นิพนธ์ต้นฉบับ • Original Article

ผลของสารเฮสเพอริดินต่อความดันเลือดและการทำงานของหลอดเลือดผิดปกติในหนูแรทที่ถูกชักนำให้เกิดความดันเลือดสูงด้วยสารแอลเนม

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Effect of Hesperidin on L-NAME-Induced Hypertension and Vascular Dysfunction in Rats

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หลักการและวัตถุประสงค์: เฮสเพอริดินพบมากในผลไม้ที่มีรสเปรี้ยว มีฤทธิ์ลดการอักเสบ และต้านอนุมูลอิสระ การศึกษานี้เพื่อตรวจสอบว่าเฮสเพอริดินสามารถบรรเทาความดันเลือดสูงจากสารแอลเนมในหนูแรทได้หรือไม่

วิธีการศึกษา: หนูแรทเพศผู้ถูกชักนำให้เกิดความดันเลือดสูงด้วยสารแอลเนม (40 มก./กก./วัน) ในน้ำกลั่น หรือได้รับร่วมกับการป้อนเฮสเพอริดิน (15 และ 30 มก./กก./วัน) เป็นเวลา 5 สัปดาห์ ความดันซิสโทลิกถูกวัดสัปดาห์ละครั้ง และประเมินการทำงานของหลอดเลือดเอออตาร์และมีเซนเทอริก

ผลการศึกษา: หนูทดลองที่ได้รับสารแอลเนมมีความดันเลือดสูงและลดการตอบสนองของหลอดเลือดต่อสารอะซิติลโคลีน การตอบสนองต่อสารโซเดียมไนโตรปรัสไซด์ไม่มีความแตกต่างระหว่างกลุ่ม เฮสเพอริดินป้องกันการเพิ่มขึ้นของความดันเลือดและบรรเทาการทำงานของหลอดเลือดที่ผิดปกติจากสารแอลเนมอย่างมีนัยสำคัญทางสถิติ ($p<0.05$)

สรุป: เฮสเพอริดินป้องกันการเกิดความดันเลือดสูงโดยสารแอลเนมได้บางส่วน ซึ่งผลนี้เกี่ยวข้องกับการเพิ่มขึ้นของการขยายตัวของหลอดเลือดซึ่งขึ้นกับเซลล์เอนโดทีเลียมในหนูแรทที่ถูกชักนำให้เกิดความดันเลือดสูงด้วยสารแอลเนม

คำสำคัญ: เฮสเพอริดิน, หนูแรทที่ถูกชักนำให้เกิดความดันเลือดสูงด้วยสารแอลเนม, การทำงานของหลอดเลือดที่ผิดปกติ

Background and Objectives: Hesperidin is a flavonoid found in peels or juices of citrus fruits. The beneficial effects of hesperidin have been reported such as anti-inflammation and antioxidation. This study was to investigate whether hesperidin could alleviate N^{ω}-Nitro-l-arginine methyl ester (L-NAME)-induced hypertension in rats.

Methods: Male Sprague-Dawley rats were induced hypertension by L-NAME administration. Rats were treated with L-NAME (40 mg/kg/day) in drinking water only or together with hesperidin (15 and 30 mg/kg BW per day) for five weeks while control rats received distilled water. Systolic blood pressure was measured weekly. Vascular function test was performed in aortic rings and mesenteric vascular beds.

Results: Rats treated with L-NAME had high blood pressure and a decrease in acetylcholine (ACh)-induced vasorelaxation in isolated aortic rings and mesenteric vascular beds. Vascular response to sodium nitroprusside (SNP) did not differ among groups. Interestingly, hesperidin significantly prevented L-NAME-induced hypertension and alleviated L-NAME-induced vascular dysfunction ($p < 0.05$).

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Conclusion: These findings suggested that hesperidin partially prevent the development of hypertension induced by L-NAME. This protective effect could involve the improvement of endothelium-dependent vasorelaxation in rats treated with L-NAME.

Keywords: Hesperidin, L-NAME-induced hypertensive rats, Vascular dysfunction

ศรีนครินทร์เวชสาร 2560; 32(1): 24-9. • Srinagarind Med J 2017; 32(1): 24-9.

Introduction

Hypertension or high blood pressure is the major risk factor for cardiovascular disease leading to cause of death. L-NAME, a nitric oxide (NO) synthase inhibitors, promoted a persistent high blood pressure associated with potent peripheral vasoconstriction and the consequent increase in peripheral vascular resistance[1]. Furthermore, NO deficiency contributes to vascular endothelial dysfunction, a major mechanism of essential hypertension. Several studies indicated that chronic administration of L-NAME impaired endothelium-dependent vasorelaxation in both conduit and resistance arteries[2-4].

