นิพนธ์ต้นฉบับ . Original Article

สาร Naringin ปรับปรุงการทำงานของหลอดเลือดในหนูแรทความดันโลหิตสูง

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Naringin Improves Vascular Function in Hypertensive Rats

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บทคัดย่อ

หลักการและวัตถุประสงค์: Naringin เป็นสารฟลาโวนอยด์ มีฤทธิ์ต้านอนุมูลอิสระและการอักเสบ การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบ Naringin มีผลต่อการตอบสนองต่อระบบประสาทซิมพาเทติกในหนูแรทความดันโลหิตสูง

วิธีการศึกษา: หนูแรทเพศผู้ได้รับแอลเนม 40 มก./กก./วัน และป้อนสาร Naringin 40 มก./กก./วัน เป็นเวลา 5 สัปดาห์ ส่วนหนูกลุ่มควบคุมได้รับน้ำดื่ม วัดความดันโลหิตและการทำงานของหลอดเลือด

ผลการศึกษา: หนูที่ได้รับแอลเนมมีความดันโลหิตสูงกว่าเมื่อเทียบกับกลุ่มควบคุม (155.95 ± 2.55 vs. 100.80 ± 1.75 มิลลิเมตรปรอท $p<0.05$) Naringin ป้องกันความดันโลหิตสูงที่เหนี่ยวนำโดยแอลเนม (101.87 ± 3.52 มิลลิเมตรปรอท $p<0.05$) การหดตัวต่อการกระตุ้นด้วยไฟฟ้าเพิ่มขึ้นในกลุ่มที่ได้แอลเนม ($p<0.05$) และถูกกดในกลุ่มที่ได้ Naringin ($p<0.05$) การหดตัวต่อนอร์อิพิเนฟรินไม่ต่างกัน Naringin เพิ่มการตอบสนองของหลอดเลือดต่ออะซิติลโคลีน ($p<0.05$) ผลของ Naringin ต่อการทำงานของหลอดเลือดเหล่านี้สัมพันธ์กับการเพิ่มขึ้นของไนตริกออกไซด์ ($p<0.05$)

สรุป: Naringin ป้องกันความดันโลหิตสูงเหนี่ยวนำจากสารแอลเนม ที่เป็นผลมาจากการปรับปรุงการทำงานของหลอดเลือดผ่านการเพิ่มขึ้นของไนตริกออกไซด์ในหนูแรทความดันโลหิตสูงที่เหนี่ยวนำจากสารแอลเนม

คำสำคัญ : ความดันโลหิตสูง, การทำงานของหลอดเลือดที่ผิดปกติ

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Abstract

Background and Objective: Naringin is a flavonoid. The beneficial effects of naringin have been reported including anti-oxidation and anti-inflammation. This study investigated whether naringin could reduce sympathetic nerve-mediated contractile responses in hypertensive rats.

Methods: Male Sprague-Dawley rats were treated with L-NAME 40 mg/kg/day and orally administered with naringin 40 mg/kg/day for five weeks while control rats received distilled water. Blood pressure and vascular function were measured.

Result: Rats received L-NAME had higher blood pressure (155.95 ± 2.55 vs. 100.80 ± 1.75 mmHg) compared to those of control group ($p<0.05$). Naringin prevents the development of hypertension induced by L-NAME (101.87 ± 3.52 mmHg, $p<0.05$). Contractile responses to electrical field stimulation (EFS) in mesenteric vascular beds was enhanced in L-NAME group ($p<0.05$) and these were suppressed in the naringin treated group ($p<0.05$). The contractile responses to exogenous norepinephrine was not different between groups. Naringin also improved the vasorelaxation responses to acetylcholine (ACh) ($p<0.05$). These vascular effects of naringin were consistent with raising nitric oxide levels in hypertensive rats ($p<0.05$).

Conclusion: Naringin prevented the development of hypertension induced by L-NAME. These preventive effects were associated with improvement of vascular function through increasing Nitric oxide metabolites in L-NAME-induced hypertension.

