Tangeretin Alleviates L-NAME Induced Vascular Dysfunction in Rats

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Background and Objective: Tangeretin is a flavonoid compound found in citrus fruits. Several biological activities of tangeretin have been revealed including; anti-neurodegeneration, anti-inflammation, and anti-oxidation. This study was to investigate the effects of tangeretin on vascular function in L-NAME-treated rats.

Methods: Rats were divided into 4 groups. Groups I; rats treated with L-NAME (40 mg/kg) in drinking water only, Groups II and III; rats treated with L-NAME together with tangeretin (15 and 30 mg/kg, respectively), Groups IV; control rats were received only distilled water (n=7/each group). At the end of experiment, vascular responses to electrical field stimulation (EFS) and vasoactive agents in mesenteric vascular beds and aortic rings were evaluated.

Results: A significant increase in contractile response to electrical EFS was observed in the mesenteric vascular bed isolated from L-NAME-treated rats (p<0.05). Tangeretin reduced the augmented response to EFS in L-NAME-treated rats comparing to those of untreated rats (p<0.05). However, the contractile response to exogenous norepinephrine was not differ-
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Materials and Methods

Animals

Male Sprague-Dawley rats weighing 220-240 g were obtained from Nomura Siam International Co., Ltd., Bangkok, Thailand. They were housed at 25 ± 2°C with a 12 h dark–light cycle at Northeast Laboratory Animal Center, Khon Kaen University, Khon Kaen, Thailand. All procedures complied with the standards for the care and use of experimental animals and were...
Experimental protocols
After a week of acclimatization, rats were treated with L-NAME (40 mg/kg/day) in their drinking water for 5 weeks whereas control rats were received distilled water. The rats were randomly divided into 4 groups of 7 rats each. Group I control + vehicle or propylene glycol (PG) (1.5 ml/kg; p.o.); Group II L-NAME + vehicle or PG (1.5 ml/kg; p.o.); Group III L-NAME + tangeretin (15 mg/kg; p.o.); Group IV L-NAME + tangeretin (30 mg/kg; p.o.). Tangeretin and PG were intragastrically administered daily for the last 2 weeks of the study.

Vascular Function Study
Experimental protocols in isolated mesenteric vascular beds
After exsanguination, mesenteric vascular beds were carefully isolated and then placed on a stainless steel grid in a humid chamber. The preparations were perfused with physiological Krebs’ solution at a constant flow rate of 5 ml/min, using a peristaltic pump. Electrical field stimulation (EFS) (5-40 Hz, 90 V, 1 ms, for 30 s at 5-min intervals) was performed. Contractile responses to EFS were detected as changes in mean perfusion pressure (mmHg). After that norepinephrine (NE) (0.15 nmol-15 nmol) was applied to evaluate the contractile responses to exogenous NE. To determine vasoactive performance of resistance small arteries, methoxamine (5-7 µM) was added into Kreb’s solution to raise tone (70-90 mmHg above baseline). Subsequently, different doses of vasoactive agents, ACh (ACh, 0.1 nM–0.1 mM) or sodium nitroprusside (SNP, 0.1 nM–0.1 mM) were applied, respectively.

Experimental protocols in isolated aortic rings
The thoracic aorta was rapidly removed and cut into rings 2-3 mm long for tension measurement. They were mounted in 15 ml baths containing Krebs’ solution at 37 ºC and gassed with a 95% O2 and 5% CO2 gas mixture. Isometric contractions were recorded with a resting tension of 1 g using a transducer connected to a 4-channel bridge amplifier and a PowerLab A/D converter and a PC running Chart v5 (PowerLab System, AD Instruments, Australia). ACh (0.001 µM–3 µM) induced endothelial mediated-relaxations and SNP (0.001 µM–3 µM) were assessed by pre-contracting with phenylephrine (10 µM) and relaxation expressed as % of relaxation.

Statistical analysis
Results were reported as means ± S.E.M. Comparisons between groups were performed using one-way ANOVA followed by Fisher’s Least Significant Difference tests. A probability value of less than 0.05 was considered statistically significant.

Results
Effects of tangeretin on contractile responses to EFS and exogenous NE in mesenteric vascular beds
EFS at 5-40 Hz produced an increase in perfusion pressure that was frequency-dependent vasoconstriction in all preparations. A significant increase in contractile responses to EFS was observed in the mesenteric vascular bed isolated from L-NAME-treated rats compared to the responses in control rats (p<0.05) (Figure 1A). Contractile response to EFS in L-NAME-treated rats treated with tangeretin was reduced compared to those of untreated rats (p<0.05). However, the contractile response to exogenous NE (0.15 nmol-15 nmol) was not different among groups (Figure 1B).