Nowadays, several lines of evidence indicated that naturally medicinal plants that have antioxidant properties can prevent and treat hypertension by improving vascular dysfunction[5,6]. Hesperidin is a flavonones, a subclass of flavonoids, abundantly found in citrus fruits such as lemon, orange peel or juice[7]. The beneficial effects of hesperidin have been reported including anti-inflammation, antioxidant and neuroprotection. However, little information regarding to antihypertensive effect of hesperidin has been reported.

The present study is aimed to investigate the effects of hesperidin on L-NAME-induced hypertension and vascular dysfunction in rats.

Methods

Animals

Male Sprague-Dawley rats (220-250 g) were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. Rats were maintained in an air-conditioned room (25 ± 2 C°) with a 12 h dark-light cycle at Northeast Laboratory Animal Center. All procedures are 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 (AEKKU-NELAC 37/2559).

Drugs and chemical

Hesperidin (purity ≥ 98%) was purchased from ChemFaces (Hubei, China). Acetylcholine chloride (ACh) and sodium nitroprusside (SNP) were obtained from FlukaChemika (Buchs, Switzerland). Polyethylene glycol (PG) was obtained from Ajax Finechem Pty Ltd. (NSW, Australia). Capsaisin, methoxamine, phenylephrine hydrochloride (Phe), norepinephrine (NE) were obtained from Sigma-Aldrich Corp (St Louis, MO, USA).

Experimental design

Over the five weeks study course, the animalswere randomly divided into 3 groups with 5-6 animals in each group; control group, rats received drinking water (0.5 ml/100 mg; p.o.); L-NAME hypertensive group, rats received L-NAME (40 mg/kg/day; p.o.) in drinking water; L-NAME treated group, rats received L-NAME (40 mg/kg/day; p.o.) and hesperidin (15 and 30 mg/kg/day; p.o.). Hesperidin was dissolved with polyethylene glycol.

Indirect measurement of blood pressure in conscious rats

Systolic blood pressure (SP) of all animals was measured weekly using non-invasive tail-cuff plethysmography (IITC/Life Science Instrument model 229 and model 179 amplifiers, Woodland Hills, CA, USA). In brief, conscious rats were placed in a restrainer and allowed to calm prior to blood pressure measurement. The tail of each rat was placed inside the tail cuff, and the cuff was automatically inflated and released. For each rat, blood pressure was recorded as the mean value from the three measurements with

15 min. intervals.

Experimental protocols in isolated mesenteric vascular beds

Mesenteric vascular beds were carefully isolated and then placed on a stainless steel grid (7x5 cm) 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 preparations were allowed to equilibrate for 30 min before the next trial. Thereafter, methoxamine (5-7 μM) was added into Kreb's solution to raise tone (70-90 mmHg above baseline). To determine vasoactive performance of resistance small arteries, different doses of vasoactive agents, acetylcholine (Ach, endothelium-dependent vasodilator, 1 nM-0.01 μM) or SNP (NO donor, 1 nM-0.01 μM), were injected through neoprene rubber tubing proximal to the tissue. The relaxation response was expressed as decrease in perfusion pressure (mmHg).

Experimental protocols in isolated aortic rings

The thoracic aorta (conduit artery) was rapidly removed for tension measurement. The tissue samples were mounted in a 15 mL bath containing Krebs' solution at 37 °C and gassed with a 95% O_2 and 5% CO_2 gas mixture.After equilibrium period, the rings were pre-contracted with phenylephrine (10 μM). To determine vasoactive performance, different doses of vasoactive agents, Ach (0.01-3 μM) or SNP (0.01-3 μM) was added to the baths. The relaxation response was expressed as percent of relaxation.

Statistical analysis

Data are expressed as mean ± S.E.M. The differences among treatment groups were analyzed by one-way analysis of variance (ANOVA). A p-value of less than 0.05 was considered statistically significant.

Results

Effect of hesperidin on L-NAME induced high blood pressure in rats

At baseline, SP was not significant difference among experimental groups. Daily administration of L-NAME for five weeks caused significant increase in SP (200.4 ± 8.2 mmHg), comparing to those of control group (124.2 ± 2.6 mmHg) ($p<0.05$). Concomitant treatment with hesperidin (15 and 30 mg/kg/day) significantly prevented the development of high SP in a dose-response manner (178.3 ± 4.6 and 160.9 ± 3.1 mmHg, respectively, $p<0.05$) comparing to those of L-NAME treated group (Figure 1).

