แอลอาร์จินีนลดความดันเลือดและปรับปรุงการทำงานของเซลล์ชั้นเอนโดทีเลียมของหลอดเลือด ในหนูแรทความดันเลือดสูงจากภาวะพร่องไนตริกออกไซด์

สราวุธ บรรบุผา[1], พัชรวิภา มณีไสย[2,4], เทวฤทธิ์ เบิกบาน[1], ปาริฉัตร ประจะเนย์[3,4], ยุพา คู่คงวิริยพันธ์[2,4] , พวงรัตน์ ภักดีโชติ[2,4]*

[1]คณะแพทยศาสตร์ มหาวิทยาลัยมหาสารคาม, จ.มหาสารคาม, ประเทศไทย

[2]ภาควิชาสรีรวิทยา [3]ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น, จ.ขอนแก่น, ประเทศไทย

[4]กลุ่มวิจัยหัวใจและหลอดเลือด มหาวิทยาลัยขอนแก่น, จ.ขอนแก่น, ประเทศไทย

L-arginine Reduces Blood Pressure and Improves Vascular Endothelial Function in Nitric Oxide-Deficient Hypertensive Rats

Sarawoot Bunbupha[1], Putcharawipa Maneesai[2,4], Thewarid Berkban[1], Parichat Prachaney[3,4], Upa Kukongviriyapan[2,4], Poungrat Pakdeechote[2,4]*

[1] Faculty of Medicine, Mahasarakham University, Maha Sarakham, Thailand

[2]Department of Physiology, [3]Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

[4]Cardiovascular Research Group, Khon Kaen University, Khon Kaen, Thailand

หลักการและวัตถุประสงค์: แอลอาร์จินีนเป็นสารตั้งต้นของการสร้างไนตริกออกไซด์ แอลอาร์จินีนมีฤทธิ์ต้านความดันเลือดสูง ต้านอนุมูลอิสระ และต้านการอักเสบ การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบผลของแอลอาร์จินีนต่อความดันเลือด และการทำงานของเซลล์บุผนังหลอดเลือด ในหนูแรทความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม

วิธีการศึกษา: หนูแรทเพศผู้สายพันธุ์ Sprague-Dawley ได้รับสารแอลเนม (40 มก./กก./วัน) ในน้ำดื่มเป็นเวลา 5 สัปดาห์ เพื่อเหนี่ยวนำให้เกิดภาวะความดันเลือดสูง หนูทดลองที่มีภาวะความดันเลือดสูงจะได้รับการป้อนด้วยแอลอาร์จินีน (100 มก./กก./วัน) หรือสารหลอกใน 2 สัปดาห์สุดท้าย ความดันซิสโทลิกถูกวัดสัปดาห์ละครั้ง ทำการประเมินการทำงานของหลอดเลือด ระดับ NOx ในพลาสมา และวัดการสร้าง $O_2^{\bullet-}$ ในหลอดเลือด

ผลการศึกษา: หนูทดลองที่ได้รับสารแอลเนมมีความดันเลือดสูงและการทำงานที่ผิดปกติของชั้นเอนโดทีเลียมของหลอดเลือด ซึ่งมีความสัมพันธ์กับการลดลงของระดับพลาสมา NOx และการสร้าง $O_2^{\bullet-}$ ในหลอดเลือดเพิ่มขึ้นเมื่อเปรียบเทียบกับกลุ่มควบคุม ($p<0.05$) แอลอาร์จินีนลดความดันเลือด

Background and Objectives: L-arginine is the substrate for vascular nitric oxide (NO) formation. It has antihypertensive, antioxidant, and anti-inflammatory activities. This study was to investigate the effect of L-arginine on blood pressure and vascular endothelial function in N^G-Nitro-L-arginine methyl ester hydrochloride (L-NAME)-induced hypertensive rats.

Methods: Male Sprague-Dawley rats were administered with L-NAME (40 mg/kg/day) in drinking water for 5 weeks in order to induce hypertension. Hypertensive rats were treated with L-arginine (100 mg/kg/day) or vehicle for the last 2 weeks. Systolic blood pressure was measured weekly. Vascular endothelial function test, plasma NO metabolite (NOx), and vascular superoxide ($O_2^{\bullet-}$) production were evaluated.