Keyword: naringin, hypertension, vascular dysfunction Introduction line 12: N$^{\omega}$-nitro-L-arginine methyl ester

Introduction

Hypertension is the chronic disease and a major cause of cardiovascular disease. The essential cause hypertension is unknown however, vascular alterations is one of the possible causes. Vascular dysfunction is associated with sympathetic overactivity and nitric oxide (NO) deficiency that have been involved in development of hypertension. NO is a vasodilator gas, it plays an important role in controlling vascular tone[1]. Impairment of NO bioavailability was related to endothelial dysfunction and high blood pressure[2]. In rodent model of hypertension, N^{ω}-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, is widely used to induce vascular dysfunction and hypertension. Potue and coworkers report that high blood pressure and endothelial dysfunction in L-NAME hypertensive rats are associated with decreased plasma nitric oxide metabolites (NOx) level[3]. Furthermore, L-NAME-induced hypertension is characterized by increased sympathetic overactivity that mediates high vascular tone[4]. Therefore, stabilization of NO, endothelial function and sympathetic nerve activity might be the targets for controlling hypertension.

Nowadays, plants containing high flavonoid compounds are widely recommended in management and treatment of hypertension[5]. Naringin is a flavonoid glycoside that highly found in citrus fruits. The beneficial effects of naringin have been reported including anti-inflammation, anticancer, neuroprotection and free radical scavenging activity[6-8]. However, the effect of naringin on vascular function in L-NAME-induced hypertensive rats remain unclear. This study aimed to investigate the preventive effects of naringin on vascular dysfunction induced by L-NAME in rats.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 200-220 g were purchased from Nomura Siam International Co., Ltd, Bangkok, Thailand. Rats were raised in an HVAC (heating, ventilation, and air-conditioning)-equipped room (23 ± 2 °C) with a 12 h dark–light cycle at the Northeast Laboratory Animal Center. All animal procedures were complied with the standards for the care and use of experimental animals and approved by Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (IACUC-KKU-38/65).

Drugs and chemicals

Naringin and L-NAME were purchased from Sigma-Aldrich (St Louis, MO, USA). Acetylcholine chloride (ACh) and sodium nitroprusside (SNP) were purchased from Fluka Chemika (Buchs, Switzerland). Polyethylene glycol (PG) was obtained from AjaxFinechem PtyLtd. (NSW, Australia). Thiopental sodium was purchased from animal hospital of faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand.

Experimental protocols

After a week of habituation, the rats were divided into 3 groups which consist of 5 rats in each group; as follow; control group was received vehicle (5% dimethyl sulfoxide, 1.5 mL/kg) by oral gavage, L-NAME group was received L-NAME 40 mg/kg/day dissolved in distilled water and L-NAME + naringin group was received L-NAME 40 mg/kg/day in drinking water and naringin 40 mg/kg/day by intragastrical administration for five weeks of the study. Naringin was dissolved with 5% dimethyl sulfoxide.

Mean arterial pressure assessment in conscious rats

Mean arterial pressure (MAP) was determined at the end of experimentation. Briefly rats will be anesthetized by intra-peritoneal administration of thiopental sodium at dose 60 mg/kg. A polyethylene tube was inserted into the femoral artery for direct blood pressure measurement was monitored by pressure transducers and recorded using the Acknowledge Data Acquisition and Analysis Software (BIOPAC system Inc., California, USA).

Vasoactive assessment in resistance artery

Mesenteric vascular beds were isolated and then placed on a stainless-steel grid (7x5cm) 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. The solution was maintained at 37 °C and continually gassed with a mixture of 95% O_2 and 5% CO_2 gas. The mesenteric vascular beds were washout by 30 minutes. After that, electrical field stimulation (EFS) (5-40 Hz. 90 V. 1 ms, for 30 second at 5-min intervals) was performed. Contractile responses to EFS were detected as changes in mean perfusion pressure (mmHg) using a

pressure transducer and data recorded via the BIOPAC System (BIOPAC Systems Inc., California, USA). The preparations were allowed to equilibrate for 30 minutes before the next trial. After the resting period, the mesenteric vascular beds were injected with bolus doses of exogenous norepinephrine (NE, 0.15 nmol-15 nmol) 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, 1 μM-0.1 mM) or sodium nitroprusside (SNP, 0.1 μM-0.1 mM) were injected through neoprene rubber tubing proximal to the tissue, respectively.

Statistical analysis

Data are expressed as mean ± S.E.M. Statistical analysis is used one-way ANOVA analysis of variance follow by Tukey's post-hoc tests for comparing between groups. A probability value < 0.05 is considered statistical significance.

