

ผลของลิซิโนพริลต่อการทำงานของหัวใจห้องล่างซ้ายและหลอดเลือดในหนูที่มีภาวะพร่องไนตริกออกไซด์

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Effect of Lisinopril on Left Ventricular and Vascular Function in Nitric Oxide-Deficient Rats

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หลักการและวัตถุประสงค์: ลิซิโนพริลตัวยับยั้งแองจิโอเทนซิน-คอนเวอร์ติง เอนไซม์ ใช้รักษาภาวะความดันเลือดสูงและหัวใจล้มเหลว การศึกษานี้ได้ทำการตรวจสอบผลของลิซิโนพริลต่อการป้องกันแอลเนมชักนำความดันเลือดสูงและการทำงานของหลอดเลือดของหัวใจห้องล่างซ้ายและหลอดเลือดในหนูแรท

วิธีการศึกษา: หนูแรทเพศผู้ได้รับแอลเนม (40 มก./กก./วัน) ในน้ำดื่มหรือร่วมกับการป้อนลิซิโนพริล (2.5 มก./กก./วัน) เป็นเวลา 5 สัปดาห์ หนูกลุ่มควบคุมได้รับน้ำ (5ตัว/กลุ่ม) ความดันเลือดและการทำงานของหัวใจถูกวัด และการทำงานของหลอดเลือดเอออดาร์กูปรีมิน

ผลการศึกษา: หนูที่ได้รับแอลเนมมีความดันเลือดสูง การทำงานของหัวใจและการตอบสนองของหลอดเลือดต่อสารอะซิติลโคลีนลดลง การตอบสนองของหลอดเลือดต่อโซเดียมไนโตรพรัสไซด์ไม่แตกต่างระหว่างกลุ่ม เป็นที่น่าสนใจว่าลิซิโนพริลสามารถป้องกันแอลเนมชักนำให้เกิดความดันเลือดสูงและบรรเทาแอลเนมชักนำให้เกิดการทำงานของหัวใจและหลอดเลือดที่ผิดปกติ ($p < 0.05$)

สรุป: สรุปได้ว่าลิซิโนพริลมีผลป้องกันการเพิ่มขึ้นของความดันเลือดสูง และป้องกันการทำงานที่ผิดปกติของหัวใจและเอนโดทีเลียมในหลอดเลือดที่ถูกชักนำด้วยสารแอลเนม

คำสำคัญ: ลิซิโนพริล, แอลเนมชักนำให้เกิดความดันเลือดสูงในหนูแรท, การทำงานที่ผิดปกติของหัวใจและหลอดเลือด

Background and Objective: Lisinopril, angiotensin-converting enzyme inhibitors (ACEIs), is widely used to treat hypertension and heart failure. This study investigated whether lisinopril could prevent N^w-Nitro-L-arginine methyl ester (L-NAME)-induced hypertension, left ventricular (LV) and vascular dysfunction in rats.

Methods: Male Sprague-Dawley rats were treated with L-NAME (40 mg/kg/day) in drinking water only or together with lisinopril (2.5 mg/kg/day) for five weeks while control rats received distilled water (n=5). Blood pressure and cardiac function were measured. Aortic rings were isolated for vascular function test.

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

Conclusion: These findings suggested that lisinopril prevented the development of hypertension and partially prevented cardiac and vascular endothelial dysfunction induced by L-NAME.

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Key words: Lisinopril, L-NAME-induced hypertensive rats, Cardiac and vascular dysfunction

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Introduction

Hypertension is the major health problem and cause of death worldwide since it contributes to other cardiovascular disease, such as heart failure, peripheral arterial disease and stroke^{1, 2}. Several factors, including nitric oxide deficiency, renin-angiotensin system (RAS) activation and sympathetic nerve overactivation have been reported to mediate the development of hypertension^{3, 4}. Nitric oxide (NO) is the most powerful vasodilator and maintains vascular function and homeostasis⁵. NO deficiency reduces vasodilation activity and increases vascular resistance resulting in high blood pressure^{3, 6}. L-NAME, a NO synthase inhibitor, has been used to induce hypertension which associates with vascular endothelial cell dysfunction⁷. Furthermore, several lines of evidence showed that L-NAME hypertensive rats had an impairment of ventricular dysfunction and RAS activation^{8, 9}.

