight of the proanthocyanidin by atomospheric pressure ionization mass spectra (API-MS), and confirmed them. API-MS were obtained with a PE SCIEX API III biomplecular mass spectrometer.

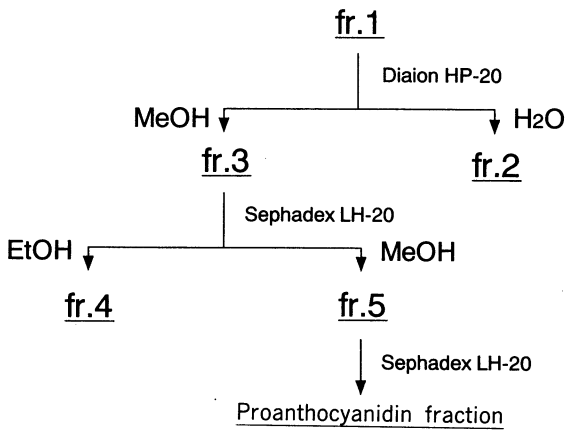

Fig. 1 Fractionation of the water extract of Cinnamonomi Cortex.

Relaxation experiments : Male Wistar rats weighing 300-400 g were anesthetized with Nembutal (50 mg/kg *i.p.*) and killed by exsanguination from the abdominal aorta. A section of the thoracic aorta was carefully cleaned of fat and connective tissues, and rings 3 mm wide were cut. The endothelial lining of some rings was removed by compression and gentle rolling on filter paper a few times. Removal of the endothelium was functionally confirmed.

The aortic rings were mounted on steel hooks in a Magnus chamber (Kishimoto UC-5TD, Kyoto, Japan). One end of each ring was attached to a force-displacement transduer (Kishimoto UM-203) so that isometric contraction could be recorded (Niko Bioscience T-634, Tokyo, Japan). The chamber was filled with 5 ml of Krebs solution with the following composition (mM) : NaCl 120, KCl 4.7, $NaHCO_3$ 25.0, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2$ 2.5, and glucose 10.0. The medium was maintained at 37°C and bubbled continuously with 5 % CO_2 in O_2 at a pH of 7.4.

Riings were equilibrated for 40 min at an initial resting tension of 1 g. During this time, the Krebs solution in the chamber was replaced every 15 min.

Table I Tannin from Cinnamonomi Cortex

Procyanidin B-2 (dimer) Pale yellow amorphous powder. $[\alpha]_D+32.2°$ (c=1.0, acetone). API-MS (negative) m/z : 577 $[M-H]^-$.
Procyanidin C-1 (trimer) Pale yellow amorphous powder. $[\alpha]_D+72.5°$ (c=1.0, acetone). API-MS (negative) m/z : 865 $[M-H]^-$.
Cinnamtannin A2 (tetramer) Pale yellow amorphous powder. $[\alpha]_D+83.5°$ (c=1.0, acetone). API-MS (negative) m/z : 1153 $[M-H]^-$.
Cinnamtannin A3 (pentamer) Pale yellow amorphous powder. $[\alpha]_D+98.5°$ (c=1.0, acetone). API-MS (negative) m/z : 1441 $[M-H]^-$.

The rings were precontracted with 60 mM KCl. When contraction reached a steady maximal response, 10^{-6} M Ach was added. When the endothelium had been removed, no relaxation was induced by Ach. The Krebs solution was again replaced every 15 min for 60 min, and then the experiments were carried out.

Each aortic strip was contracted by exposure to 3×10^{-6}M PGF2α. When this contraction reached a plateau, TCC (fr.5) was added at concentrations ranging from 10^{-9} to 10^{-4} g/ml. TCC was also obtained in the presence of indomethacin (3μM, a cyclo-oxygenase inhibitor) and incubated with the tissue 20-30 min before drug addition. In other experiments without precontraction, TCC was added at 10^{-3} g/ml. Relaxation was expressed as the percent decrease in the maximal tension induced by PGF2α.