Result

Effect of naringin on blood pressure and plasma NOx in L-NAME induced hypertensive rats MAP in L-NAME group was significantly increased when compared to those of control group (155.95 ± 2.55 vs 100.80 ± 1.75 mmHg; p<0.05). Naringin was significantly reduced MAP in L-NAME-induced hypertensive rats compared to untreated L-NAME group. (101.87 ± 3.52 vs 155.95 ± 2.55 mmHg; p<0.05) (Figure 1A). Plasma NOx concentration was significantly decreased in L-NAME group when compared to those of control group (10.05 ± 0.24 vs 14.30 ± 1.18 μM; p<0.05). The level of plasma NOx was significantly restored in L-NAME treated naringin compared to those of untreated group (14.02 ± 0.17 vs 10.05 ± 0.24 μM) (p<0.05; Figure 1B).

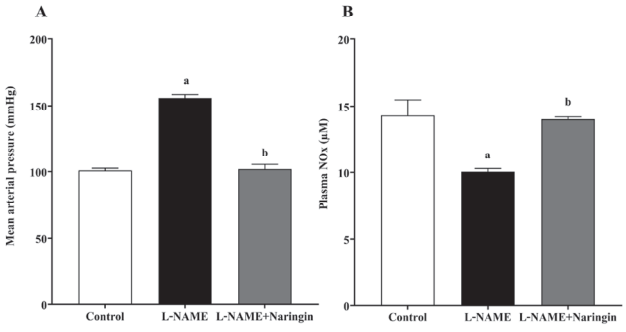

Figure 1 Effect of naringin on mean arterial pressure and plasma NOx. Data are expressed as mean ± S.E.M. (n=5/group). A; p<0.05 vs control, B; p<0.05 vs L-NAME. L-NAME = hypertensive rats, L-NAME+Naringin = hypertensive rats treated with naringin 40 mg/ kg/ day.

Effect of naringin on contractile responses to electrical field stimulation (EFS), exogenous norepinephrine (NE) and vascular reactivity in mesenteric vascular beds

The contractile responses stimulated by sympathetic nerve were enhancer in the mesenteric vascular bed isolated from L-NAME group than those control group (at 40 Hz, 121.41 ± 6.71 vs 58.45 ± 6.49 mmHg, p<0.05). L-NAME rats treated with naringin significantly decreased contractile responses to EFS compared to those of untreated L-NAME group (at 40 Hz, 69.06 ± 4.17 vs 121.41 ± 6.71 mmHg) (p<0.05; Figure 2A). The contractile responses to exogenous norepinephrine (0.15 nmol-15 nmol) showed no significant difference among groups (Figure 2B). Vasorelaxation response to ACh and SNP were expressed as a reduction of perfusion pressure. Vasorelaxation response to ACh (0.1 μM-0.1 mM) in mesenteric vascular beds were significantly blunted in L-NAME rats when compared to those of control group (0.1 mM ACh, 10.32 ± 6.16 vs. 48.23 ± 6.15 mmHg). Treatment with naringin significantly improved the response to ACh when compared to L-NAME group (0.1 mM ACh, 47.08 ± 7.39 vs. 10.32 ± 6.16 mmHg) (p<0.05; Figure 2C). However, there was no significant difference in the vasorelaxation responses to SNP among groups, indicating that normal vascular smooth muscle cell function (Figure 2D).