Angiotensin-converting enzyme inhibitors (ACEIs) is one of the first line drugs recommended for hypertension management¹⁰. ACEIs act as the potent competitive inhibitors of angiotensin-converting enzyme that cause the reduction of angiotensin II (Ang II) synthesis. It has been reported that ACEIs reduce blood pressure and cardiac work¹¹. Lisinopril is one of ACEIs that has widely used to treat essential hypertension, renovascular hypertension and chronic heart failure¹². Little information has shown the effect of lisinopril on cardiac and vascular function in hypertensive rats. This study aimed to investigate the protective effect of lisinopril on cardiac and vascular dysfunction on L-NAME-induced hypertensive rats.

Methods

Animals

Male Sprague-Dawley rats weighing 220-250 g were purchased from Nomura Siam International Co, Ltd., Bangkok, Thailand. Rats were housed in the HVAC (Heating, Ventilation and Air-Conditioning) System (23 ± 2°C) with a 12 h dark-light cycle at 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 (AEKKU-NELAC 72/2561).

Drugs and Chemicals

Lisinopril and Phenylephrine (PE) were purchased from Sigma-Aldrich Corp (St Louis, MO, USA). Acetylcholine chloride (ACh) and sodium nitroprusside (SNP) were purchased from Fluka Chemika (Buchs, Switzerland). Thiopental sodium was purchased from animal hospital of faculty of Veterinary Medicine, KKU (Khon Kaen, Thailand).

Experimental designs

All rats were randomly divided into 3 groups which consist of 5 rats in each group; control group: rats orally fed with vehicle (distilled water) (1.5 mL/kg; p.o.), L-NAME hypertensive group: rats received L-NAME (40 mg/kg/day; p.o.) in drinking water and orally fed with distilled water (1.5 mL/kg; p.o.), L-NAME treated group, rats received L-NAME (40 mg/kg/day; p.o.) and orally fed with lisinopril (2.5 mg/kg/day; p.o.) for five weeks.

Cardiac Function Study

At the end of experiment, all rats were anesthetized with thiopental sodium (70 mg/kg, i.p.). Their chests were shaved and cleaned, and they were placed on one side. Echocardiogram was performed by veterinarian using Model LOGIQ S7 (GE Healthcare, WI, USA). LV structure and function were assessed from two-dimensional short-axis view and then M-mode tracings were recorded for end diastolic volume (EDV), end systolic volume (ESV), stroke volume (SV) and ejection fraction (EF) from three consecutive cardiac cycle.

Direct measurement of blood pressure

After cardiac function measurement, the left femoral artery was identified and cannulated by a polyethylene tube. Baseline values of systolic blood pressure (SP) and heart rate (HR) were continuously monitored for 20 min by a way of a pressure

transducer and recorded using Acknowledge Data Acquisition software (Biopac Systems Inc., Santa Barbara, CA, USA).

Experimental protocols in isolated aortic rings

To assess vasoactive performance of the large arteries, the thoracic aorta was rapidly removed and cut to rings 2-3 mm in length for tension measurement. They were mounted in 15 ml baths containing Krebs' solution at 37 °C 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, ADInstruments, Australia). ACh (0.001 μM-3 μM) induced endothelial mediated-relaxations and vascular response to SNP (0.001 μM-3 μM) were assessed by pre-contracting with PE (10 μM) and relaxation expressed as % of the PE-induced contraction.

Statistical analysis

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

Results

Effect of lisinopril on SP and HR in L-NAME induced hypertensive rats

L-NAME hypertensive group significantly increased SP and HR (188.46 ± 4.11 mmHg and 396.75 ± 16.78 beats/min, respectively) when compared to the control group (120.25 ± 1.78 mmHg and 323.47 ± 26.36 beats/min, respectively, p<0.05). Treated with lisinopril significantly decreased SP and HR (126.52 ± 4.77 mmHg and 323.1 ± 14.98 beats/min, respectively) when compared to untreated hypertensive group (p<0.05) (Figure 1).