To investigate the effect of nitric oxide, preparations with endothelium were exposed to 10^{-4}M L-NAME for 60 min before precontraction, L-NAME is known to inhibit the synthesis of nitric oxide.

Dimer, trimer, tetramer, and pentamer forms of TCC were examined similarly at concentrations ranging from 10^{-9} to 10^{-4}M. (−)-Epicatechin, which is simple condensed tannin, was also added to the preparations at the same concentrations.

Statistical analysis : Data are reported as the mean±standard error of the mean (S.E.M.). One-way analysis of variance (ANOVA) was used for statistical analysis. A P value <0.05 was regarded as significant.

Results

Rat aorta with intact endothelium showed relaxation after addition of 10^{-7} g/ml TCC, which reached a maximum at 10^{-5} g/ml and then decreased at 10^{-4} g/ml (n=6) (Fig. 2a). Indomethacin (3μM) had no significant effect. In contrast, rings without endothelium showed no relaxation (n=6) (Fig. 2b). Rings with intact endothelium also displayed no relaxation in the presence of 10^{-4}M L-NAME (n=6) (Fig. 3). When non-precontracted preparations were used, TCC caused contraction at 10^{-3} g/ml both with and without endothelium (Fig. 2c). About 25 % contractions of TCC were expressed as the percentage in maximal tension obtained by 60 mM KCl-induced contraction. When 3×10^{-6}M indomethacin was added to TCC, there was no contraction of non-precontracted preparations (n=4) both with and without endothelium.

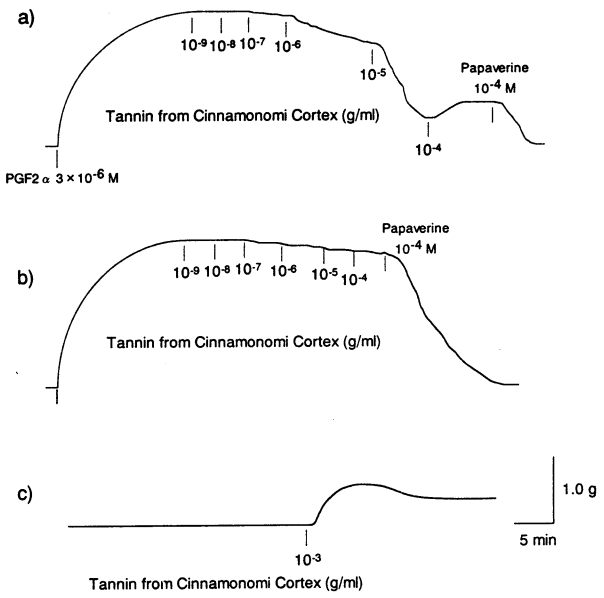

Fig. 2 Typical tracing showing the effect of TCC on isolated rat aorta. a) A ring precontracted with 3×10^{-6}M PGF2α with intact endothelium. b) without endothelium. c) A ring without precontraction.

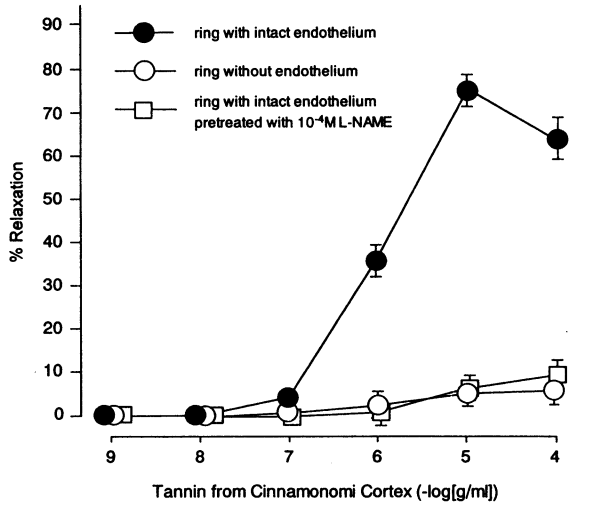

Fig. 3 Concentration-response curves for TCC-induced relaxation of rat aortic rings with intact endothelium. Values are expressed as the percent decrease in the maximal tension caused by 3×10^{-6}M PGF2α. The mean± S.E. of 6 determinations is shown.