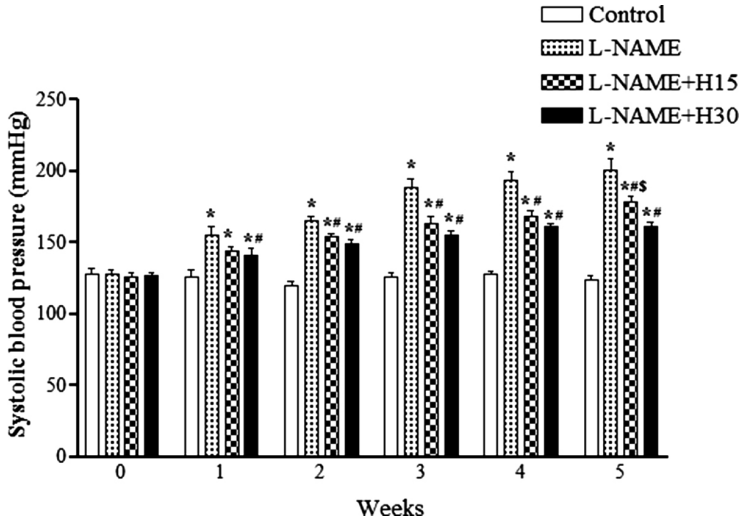

Figure 1 Effects of hesperidin on systolic blood pressure in L-NAME induced hypertension. Data are expressed as mean ± S.E.M. (n = 6 / group). * $p<0.05$ vs. control, # $p<0.05$ vs. L-NAME, $ $p<0.05$ vs. L-NAME+H30.

Effect of hesperidin on vascular reactivity in mesenteric vascular beds

Vasorelaxation response to Ach (0.1 μM-0.1 mM) in the mesenteric vascular bed was significantly blunted in L-NAME treated group compared to those of control group (0.1 μM Ach, 17.1 ± 1.8 *vs.* 53.5 ± 9.3 mmHg) ($p<0.05$). Concomitant treatment with hesperidin at dose 30 mg/kg/day significantly improved the response to Ach compared to those of L-NAME treated group (0.1 μM Ach, 43.9 ± 8.2 mmHg) ($p<0.05$; Figure 2A). However, there was no significant difference in the vasorelaxation responses to SNP among groups, indicating normal vascular smooth muscle cell function (Figure 2B).

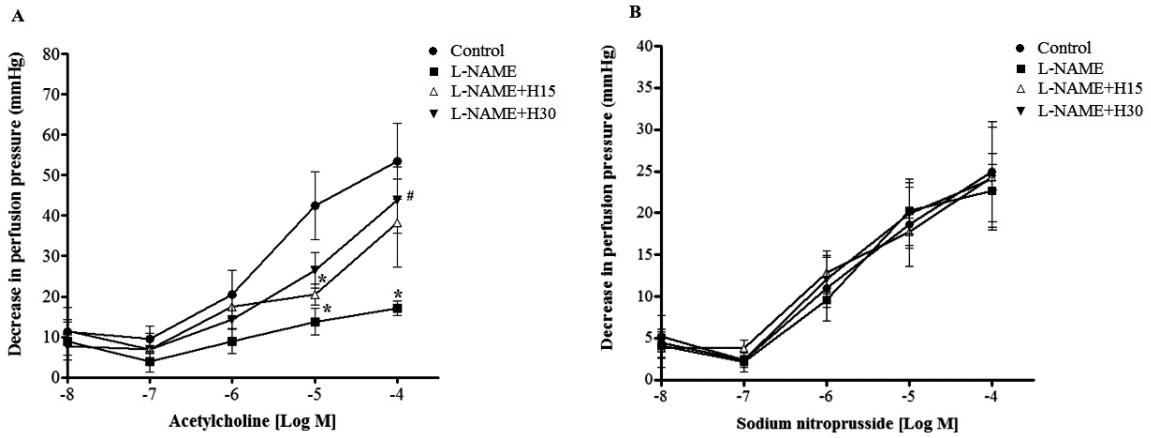

Figure 2 Effect of hesperidin on vascular responses to acetylcholine (A) and sodium nitroprusside(B) in mesenteric vascular beds. Data are presented as mean ± S.E.M. (n = 4-6/group). * $p<0.05$ vs. control, # $p<0.05$ vs. L-NAME.