Results: Rats treated with L-NAME had high blood pressure and endothelial dysfunction associated with decreased plasma NOx level and increased vascular

*Corresponding author : *Poungrat Pakdeechote, Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. E-mail: ppoung@kku.ac.th*

ปรับปรุงการการทำงานของเซลล์บุผนังหลอดเลือดในหนูแรทความดันเลือดสูงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) นอกจากนี้แอลอาร์จินีนสามารถเพิ่มชีวปริมาณออกฤทธิ์ของไนตริกออกไซด์ โดยการฟื้นฟูระดับพลาสมา NOx และลดการสร้าง $O_2^{\bullet-}$ ($p<0.05$)

สรุป: จากผลการศึกษาแสดงให้เห็นว่าแอลอาร์จินีนลดความดันเลือด บรรเทาการทำงานที่ผิดปกติของชั้นเอนโดทีเลียมของหลอดเลือด ในหนูแรทความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม ผลดังกล่าวมีความสัมพันธ์กับการเพิ่มชีวปริมาณออกฤทธิ์ของไนตริกออกไซด์

คำสำคัญ: แอลอาร์จินีน, ภาวะความดันเลือดสูง, ภาวะเครียดออกซิเดชัน, ชีวปริมาณออกฤทธิ์ของไนตริกออกไซด์

Conclusion: Our results indicated that L-arginine decreased blood pressure and alleviated vascular endothelial dysfunction in L-NAME-induced hypertensive rats. This was associated with increasing in NO bioavailability.

Keywords: L-arginine, hypertension, oxidative stress, NO bioavailability

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Introduction

The endothelium plays a key role in regulating vascular tone by the balanced release of various vasodilators and vasoconstrictors[1]. In the vascular endothelium, nitric oxide (NO) is synthesized from L-arginine by endothelial NO synthase (eNOS) and mediated vasorelaxation[2]. N^G-Nitro-L-arginine methyl ester hydrochloride (L-NAME), an L-arginine analogue, is NO synthase inhibitor that reduces NO production and causes vascular endothelial dysfunction[3]. Several reports have shown that L-NAME-induced systemic arterial hypertension together with low levels of plasma NO, and reduced eNOS protein expression and activity, and impairment of vascular endothelial function[4,5].

L-arginine is a semi-essential amino acid derived from dietary intake, body protein breakdown, or endogenous de novo arginine production[6]. It is the sole substrate of the enzyme eNOS in the biosynthesis of potent vasodilator NO. Several studies have shown a variety of biological effects of L-arginine such as antihypertensive, antioxidant, anti-inflammatory, and antihyperlipidemic activities[7-9]. Tay and coworkers demonstrated that L-arginine treatment reduced blood pressure and improved insulin sensitivity in fructose-hypertensive rats[10]. Chronic L-arginine supplementation also inhibited the pulmonary vascular structural remodeling in pulmonary hypertensive rats[11]. Furthermore, L-arginine restored endothelium-dependent vasodilator response in isolated pulmonary arteries from pulmonary hypertensive exercise trained rats[12].

Although a wide range of potentially therapeutic effects of L-arginine have been reported, the effects of L-arginine on vascular endothelial function and oxidative stress in L-NAME-treated rats remain unknown. Therefore, the aim of this study was to investigate whether L-arginine can reduce blood pressure and alleviate endothelial oxidative stress and dysfunction, and improve NO bioavailability in L-NAME-induced hypertensive rats.

Methods

Chemicals

L-arginine, L-NAME, N-(1-Napthyl)ethylenediamine dihydrochloride (NED), and sulfanilamide were obtained from Sigma-Aldrich (St Louis, MO, USA). Nitrate reductase, nicotinamide adenine dinucleotide phosphate (NADPH), glucose-6-phosphate disodium and glucose-6-phosphate dehydrogenase were obtained from Roche Applied Sciences (Mannheim, Germany). Lucigenin, acetylcholine chloride (ACh),

and sodium nitroprusside (SNP) were obtained from Fluka Chemika Co., Ltd (Buch, Switzerland). All chemicals used were of analytical grade quality.