Effects of tangeretin on vasorelaxation responses to vaso-dilator agents in mesenteric vascular beds
Vasorelaxation response to ACh (0.1 nM–0.1 mM) in the mesenteric vascular bed was significantly blunted in aortic rings from L-NAME-treated rats compared to control rats (p<0.05) (Figure 2A). Treatment with tangeretin improved the response to ACh in L-NAME-treated rats compared to untreated rats (p<0.05). There was no significant difference in the vasorelaxation responses to SNP among groups (Figure 2B), indicating normal vascular smooth muscle cell function.

Effects of tangeretin on vasorelaxation responses to vaso-dilator agents in aortic rings
Endothelium-dependent vasorelaxation responses to ACh (0.001 µM–3 µM) were significantly blunted in aortic rings from L-NAME-treated rats compared to control rats (p<0.05) (Figure 3A). Tangeretin at dose 30 mg/kg improved vascular response to ACh compared to untreated rats (p<0.05). However, vasorelaxation response to SNP, an NO donor, did not differ significantly among groups (Figure 3B).
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Figure 1 Effect of tangeretin on contractile responses to EFS (A) and exogenous NE (B) in mesenteric vascular beds. Data are presented as mean ± S.E.M. (n = 7/group). *p<0.05 vs. control, #p<0.05 vs. L-NAME.

Figure 2 Effect of tangeretin on vascular responses to acetylcholine (A) and sodium nitroprusside (B) in mesenteric vascular beds. Data are presented as mean ± S.E.M. (n = 7/group). *p<0.05 vs. control, †p<0.05 vs. L-NAME, ∆p<0.05 vs. L-NAME+T15.

Figure 3 Effect of tangeretin on vascular responses to acetylcholine (A) sodium nitroprusside (B) in aortic rings. Data are expressed as mean ± S.E.M. (n = 7/group). *p<0.05 vs. control, ‡p<0.05 vs. L-NAME.
Discussion

This study demonstrated that chronic administration of L-NAME in rats increased sympathetic nerve activation as evidenced by increased contractile response to EFS and impaired endothelium dependent vasorelaxation. Tangeretin improved endothelial dysfunction and reduced sympathoexcitation in L-NAME-treated rats.

Sympathetic activation is an important factor to stimulate and maintain high blood pressure via vascular tone regulation. Previous study demonstrated that L-NAME can induce sympathetic activation in rats. In rat mesenteric arteries, vascular tone is mediated by the integrated action of different neurotransmitters, mainly NE from sympathetic nerve terminals. The result of this study showed that there was an enhancement of vasoconstriction responses to sympathetic nerve stimulation without affecting the response to exogenous NE in L-NAME-treated rats, indicating the augmentation of NE release from pre-junctional sites. However, treatment with tangeretin suppressed the nerve mediated contractile response in L-NAME-treated rats. It is possible that tangeretin has antioxidant effect to raise NO bioavailability, which can suppress NE release from sympathetic nerve terminal.

The results of this study showed an impairment of endothelium-dependent vasodilation in isolated aortic rings and mesenteric vascular beds of L-NAME-treated rats. Oxidative stress is also involved in L-NAME mediated endothelial dysfunction. Fu and co-workers established that treatment with L-NAME impaired ACh-induced endothelium-dependent vasorelaxation, which mediated by increasing oxidative stress. Previous study demonstrated that L-NAME-treated rats showed impairment of ACh-induced vasorelaxation, and decreased NO bioavailability. NO is also known as a potent vasodilator.

High ROS concentrations reduce the quantity of bioactive NO by rapidly reaction with NO to form the potent cytotoxic peroxynitrite which was induced vascular oxidative stress and endothelial dysfunction. Tangeretin also improved endothelial dysfunction in L-NAME-treated rats, probably as an anti-oxidant effect of tangeretin. Previous study showed that tangeretin inhibited ROS production and suppressed the mRNA expression of p47phox, p67phox, and gp91phox in LPS-stimulated microglia, indicating tangeretin exhibits strong antioxidant activity. Therefore, antioxidative activity of tangeretin might be partially responsible for the alleviation of endothelial dysfunction in NO deficiency rats.

Conclusion

In summary, tangeretin attenuates L-NAME-induced sympathoexcitation and endothelial dysfunction in rats. This might be involved with its antioxidant to increase NO bioavailability in L-NAME-treated rats.

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