Asiatic Acid Reduces Left Ventricular Remodeling in L-NAME-induced Hypertensive Rats
เอเชียติก แอซิด ลดการปรับเปลี่ยนโครงสร้างหัวใจห้องล่างซ้าย ในหนูขาวความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม

Sarawoot Bunbupha (สราวุธ บรรบุผา)* Dr.Poungrat Pakdeechote (ดร.พวงรัตน์ ภักดีโชติ)[1]**
Dr.Upa Kukongviriyapan (ดร.ยุพา คู่คงวิริยะพันธุ์)*** Dr.Parichat Prachaney (ดร.ปาริฉัตร ประจะเนย์)****
Pattanapong Boonprom (พัฒนพงษ์ บุญพรม) *****

ABSTRACT

Asiatic acid is a triterpenoid isolated from Centella asiatica. This study aimed to investigate whether asiatic acid could alleviate left ventricular (LV) remodeling in N-nitro-L-arginine-methylester (L-NAME)-induced hypertensive rats. Chronic administration of L-NAME (40 mg/kg/day) in male Sprague-Dawley rats for 5 weeks showed significant increases in mean arterial pressure (MAP) and LV hypertrophy ($p<0.05$). However, treatment with asiatic acid (20 mg/kg/day) for the last 2 weeks significantly reduced MAP, and the remodeling of the LV ($p<0.05$) in L-NAME-treated rats. This study suggests that asiatic acid reduced blood pressure and cardiac hypertrophy in LNAME-induced hypertensive rats.

บทคัดย่อ

สารเอเชียติก แอซิด เป็นกลุ่มสารไตรเตอร์ปินนอยด์ที่สกัดจากใบบัวบก การศึกษานี้มีวตถุประสงค์เพื่อศึกษาสารเอเชียติก แอซิ ด สามารถลดการปรับเปลี่ยนโครงสร้างของหัวใจห้องล่างซ้ายในหนูขาวความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนมได้หรือไม่ หนูทดลองสายพันธุ์ Sprague-Dawley ได้รับสารแอลเนม (40 มก./กก./วัน)ต่อเนื่องเป็นเวลา 5 สัปดาห์ มีการเพิ่มขึ้นของความดันเลือดและเกิดภาวะหัวใจห้องล่างซ้ายโต ($p<0.05$) อย่างไรก็ตามการให้เอเชียติก แอซิ ด (20 มก./กก./วัน) ในช่วงเวลา 2 สัปดาห์สุดท้าย สามารถลดระดับความดันเลือดและการปรับเปลี่ยนโครงสร้างของหัวใจห้องล่างซ้าย ($p<0.05$) ในหนูทดลองที่ได้รับสารแอลเนม การศึกษาครั้งนี้แสดงให้เห็นว่า เอเชียติก แอซิ ด สามารถลดภาวะความดันเลือดสูงและภาวะหัวใจโต ในหนูขาวความดันเลือดสูงที่ถูกเหนี่ยวนำด้วยสารแอลเนม

Key Words: Asiatic acid, Hypertension, Left ventricular hypertrophy
คำสำคัญ: เอเชียติก แอซิด ภาวะความดันเลือดสูง ภาวะหัวใจห้องล่างซ้ายโต

[1] *Correspondent author: ppoung@kku.ac.th*
** Student, Doctor of Philosophy in Medical Physiology, Department of Physiology, Faculty of Medicine, Khon Kaen University*
*** Asst. Prof. Department of Physiology, Faculty of Medicine, Khon Kaen University*
**** Assoc. Prof. Department of Physiology, Faculty of Medicine, Khon Kaen University*
***** Asst. Prof. Department of Anatomy, Faculty of Medicine, Khon Kaen University*
****** Research assistant, Department of Anatomy, Faculty of Medicine, Khon Kaen University*

Introduction

Left ventricular (LV) hypertrophy is an adaptive reaction to increased haemodynamic load. It represents an independent risk factor of increased cardiovascular morbidity and mortality [1]. Many studies reported that chronic administration of NG-nitro-L- arginine methyl ester (L-NAME), a nonspecific inhibitor of all three nitric oxide synthase (NOS), induces systemic arterial hypertension [2, 3]. Moreover, drinking water containing with L-NAME can increase LV fibrosis resulting in cardiac hypertrophy [4]. Asiatic acid is a triterpenoid compound derived from the medicinal plant *Centella asiatica*. The pharmacological activities of asiatic acid such as antioxidant [5], antihyperlipidemic [6], antidiabetic [7] and anti-inflammatory [8] properties have been demonstrated. In addition, our previous study found that asiatic acid decreased blood pressure in L-NAME-treated rats [9]. However, the effects of asiatic acid supplementation on cardiac remodeling in chronic nitric oxide-deficient hypertensive rats has not been previously reported.