Animals and experimental protocols

Male Sprague–Dawley rats (220–240 g) were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. They were housed at 25 ± 2 ℃ 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 approved by the Animal Ethics Committee of Khon Kaen University (AEKKU 37/2555). After a week of acclimatization, rats were randomly divided into three groups of 6 rats each: Group I. control + vehicle (distilled water, 0.15 mL/100 g), Group II. L-NAME + vehicle (distilled water, 0.15 mL/100 g), and Group III. L-NAME + L-arginine (100 mg/kg/day). Over the 5-week study course, control rats received distilled water, while hypertensive rats received L-NAME (40 mg/kg/day) in their drinking water in order to induce hypertension. L-arginine or vehicle was intragastrically administered daily for the last 2 weeks of the study. The concentrations of L-arginine used in the present study were influenced by the previous study[13].

Indirect measurement of blood pressure in conscious rats

Systolic blood pressure (SBP) 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.

Vascular responsiveness measurements

On the last day of the study, the animals were anesthetized by peritoneal injection of pentobarbital sodium (60 mg/kg). Body temperature was monitored using a rectal probe and maintained at 37 ± 2 ℃ throughout the study using a heating pad. A femoral artery was identified, cleaned of a connective tissue, and cannulated with a polyethylene tube. Direct blood pressure were recorded by using Acknowledge Data Acquisition software (Biopac Systems Inc., Santa Barbara, CA, USA). After obtaining stable baseline of blood pressure, a femoral vein was cannulated with a polyethylene tube. To test vascular endothelial and smooth muscle cell function, a vascular responsiveness test was carried out by intravenous infusion of ACh, an endothelium-dependent vasodilator (3, 10, and 30 nmol/kg) and SNP, an endothelium-independent vasodilator (1, 3, and 10 nmol/kg). Each vasoactive agent was infused in stepwise concentration increases at 5-min intervals, with 5-min intervals between doses. At the end of study, rats were killed by over dosage of the anesthetic drug. Blood samples were collected from the abdominal aorta into EDTA tubes for plasma NO metabolite (NOx) assays. A carotid artery was rapidly excised for analysis of superoxide ($O_2^{\bullet-}$) production.

Assay of superoxide production

Vascular $O_2^{\bullet-}$ production was measured using a lucigenin-enhanced chemiluminescence method[14] with some modifications[15].

Assay of NO metabolite

The concentration of plasma NO metabolite (NOx) was measured using an enzymatic conversion method[16] with some modifications[15].

Statistical analysis

Data are expressed as mean ± standard error of mean (SEM). 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

Effects of L-arginine on SBP in conscious rats

At the beginning of the study, average baseline values of SBP among all groups of rats were not significantly different (Figure 1). In the control group, the SBP did not change throughout the 5 weeks of the experiment. Administration of L-NAME for 5 weeks caused a progressively increase in SBP compared to SBP levels in control rats (SBP at 5th week, 212.8 ± 5.5 mmHg vs. 121.3 ± 3.2 mmHg) ($p<0.05$). Treatment with L-arginine for two weeks significantly reduced SBP (162.1 ± 2.9 mmHg) in L-NAME hypertensive rats compared to untreated rats ($p<0.05$).

Effect of L-arginine on vascular responsiveness

Vasodilation responses to ACh were significantly blunted in L-NAME hypertensive rats compared to control rats; 28.4 ± 2.2% vs. 49.3 ± 2.2% at 3 nmol/kg, 41.0 ± 1.4% vs. 56.8 ± 1.5% at 10 nmol/kg, and 48.2 ± 2.5% vs. 65.9 ± 0.8% at 30 nmol/kg ($p<0.05$) (Figure 2A). However, there was no significant difference in vascular responses to the endothelium-independent vasodilation agent SNP across all groups of rats (Figure 2B), which may indicate that there was an endothelial dysfunction in L-NAME-induced hypertensive rats. Interestingly, Oral supplementation of L-arginine markedly restored ACh-induced vasorelaxation in L-NAME hypertensive rats (49.4 ± 1.3% at 10 nmol/kg and 58.2 ± 1.2% at 30 nmol/kg) ($p<0.05$).