Effect of lisinopril on cardiac function on L-NAME induced hypertensive rats.

L-NAME hypertensive group significantly reduced EDV, SV, and EF (0.43 ± 0.09 ml, 0.28 ± 0.06 ml, and 67.34 ± 2.78 %, respectively) when compared with control group (0.64 ± 0.06 ml, 0.5 ± 0.04 ml, and 77.41 ± 1.83%, respectively, p<0.05). There was no significant

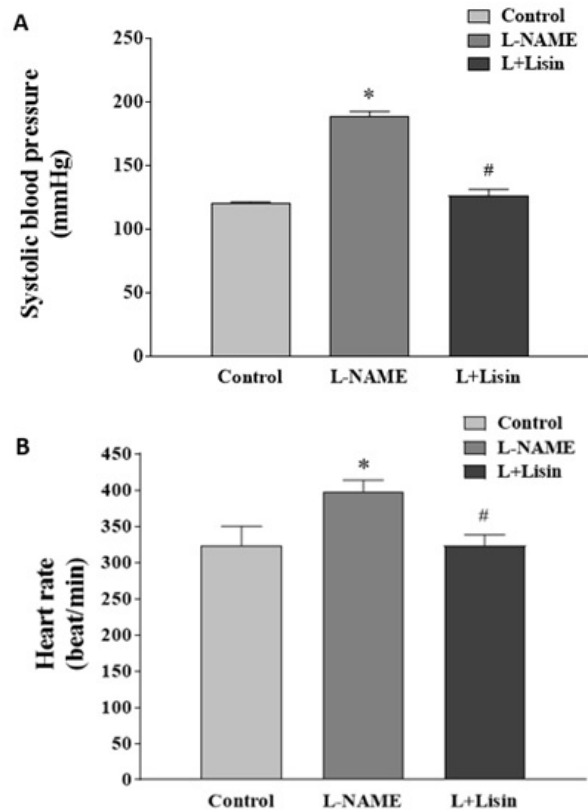


Figure 1 Effects of lisinopril on systolic blood pressure (A) and heart rate (B) in L-NAME-induced hypertension. Data are expressed as mean ± S.E.M. (n = 5 / group). * p<0.05 vs. control, # p<0.05 vs. L-NAME

difference of ESV among group. Treated with lisinopril significantly improved EF (80.76 ± 3.14%) while it was not effect on EDV, ESV, and SV (0.43 ± 0.05 ml, 0.09 ± 0.02 ml, and 0.34 ± 0.03 ml, respectively) when compared with untreated group (p<0.05) (Figure 2).

Effect of lisinopril on vascular reactivity in aortic rings

Endothelium-dependent vasorelaxation responses to ACh (0.001 μM-3 μM) were significantly blunted in aortic rings from L-NAME hypertensive rats compared to those of control rats (3 μM ACh, 12.13 ± 2.43 vs. 71.31 ± 5.51% of relaxation) (p<0.05). Treated with lisinopril significantly improved vascular response to ACh compared to untreated group (3 μM ACh, 40.34 ± 3.95 % of relaxation; p<0.05) (Figure 3A). In addition, vasorelaxation response to SNP (0.001 μM-3 μM), a NO donor, did not differ significantly among groups (Figure 3B).