Objectives of the study

This study aimed to evaluate whether asiatic acid could reduce LV remodeling and cardiac hypertrophy in L-NAME-induced hypertensive rats.

Methodology

Animal and experimental protocols

Male Sprague-Dawley rats (220-240 g) were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. Rats were maintained in an air-conditioned room (25 ± 2 Co) 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 37/2555).

After one week of acclimatization, the animals were randomly divided into 2 main groups. Group 1 is a normal control group which received tap water for 5 weeks. Group 2 is an L-NAME-treated group which received L-NAME (40 mg/kg/day) in their drinking water for 5 weeks to induce hypertension. The animals in all experimental groups were fed with a standard chow diet (Chareon Pokapan Co. Ltd., Thailand). After 3 weeks of study, normal control rats were divided in to 2 groups (n = 6/group); control rats treated with vehicle (propylene glycol) and control rats treated with asiatic acid (20 mg/kg/day) for the last 2 weeks. Hypertensive rats were divided in to 2 groups (n = 6/group); hypertensive rats treated with vehicle (propylene glycol) and hypertensive rats received asiatic acid (20 mg/kg/day) for the last 2 weeks.

Blood pressure measurement

At the end of study, body weight (BW) was measured and then 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 °C throughout the study using a heating pad. A femoral artery was identified, cleaned of connective tissue and cannulated with a polyethylene tube. Baseline values of mean arterial blood pressure (MAP) were continuously monitored for 20 min by a way of a pressure transducer and recorded using the Acknowledge Data Acquisition with analysis software (Biopac Systems Inc., Santa Barbara, CA, USA).

Histomorphometric study of the heart

The hearts were rapidly removed, then heart weight (HW) and left ventricle weight (LVW) were determined and the LVW/100 g BW ratio were calculated. Left ventricle was bisected coronally at the midventricular position, equidistant between base and apex. Then, the tissues were fixed 24 h in 10% formalin, routinely processed in paraffin and 5 μm thick slides from the midventricular surface, either to the base or to the apex were stained with Hematoxylin and Eosin (H&E). The heart sections were captured with stereoscope (Olympus SZH-ILLD with NIS elements software). Morphometric evaluations of LV wall thickness and cross section area were evaluated with Image-J NIH image analysis software as follows:

1. The LV wall thickness was measured every 45o interval around the cardiac circumference. The average value was calculated for each section.

2. Cross sectional area was calculated by using the difference between the value of the external circumferential area of the heart and the chamber area.

Statistical analysis

Data are presented as means ± standard error of mean (SEM). Statistical comparisons among groups were made using one-way analysis of variance (ANOVA) with a Student Newman-Keul's test. All analysis was performed using SigmaStat software version 3.1. Statistical significance was determined at a level of $p<0.05$.

Results

Effects of asiatic acid on blood pressure

After 5 weeks of L-NAME treatment, MAP was significantly increased (174.8 ± 7.4 mmHg) when compared to those of the normal control group (91.8 ± 2.3 mmHg) ($p<0.001$) (Figure 1). However, concomitant treatment with asiatic acid (20 mg/kg/day) for the last 2 weeks in L-NAME-treated rats significantly reduced MAP in a comparing to those of the hypertensive group without asiatic acid treatment (131.4 ± 3.4 mmHg) ($p<0.001$). Moreover asiatic acid had no effect on blood pressure in normal control rats.

Effect of asiatic acid on body weight and cardiac parameters

At the end of the study BW was not significantly different among groups (Table 1). HW, LVW and LVW/100 g BW ratio were significantly increased in L-NAME-treated rats when compared to those of the normal control group ($p<0.05$) (Table 1). Treatment with asiatic acid (20 mg/kg/day) significantly reduced HW, LVW and LVW/100 g BW comparing to those of the hypertensive group ($p<0.05$).

Effect of asiatic acid on wall thickness and cross sectional area of left ventricle

Administration of L-NAME caused a significant increase in wall thickness and cross sectional area of LV when compare to the control group ($p<0.05$) (Table 2). Wall thickness and cross sectional area in L-NAME receiving asiatic acid were significantly reduced ($p<0.05$).