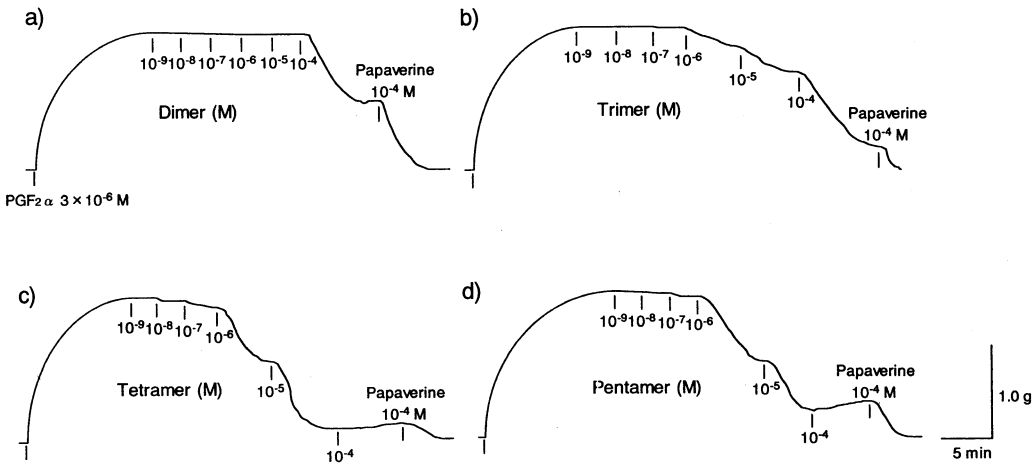

Fig. 4 Typical tracings of the effects of tannin fractions on isolated rat aortic rings with intact endothelium precontracted with 3×10^{-6}M $PGF_{2\alpha}$. a) dimer, b) trimer, c) tetramer, d) pentamer.

(−)-Epicatechin failed to induce relaxation at concentrations of 10^{-9} to 10^{-4}M. DMSO, which was used for the solvent of (−)-epicatechin, also failed to induce relaxation by itself. The relaxation of rat aorta with intact endothelium in response to a 10^{-4}M solution of the dimer was about 50 % (Fig. 4a and 5). Rat aorta with intact endothelium showed relaxation when exposed to 10^{-6}M trimer, reaching a maximum of about 90 % at 10^{-4}M (Fig. 4b and 5). The tetramer and pentamer caused relaxation at 10^{-8} M, and induced significantly greater relaxation than the dimer at concentrations of 10^{-6} and 10^{-5}M (Fig. 4c, 4d and 5). TCC showed vasorelaxation responses in rings of rat aorta with intact endothelium and precontracted with $PGF_{2\alpha}$ (3×10^{-6}M) at a mean EC50 value of 84.6 ± 6.3 μM (dimer), 10.4 ± 1.1 μM (trimer), 4.32 ± 0.75 μM (tetramer) and 0.85 ± 0.14 μM (pentamer), respectively. The relaxation response to dimer, trimer, tetramer, and pentamer was reduced in specimens without endothelium, as well as those with endothelium by the addition of 10^{-4}M L-NAME.