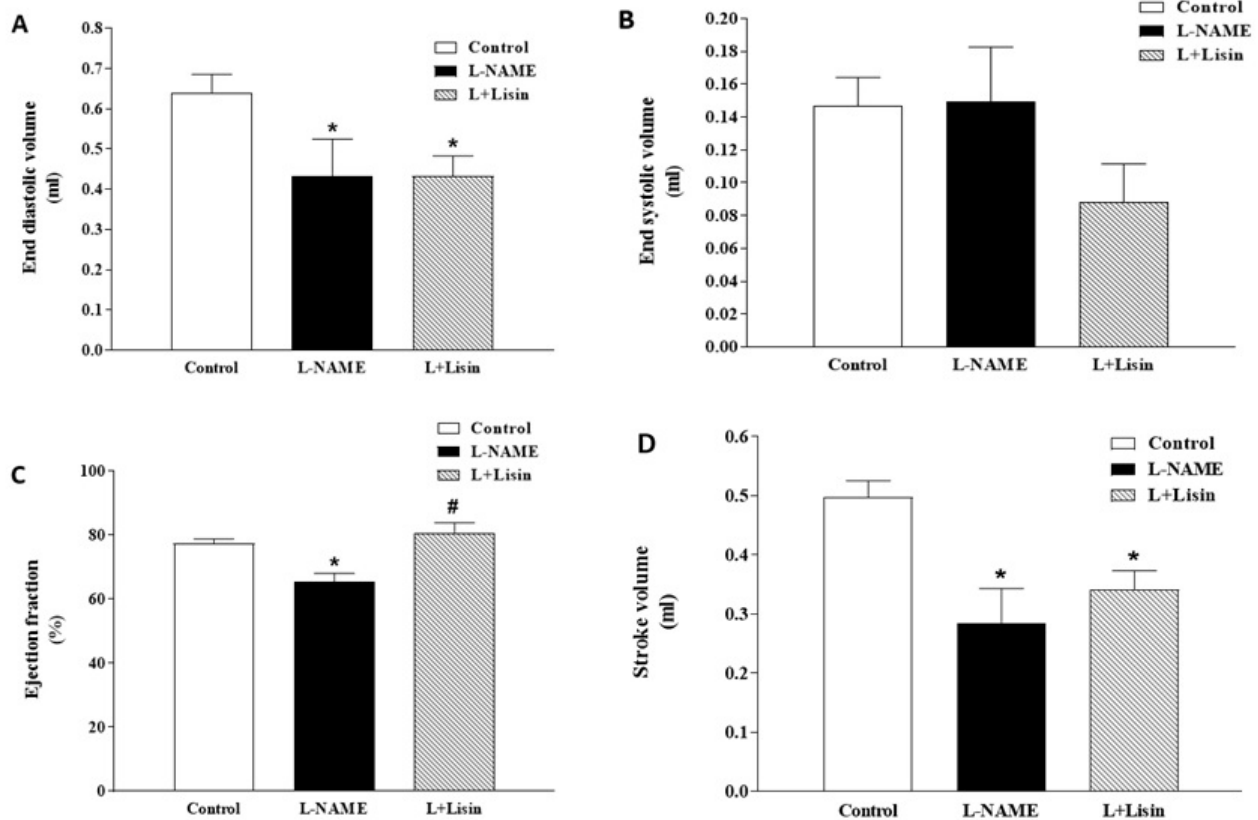


Figure 2 Effects of lisinopril on left ventricular function; end-diastolic function (A), end-systolic function (B), ejection fraction (C), and stroke volume (D) in L-NAME-induced hypertension. Data are expressed as mean \pm S.E.M. (n = 5 / group). * p < 0.05 vs. control, # p < 0.05 vs. L-NAME