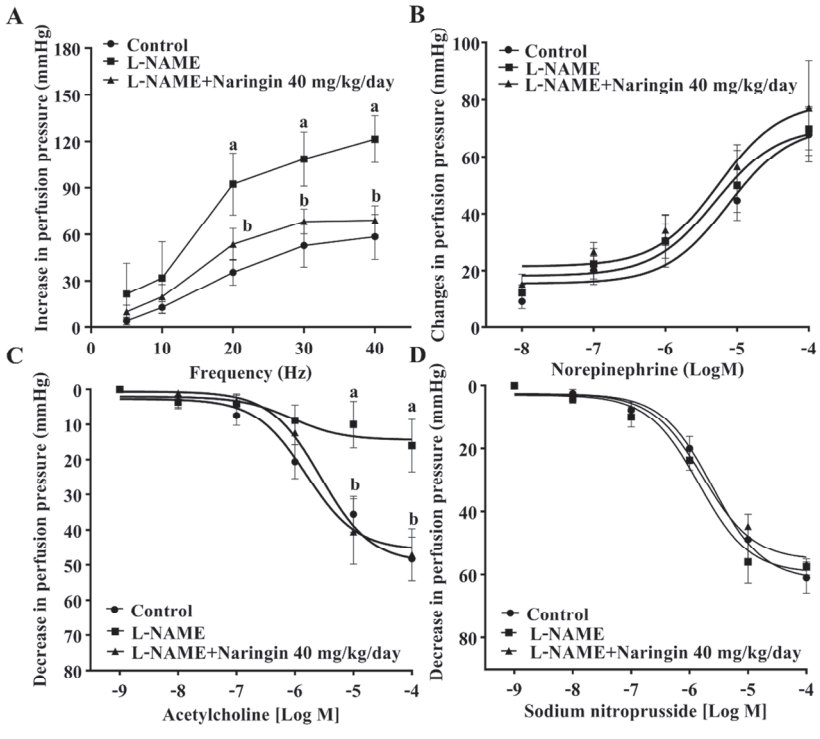

Figure 2 Effect of naringin on contractile responses to sympathetic nerve stimulation (A), exogenous norepinephrine (B), vascular responses to acetylcholine (C) and sodium nitroprusside (D) in mesenteric vascular beds. Data are expressed as mean ± S.E.M. (n=5/group). a; $p<0.05$ vs control, b; $p<0.05$ vs L-NAME. L-NAME = hypertensive rats, L-NAME+Naringin 40 mg/ kg/ day = hypertensive rats treated with naringin 40 mg/ kg/ day.

Discussion

The main findings of this study are that naringin prevented the development of hypertension, improved endothelium-dependent vasorelaxation and attenuated contractile responses in mesenteric vascular beds in L-NAME-induce hypertension rats. These effects were consistent with increased NO production. We demonstrate that rats received L-NAME developed high blood pressure associated with impairment of endothelium-dependent vasorelaxation and enhancement of contractile responses to sympathetic stimulation has been demonstrated. Our findings were consistent with several studies that endothelial dysfunction observed in L-NAME hypertension was associated with low levels of circulating NO[3,9]. In fact, increasing blood pressure, sympathetic overactivity and endothelial dysfunction were consequences of decreased NO bioavailability[10,11]. In addition, our study confirmed the sympathetic overactivity in L-NAME hypertensive rats by elevation of contractile responses to EFS. The enhancement of contractile responses to EFS might be mediated by elevation of NE releasing from presynaptic sites of sympathetic nerve. NE binds to its receptor on vascular smooth muscle cells to promote vasoconstriction, which increase total peripheral resistance and finally hypertension. Endothelial dysfunction as indicated by the reduction of vasorelaxation responses to ACh in L-NAME-induced hypertensive rats. Our results were supported by several previous studies that L-NAME administration is associated with decreased NO bioavailability, sympathetic overactivity and subsequently hypertension[3, 4, 12, 13].

Naringin significantly prevented the development of hypertension induced by L-NAME in rats. These results are congruent with many studies that naringin acts as a potential antihypertensive agent[14,15]. Moreover, our study found that antihypertensive effect of naringin was linked to suppression of vasoconstriction response to sympathetic nerve stimulation. This effect might act on the pre-synaptic site since the response to NE did not alter in all groups. Furthermore, naringin also raised the response to acetylcholine while the response to SNP was not different among groups,

suggesting improvement of endothelial function. These results were consistent with the previous findings that L-NAME had no effect on vascular responses to SNP. They suggested the normal function of vascular smooth muscle cells in this animal model[12]. The vascular effect of naringin in the present study might be mediated by restoring systemic NO level in L-NAME rats. There is evidence supported our study that naringin can reduce systolic blood pressure, increase nitric oxide bioavailability and ameliorate endothelial dysfunction in stroke-prone spontaneously hypertensive rats[16] Thus, naringin prevented hypertension and alleviated vascular dysfunction via improvement of endothelium-dependent vasorelaxation in L-NAME treated rats.

Conclusion

Naringin had an antihypertensive effect associated with improvement vascular function through increasing NO metabolites in L-NAME-induced hypertension.

Acknowledgement

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