Discussion and Conclusions

The present study demonstrates the effect of the asiatic acid on blood pressure and cardiac wall changes in L-NAME-induced hypertension. Chronic L-NAME treatment caused an increase in MAP and hypertrophy of the LV as increases in LVW, wall thickness and cross sectional area of left ventricle. There were reductions of blood pressure, LVW, wall thickness and cross sectional area of left ventricle in hypertensive rats treated with asiatic acid.

Our results confirm previous studies that chronic inhibition of NO synthesis with L-NAME induces a systemic arterial hypertension [2, 3]. Treatment with asiatic acid attenuated high blood pressure induced by L-NAME without having any effects in normotensive rats. In our previous study found that asiatic acid supplementation reduced blood pressure and oxidative stress biomarkers in hypertensive rats induced by chronic inhibition of NO synthesis with L-NAME [9]. The antihypertensive effect of asiatic acid may involve its antioxidant capacity. There is evidence support that asiatic acid supplementation increased the activities of catalase, superoxide dismutase, and glutathione peroxidase in the liver tissue and attenuated tissue MDA concentration in λ-carrageenan induced edema in mice [8]. In addition, asiatic acid from Potentilla chinensis remarkably alleviated oxidative stress by reduced malondialdehyde and restored impairment of antioxidants enzymes in chronic ethanol-induced hepatic injury rats [5].

In general, L-NAME-induced hypertension in rats is characterized by an increased in blood pressure and associated with cardiac fibrosis and hypertrophy [10, 11]. This present study revealed that the LVW, LV wall thickness and the cross-sectional area in the L- NAME-treated rats were increased. These results indicated that the left ventricle of these rats have been remodelled by hypertrophy as cardiac adaptation to maintain the normal cardiac output. Asiatic acid supplementation reduced LVW and LV dimension in hypertensive rats induced by chronic inhibition of NO synthesis with L-NAME. The mechanism involve that asiatic acid inhibit left ventricle remodeling is unknown. However, there are evidence supports that asiatic acid treatment ameliorated liver fibrosis in a rats model of CCl_4 -induced liver fibrosis [12] and tubulointerstitial fibrosis in mice with ureteral obstruction [13].

In conclusion, asiatic acid is able to attenuate the increasing in blood pressure and the myocardial hypertrophy in rats with inhibition of NO synthesis by L-NAME. Our study is suggestive of asiatic acid is present

in Centella asiatica, it is feasible to develop those plants to diminish blood pressure and cardiac hypertrophy in hypertensive people.

Acknowledgements

This work was supported by a grant from Khon Kaen University, Under Incubation Researcher Project, Thailand. Sarawoot Bunbupha holds a scholarship from Graduate School, Khon Kaen University, Thailand.

References

1. Simko F. Physiologic and pathologic myocardial hypertrophy--physiologic and pathologic regression of hypertrophy? Med Hypotheses. 2002; 58(1): 11-4.
2. Baylis C, Mitruka B, Deng A. Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. J Clin Invest. 1992; 90(1): 278-81.
3. Krier JD, Romero JC. Systemic inhibition of nitric oxide and prostaglandins in volume-induced natriuresis and hypertension. Am J Physiol. 1998; 274(1 Pt 2): R175-80.
4. Paulis L, Matuskova J, Adamcova M, Pelouch V, Simko J, Krajcirovicova K, et al. Regression of left ventricular hypertrophy and aortic remodelling in NO-deficient hypertensive rats: effect of L-arginine and spironolactone. Acta Physiol (Oxf). 2008; 194(1): 45-55.
5. Wei J, Huang Q, Huang R, Chen Y, Lv S, Wei L, et al. Asiatic acid from Potentilla chinensis attenuate ethanol-induced hepatic injury via suppression of oxidative stress and Kupffer cell activation. Biological & pharmaceutical bulletin. 2013; 36(12): 1980-9.
6. Pakdeechote P, Bunbupha S, Kukongviriyapan U, Prachaney P, Khrisanapant W, Kukongviriyapan V. Asiatic acid alleviates hemodynamic and metabolic alterations via restoring eNOS/iNOS expression, oxidative stress, and inflammation in diet-induced metabolic syndrome rats. Nutrients. 2014; 6(1): 355-70.
7. Ramachandran V, Saravanan R, Senthilraja P. Antidiabetic and antihyperlipidemic activity of asiatic acid in diabetic rats, role of HMG CoA: in vivo and in silico approaches. Phytomedicine. 2014; 21(3): 225-32.
8. Huang SS, Chiu CS, Chen HJ, Hou WC, Sheu MJ, Lin YC, et al. Antinociceptive activities and the mechanisms of anti-inflammation of asiatic Acid in mice. Evid Based Complement Alternat Med. 2011; 2011: 895857.
9. Bunbupha S, Pakdeechote P, Kukongviriyapan U, Prachaney P, Kukongviriyapan V. Asiatic Acid Reduces Blood Pressure by Enhancing Nitric Oxide Bioavailability with Modulation of eNOS and p47 Expression in l-NAME-induced Hypertensive Rats. Phytother Res. 2014.