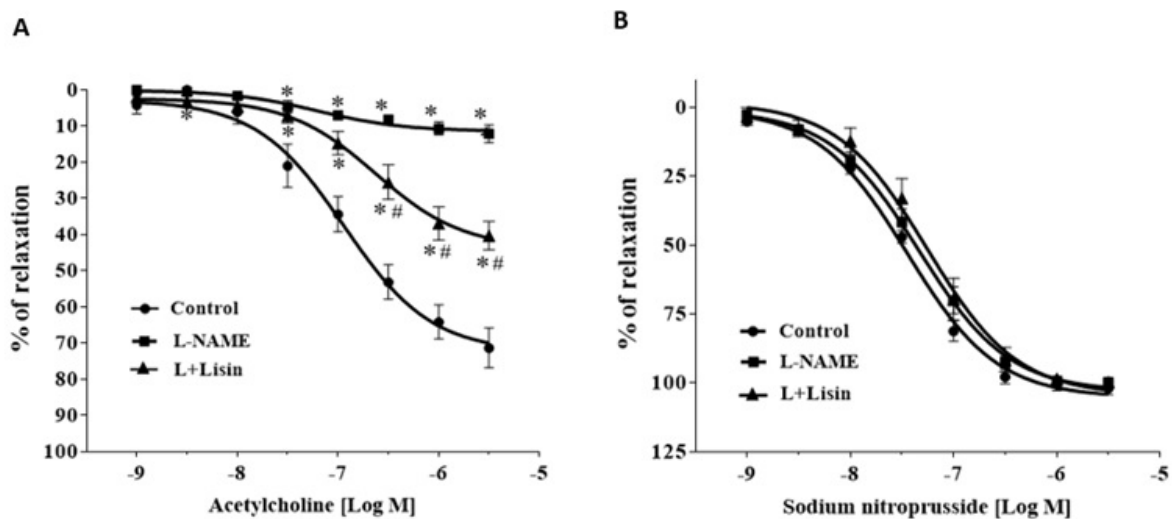


Figure 3 Effect of lisinopril on vascular responses to acetylcholine (A) and sodium nitroprusside (B) in aortic rings. Data are expressed as mean \pm S.E.M. (n = 5/group). * p < 0.05 vs. control, # p < 0.05 vs. L-NAME.

Discussion

The main findings of this study are that lisinopril prevented the development of hypertension, and improved EF and endothelium-dependent vasorelaxation in L-NAME-induced hypertensive rats. L-NAME-induced hypertension is widely used to

mimic hypertension. Rats that received L-NAME developed high blood pressure associated with cardiac and vascular dysfunction has been demonstrated⁸. Furthermore, increased HR was observed in L-NAME hypertensive rats since L-NAME can increase central sympathetic outflow^{14, 15}. Additionally, RAS activation was predominant in this

animal model¹³. In fact, reduction of NO production and NO bioavailability produces vascular endothelial dysfunction, systemic vasoconstriction and high vascular resistance, resulting in increased blood pressure⁹. Our results confirmed the endothelial dysfunction in aortic ring as vascular responses to ACh were blunted in L-NAME hypertensive rats. These findings were consistent with the recent study that inhibition of NO production by L-NAME was associated with high level of oxidative stress, impairment of ACh-induced vasorelaxation, increased vascular resistance and development of hypertension¹⁶. Increases in systemic vasoconstriction and vascular resistance enhance workload of the heart and promotes cardiac dysfunction as Jin and coworkers reported an impairment of LV function L-NAME-induced hypertensive rats¹⁷. We also found that rats treated with L-NAME for five weeks significantly reduced cardiac function indicating by low level of EF.

Lisinopril prevented the development of hypertension induced by L-NAME in rats. This was associated with the finding that lisinopril also alleviated the impairment of endothelium-dependent vasorelaxation. These findings were supported by several studies. For instances, Jan-On and coworkers found that lisinopril improved hemodynamic parameters and endothelial function and reduced oxidative stress markers in L-NAME-induced rats¹⁸. Powers and coworkers found that lisinopril improved EF and attenuated signs and symptoms of CHF individuals¹². It is well established that lisinopril reduce Ang II synthesis, enhances vasodilation, and lowers blood pressure and heart work^{11,19}. This study found that lisinopril partially prevented L-NAME-induced ventricular dysfunction. The improvement of EF was seen in lisinopril treated group while other cardiac parameters, EDV, ESV and SV did not restore. Thus, a higher dose or longer treatment of lisinopril to restore SV and LV diameter is required.

Conclusion

In summary, lisinopril prevents the development of hypertension in NO-deficient rats. It also partially prevents ventricular dysfunction and an impairment of endothelium-dependent vasorelaxation induced by L-NAME in rats.

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