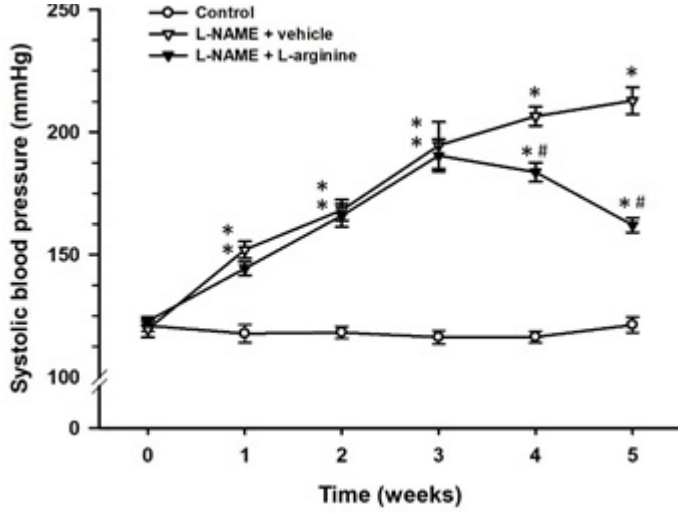

Figure 1 Systolic blood pressure (indirect measurement) during treatments in all experimental groups. Results are expressed as mean ± SEM. *p<0.05 vs. control group, #p<0.05 vs. L-NAME + vehicle group (n = 6).

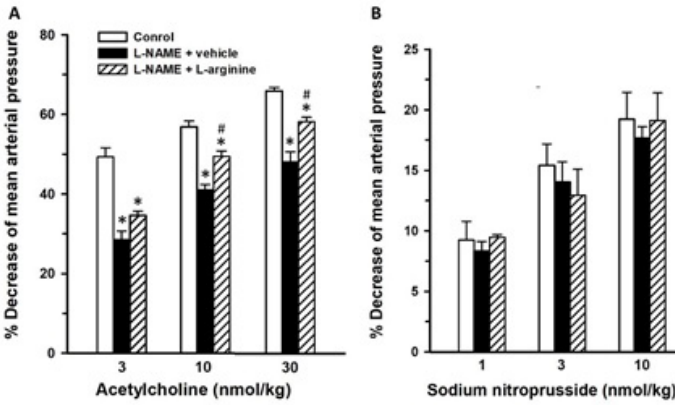

Figure 2 Effect of L-arginine on vascular responses to (A) acetylcholine and (B) sodium nitroprusside in hypertensive rats. Results are expressed as mean ± SEM. *p<0.05 vs. control group, #p<0.05 vs. L-NAME + vehicle group (n = 6).

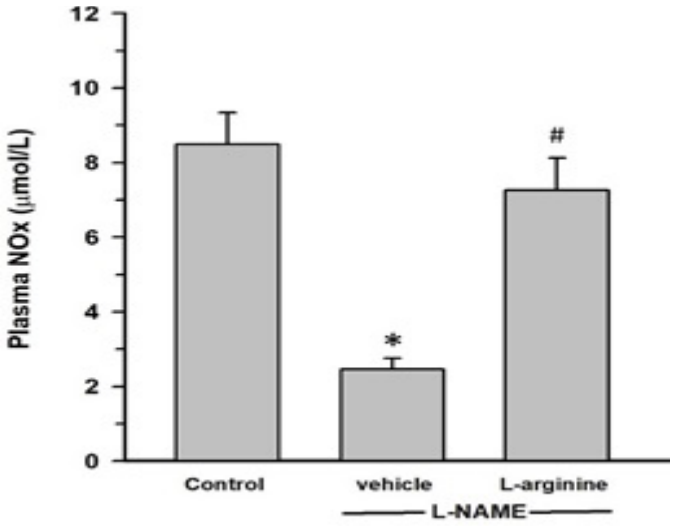

Figure 3 Effect of L-arginine on plasma nitric oxide metabolites (NOx) in hypertensive rats. Results are expressed as mean ± SEM. *p<0.05 vs. control group, #p<0.05 vs. L-NAME + vehicle group (n = 6).

Effect of L-arginine on plasma NOx levels

Plasma NOx levels from the different experimental groups are shown in Figure 3. In L-NAME hypertensive rats, plasma NOx concentrations were significantly decreased (2.4 ± 0.3 μmol/L) compared to control rats (8.5 ± 0.8 μmol/L) ($p<0.05$). However,