10. Bernatova I, Pechanova O, Kristek F. Mechanism of structural remodelling of the rat aorta during long-term NG-nitro-L-arginine methyl ester treatment. Jpn J Pharmacol. 1999; 81(1): 99-106.
11. Pechanova O, Bernatova I, Babal P, Martinez MC, Kysela S, Stvrtina S, et al. Red wine polyphenols prevent cardiovascular alterations in L-NAME-induced hypertension. J Hypertens. 2004; 22(8): 1551-9.
12. Tang LX, He RH, Yang G, Tan JJ, Zhou L, Meng XM, et al. Asiatic acid inhibits liver fibrosis by blocking TGF-beta/Smad signaling in vivo and in vitro. PLoS One. 2012; 7(2): e31350.
13. Xu C, Wang W, Xu M, Zhang J. Asiatic acid ameliorates tubulointerstitial fibrosis in mice with ureteral obstruction. Exp Ther Med. 2013; 6(3): 731-6.

Table 1 Effect of asiatic acid (AA) (20 mg/kg/day) on general biological parameters of heart

	BW (g)	HW (mg)	LVT (mg)	LVT/100 g BW (mg/g)
Control	405.6 ± 6.71	1362.7 ± 16.8	936.1 ± 8.82	21.1 ± 5.6
Control + AA	404.2 ± 9.21	1378.8 ± 30.4	910.6 ± 16.62	35.8 ± 5.0
L-NAME	388.7 ± 11.81	1669.9 ± 65.0*	1261.2 ± 32.5*	315.4 ± 10.1*
L-NAME + AA	401.8 ± 9.41	1464.9 ± 25.8#	997.6 ± 11.7#	252.3 ± 8.4#

Results are expressed as mean ± SEM. *$p<0.05$ vs. control group, #$p<0.05$ vs. L-NAME group (n=6/group).

Table 2 Effect of asiatic acid (AA) (mg/kg/day) on wall thickness and cross sectional area of left ventricle

	Wall thickness (mm)	Cross sectional area (mm^2)
Control	2.4 ± 0.11	50.2 ± 2.6
Control + AA	2.5 ± 0.10	50.9 ± 1.1
L-NAME	3.2 ± 0.11*	76.4 ± 4.1*
L-NAME + AA	2.6 ± 0.04#	53.4 ± 0.5#

Results are expressed as mean ± SEM. *$p<0.05$ vs. control group, #$p<0.05$ vs. L-NAME group (n=6/group).

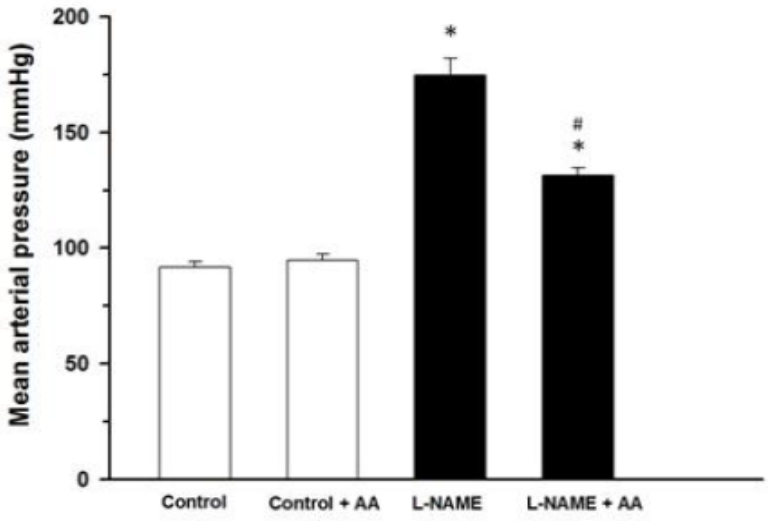

Figure 1 Effect of asiatic acid (AA) (20 mg/kg/day) on MAP in all experimental groups. Results are expressed as mean ± SEM. *$p<0.05$ vs. normal control group, #$p<0.05$ vs. L-NAME group (n=6/group).