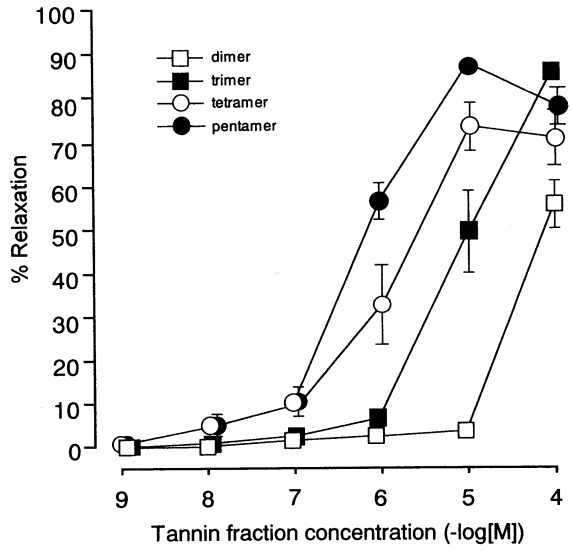

Fig. 5 Concentration-response curves for the relaxation of rat aortic rings with intact endothelium induced by tannin fracions. Results are the mean±S.E. of 6 determinations.

Discussion

The present study clearly demonstrated that TCC produced endothelium-dependent vasodilation in the rat aorta, as was reported by Furchgott *et al.*[8] Because treatment with L-NAME inhibited this vasodilator response, nitric oxide (NO) was thought to be involved.

When indomethacin was added to TCC, there was no contraction of non-precontracted aortic rings with or without endothelium, indicating that TCC-mediated contraction was affected by eicosanoids.

(−)-Epicatechin, which is simple condensed tannin, failed to induce relaxation. Dimer, trimer, tetramer, and pentamer forms of TCC, produced endothelium-dependent vasodilation involving NO.

Stronger relaxation was observed with higher molecular weight tannins, and endothelium-dependent vasodilatation also occurred at lower concentrations. With regard to vasomotion, TCC has been reported to inhibit ACE activity, and this inhibitory effect on ACE has been shown to be greater with higher molecular weight tannins.[6] ACE inhibitors cause vasodilation and protect the vascular endothelium by inducing NO production.[9] ACE inhibitors have also been reported to act as scavengers for superoxide radicals, thereby modulating NO degradation.[10] Likewise, condensed tannins have been reported to have a radical scavenging action, which is stronger with higher molecular weight tannins,[11] and our results were in strong agreement with such findings. TCC is a condensed tannin, so it may also have a radical scavenging action *in vivo* and thus modulate NO degradation. On the other hand (−)-epicatechin also has been reported to have a radical scavenging action,[12] but it failed to induce relaxation in our investigation. This result suggests that TCC has the direct action of producing EDRF/NO to endothelium. The relaxation of TCC caused not only a scavenging action but also the effect of producing EDRF/NO.

Other tannins, such as galloylglucose from Paeoniae Radix and condensed tannin from Areca Semen, are also reported to have an endothelium-dependent vasodilator effect.[13,14] The present study demonstrated that TCC had such a vasodilator effect, and that higher molecular weight tannins showed increased activity.

Conclusions

TCC exhibits an endothelium-dependent vasodilator effect on the isolated rat aorta. Higher molecular weight tannins have a stronger activity than those of lower molecular weight.

Acknowledgements

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和文抄録

桂皮の血流改善作用については古くから知られており，これに関連した報告はあるものの，その詳細な検討はなされていない。今回我々はマグヌス法を用いて，ラット胸部大動脈輪状標本における桂皮含有タンニンの血管作動性について検討した。桂皮含有タンニンは，プロスタグランディン $F_{2\alpha}$（$PGF_{2\alpha}$）の血管収縮に対し，内皮保存血管において濃度依存性に血管弛緩作用が認められた。しかし，内皮除去血管及び N^G-nitro-l-arginine methyl ester（L-NAME）前処置内皮保存血管においては，血管弛緩作用はほぼ消失した。以上より，桂皮含有タンニンの血管弛緩作用は内皮依存性であることが明らかとなった。桂皮含有タンニンをさらに二量体から五量体までのタンニン画分に分取し検討したところ，二量体以上の重合したタンニンにおいて血管弛緩作用が認められた。また，重合度が増すに従い血管弛緩作用はより低い濃度で発揮され，作用も増強されることが明らかとなった。

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