Effect of hesperidin on vascular reactivity in aortic rings

Similarly, Endothelium-dependent vasorelaxation responses to Ach (0.01 μM-3 μM) were significantly blunted in aortic rings from L-NAME treated rats compared to those of control rats (3 μM Ach, 6.7 ± 3 *vs.* 44.9 ± 4.9% of relaxation) ($p<0.01$). Hesperidin at dose 15 and 30 mg/kg significantly improved vascular response to Ach compared to untreated group (3 μM Ach, 24.4 ± 3.3 and 30.1 ± 2.8% of relaxation; $p<0.05$) (Figure 3A). In addition, vasorelaxation response to SNP, an NO donor, did not differ significantly among groups (Figure 3B).

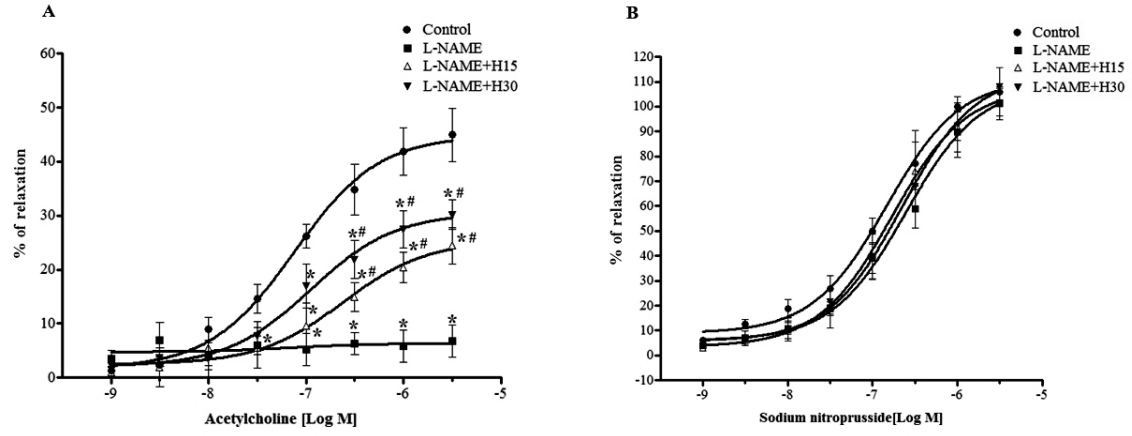

Figure 3 Effect of hesperidin on vascular responses to acethylcholine(A) sodium nitroprusside (B) in aortic rings. Data are expressed as mean ± S.E.M. (n = 4-6/group). * $p<0.05$ vs. control, # $p<0.05$ vs. L-NAME.

Discussion

The main findings of this study are that hesperidin prevented the development of hypertension and improved endothelium-dependent vasorelaxation in aortic rings and mesenteric vascular beds of L-NAME-induced hypertensive rats. We demonstrated that rats received L-NAME developed high blood pressure. It is well established that NO is a potent vasodilator and regulates vascular diameter[8]. L-NAME administration produced a systemic vasoconstriction, an increase in vascular resistance and sustained high blood pressure[1,9,10].

Moreover, the increase in blood pressure after L-NAME administration was accompanied by vascular dysfunction since the present study showed a significantly blunted response to Ach in both conduit and resistance arteries. It is known that endothelial dysfunction is characterized by impaired NO bioavailability because of a reduced production of NO[11]. In addition, our findings were consistent with several studies that endothelial dysfunction observed in L-NAME hypertension is associated with a decrease of NO level, NO metabolites and eNOS expression[2,9,12]. Recently, Yang and coworkers reported that flavonoids, polyphenols, isolated from sea buckthorn berries can alleviate vascular impairment in rats with hyperlipidemia[13].

Hesperidin significantly prevented the development of hypertension induced by L-NAME. This was associated with the finding that hesperidin also alleviated the impairment of endothelium-dependent vasorelaxation induced by L-NAME. Hesperidin has antioxidant activity and depletes reactive oxygen species to increase NO bioavailability[14]. Rizza and coworkers found that hesperidin stimulates phosphorylation of Akt, AMPK, and eNOS to produce NO in endothelial cells[15]. There is evidence to support our finding that hesperidin improved endothelial dysfunction in spontaneous hypertensive rats[16]. Thus, hesperidin prevented hypertension and improved endothelium-dependent relaxation in L-NAME treated rats. This could involve its antioxidant properties.Therefore, a decreased in systolic blood pressure after treatment with hesperidin maybe involved the ability to improve vasorelaxation responses.

Conclusion

In summary, hesperidin prevents the development of hypertension. This could associate with its vascular protective effect in L-NAME-induced hypertensive rats.

Acknowledgments

This study was supported by Invitation Research Fund (IN60131), Faculty of Medicine, Khon Kaen University, Thailand.

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