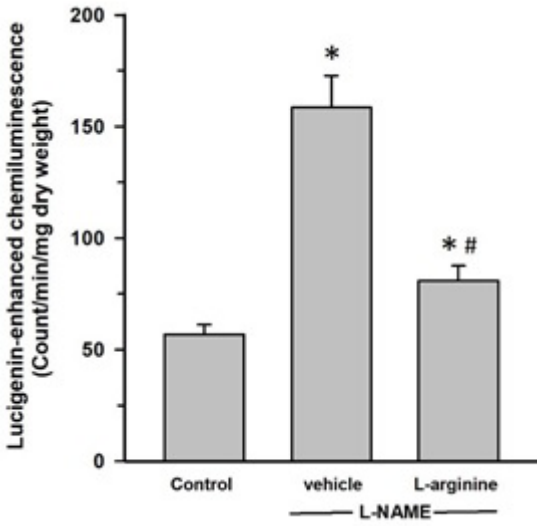

Figure 4 Effect of L-arginine on vascular superoxide production in hypertensive rats. Results are expressed as mean ± SEM. *p<0.05 vs. control group, #p<0.05 vs. L-NAME + vehicle group (n = 6).

treatment with L-arginine completely restored plasma NOx concentration in L-NAME hypertensive rats (7.2 ± 0.9 µmol/L) (p<0.05).

Effect of L-arginine on vascular $O_2^{\bullet-}$ production

Increased $O_2^{\bullet-}$ production in carotid arteries was found in the L-NAME hypertensive group (158.5 ± 14.3 counts/min/mg dry weight) compared to control group (56.9 ± 4.3 counts/min/mg dry weight) (p<0.05) (Figure 4). Administration of L-arginine to L-NAME hypertensive rats significantly attenuated $O_2^{\bullet-}$ production in carotid arteries (80.9 ± 6.6 counts/min/mg dry weight) (p<0.05).

Discussion

The present study demonstrates the effect of L-arginine on blood pressure, vascular endothelial function and vascular oxidative stress in L-NAME-induced hypertensive rats. L-NAME administration for 5 weeks produced an increase in blood pressure (SBP), together with impaired endothelium-dependent vasodilation, and these effects were restored by L-arginine treatment. Furthermore, an impairment of vascular endothelial function was accompanied by reducing plasma NOx concentration and increasing in vascular $O_2^{\bullet-}$ formation. These diminishing of NO synthesis and increasing in oxidative stress markers observed in L-NAME hypertensive rats were also alleviated by L-arginine supplementation.

It is well established that chronic administration of a NO synthase inhibitor in rats is widely used as an animal model to induce a sustained hypertension with increased total peripheral resistance[2]. Our result showed that the blunted response to ACh, and the normal response to the NO donor SNP in L-NAME-treated rats, indicates impairment of endothelium-dependent vasodilation. This confirms and extends previous reports that the increase blood pressure in experimental L-NAME-induced hypertension are associated with endothelial dysfunction[4,17]. Treatment of L-NAME-induced hypertensive rats with L-arginine reduced blood pressure and restored vascular response to ACh, suggesting that L-arginine improved vascular endothelial function. This result confirms a previous report that L-arginine restored ACh-induced vasorelaxation in pulmonary artery rings from pulmonary hypertensive exercise trained rats[12]. Furthermore, long-term L-arginine supplementation also improved small-vessel coronary endothelial function in humans with nonobstructive coronary artery disease[18]. It is likely that L-arginine ameliorates hypertension caused by L-NAME suppression of NO synthesis by improving vascular endothelial function. The low levels of plasma NO observed in L-NAME-induced hypertensive rats may not only be the result of a failure to generate sufficient NO via NO synthase, it may also be enhanced inactivation of NO resulting from increased oxidation of NO by $O_2^{\bullet-}$ to peroxynitrite ($ONOO^-$)[19]. In our experimental animals, we also found that increased $O_2^{\bullet-}$ production accompanied by decreased plasma NOx. Interestingly, treatment of L-NAME-induced hypertensive rats with L-arginine completely raised plasma NOx concentration and decreased vascular $O_2^{\bullet-}$ production, indicating improvement of endothelial NO bioavailability. Our results support a previous study that the dietary L-arginine supplementation enhanced endothelial NO generation in diabetic rats[20]. L-arginine also has been shown good radical scavenging activity by inhibiting linoleic acid peroxidation in in vivo study[8]. Additionally, L-arginine decreases oxidative stress by

reduction of the vascular $O_2^{\bullet-}$ production with improvement of endothelial function in hypercholesterolaemic subjects[21].

Conclusion

In conclusion, L-arginine reduced blood pressure and improved vascular endothelial function in L-NAME-induced hypertensive rats. The underlying mechanism might involve with reducing vascular $O_2^{\bullet-}$ production leading to enhance NO bioavailability.

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